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Toxicity Effects of *N. oleander* and *E. terracina* on *M. tuberculata* and *B. alexandrina* Snails

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تأثير سُمية نبات الدفلة واللبينة على قوقعي *M. tuberculata* و *B. alexandrina*

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Abstract

The molluscicidal activity of different extracts of two species belonging to many families (*Nerium oleader* and *Euphorbia terracina*) was evaluated against two types of snails (*Melonides tuberculata* and *Biomphalaria alexandrina*) in the agricultural area of Taourghaa region, Libya. The results clearly showed that (The Lc50 – Lc90 for two snails and two plants of ethanol extract between (1.26 and 2143.2 *ppm*) interference to ethanol extract, also the $Lc_{50}$and Lc_{90} for two snails and two plants of acetone extract between (100 and 924.6) *ppm*.

Keywords: Molluscicidal, *N. oleader, E. terracina, B. alexandrina, M. tuberculata, Taourghaa region*.

الملخص

دراسة عن المستخلص الكحولي والاسيتوني لنباتي الدفلة واللبينة على قوقعي *Melonides tuberculata* و *Biomphalaria alexandrina* والموجودة بمنطقة عين تاورغاء بليبيا. أظهرت النتائج أنَّ قيمة Lc 50 و Lc 90 للمستخلص الكحولي للنباتين على القوقعين كانت ما بين (1.26 – 2143.2 جزء في المليون) وأن قيمة Lc_{50} و Lc_{90} لنفس القوقعين ونفس النباتين للمستخلص الاسيتوني كانت بين (100 – 924.6 جزء في المليون).

الكلمات الدالة: الدفلة، اللبينة، قوقع *E. terracina*، قوقع *B. alexandrina*، منطقة تاورغاء.

1. Introduction

Human schistosomiasis is a parasitic disease caused by digenetic trematode species of the genus *Schistosoma* which co-habitate the venous plexuses of the mammalian viscera and transmitted by freshwater gastropod molluscs which serve as intermediate hosts (Smith *et al.*, 1989; and Lockyer *et al.*, 2003). In the tropics and subtropics, schistosomiasis is the second most important parasitic disease after malaria in terms of prevalence, public health and socio-

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economic importance (James and Colley, 1995; and Steinmann *et al.*, 2006). *Biomphalaria alexandrina* is a species of air-breathing freshwater snail, an aquatic pulmonate gastropodmollusk in the family Planorbidae. *Biomphalaria alexandrina* serves as an intermediate host for *Schistosoma mansoni*. Man, the definitive host, acquires infection by contact with freshwater infested with *schistosoma* cercariae, which actively penetrate his intact skin (El Ridi, 2002; and Fayez, 2009), and subsequently develop to the adult worms. These cercariae are released into water by infected snails, in which the parasite undergoes asexual larval multiplication. The snails in turn become infected by miracidia released from *schistosome* eggs which reach freshwater with human excrement (Benson, 2008; and Fayez, 2009). *Melanoida stuberculata* is a species of freshwater snail with an operculum, a parthenogenetic, aquatic gastropodmollusk in the family Thiaridae. The common name comes from the presence of reddish spots on the otherwise greenish-brown shell. This species has an elongate, conical shell, which is usually light brown, marked with rust-colored spots. This species is native to subtropical and tropical northern Africa and southern Asia. In Africa it present in Algeria, Burundi, The Democratic Republic of the Congo, Egypt, Eritrea, Ethiopia, Kenya, Libya, Malawi, Morocco, Mozambique, Namibia, Niger, South Africa, Sudan, Swaziland, Tanzania, Tunisia, and Zimbabwe (Benson, 2008). *Melanoides tuberculatus* is known to carry certain parasites which can be dangerous to humans. These snails serve as first intermediate host for parasites which include: *Clonorchissinensis* (Chinese liver fluke), *Paragonimus westermani* (Oriental lung fluke), *Metagonimus*, *Diorchitrema formosanum*, *Opisthor chissinensis*, *Philophthalmus* sp., *Haplorchis* sp., *Centrocestus formosanus* and *Schistosoma* sp. (Nakano *et al.*, 2003). In addition to this species is a host for a trematode parasite which has been found to infect an endangered species of fish in Texas. There is an increased attention for the use of new molluscicides which are highly effective, rapidly biodegradable, less toxic readily available and easily applicable than synthetic molluscicides, so, plant molluscicides could be appropriate for snails, especially in developing countries (Sushma, 1998; and Osman *et al.*, 2007). It was found that many plants are growing in Tourgha region like *Lantana camara*, *Neriumoleader*, *Ricinus comminus*, *Euphorbia terracina*, *Chrozophora tinctoria* and *Hyoscya musalbus* which have may biological activities as antimicrobial, treatment of malaria, rheumatism, and skin rashes (Abdalla *et al.*, 2009; and Sharma *et al.*, 2009).

