Original Article

Larvicidal, Biological and Genotoxic Effects, and Temperature-Toxicity Relationship of Some Leaf Extracts of *Nerium oleander* (Apocynaceae) on *Culex pipiens* (Diptera: Culicidae)

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Abstract

Background: The present study was undertaken to study the larvicidal activity of different extracts of *Nerium oleander* leaves, and post-treatment temperature- toxicity relationship of these extracts against *Culex pipiens*. Further, the most potent extract was used to evaluate its biological and genotoxic activities.

Methods: Crude extracts of *N. oleander* leaves were prepared using water, chloroform, acetone and diethyl ether as solvents. Extraction was carried out using soxhlet apparatus. Bioassay test was carried out on the larvae, and the LC_{50} of each extract was determined. Thus, newly hatched first instar larvae were treated, and the mortality count was recorded daily till pupation (accumulated mortality). The LC_{50} of diethyl ether extract, as the most potent extract, was used for the further biological and genotoxic studies.

Results: The results obtained indicated that diethyl ether extract of *N. oleander* leaves was the most potent extract, with LC_{50} of 10500 mg/l. The toxicity of the four extracts, using the LC_{50}, at 10 °C was higher than that at 35 °C. The LC_{50} of diethyl ether extract significantly decreased the larval duration, pupal duration, percentage of pupation, percentage of adult emergence, longevity of females, fecundity, and oviposition activity index, whereas the growth index and the percentage of development per day of larvae and pupae were significantly increased compared to non-treated insects. Moreover, treatment with this extract induced significant dominant lethality in both male and female adults.

Conclusion: It appears that diethyl ether extract of *N. oleander* leaves is potential control agent to *Cx. pipiens*.

Keywords: *Culex pipiens*, Genotoxicity, Larvicidal activity, *Nerium oleander*, Temperature-toxicity relationship

Introduction

Culex pipiens complex inhabits Europe, mainly the extra-tropic parts of Asia and Africa, the middle part of North America, the southern one-third of South America and Australia. It is wide spread in Egypt, where both autogenous and anautogenous forms co-exist (Vinogradova 2000). This mosquito transmits many diseases to man and animals including Rift Valley fever, lymphatic filariasis (*Wuchereria bancrofti*), St Louis encephalitis and West Nile virus (Vinogradova 2000).

Prevalence of mosquito-borne diseases is one of the world's most health hazardous problems. In the absence of an effective vaccine/antiviral therapy, at present vector control is the only way to limit the mosquito-borne diseases (Gautam et al. 2013). In this scenario, conventional pesticides such as organophosphorous and pyrethroids are generally used for mosquito control but their indiscriminate usage may cause environmental pollution, residual effect, resistance in mosquito species and adverse effects on human health. These problems forced to search for new control measures especially from plant sources, as plant-derived molecules are eco-friendly, biodegradable, target specific and development of resistance by vectors against them has not been reported so far (Gautam et al. 2013). Extracts of *N. oleander* leaves, an

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evergreen flowering shrub which grows in the Mediterranean tropical and subtropical regions (Raveen et al. 2014), have shown potential in this respect against mosquitoes (Komalamisra et al. 2005, Lokesh et al. 2010, Kumar et al. 2012, Roni et al. 2013, Madhuri et al. 2013, Raveen et al. 2014).

The present study aimed to elucidate the larvicidal activity of water, chloroform, acetone and diethyl ether extracts of *N. oleander* leaves and post-treatment temperature-toxicity relationship to *Cx. pipiens*. Further, the most potent extract was used to evaluate its biological and genotoxic activities.

Materials and Methods

Insect rearing

The stock colony of *Cx. pipiens* complex was maintained in the laboratories of Entomology Department, Faculty of Science, Cairo University, Egypt, at 25±2 °C, 65±5% RH and 12: 12 h (L: D) photoperiod for several years without exposure to insecticides. Adults were kept in wooden cages (30× 30× 30 cm) and offered 10% sucrose solution through a piece of sponge for a period of 3–4 days after emergence. Females were then allowed to take a blood meal from a pigeon for 2–3 h for egg production. Oviposition occurred in a plastic cup (150 ml) containing distilled water (Das et al. 2007). The resulting egg rafts were picked up and transferred into plastic dishes (25× 30×15 cm) containing distilled water and covered with muslin cloth. The hatching larvae were fed daily on the tropical fish food (Tetramin®). Water was aerated daily using an air bubbler to avoid scum formation, and evaporated water was replaced as needed to maintain volume.

