

## ACUTE TOXICITY OF *Euphorbia royleana* Boiss (Euphorbiaceae) LATEX ON FRESHWATER CATFISH, *Heteropneustes fossilis* (Siluriformes, Heteropneustidae)

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**Abstract:** ACUTE TOXICITY OF *Euphorbia royleana* Boiss (Euphorbiaceae) LATEX ON FRESHWATER CATFISH, *Heteropneustes fossilis* (Siluriformes, Heteropneustidae). ManiRam Prasad; Abhishek Kumar, Diwakar Mishra, Sunil K. Srivastav and Ajai K. Srivastav. *Acta Toxicol. Argent. (2010) 18 (1): 5-9*. An acute toxicity test was performed by using a four-day static renewal test to determine the LC<sub>50</sub> value of aqueous extract of *Euphorbia royleana* latex for the freshwater fish, *Heteropneustes fossilis*. The LC<sub>50</sub> values, their upper and lower confidence limits and slope functions were calculated. The LC<sub>50</sub> values for aqueous extract of *Euphorbia royleana* latex at various exposure periods were 7.758 mg/L for 24 h, 5.847 mg/L for 48 h, 4.474 mg/L for 72 h and 3.090 mg/L for 96 h. The regression coefficient showed that there was significant negative correlation between exposure time and different LC values. Hence, it is concluded that the concentration to produce toxicity of latex of *Euphorbia royleana* is comparable and close to the concentration to produce toxicity of synthetic organophosphates pesticides for the fish *H. fossilis*. Therefore, adequate precautions must be taken when *Euphorbia royleana* latex is being used near fish-inhabited areas.

**Key words:** Fish; *Euphorbia royleana*; Toxicity; LC<sub>50</sub>

**Resumen:** TOXICIDAD AGUDA DEL LÁTEX DE *Euphorbia royleana* Boiss (Euphorbiaceae) EN EL BAGRE DE AGUA DULCE, *Heteropneustes fossilis* (Siluriformes, Heteropneustidae). ManiRam Prasad; Abhishek Kumar, Diwakar Mishra, Sunil K. Srivastav and Ajai K. Srivastav. *Acta Toxicol. Argent. (2010) 18 (1): 5-9*. La prueba de la toxicidad aguda fue realizada utilizando un test estático con renovación, de cuatro días de duración, para determinar el valor de la CL<sub>50</sub> de un extracto acuoso del látex de *Euphorbia royleana*, en el pez de agua dulce *Heteropneustes fossilis*. Se calcularon el valor de la CL<sub>50</sub>, los límites de confianza máximo y mínimo y la pendiente. Los valores de la CL<sub>50</sub> para el extracto acuoso del látex en varios períodos de exposición fueron 7,758 mg/L para 24 h, 5,847 mg/L para 48 h, 4,474 mg/L para 72 h y 3,090 mg/L para 96 h. El coeficiente de regresión mostró una correlación negativa significativa entre el tiempo de exposición y diferentes valores de la CL<sub>50</sub>. Se concluye que la concentración del látex de *E. royleana* que produce toxicidad es comparable y cercana a la de los plaguicidas sintéticos organofosforados para el pez *H. fossilis*. Por lo tanto, se deben tomar precauciones adecuadas cuando el látex de *E. royleana* es utilizado cerca de áreas donde habita el pez *H. fossilis*.

**Palabras clave:** Peces; *Euphorbia royleana*; Toxicidad; CL<sub>50</sub>

### INTRODUCTION

Now-a-days, botanical compounds are being used as an alternative to synthetic pesticides. These botanicals have been considered as less toxic, less persistent and safe to non-target organisms. At present there are scores of botanical compounds for which there exists no toxicological data. A compound should not be treated as safe only by considering that it is a natural product.