2. Materials and Methods

2.1. Study Area

This study is conducted in Agricultural Taourghaa region at 240 km east from Tripoli and 38 km from east of Misurata city.

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2.2. Plant Materials

Whole plant materials are collected during October/November 2013, shown in Figures (1 and 2). The plants are identified at the Department of Botany, Faculty of Science, University of Tripoli.

Figure 1. *Nerium oleander* plant

Figure 2. *Euphorbia terracina* plant

2.3. Preparation of Extracts

120 *g* of each coarsely powdered plant is macerated in 300-400 *ml* of methanol (95% vol.) and acetone for 24 *hrs* at room temperature (26-29 ^{o}C). The extract is filtered and the solvents were evaporated under reduced pressure at 40 ^{o}C. Serial dilutions were done from each extract to which snails are exposed (*i.e.* 1000, 850, 700, 500, 300, 150 and 75 *ppm*).

2.4. Snail Collection and Exposure

Melanoides tuberculata and *Biomphalaria alexandrina* which is the intermediate host for *Schistosoma mansoni*. They are examined in the laboratory for patent trematode infections by being placed in glass beakers with clean water, leaves of lettuce, some stones and pump of air, then kept the laboratory for a period up to four weeks and rescreened again (at the end of this period we found that the diameter of snails increased from 0.4 to1.2 *cm*). Only those snails free from any infection and measuring 8-10 *mm* in diameter are used in the laboratory experiments.

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10 snails of each type were placed in alcohol 50 *ml* volume beaker containing 50 *ml* of each concentration. ten snails are put in a separate beaker containing 50ml of distilled water as a control. The beakers are left in the laboratory for 24 *hrs* and then snails were transferred into beakers containing 50 *ml* of distilled water for recovery. They are examined after 24 *hrs* noting the dead as well as live ones. A snail is confirmed dead if it was remained immobile after having been observed for five minutes with the aid of 10 magnification hand lens and either retracted well into or hanged out of the shell, with the body and shell discoloured.

The experiments were carried out at room temperature (26-29 ^{o}C), using a mercuric thermometer. Each experiment was repeated three times.

2.5. Methods of Analysis

Probit regression analysis (by using SPSS) was carried out for all the plants tested to determine the Lc_{50} and Lc_{90} values. The slope of the regression line was used to assess the effect of the extract; the steeper the slope, the more lethal the plant molluscicide effect.

3. Results and Discussion

3.1. Molluscicidal Activity of *Nerium Oleander*:

The comparative susceptibility of the snails: *Melanoides tuberculata*, *Biomphalaria alexandrina* to the action of different extracts (ethanol, and acetone,) from *N. oleander* has been determined.

3.1.1. Ethanol Extract

The effect of various concentrations of ethanol extract of *N. oleander* on adults of *M. tuberculata*, *B. alexandrina* snails after 24 *hrs* exposure are listed in Tables (1, 3, 5, 7, 8, and 10). The results of mortality were statistically analyzed using Probit analysis. The LC_{50} and LC_{90} of this extract against *M. tuberculata* were 1.3 and 256.6 *ppm* respectively. The LC_{50} and LC_{90} of the same extract against *B. alexandrina* were 178.7 There was a difference between molluscicidal activities of ethanol extract of *N. oleander* against three tested snails. *M. tuberculata* were more sensitive to *N. oleander* extract than two other snails.

3.1.2. Acetone Extract

The effect of various concentrations of acetone extract of *N. oleander* on adults of *M. tuberculata*, *B. alexandrina* snails after 24 *hrs* exposure are listed in Tables (2, 4, 6, 9, 11, and 12). The results of mortality were statistically analyzed using Probit analysis. The LC_{50} and LC_{90} of this extract against *M. tuberculata* were 103.3 and 229.0 *ppm* respectively. The LC_{50} and LC_{90} of the same extract against *B. alexandrina* were 100 and 287.7 *ppm* respectively.

3.2. Molluscicidal activity of *Euphorbia terracina*

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The comparative susceptibility of the snails: *Melanoides tuberculata*, *Biomphalaria alexandrina* to the action of different extracts (ethanol, acetone) from *E. terracina* has been determined.