Preparation of stock extracts

The leaves of *N. oleander* were collected from Giza Governorate, Egypt, and identified in Botany Department, Faculty of Science, Cairo University, in comparison with herbarium sheets of the authentic sample. The plant leaves were thoroughly washed with distilled water, shade-dried at room temperature for about two weeks and powdered finely using a blender. The resulting powder was soaked separately, for a week at room temperature, in a tightly sealed conical flask (one liter) containing water, chloroform, acetone and diethyl ether at a ratio of 1: 4 (w/ v). The solution was occasionally stirred using a magnetic stirrer and extracted using soxhlet apparatus. The extracts were concentrated under the rotary vacuum apparatus, at an evaporator, until the complete solvents evaporated (at 45 °C) to get semi-solid mass of crude extracts. The collected concentrated extracts (stock extracts) were lyophilized (at -80 °C) to obtain solid residue. The extracts were labelled and stored in a refrigerator at 4 °C till use (Kumar and Yadav 2013, Raveen et al. 2014).

Larvicidal bioassay

Bioassay of water, chloroform, acetone and diethyl ether extracts of *N. oleander* leaves was performed to determine the LC_{50} of each extract against *Cx. pipiens* larvae. Five to six serial concentrations of each extract were prepared: 0.50×10^4, 1.00×10^4, 2.00×10^4, 4.00×10^4 and 8.00×10^4 mg/l for larvae treated with water extract, 0.25×10^4, 0.50×10^4, 1.00×10^4, 2.00×10^4 and 4.00×10^4 mg/l for larvae treated with chloroform extract, 0.25×10^4, 0.50×10^4, 1.00×10^4, 2.00×10^4, 4.00×10^4 and 8.00×10^4 mg/l for larvae treated with acetone extract, 0.25×10^4, 0.50×10^4, 1.00×10^4, 2.00×10^4 and 4.00×10^4 mg/l for larvae treated with diethyl ether extract. Bioassay was carried out on the first larval instars (Ibarra and Federici 1987), with minor modifications. Twenty five newly hatched first instar larvae were released into each 300 ml plastic cup containing 200 ml of distilled water and test concentration. The extracts were diluted directly in the rearing water (w/v) without sol-

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vents. A parallel group of non-treated larvae was used as the control. Three replicates for each concentration (total n= 75 larvae/ concentration) were undertaken. All experiments were incubated at 25 ˚C. The mortality count was recorded daily until pupation i e, accumulated mortality.

Effect of temperature on the toxicity of extracts

To elucidate the relationship between post-treatment temperature and the toxicity of the above mentioned four extracts of *N. oleander* leaves to *Cx. pipiens* larvae, two groups per extract of twenty five newly hatched first instar larvae each were released in a plastic cup, as described above, containing the LC_{50} of each extract (4.82×10^4, 1.91×10^4, 3.00×10^4 and 1.05×10^4 mg/l for water, chloroform, acetone and diethyl ether extract, respectively), as previously determined in the present study (Table 1). The two groups were incubated at 10 and 35 ˚C, respectively. A parallel control of non-treated larvae was also run. Each experiment was repeated three times. The percentage of accumulated mortality was recorded, as mentioned above.

Biological effect of diethyl ether extract

As the present larvicidal bioassay revealed that diethyl ether extract of *N. oleander* leaves was the most potent extract (Table 1), this extract will be used for the further experiments. Twenty five newly hatched first instar larvae of *Cx. pipiens* were treated with the LC_{50} of this extract (10500 mg/l) and incubated at 25 ˚C. The control experiment consisted of non-treated larvae. The experiment was repeated three times. Prior to the end of pupation, the pupae were transferred into adult rearing cages for adult emergence at room temperature, as previously described. The following biological activities were determined for the survivors: larval duration, pupal duration, female fecundity (number of eggs/female), based on the cross mate treated unmated males × treated virgin females, using a fixed number, and longevity of both sexes. Moreover, certain biological indices were calculated as follows:

Growth index= a/b, where: a= % Instar survival, b= Mean developmental period of the instar in days (Moonis 1979), Survival index= a/b, where: a= % Instar survival, b= Maximum survival of the instar in all treatments (Moonis 1979), Oviposition activity index (OAI) = $N_t - N_c / N_t + N_c$, where: N_t= Number of eggs in treatment, N_c = Number of eggs in the control, the index values lie within the range -1 to +1 (Kuppusamy and Murugan 2012). They differentiated the OAI according to the resulting values, where negative values indicate that more eggs were deposited in the control than in the treatment, ie the extract is deterrent. Conversely, positive values indicate that more eggs were deposited in the treatment than in the control, i e the extract is attractant, % Development/day= (1/a) 100, where: a= Mean developmental period of the instar in days (Powers and Oatman 1984).