The chemical constituents of plants of the Euphorbiaceae family include triterpenoids and related compounds (sterols, alcohols and hydrocarbons), phenolic compounds (flavonoids, lignans, coumarins, tannins, phenanthrenes, quinones, phenolic acids, etc.), alkaloids,

cyanogenic glucosides and glucosinolates (Abdel-Fattah 1987). Various parts of *Euphorbia royleana* have insecticidal and molluscicidal properties (Abdel-Hamid 2003; Srivastava et al. 2003; Tiwari et al. 2004). The latex of *Euphorbia royleana* has been reported as an irritant to the skin and eye (Basak et al. 2009). Bani et al. (1998) have shown that *Euphorbia royleana* has analgesic and antipyretic properties in rats and rabbits. There exists a single study which determined the acute toxicity of *Euphorbia royleana* for the fish *Channa punctatus* (Singh and Singh 2002). Keeping in view the wide use (Bani et al. 1998; Abdel-Hamid 2003; Srivastava et al. 2003; Tiwari et al. 2004)

of *Euphorbia royleana* (Family: Euphorbiaceae), the present study was designed to assess the toxicity of latex of *Euphorbia royleana* on a freshwater catfish *Heteropneustes fossilis*. *H. fossilis* was selected because it is hardy, readily available, easy to handle and can be kept alive for longer duration in the aquaria. This is a common edible fish and constitutes an important species in many water resources mainly ponds, ditches, swamps, marshes and sometimes occurs in muddy rivers (Rainboth 1996). This species is found in India, Pakistan, Sri Lanka, Nepal, Bangladesh, Burma, Thailand and Vietnam (Berra 2007).

### MATERIAL AND METHODS

Adult freshwater catfish *Heteropneustes fossilis* (both sexes; body wt 24-36 g) were collected locally (from Ramgarh lake, Gorakhpur) and acclimatized to laboratory conditions for 15 days in plastic tanks. They were fed 2-3 times a day with wheat flour pellets and ground dried shrimps.

The white-milky latex of *Euphorbia royleana* was drained into glass tube by cutting the stem and bark. The latex was lyophilized at -40°C and lyophilized powder was stored for further use. For the determination of LC<sub>50</sub> (Median Lethal Concentration i.e. concentration at which 50% mortality occurs) value of lyophilized latex of *Euphorbia royleana* on *H. fossilis* the four-day static renewal acute toxicity test (APHA et al. 1998) was used. 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 (2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0 mg/L) of lyophilized latex of *Euphorbia royleana*. The dried latex powder of *Euphorbia royleana* was weighed and a stock solution (4 mg/ml) 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 of ten fish kept in 30L tap water containing 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. The physicochemical conditions of the tap water used in the experiment were: temperature 25.4

± 2.3 °C; pH 7.32 ± 0.06; hardness 164.56 ± 5.09 mg/L as CaCO<sub>3</sub>; dissolved oxygen 8.15 ± 0.39 mg/L; electrical conductivity 308.18 ± 66.31 mmho/cm and no free chlorine.

At different exposure periods (24, 48, 72 and 96 h), the mortality of the fish was subjected to Probit analysis with the POLO-PC software (LeOra Software) to calculate the LC<sub>10</sub>, LC<sub>50</sub> and LC<sub>90</sub> 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 lyophilized latex of *Euphorbia royleana* for 24, 48, 72 and 96 h has been depicted in Figures 1 to 4. The LC<sub>50</sub> values at the different exposure periods were 7.758 mg/L at 24 h, 5.847 mg/L at 48 h, 4.474 mg/L at 72 h, and 3.090 mg/L at 96 h. The LC<sub>10</sub>, LC<sub>50</sub> and LC<sub>90</sub> values, their upper and lower confidence limits and slope functions are given in Table 1.

In this study, the toxicity of lyophilized latex 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.

