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Toxicity of *Nerium oleander* extracts against *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae)

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Abstract

Laboratory evaluation of 70% Hydroethanolic extracts of *Nerium oleander* (leaves, stems and flowers) against the 1st instar larvae of *Pectinophora gossypiella* (Suanders). Stem extract was the most toxic on the tested insect. Leaves and stems prolonged the larval period while leaves and flowers prolonged the pupal period. The three extracts decrease the larval and pupal weight compared with the control weight. The contents of some heavy metals in the extracts were measured by ICP. The highest metal content of leaves was the Hg (16.27 mg/ 0.5g extract), but the highest metal content was Fe (0.689 mg/ 0.5g extract) in the steam and Ni (8.177 mg/ 0.5g extract) in the flowers.

Keywords: Heavy metals, *Nerium oleander*, *Pectinophora gossypiella*, Toxicity.

Introduction

Nerium oleander is an evergreen shrub or small tree in the dogbane family Apocynaceae. It is known as oleander from its superficial resemblance to the unrelated plant *Olive olea* but has many other names like *Nerium indicum* Mill., and *Nerium odorum* Soland. The white and red flowered variety is equated with *Nerium indicum* (**Chaudhary, *et al.*, 2015**).

The pink bollworm (PBW), *P. gossypiella* (Saunders) (Lepidoptera: Gelechiidae) is considered a destructive and key pest of the bolls of cotton crop in Egypt and most cotton producing countries which causes great damage to the quality and quantity of cotton yield (**El-Naggar, 2003**).

The metal which has a relatively high density and toxic at low quantity is referred as 'heavy metal', e.g., arsenic (As), lead (Pb), mercury (Hg), cadmium (Cd), chromium (Cr), thallium (Tl), etc. Some 'trace elements' are also known as heavy metals, e.g., copper (Cu), selenium (Se) and zinc (Zn) (**Suganya *et al.*, 2016).**

The present work aimed to investigate and find the relation between the content of some heavy metal elements (As, Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Zn) in 70% ethanolic extracts of *Nerium oleander* and the degree of toxicity against the Pink Bollworm, *P. gossypiella.*

Materials and Methods

Plant material

The plant *N. oleander* was collected from Khulias, Jeddah, Kingdom of Saudi Arabia.

Preparation of extracts

The fresh flowers, stems and leaves of *N. oleander* (50 g) were grounded separately and then macerated in 300 ml of 70% aqueous ethanol solution. After leaving the solutions 7days, they were filtered through Whatman No. 40 filter paper. The solvents were removed under reduced pressure using a rotary evaporator to obtain 3.47g extract for the flower, 2 g extract for the stems and 2.6g extract for leaves.

Insect used

The 1st instar larvae of PBW, used in this study, was obtained from a standard laboratory colony, reared at Bollworm Department, Plant Protection Research Institute; Agriculture Research Centre (ARC), Giza, Egypt on an artificial diet for several generations away from any insecticidal contamination (**Rashad and Ammar, 1985**).

Toxicity assay

Response of the newly hatched larvae of *P. gossypiella* was studied. Serial concentrations of 20, 10, 5 and 2.5 (g extract/ 100g diet) were prepared. Fourty newly hatched larvae were transferred individually to the surface of the treated diet, the live larvae transferred after 6 hours on untreated diet and kept in glass tubes (2 x 7.5 cm). The same procedure was done against the newly hatched larvae with untreated diet and used as control. All tubes were capped with cotton stopper and incubated at 26±2 ºc and 70-85% RH and inspected daily until pupation.

Mortality was recorded at intervals after 1, 5 and 7 days after larval feeding. Mortalities percentages were corrected according to Abbott formula in each case (**Abbott, 1925**).

The LC_{50} concentration was used to investigate the effect of *Nerium oleander* extracts on the development stages of *P. gossypiella* larval and pupal duration, weight, and their reduction in weight were calculated as follow: Weight reduction = (weight of control – weight of treatment / weight of control) X 100.

Digestion of Plant extract and Analysis of Heavy Metals

In brief, 0.5 gm of the extract was digested by using 5 ml of concentrated HNO3. The sample was digested until the clear solution was obtained. The digested solution was diluted to the final volume of 100 ml with distilled water and then heated with continuous shaking for 30 min. The sample solution was then filtered through membrane (0.45micron) filter. Finally, the digested samples were used for metal analysis using Inductively Coupled Plasma Mass Spectrometry (Optima 8300 ICP-OES).

Results and Discussion

The insecticidal activity of the leave extract against the 1st instar larvae of *P. gossypiella* was recorded in table (1). The results showed that the toxic effect of the extract against the insect increased with the increase of concentration. The highest percentage of mortality was 80 (g extract/ 100g diet) at the concentration 20 (g extract/ 100g diet).

Table (1): Toxic effect of *N. oleander* leave extract against the 1st instar larvae of *P. gossypiella*

	Corrected Mortality %		
Conc.	**After 1day**	**After 5 days**	**7 days**
20	80	80	80
10	65	65	70
5	55	60	60
2.5	25	25	25
control	0.00	0.00	0.00
LC_{50}	5.47	5.16	4.95
Slope	1.55±0.99	1.52±0.92	1.61±0.94

Values in the same column with different letters were significantly different (ANOVA, Duncan's multiple range test $P < 0.05$).

Data in table (2) describe the toxicity of stems extract against the 1st instar larvae of *P. gossypiella*. The concentration 20% was the most effective against the tested insect.