Genotoxic effect of diethyl ether extract

To evaluate the genotoxic effect of diethyl ether extract of *N. oleander* leaves on *Cx. pipiens* adults surviving larval treatment with the LC_{50}, the following crossing combinations were tested, using a fixed number: Treated unmated males × Treated virgin females, Normal unmated males × Treated virgin females, Treated unmated males × Normal virgin females, and Normal unmated males × Normal virgin females. The latter combination was used as the control. Each resulting egg raft was collected, allowed to hatch and carefully examined under a suitable magnification. Twenty egg rafts per crossing combination was examined. Eggs with open opercula were considered as hatched, while those with closed ones were taken as unhatched. The number of unhatched eggs per egg raft was taken as the measure for cal-

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culating the dominant lethality, as follows:

% Frequency of induced dominant lethality= (a/b) 100, where: a= Number of unhatched eggs in one egg raft, b = Total number of eggs in the egg raft (Bhinder and Chaudhry 2013).

Statistical analysis

The crude mortality obtained from the larvicidal bioassay was corrected using Abbott's formula (1925). The corrected mortality was then subjected to probit analysis (Finney 1971) for determining the LC_{50} of each extract. In the experiments of temperature-toxicity relationship, Chi-square test (χ^2) was carried out for each temperature regime, assumed that the applied LC_{50} will give 50 % mortality at the two temperatures tested. In the experiments of biological and genotoxic effects, Student's *t*-test was undertaken between treated and non-treated (control) experiments. All of the analyses were carried out using Statistical Package Social Science (SPSS) software version 11.5 (SPSS 2007). Significant level was set at $P< 0.05$.

Results

Larvicidal activity

The insecticidal activity of water, chloroform, acetone and diethyl ether crude extracts of *N. oleander* leaves to *Cx. pipiens* larvae revealed that diethyl ether extract was the most potent extract, with LC_{50} of 10500 mg/l (Table 1). This extract was about 4.59, 1.82 and 2.86 folds as toxic as water, chloroform and acetone extract, respectively. In contrast, water extract was the least toxic extract, with the LC_{50} value of 48200 mg/l. This indicates that water extract of *N. oleander* leaves might be unsuitable for controlling *Cx. pipiens*.

Effect of post-treatment temperature

Table 2 shows that exposure of the LC_{50} of water, chloroform, acetone and diethyl ether extracts of *N. oleander* leaves to 10 °C enhanced the mortality of *Cx. pipiens* larvae by about 24.10, 39.16, 47.80 and 78.80 % of the expected mortality (50%), respectively. This enhancement was significant ($P< 0.05$) in case of acetone and diethyl ether extracts. On the contrary, exposure of the LC_{50} of water, chloroform and acetone extracts to 35 °C showed about 23.34, 28.34 and 21.68 % decline in the larval mortality, while exposure of the LC_{50} of diethyl ether extract to the same temperature regime increased (~3.32%) the larval mortality relative to the expected mortality. Chi-square test (χ^2) indicated that the change in the larval mortality at 35 °C was insignificant.

Biological effect of diethyl ether extract

Application of the LC_{50} of diethyl ether extract of *N. oleander* leaves, as the most potent extract, on the first instar larvae of *Cx. pipiens* significantly decreased ($P< 0.05$) the resulting larval duration, pupal duration, percentage of pupation, percentage of adult emergence, and fecundity compared to the control (Table 3). The reduction in fecundity was about 36.57 % of the control. The longevity of treated adults differed with the sex, where the longevity of males was insignificantly increased, whereas that of females was significantly shortened ($P< 0.05$) to about 45.58 % of the control.