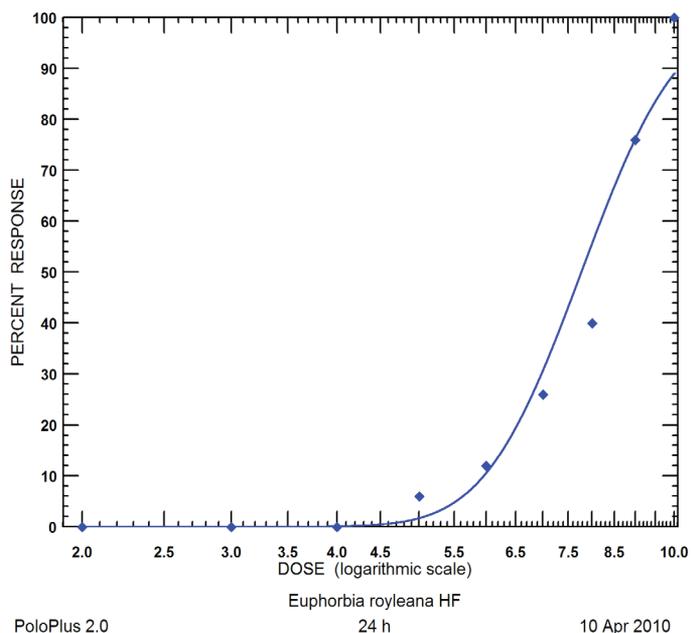
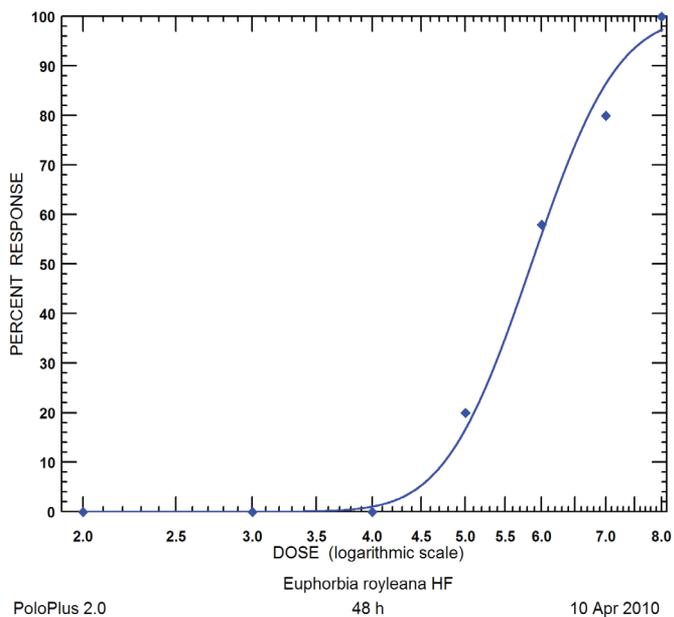
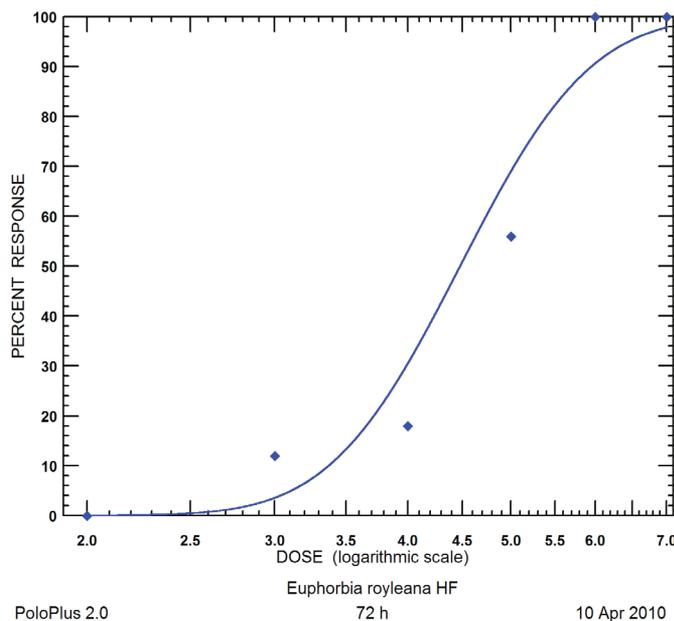


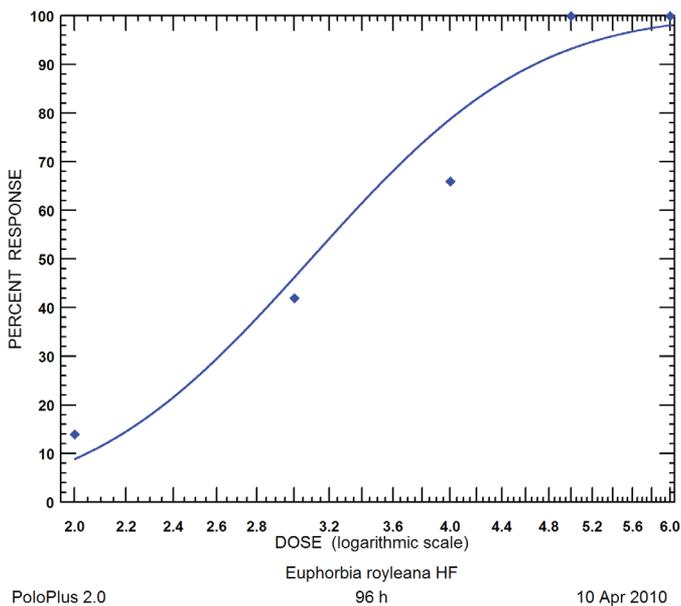
Figure 1. Percent mortality of the fish *Heteropneustes fossilis* after a 24 h exposure to different concentrations of *Euphorbia royleana* latex (mg/L).



