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Original Article

Acute toxicity of *Euphorbia turcomanica* on *Aphanius dispar*

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Abstract: Piscicidal and molluscicidal activities of aqueous extracts of many members of the family Euphorbiaceae are well-known, but the toxicity potential of *Euphorbia turcomanica* was not yet studied on any aquatic animals. An acute toxicity test was performed by using a four-day static renewal test to determine the LC_{50} value of dried powder of *E. turcomanica* for the euryhaline fish, *Aphanius dispar*. The LC_{50} values at various exposure periods are 0.177±0.039 g/L for 24 hrs, 0.131±0.030 g/L for 48 hrs, 0.073±0.018 g/L for 72 hrs, and 0.052±0.013 g/L for 96 hrs. The toxicity of dried powder of *E. turcomanica* exhibits a positive correlation between fish mortality and exposure periods. As this is the first report about toxicity of *E. turcomanica* on *A. dispar*, the results could be only compared to that of other Euphorbiaceae as well as other fishes. It is concluded that the toxicity potential of *E. turcomanica* is comparable and close to that of well-documented Euphorbiaceae. It has been suggested that *E. turcomanica* products cannot be used directly in fish-inhabiting water reservoirs.

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Introduction

Increased awareness of the negative effects caused by overexposure to synthetic organo-piscicides (Reidinger and Russell, 1976) has led to efforts for finding products from plant origin to substitute. Being the products of biosynthesis, they are potentially biodegradable (Marston and Hostettmann, 1985). The Euphorbiaceae is a large families with about 300 genera and 7500 species (Vasas and Hohmann, 2014). Of the member of this family, the genus *Euphorbia* with about 2000 species (Frodin, 2004), is one of the five most species-rich genera of flowering plants (Govaerts et al., 2000). They have a poisonous milky white latex-like sap and unique kind of floral structures. The chemical constituents of these plants, 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; Neuwinger, 2004; Kumar et al., 2010) that are poisonous to target and non-target aquatic organisms (Singh et al., 1996; Ebenso, 2004; Prasad et al., 2010). Many of these plants are cosmopolitan distributing in subtropics and temperate regions (Horn et al., 2012). In the flora of Iran, this genus comprises 70 species, of which 17 species are endemic (Mozaffarian, 1996). *Euphorbia turcomanica* Boiss, is an annual herb, which grows wild in plains of Iran (Mozaffarian, 1996; Pahlevani and Riina, 2011).

Different species of the genus *Euphorbia* are used as insecticide, piscicide, and molluscicide (Sastry and Siddiqui, 1983; Oliveira-Filho and Paumgartten, 2000; Tiwari and Singh, 2004; Tiwari et al., 2004; Singh et al., 2005; Oliveira-Filho et al., 2010; Hassan et al., 2011). Since, there is no information available

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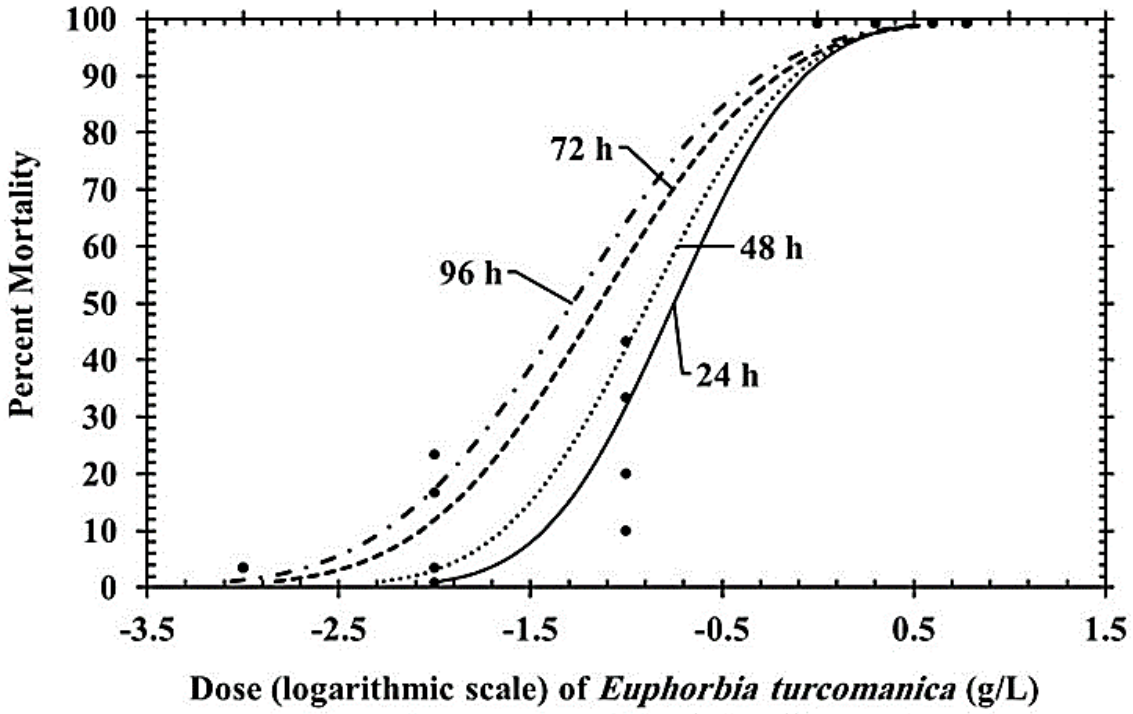