3.2.1. Ethanol Extract

The effect of various concentrations of ethanol extract of *E. terracina* on adults of *M. tuberculata*, *B. alexandrina* snails after 24 *hrs* exposure are listed in Table (1). The results of mortality were statistically analyzed using Probit analysis. The Lc_{50} and Lc_{90} of this extract against *M. tuberculata* were 56.2 and 243.22 *ppm* respectively. The Lc_{50} and Lc_{90} of the same extract against *B. alexandrina* were 44.2 and 176.2 *ppm* respectively as shown in Table (3).

Table 1. Mortality rates among *M.tuberculata* and *B.alexandrina* snails (N=10) exposed to different concentrations of alcoholic extract of *Euphorbia terracina*

Dose of extract in (ppm)	***Melanoides tuberculata***				***Biomphlaria alexandrina***			
	Mortality %				***Mortality %***			
	Exp.1	***Exp.2***	***Exp.3***	***mean***	***Exp.1***	***Exp.2***	***Exp.3***	***mean***
75	70	60	70	66.6	70	80	70	73.3
150	70	80	80	76.6	80	80	90	83.3
300	100	100	100	100	100	90	100	96.6
500	100	100	80	93.3	100	100	100	100
700	100	100	100	100	100	100	100	100
850	100	100	100	100	100	100	100	100
1000	100	100	100	100	100	100	100	100

Table 2. Mortality rates among *M.tuberculata* and *B.alexandrina* snails (N=10) exposed to different concentrations of acetone extract of *Euphorbia terracina*

Dose of extract in (ppm)	***Melanoides tuberculata***				***Biomphlaria alexandrina***			
	Mortality %				***Mortality %***			
	Exp.1	***Exp.2***	***Exp.3***	***mean***	***Exp.1***	***Exp.2***	***Exp.3***	***mean***
75	20	20	20	20	30	20	30	26.6
150	10	20	10	13.3	10	30	20	20
300	20	10	20	16.6	20	20	10	16.6
500	30	20	20	23.3	20	30	30	26.6
700	90	100	90	93.3	90	100	100	96.6
850	100	100	100	100	100	100	100	100
1000	100	100	100	100	100	100	100	100

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3.2.2. Acetone Extract

The effect of various concentrations of acetone extract of *E. terracina* on adults of *M. tuberculata*, *B. alexandrina* snails after 24 *hrs* exposure are listed in Table (2). The results of mortality were statistically analyzed using Probit analysis. The Lc_{50} and Lc_{90} of this extract against *M. tuberculata* were 311.9 and 924.7 *ppm* respectively. The LC_{50} and LC_{90} of the same extract against *B. alexandrina* were 273.5 and 857.0 *ppm* respectively as shown in Table (4).

Table 3. Mortality rates among *M. tuberculata* and *B. alexandrina* snails (N=10) exposed to different concentrations of alcoholic extract of *Nerium oleander*

Dose of extract in (ppm)	Melanoides tuberculata				Biomphlaria alexandrina			
	Mortality %				Mortality %			
	Exp.1	Exp.2	Exp.3	mean	Exp.1	Exp.2	Exp.3	mean
75	10	20	10	13.3	0.0	10	0.0	3.3
150	70	80	70	73.3	50	60	50	53.3
300	90	60	80	76.6	70	50	60	60
500	100	100	100	100	100	100	100	100
700	100	100	100	100	100	100	100	100
850	100	100	100	100	100	100	100	100
1000	100	100	100	100	100	100	100	100

Table 4. Mortality rates among *M. tuberculata* and *B. alexandrina* snails (N=10) exposed to different concentrations of acetone extract of *Nerium oleander*

Dose of extract in (ppm)	Melanoides tuberculata				Biomphlaria alexandrina			
	Mortality %				Mortality %			
	Exp.1	Exp.2	Exp.3	mean	Exp.1	Exp.2	Exp.3	mean
75	40	50	40	43.3	50	40	50	46.6
150	60	70	60	63.3	70	60	60	63.3
300	80	90	90	86.6	90	80	80	83.3
500	100	100	100	100	90	100	90	93.3
700	100	100	100	100	100	100	100	100
850	100	100	100	100	100	100	100	100
1000	100	100	100	100	100	100	100	100

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Table 5. Toxicity of the *Nerium oleander* alcohol extract against the snail Biomphalaria *alexandrina*