**Figure 2.** Percent mortality of the fish *Heteropneustes fossilis* after a 48 h exposure to different concentrations of *Euphorbia royleana* latex (mg/L).



**Figure 3.** Percent mortality of the fish *Heteropneustes fossilis* after a 72 h exposure to different concentrations of *Euphorbia royleana* latex (mg/L).



**Figure 4.** Percent mortality of the fish *Heteropneustes fossilis* after a 96 h exposure to different concentrations of *Euphorbia royleana* latex (mg/L).

**Table 1.** LC<sub>10</sub>, LC<sub>50</sub> and LC<sub>90</sub> values, slope functions and confidence limits for *Euphorbia royleana* latex at different time intervals for the fish *Heteropneustes fossilis*.

Exposure periods	Effective dose (mg/L)	*Limits (mg/L)		Slope Function	't' ratio	Heterogeneity
		LCL	UCL			
24 h	LC10=5.95a3	5.048	6.511	11.145±1.078	10.343	2.5246
	LC50=7.758	7.222	8.376			
	LC90=10.109	9.182	12.125			
48 h	LC10=4.746	4.417	4.994	14.149±1.506	9.394	0.832
	LC50=5.847	5.638	6.052			
	LC90=7.203	6.884	7.666			
72 h	LC10=3.368	1.642	4.039	10.393±1.035	10.038	6.1109
	LC50=4.474	3.515	5.481			
	LC90=5.942	5.002	11.189			
96 h	LC10=2.045	0.750	2.650	7.145±0.748	9.546	3.8656
	LC50=3.090	2.173	3.895			
	LC90=4.671	3.743	9.619			

\*The upper and lower confidence limits for LC<sub>50</sub> values calculated at 0.05 levels.

## DISCUSSION

To our knowledge only few studies exist regarding the LC<sub>50</sub> values of *Euphorbia royleana* on a single fish species *Channa punctatus* (Singh and Singh 2002; Tiwari and Singh 2003, 2004). Singh and Singh (2002) have reported the LC<sub>50</sub> value at 24 h of aqueous extract of *Euphorbia royleana* for *Channa punctatus* as 0.05 g/L. Tiwari and Singh (2003) reported the 24 h LC<sub>50</sub> value of diethyl ether, chloroform, methanol and acetone extracted *Euphorbia royleana* latex on the fish *Channa punctatus* as 16.19 mg/L, 13.34 mg/L, 11.76 mg/L, 12.88 mg/L, respectively. Tiwari and Singh (2004) observed the 96 h LC<sub>50</sub> value of diethyl ether, chloroform, methanol and acetone extracted *Euphorbia royleana* stem bark on the fish *Channa punctatus* as 31.76 mg dried weight (DW)/L, 56.26 mg DW/L, 56.80 mg DW/L, 65.77 mg DW/L, respectively. Considering the LC<sub>50</sub> values for *Channa punctatus*, it seems that *H. fossilis* is very sensitive to *Euphorbia royleana* latex 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*).

The 96 h LC<sub>50</sub> value for *Euphorbia royleana* on *Heteropneustes fossilis* has been estimated to be 3.09 mg/L. This is comparable to the reported LC<sub>50</sub> values for synthetic pesticides like organophosphates. For *H. fossilis* the 96 h LC<sub>50</sub> values for organophosphates are 2.20 mg/L for chlorpyrifos (Srivastav et al. 1997) and 6.60 mg/L for metacid-50 (Mishra et al. 2004). However, the 96 h LC<sub>50</sub> value for *E. royleana* on *H. fossilis* is higher as compared to pyrethroids, since the 96 h LC<sub>50</sub> for *H. fossilis* are 7.20 µg/L for cypermethrin (Mishra et al. 2005) and 1.86 µg/L for deltamethrin (Srivastav et al. 2002; 2009). Hence, it is concluded that the concentration to produce toxicity of latex of *Euphorbia royleana* is comparable and close to the concentration to produce toxicity of organophosphates for the fish *H. fossilis*. Therefore, adequate precautions must be taken when *Euphorbia royleana* latex is being used near fish-inhabited areas.

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