Figure 1. Percent mortality of the fish *Aphanius dispar* after 24, 48, 72 and 96 hrs exposures to different concentrations of *Euphorbia turcomanica* powder (g/L).

regarding the effect of *E. turcomanica* on fishes as piscicid. Therefore, due to wide use of various parts of the members of the family Euphorbiacea (Bani et al., 1997; Abdel-Hamid, 2003; Srivastava et al., 2004; Tiwari et al., 2004), this study was conducted to assess the acute toxicity of the lethal concentrations of aqueous extracts of *E. turcomanica*'s aerial parts on *Aphanius dispar*, an euryhaline fish of Iranian inland water.

Materials and Methods

A total of 240 individuals of *A. dispar* (both sexes: with mean body weight and total length of 2.03±0.5 g, and 47.7±0.45 mm, respectively) were collected from seasonal rivers in Bandar Abbas and transported to the Hormozgan University Fisheries laboratory. In the laboratory, healthy fish were introduced into a 100 L tank with continuous aeration, where they were acclimatize for 14 days to the laboratory conditions. The fish were considered fully acclimatized when no death was observed for seven successive days. The fish were fed 2-3 times a day during this period with commercial pellets, containing protein >28%, lipid >3%, fiber <4% and moisture <10%.

The stem, branches and leaves of *E. turcomanica* were dried in room temperature away from direct sunlight. Then, they were powdered and mixed with water to obtain the required plant concentrations. For the determination of LC_{50} of *E. turcomanica* on *A. dispar*, the four-day static renewal acute toxicity test was performed based on Clesceri et al. (1998). Fish were exposed to 0.001, 0.01, 0.1, 1, 2, 4 and 6 g/L of the dried powder of *E. turcomanica* with three replicates each containing 10 fish (kept in 4 L plastic aquaria). In addition, control group with three replicates in similar stocking density and aquaria were considered for this experiment.

The water was renewed every day and required dried plant was added after water renewal to keep experimental concentrations. The fish were not feed 24 hrs before and during the experiment. Dead fish were counted and removed from the treatments immediately. A toxic effect was determined by a statistically significant decrease in the survival of fish exposed to the plant relative to the survival of

Table 1. Effective dose, confidence limits, and slope function for aqueous extract of *Euphorbia turcomanica* at different intervals on *Aphanius dispar*.