Table (2): toxic effect of *N. oleander* stems extract against the 1st instar larvae of *P. gossypiella*

Corrected Mortality			
Conc.	**After 1day**	**After 5 days**	**7 days**
20	95	95	95
10	60	65	65
5	40	55	55
2.5	30	45	50
control	0.00	0.00	0.00
LC_{50}	5.61	3.71	3.33
Slope	1.96±0.92	1.56±0.96	1.40±0.91

Values in the same column with different letters were significantly different (ANOVA, Duncan's multiple range test $P < 0.05$)

Table (3) explains the toxic effect of *N. oleander* flower extract against the 1st instar larvae of *P. gossypiella***.** The extract was effective at all concentrations used. The toxic effect increased with the increasing of concentration.

Table (3): toxic effect of *N. oleander* flower extract against the 1st instar larvae of *P. gossypiella*

Corrected Mortality			
Conc.	After 1day	After 5 days	7 days
20	90	95	95
10	80	80	80
5	60	63.33	63.33
2.5	16.67	26.67	26.67
control	0.00	0.00	0.00
LC_{50}	4.90	4.14	4.14
Slope	2.44±0.94	1.94±0.95	2.49±0.98

In this respect, **Lokesh *et al*., (2010)** found that the leaf extract of *N. oleander* contains larvicidal activity for the most available genera in the survey. In addition, **Ali *et al*., (2008)** found that *T. granarium* larvae showed 10% mortality after 72 hours at 100mg dose level of *N. oleander* leaf extract. Also, Hussain ***et al*., (1996**) reported that treated *Spodoptera littoralis* with *N. oleander* extract induced remarkably increase mortality.

And**,** *N. oleander* ethanolic extracts showed insecticidal activity against rice weevil, Sitophilus oryzae infesting stored wheat and rice (**Satpathi *et al*., 1992**). **Fouad *et al*. (2015)** concluded that the larvicidal activity of hydroethanolic extract of *N. oleander* on *C. pipiens* could be due to the major components. This would include the flavonoids, the sterols, and the terpenes. And**, Trease and Evans (2002); Tiwari and Singh (2004**) found that the leaves of *N. oleander* contain a mixture of very toxic cardiac glycosides of cardenolides like oleandrin, oleandrigenin, digoxin, digitonin, digitoxigenin, nerizoside, neritaloside, and odoroside. **Semiz, (2017)**, indicated that *N. oleander* leaves extract can be used as a commercial insecticide against *Thaumetopoea wilkinsoni*.

The duration and weight of larvae and pupae of newly hatched larvae, treated with the LC_{50} concentrations of *N. oleander* extracts are shown in table (4). The average of larval duration for the larvae treated with *N. oleander* stems, leaves and flowers extracts was 27.15,

26.69 and 17.14 days, respectively, while the control recorded 16.01 days. The average of pupal duration for the larvae treated with *N. oleander* leaves and flowers was significantly differs than control. All the extracts recorded a significantly reduction in larval and pupal weight when compared with the control weight.

Table (4): Effect of LC_{50} of *N. oleander* on the developmental parameters of *P. gossypiella* under laboratory conditions

Treatments	Larvae duration			Pupal period		
	Duration days (Mean ±SD)	Weight (g) (Mean ±SD)	W.R %	Pupal duration (days) (Mean ±SD)	Weight (g) (Mean ±SD)	W.R%
Stems	27.15^{a}±0.23	0.027^{c}±0.0005	42.55	8.25^{c}±0.20	0.021^{c}±0.003	42.55
Leaves	26.69^{b}±0.35	0.022^{d}±0.003	53.19	12.62^{a}±0.27	0.0203^{c}±0.004	44.04
Flowers	17.14^{c}±0.21	0.039^{b}±0.004	17.02	11.90^{b}±0.37	0.0256^{b}±0.002	32.77
Control	16.01^{d}±0.58	0.047^{a}±0.005	0.00	7.97^{c}±0.23	0.041^{a}±0.0042	0.00
F value	6968.26***	568.46***		232.34***	696.94***	
LSD	0.23	0.0015		0.51	0.0012	

Values in the same column with different letters were significantly different (ANOVA, Duncan's multiple range test $P < 0.05$)

The data in table (5) represents the heavy metal contents in ethanolic extract of *N. oleander* different parts. The metals (As, Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Zn) contents were measured by ICP instrument. The highest metal content of leaves was Hg16.27 (mg/ 0.5gm extract), but the highest metal content was Fe 0.689 (mg/ 0.5gm extract) in the steam and Ni was 8.177 (mg/ 0.5gm extract) in the flowers.

Table (5): The heavy metal contents in ethanolic extracts of *N. oleander*

Element	Concentration (mg/ 0.5gm extract)		
	Leaves	Stems	Flowers
As	0.492	0.00	0.301
Cd	0.048	0.00	0.00
Co	0.098	0.012	0.027
Cr	0.110	0.458	0.099
Cu	0.808	0.00	0.00
Fe	1.111	0.689	3.356
Hg	16.27	0.102	5.475
Mn	0.338	0.090	0.127
Ni	0.187	0.019	8.177
Pb	0.00	0.00	0.00
Zn	0.746	0.00	0.250

Heavy metals received major attention because of their persistent toxic effect (**Lagrana *et al*., 2011**). The toxic effect of some heavy metals against insects was previously reported by **Suganya *et al*., (2016)**; ***Baghban* (2014)**; **El-Sheikh *et al*., (2010)** and **Kitvatanachai (2005)**. **Lagrana *et al*., 2011**, reported the toxic effect of Cu, Cd and Pb against chironomids. **Yousef *et al*., 2016** reported the toxic effect of some heavy metals present in *Calotropis procera* and *Ocimum sanctum* against *Pectinophora gossypiella* (Saunds.).

Conclusion

In conclusion, the results of the present study provide new data focusing on insecticidal efficacy of *N. oleander* extracts on *P. gossypiella*. Such findings may encourage a further research to test *N. oleander* extracts effects against non-target organisms in the environment.

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