This treatment increased the growth index of both larvae and pupae to about 13.05 and 18.01 % of the control, respectively, and the percentage of development per day of larvae and pupae to about 40.10 and 48.26 % of the control, respectively. In contrast, the survival index of larvae and pupae was reduced to 19.00 and 20.00 % of the control, respectively. Oviposition activity index was consistent with the reduction in fecundity, where it recorded about -0.22 (Table 4).

Mutagenic effect of diethyl ether extract

The egg hatchability of *Cx. pipiens* surviving

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larval treatment with the LC_{50} of diethyl ether extract of *N. oleander* leaves was significantly reduced ($P< 0.05$) in all the crossing combinations compared to the control, except for the combination containing treated males mated with untreated females, where such decrease was insignificant (Table 5). The highest decline in egg hatchability was attained in the crossing combination containing both treated sexes, followed by that containing treated females, and then that containing treated males. In turn, the percentage of unhatched eggs was significantly increased ($P< 0.05$) in all the crossing combinations compared to the control, with the highest magnitude in the crossing combination containing both treated sexes, followed by that containing treated males, and then that containing treated females (Table 5).

Table 1. Toxicity of water, chloroform, acetone and diethyl ether extracts of *Nerium oleander* leaves to *Culex pipiens* larvae at 25 °C

Extract	LC_{50} (mg/l)	95% Confidence limits (mg/l)		Slope	χ^2 (d.f.)
		Lower	Upper		
Water	4.82×10^4	3.70×10^4	13.68×10^4	0.05	3.05 (3)
Chloroform	1.91×10^4	0.21×10^4	7.71×10^4	1.09	2.02 (3)
Acetone	3.00×10^4	0.89×10^4	10.09×10^4	2.44	1.08 (4)
Diethyl ether	1.05×10^4	0.33×10^4	3.35×10^4	3.04	2.30 (3)

Table 2. Temperature-toxicity relationship of water, chloroform, acetone and diethyl ether extracts of *Nerium oleander* leaves to *Culex pipiens* larvae, previously treated with the LC_{50} of each extract at 25 °C

Extract	Temperature			
	10°C		35°C	
	% Mortality	χ^2 (d.f.)	% Mortality	χ^2 (d.f.)
Water	62.05	2.90 (4)	38.33	2.72 (4)
Chloroform	69.58	7.67 (4)	35.83	4.02 (4)
Acetone	73.90	11.42* (4)	39.16	2.35 (4)
Diethyl ether	89.40	31.05* (4)	51.66	0.06 (4)

* Significant at $P< 0.05$.

Table 3. Biological activities of *Culex pipiens* surviving larval treatment with the LC_{50} of diethyl ether extract of *Nerium oleander* leaves at 25 °C

Biological activity	Control (Mean ± S E)	Treated (Mean ± S E)
Larval duration (days)	8.32±0.43	5.94*±0.07
Pupal duration (days)	4.36±0.26	2.94*±0.07
% Pupation	95.00±0.26	76.70*±2.77
% Adult emergence	96.60±1.71	76.88*±2.56
Male longevity (days)	12.33±0.88	15.67±1.45
Female longevity (days)	19.00±1.53	10.34*±0.40
Female fecundity (No. of eggs/♀)	216.00±8.47	137.00*±8.89

* Significant at $P< 0.05$, using Student's *t*-test.

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Table 4. Growth index, survival index, percentage of development per day and oviposition activity index of *Culex pipiens* surviving larval treatment with the LC_{50} of diethyl ether extract of *Nerium oleander* leaves at 25 °C

Experiment	Growth index		Survival index		% Development/day		%Oviposition activity index
	Larvae	Pupae	Larvae	Pupae	Larvae	Pupae	
Control	11.42	22.16	1.00	1.00	12.02	22.94	-
Treated	12.91	26.15	0.81	0.80	16.84	34.01	-0.22

Table 5. Genotoxic effect of diethyl ether extract of *Nerium oleander* leaves to *Culex pipiens* male and female adults surviving larval treatment with the LC_{50} of the extract at 25 °C

Crossing combination	Egg hatchability ± S E (No. of hatched eggs/egg raft)	% Frequency of unhatched eggs ± S E
Control	172.00^{a}±8.37	5.15^{a} ±0.28
N ♂♂ × T ♀♀	123.05^{b}±11.76	8.94^{b}±1.27
T ♂♂ × N ♀♀	158.65^{a}±12.03	11.37^{b}±1.78
T ♂♂ × T ♀♀	113.33^{b}±8.68	15.91^{c}±0.71

Figures followed by different letters are significantly different from each other ($P< 0.05$), using Student's *t*-test. N= Normal, T= Treated. Control = N ♂♂ × N ♀♀.