Exposure periods	Effective dose (g/L)	SE	limits		Slope function	'*t*' ratio
			LCL	UCL		
24 hrs	LC_1=0.010	0.004	0.004	0.024		
	LC_5=0.024	0.008	0.012	0.047		
	LC_{10}=0.037	0.011	0.020	0.067		
	LC_{20}=0.063	0.017	0.038	0.106		
	LC_{50}=0.177	**0.039**	**0.115**	**0.272**	1.414±0.229	6.183
	LC_{80}=0.496	0.117	0.313	0.789		
	LC_{90}=0.851	0.228	0.503	1.439		
	LC_{95}=1.327	0.401	0.734	2.400		
	LC_{99}=3.056	1.155	1.457	6.411		
48 hrs	LC_1=0.005	0.002	0.002	0.013		
	LC_5=0.014	0.005	0.007	0.028		
	LC_{10}=0.023	0.007	0.012	0.043		
	LC_{20}=0.041	0.011	0.024	0.071		
	LC_{50}=0.131	**0.030**	**0.084**	**0.204**	1.485±0.226	6.556
	LC_{80}=0.414	0.104	0.253	0.678		
	LC_{90}=0.757	0.219	0.430	1.334		
	LC_{95}=1.246	0.409	0.654	2.373		
	LC_{99}=3.172	1.317	1.406	7.156		
72 hrs	LC_1=0.001	0.001	0.001	0.004		
	LC_5=0.005	0.002	0.002	0.011		
	LC_{10}=0.009	0.003	0.004	0.018		
	LC_{20}=0.018	0.006	0.010	0.033		
	LC_{50}=0.073	**0.018**	**0.045**	**0.118**	1.570±0.219	7.163
	LC_{80}=0.296	0.083	0.171	0.512		
	LC_{90}=0.618	0.202	0.325	1.173		
	LC_{95}=1.133	0.427	0.541	2.373		
	LC_{99}=3.540	1.714	1.371	9.144		
96 hrs	LC_1=0.001	0.000	0.000	0.003		
	LC_5=0.003	0.001	0.001	0.007		
	LC_{10}=0.005	0.002	0.003	0.012		
	LC_{20}=0.012	0.004	0.006	0.023		
	LC_{50}=0.052	**0.013**	**0.032**	**0.087**	1.675±0.228	7.332
	LC_{80}=0.230	0.067	0.130	0.407		
	LC_{90}=0.500	0.172	0.255	0.981		
	LC_{95}=0.949	0.378	0.435	2.070		
	LC_{99}=3.155	1.621	1.153	8.635		

fish in a control. The physico-chemical parameters of the water during experiment, including temperature (23.5±1ºC), dissolved oxygen (4.17±0.1 mg/L), electrical conductivity (840.41±2.46 µS/cm), and pH (8.12±0.03) were measured daily.

At different exposure periods (24, 48, 72 and 96 hrs), the mortality of the fish was subjected to probit analysis using Minitab software (Minitab®16.2.4) to calculate the LC values, their slope functions, and confidence limits.

Results

The percent mortality of the exposed *A. dispar* to

various concentrations of the plant extract of *E. turcomanica* for 24, 48, 72 and 96 hrs are depicted in Figure 1. The LC_{50} values at various exposure periods were 0.177 g/L for 24 hrs, 0.131 g/L for 48 hrs, 0.073 g/L for 72 hrs, and 0.052 g/L for 96 hrs. The LC values, their upper and lower confidence limits, and slope functions are given in Table 1.

The toxicity of dried powder of *E. turcomanica* was found to be time and dose-dependent ($P<0.05$). The regression coefficient demonstrated a significant positive correlation ($P<0.05$) between mortality percent and concentration of *E. turcomanica*. Also a significant negative correlation ($P<0.05$) was found between the exposure time and different LC values.

Discussion

The results revealed that the dried powder of *E. turcomanica* has a high piscicidal activity causing more mortality with increasing its concentration. The toxicity was both time and dose dependent. A significant negative correlation demonstrated between different LC values and exposure time. LC_{50} value of *E. turcomanica* decrease with increasing exposure time in *A. dispar* from 0.177 g/L after 24 hrs to 0.052 g/L after 96 hrs.

To my best knowledge, there is no report on the toxicity effects of *E. turcomanica* on aquatic animals. Although, some reports are present on the toxicity effects of the aqueous and latex extracts of other members of the family Euphorbiaceae on some animals, including fish and molluscs (Singh and Singh, 2002; Singh and Singh, 2005; Tiwari and Singh, 2006; dos Santos et al., 2007; Oliveira-Filho et al., 2010).

