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DISCOVERY OF A HIGHLY ACTIVE MOLLUSCICIDAL EXTRACT AGAINST LAND SNAILS, FROM *Nerium Oleander* L.

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ABSTRACT

Nerium oleander L. is the widest spread ornamental plant; it is cultivated every where in Egypt as well as in the whole world. We noticed that snails living very close to the plant do not approach it. A chloroformic cardenolide extract was isolated from the leaves of the plant after removing the other constituents with lead acetate; the isolated extract was tested for its molluscicidal activity against the harmful land snails, *Theba pisana* (Muller) and *Eobania vermiculata*. The extract was highly active against *T pisana* with LD_{50} value of 12.039 µg / gm of body weight, while *E vermiculata* was less sensitive to the extract, with LD_{50} value of more than 100 µg / gm of body weight. The results indicate that *N oleander* is a good source of natural molluscicidal extracts, or compounds which are more toxic to *T pisana* snails than the present used synthetic pesticides, methomyl and methiocarb.

INTRODUCTION

Nerium oleander L. is an ever green shrub growing to 4m by 4m, it flowers from June to October, the plant grows in different types of soils and climates and can tolerate drought. *N oleander* is the most wide spread ornamental plant in the world; in Egypt, it is cultivated in streets, in private and public gardens and on the edges of water canals and streams, it is used for informal hedging. The leaves and the flowers of the plant are cardio tonic, diaphoretic, diuretic, emetic and expectorant (Chiej, 1984). Many investigators mentioned that the plant is used as a rat poison, a parasiticide and insecticide (Chiej, 1984; Polunin, 1969). The plant is toxic to man and mammals (Al-Yahya *et al.*, 2000; Adam *et al.*, 2001; Hossain et al, 2004). *N oleander* contains many cardiac glycosidal compounds (Abe and Yamauchi, 1992; Siddiqui et al, 1986). Many insect species feed on the plant (Euw and Reichstein, 1971; Rothschild *et al.*, 1970). We have noticed that snails living on plants very close to *N oleander* do not approach the plant. Therefore, this work was carried out in the Plant Protection Research Station, Sabbahia, Baccous, Alexandria, to isolate the chemical group responsible for the molluscicidal activity of this plant. This work is a continuation to, and supports our previous efforts to discover molluscicidal plants that are more toxic to snails than some used conventional pesticides (Hussein *et al.*, 1994; Hussein *et al.*, 1999; Hussein and El-Wakeel, 1996).

MATERIALS AND METHODS

Isolation of the molluscicidal extract: Leaves of *N oleander* were air- dried and ground using an electrical mill. Ground leaves (325 gm) were extracted with 2 liters of methanol at room temperature overnight. The methanol extract was concentrated under reduced pressure to about 400 ml and kept at 4 °C for 24 h, the soluble layer was decanted and diluted with 250 ml of distilled water; the resulting precipitate was isolated by filtration and discarded. Forty ml of lead acetate (10 %) was added followed by ammonium sulfate (30 %). After filtration, the solution was adjusted to pH 7 by sodium carbonate (5 %). The neutral solution was extracted with chloroform, 200 ml. The chloroform extract was dried through a column of anhydrous sodium sulfate; the solvent was evaporated under reduced pressure to give a light, green, crystallized residue, which gave strong positive reactions with Rayamond and Kedde Reagents. This crystallized extract was used for the molluscicidal tests.

Test animals: Adult animals of *T pisana* and *E vermiculata* were collected from a small green house used for snail breeding in Sabbahia Research Station, and kept at room temperature many days before test.

Contact toxicity test: toxicity tests were performed according to our method (Hussein *et al.*, 1994), by applying the required dose dissolved in 10 % ethanol (containing 0.03 % Tween 80) to the snail's body through the orifice of the shell, by the aid of a micropipette. Control animals received solvent only; three replicates were used in every dose with 10 animals in every replicate in case of *T pisana* and 5 animals in case of *E vermiculata*. Animals were kept at room temperature in 0.5 liter plastic cups provided with lettuce leaves and dead animals were counted 24 h after treatment according to the method of WHO, 1965. Probit analysis was done according to Finney, 1971.