Discussion

Different parts of plants contain a complex of chemicals with unique biological activity (Farnsworth and Bingel 1977). Preliminary screening is a good approach to evaluate the potential larvicidal activity of plants (Sakthivadivel and Daniel 2008, Arivoli et al. 2012, Tennyson et al. 2012), and the activity of crude plant extracts subjected further to partial purification with respective solvent washed fraction is often distributed to the complex mixture of active compounds (Sakthivadivel and Daniel 2003). In this context, qualitative phytochemical analysis of *N. oleander* leaves confirmed the presence of various phytochemicals like carbohydrates, cholesterol, protein, amino acids, alkaloids, flavonoids, tannins, saponins, cardiac glycosides, terpenoids, and phlobatinins in their aqueous extract followed by ethanol, ethyl acetate, diethyl ether and chloroform (Santhi et al. 2011, Kumar and Yadav 2013). In comparison, the phytochemicals in methanolic leaf extract of *Ervatamia coronaria* (Apocynaceae) are alkaloids, saponins, tannins, steroids and flavonoids (Mathivanan et al. 2010).

The larvicidal activity of the different extracts of *N. oleander* leaves against *Cx. pipiens* in the present study is supported by the findings of Komalamisra et al. (2005) who reported larvicidal activity of ethanolic extract of the leaves of this plant against *Aedes aegypti* with LC_{50} of 197.97 mg/l. Roni et al. (2013) reported that the LC_{50}s of water leaf extract of *N. oleander* against the 1^{st}, 2^{nd}, 3^{rd} , and 4^{th} larval instars of *Anopheles stephensi* were 232.90, 273.71, 318.94, and 369.96 ppm, respectively. Also, Raveen et al. (2014) attained 24 h LC_{50} of 102.54 and 2758.87 ppm for hexane and water flower extracts of *N. oleander* against *Cx. quinquefasciatus*, respectively. Similarly, the larvicidal activity of the leaf extracts of other plants of family Apocynaceae were reported by some authors. For instance, Mathivanan et al. (2010) found that 24 h LC_{50} value of methanolic leaf extract of *E. coronaria* against *Cx. quinquefasciatus* was 72.41 mg/l, while Sakthivadivel et al. (2014) showed that 24 h LC_{50} of water and petroleum ether leaf extracts of *Wrightia tinctoria* against the

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same vector was 0.21 and 0.37 %, respectively. The differential responses induced by phytochemicals on various species of mosquitoes were influenced by extrinsic and intrinsic factors such as the species of the plant, the part of the plant, the geographical location where the plants were grown, photosensitivity of the compounds in the extract, the methods used for extraction, and the solvent used for extractions (Sukumar et al. 1991, Shaalan et al. 2005). As to the latter factor, polar solvents will extract polar molecules and non-polar solvents will extract non-polar molecules (Rawani et al. 2010).

Although oleanders contain cardenolides, and the basis for their physiological action is similar to that of the classic digital glycosides, i e inhibition of plasma membranes Na^+- K^+- ATPase, there are differences in toxic and cardiological effects between the oleanders and digital cardenolides, thus the human mortality associated with oleander ingestion is generally very low, even in cases of intensive uptake (suicide attempts) (Langford and Boor 1996). Moreover, it was pointed out that the active ingredient in the ethanolic extract of *N. oleander* leaves is the glycoside neriifolin which displays very slight mammalian cytotoxicity and negligible mutagenicity (El-Shazly et al. 2000). These findings indicate that field application of *N. oleander* leaf extracts in controlling *Cx. pipiens* should be safe.

The toxicity of the extracts of *N. oleander* leaves were affected by post-treatment temperature in a manner similar to that reported for pyrethrin and pyrethroids ie, the toxicity increased with the decrease in post-treatment temperature (Sparks et al. 1982, 1983). The relationship between temperature and the toxicity of an insecticide is a complex matter, where absorption of the insecticide, permeability through the cuticle, distribution within the insect body, detoxification and excretion are affected by the change in temperature (Hinks 1985). Temperature should be included as an important factor in the decision-making process in situations where comparable products from multiple insecticide classes are available (Musser and Shelton 2005). The increase in the toxicity of diethyl ether extract of *N. oleander* leaves in the current investigation after exposure to both 10 and 35 °C may indicate that this extract will be a potential agent in controlling *Cx. pipiens* in both winter and summer.