The LC_{50} value for 24 hrs of dried powder of *E. tirucalli* stem bark latex for *Colisa fasciatus* was 8.14 mg/L, whereas this value for *Channa punctatus* was 9.01 mg/L (Tiwari et al., 2003). In another experiment, the toxicity of four plants belonging to members of Euphorbiaceae and Apocynaceae on *C. punctatus* evaluated (Singh and Singh, 2005). In this study, 96 hrs LC_{50} values of *E. royleana*, *Nerium indicum*, *Jatropha gossypifolia*, and *Thevetia peruviana* were 0.020, 0.041, 4.34, and 3.17 g/L, respectively (Singh and Singh, 2005). This value for *E. turcomanica* was 0.052 g/L on *A. dispar*. In general, juicy and latex-bearing Euphorbiaceae are more potent in their toxic effects than rotenone-yielding plants (Neuwinger, 2004). In the present study, the dried powder of whole plant is used directly and nevertheless, the LC_{50} values of *E. turcomanica* is comparable in potent toxicity to the other studies. Also, it should be noted that the experimental fish in this study is very hardy comparing to other studies. Therefore, it is suggested that *E. turcomanica* has more toxicity than other species such as *J. gossypifolia* and *T. peruviana*.

It is concluded that dried powder of *E. turcomanica* has a potent piscicidal activity on *A. dispar*. Therefore, enough precautions must be exercised when derivatives of *E. turcomanica* is being used near fish-inhabiting water reservoirs.

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چکیده فارسی

تعیین غلظت های کشنده گیاه فرفیون ترکمنی (*Euphorbia turcomanica*) بر روی ماهی آفانیوس گورخری (*Aphanius dispar*)

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چکیده:
تاکنون تاثیر عصاره‌های آبی حاصل از بسیاری از گونه‌های متعلق به خانواده Euphorbiaceae به‌عنوان سم کشنده ماهی و نرم‌تنان مورد مطالعه قرار گرفته است، با این وجود اطلاعاتی در مورد توان سمیت و کشندگی گیاه فرفیون ترکمنی *E. turcomanica* بر روی موجودات آبزی در دسترس نمی‌باشد. آزمون تعیین غلظت‌های کشنده این گیاه با استفاده از روش آزمایش چهار روزه در محیط محصور به منظور تعیین غلظت‌های LC_{50} پودر خشک شده گیاه *E. turcomanica* بر روی ماهی آفانیوس گورخری *A. dispar*، یک گونه با دامنه تحمل بالای شوری، به اجرا درآمد. مقادیر LC_{50} در زمان‌های مختلف در معرض قرارگیری به ترتیب ۰/۱۷۷±۰/۰۳۹ گرم بر لیتر برای ۲۴ ساعت، ۰/۱۳۱±۰/۰۳۰ گرم بر لیتر برای ۴۸ ساعت، ۰/۰۷۳±۰/۰۱۸ گرم بر لیتر برای ۷۲ ساعت و ۰/۰۵۲±۰/۰۱۳ گرم بر لیتر برای ۹۶ ساعت به دست آمد. سمیت پودر خشک شده گیاه *E. turcomanica* همبستگی مثبتی را بین تلفات ماهی و مدت زمان تاثیرگذاری نشان داد. با توجه به اینکه نتایج این تحقیق اولین گزارش در مورد سمیت گیاه *E. turcomanica* بر روی ماهی *A. dispar* می‌باشد، این نتایج تنها قابل مقایسه با نتایج سایر گونه های Euphorbiaceae بر روی ماهیان دیگر است. از نتایج حاصل می‌توان استنباط نمود که توان سمیت گیاه *E. turcomanica* قابل مقایسه و نزدیک به سایر گونه‌های Euphorbiaceae است که تاکنون مورد بررسی قرار گرفته‌اند. همچنین پیشنهاد می‌شود که ترکیبات تولیدی از گیاه *E. turcomanica* با توجه به سمیت بالای آن به‌طور مستقیم در محیط‌های آبی که محل زیست ماهیان مختلف است، استفاده نگردد.

کلمات کلیدی: فرفیون ترکمنی، پودر خشک، سم کشنده ماهی، ماهی گورخری.