RESULTS AND DISCUSSION

The results shown in Tables 1 and 2 indicate the strong, very promising molluscicidal activity of the cardenolide extract isolated from the leaves of *N oleander* against *T pisana*. *E vermiculata* was less sensitive to the extract with LD_{50} value of > 100 µg / gm of body weight (Tables 1 and 2). The LD_{50} of the extract against *T pisana* was 12.039 µg / gm of body weight and the LD_{95} was 34.76 µg / gm of body weight. These results show that the tested extract is more toxic to *T pisana* than the most famous commercial molluscicides, methomyl, which had LD_{50} value of 114.26 µg / gm of body weight (Hussein and El-Wakeel, 1996), and methiocarb which had LD_{50} value of 107.34 µg / snail (El-Zemity, 2001). This means that the tested extract is 9.5 times more toxic to *T pisana* than methomyl and 8.9 times more toxic than methiocarb. Methomyl caused 20 % mortality in *T pisana* after 24 h at 100 µg / snail (Abdelgaleil, 2005), while the tested extract caused 90 % mortality at 30 µg / gm (Table 1).

Up to date, there is not any report on any commercial pesticide that has molluscicidal activity equal to uscharin (Hussein *et al.*, 1994), or equal to the activity of the tested extract in this study. The results of this study provide

the bases for a new source of highly active, natural molluscicides that could be exploited in controlling the harmful snails, *T pisana* in the form of crude extracts, or after separating and isolation of the active components found in these extracts. The results also agree well with our previous findings on the strong molluscicidal activity of the cardenolide compounds or extracts derived from some plant species (Hussein *et al.*, 1994; Hussein and El-Wakeel, 1996; Hussein *et al.*, 1999).

Table 1. Mortality percent versus doses (µg / gm) of tested *N oleander* extract against *T pisana* and *E vermiculata*.

Species	Dose (µg / gm)	Mortality %
T pisana	6.25	16.6
	12.5	46.6
	20	85
	30	90
E vermiculata	100	0.0

Table2. Probit analysis for toxicity of tested *N oleander* extract against *T pisana and E vermiculata*

Species	LD_{50} (95 % FL) (µg / gm)	LD_{95} (95 % FL) (µg / gm)	Slope ± SE
T pisana	12.039 (10.87-13.21)	34.76 (29.59-43.24)	3.57 ± 0.317
E vermiculata	> 100		

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اكتشاف مستخلص عالى الفاعلية ضد القواقع الأرضية من نبات الدفلة

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ينتشر نبات الدفلة فى كل مكان فى مصر وفى كل أنحاء العالم كنبات زينة. وقد لوحظ أن القواقع الارضية لا تقترب من أوراق النبات . تم عزل مستخلص يحتوى مجموعة الجليكوسيدات القلبية من أوراق النبات بعد التخلص من مكونات النبات الاخرى بواسطة خلات الرصاص . تم اختبار فعالية هذا المستخلص ضد القواقع الارضيه تيبا بيسانا و أوبانيا فيرمكيولاتا و كان المستخلص عالى الفاعليه ضد قوقع تيبا بيسانا حيث كانت قيمة الجرعة القاتلة النصفية تساوى ٠,٣٩ و ١٢ ميكروجرام/ جم من وزن الجسم بينما كان قوقع اوبانيا فيرمكيولاتا اقل حساسية كانت قيمة الجرعة القاتلة النصفية أكبر من ١٠٠ ميكروجرام/ جم من وزن الجسم. وتدل النتائج على أن نبات الدفلة يعتبر مصدر هام لمستخلصات او مركبات أكثر سمية ضد قوقع تيبا بيسانا ، من المبيدات المصنعة مثل الميثوميل و المثيوكارب.