The decrease in the larval duration of *Cx. pipiens* after treatment with diethyl ether extract of *N. oleander* leaves is in agreement with the results of EL-Sheikh et al. (2011), who reported a significant decrease in the larval duration of the same vector after treatment with diethyl ether extract of *Cupressus sempervirens* leaves. On the contrary, prolonged larval duration of *Ae. aegypti* was reported after treatment with methanolic extract of *Nerium indicum* leaves (Mohtar et al. 1999). The dramatic reduction in the female longevity of *Cx. pipiens* to about half of the control (45.58%) in the present investigation might render this species less potential vector.

The suppression of fecundity due to treatment with diethyl ether extract of *N. oleande*r leaves may be attributed to the suppression of uptake of vitellogenin from the haemolymph. Elevation in oostatic hormone in mosquitoes suppresses trypsin biosynthesis in the cells of the mosquito's midgut, inhibits the action of juvenile hormone on vitellogenic follicle cells and prevents the ovary from accumulating vitellogenin from the haemolymph during yolk deposition (Borovsky 1988). The latter process in mosquitoes includes a series of metabolic changes that is occurred by changing in the composition of haemolymph proteins (Clements 1992).

The increase in the growth index and the percentage of development per day of *Cx. pipiens* larvae and pupae treated, starting from the first instar larvae, with the LC_{50} of diethyl ether extract of *N. oleander* leaves in

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the current study is suggestive of an attempt by the treated insects to avoid/tolerate the toxic effect of this extract. In this context, it was found that as *An. stephensi* larvae increased in age and weight, starting from the first larval instars to pupae, their tolerance to water extract of *N. oleander* leaves increased (Roni et al. 2013). The increase in the percentage of development per day in *Cx. pipiens* larvae and pupae treated with diethyl ether extract of *N. oleander* leaves might be attributed to their lower durations compared to the control, where the development per day is the inverse of the duration (Powers and Oatman 1984). On the other hand, the decrease in the survival index of treated larvae and pupae might reflect the decrease in the percentage of pupation and adult emergence, respectively. The oviposition activity index indicated that diethyl ether extract of *N. oleander* leaves was oviposition deterrent, according to the formula of Kuppusamy and Murugan (2012).

It seems likely that diethyl ether extract of *N. oleander* leaves is potential mutagen for the genome of *Cx. pipiens*, where it induced significant dominant lethality. Similarly, water leaf extract of *Azadirachta indica*, *Momodrica charantia*, *Murraya exotica*, and *Caspicum frutescens* decreased the egg hatchability in *Cx. quinquefasciatus* (Bashar et al. 2011), and the organophosphate insecticides, acephate and chlorpyrifos induced significant dominant lethality in the same mosquito species (Bhinder and Chaudhry 2013). The genetic basis of dominant lethality is mainly the induction of structural and numerical chromosomal anomalies which tend to induce non-viable zygotes, early embryonic death, sterility and semi-sterility in the offsprings of effected parents (Chaubey et al. 1999). Pesticides significantly increase the cellular reactive oxygen species production which causes chemical modifications and alterations in DNA and nucleoproteins, including modified bases and sugars and even strand breaks leading to chromosomal aberrations (Hreljac and Filipic 2009).

Although the second and third most highest decrease in the egg hatchability was obtained in the crossing combination containing treated females and that containing treated males, respectively, these two combinations showed the third and second most highest increase in the percentage of frequency of unhatched eggs, respectively (Table 5). This apparent conflict might be due to the variations in the total number of eggs in the egg rafts, based on the formula of Bhinder and Chaudhry (2013).

Conclusion

The present study explores the potential role of the different leaf extracts of *N. oleander* as control agents to *Cx. pipiens*. Diethyl ether extract of *N. oleander* leaves should reduce the population dynamics of *Cx. pipiens*, either directly through larval kill, or indirectly through its latent effects expressed in reduction of survival, fecundity and hatchability, and induction of dominant lethality in the subsequent generation. This extract can be considered for use in future integrated management strategies of *Cx. pipiens*.

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