Concentration	*Mortality (%)*	*Log conc. (X)*	*Probit (Y)*
75	10	1.875	3.72
150	53.3	2.176	5.08
300	60	2.477	5.25
500	100	2.699	8.09

b=4.489556, Lc_{50} = 1.269 *ppm*, and Lc_{90} = 178.66 *ppm*

Table 6. Toxicity of the *Nerium oleander* acetone extract against the snail *Biomphalaria alexandrina*

Concentration	*Mortality (%)*	*Log conc. (X)*	*Probit (Y)*
75	46.7	1.875	4.90
150	63.3	2.176	5.33
300	83.3	2.477	5.95
500	93.3	2.699	6.48
700	100	2.845	8.09

b=3.68873, Lc_{50} = 100 *ppm*, and Lc_{90} = 287.74 *ppm*

Table 7. Toxicity of the *Nerium oleander* alcohol ext1ract against the snail *Melanoides tuberculatum*

Concentration	*Mortality (%)*	*Log conc. (X)*	*Probit (Y)*
75	13.3	1.875	3.87
150	73.3	2.176	5.61
300	76.2	2.477	5.71
500	100	2.699	8.09

b = 4.489556, Lc_{50} = 1.269 *ppm*, and Lc_{90} = 256.567 *ppm*

Table 8. Toxicity of the *Euphorbia terracina* alcohol extract against the snail *Biomphalaria alexandrina*

Concentration	*Mortality (%)*	*Log conc. (X)*	*Probit (Y)*
75	73.3	1.875	5.61
150	83.3	2.176	5.95
300	96.7	2.477	6.75
500	100	2.699	7.33

b = 2.131, Lc_{50} = 44.157 *ppm*, and Lc_{90} = 176.16 *ppm*

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Table 9. Toxicity of the *Euphorbia terracina* acetone extract against the snail Biomphalaria *alexandrina*

Concentration	*Mortality (%)*	*Log conc. (X)*	*Probit (Y)*
75	26.7	1.875	4.36
150	20	2.176	4.16
300	16.7	2.477	4.01
500	26.7	2.699	4.36
700	96.7	2.845	6.75
850	100	2.929	7.33

b = 2.583, Lc_{50} = 273.527 *ppm*, and Lc_{90} = 857.04 *ppm*

Table 10. Toxicity of the *Euphorbia terracina* alcohol extract against the snail *Melanoides tuberculata*

Concentration	*Mortality (%)*	*Log conc. (X)*	*Probit (Y)*
75	66.7	1.875	5.41
150	76.7	2.176	5.71
300	96.7	2.477	6.75
500	93.3	2.699	6.78
700	100	2.845	7.33

b=2.1011, Lc_{50} = 156.234 *ppm*, and Lc_{90} = 2143.22 *ppm*

Table 11. Toxicity of the *Nerium oleander* acetone extract against the snail *Melanoides tuberculata*

Concentration	*Mortality (%)*	*Log conc (X)*	*Probit (Y)*
75	43.3	1.875	4.82
150	63.3	2.176	5.33
300	86.7	2.477	6.08
500	100	2.699	8.09

b= 3.68873, Lc_{50} = 103.276 *ppm*, and Lc_{90} = 229.01 *ppm*

The toxicity values of ethanol extracts from different plants are arranged in a decreasing order as follows, *Letium. camara Euphnorbia terracina*, and the toxicity value of the acetone extracts from two plants is *Nerium oleander*.

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Table 12. Toxicity of the *Euphorbia terracina* acetone extract against the snail *Melanoides tuberculata*

Concentration	*Mortality (%)*	*Log conc. (X)*	*Probit (Y)*
75	20	1.875	4.16
150	13.3	2.176	3.87
300	16.7	2.477	4.01
500	23.3	2.699	4.26
700	93.3	2.845	6.48
850	100	2.929	7.33

b = 2.1709, Lc_{50} = 311.889 *ppm*, and Lc_{90} = 924.698 *ppm*

The molluscicidal activity of most the tested plants extracts is probably due to the presence of alkaloids, flavonoids terpenoids as well as phorble esters in *E. terracina* possess molluscicidal, properties (Abdel Gawad *et al*., 2000; Mohamed and Refahy, 2001; Refahy, 2002; and Shi *et al*., 2008).

In the potential molluscicides derived from local plants have attracted the attention due to high costs of imported synthetic molluscicides. Treatment of *Schistosoma* and *Fasciloa* infections remains highly problematic. In *schistosomiasis*, praziquantel is faced with failure to prevent reinfection as a result of development of drug resistance *schistosoma* strain and serious side effects, treatment of Fasciloe requires high or multiple doses of drug with frequent side effects (Refhy, 2002) These studies are usually in accordance with our study.

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