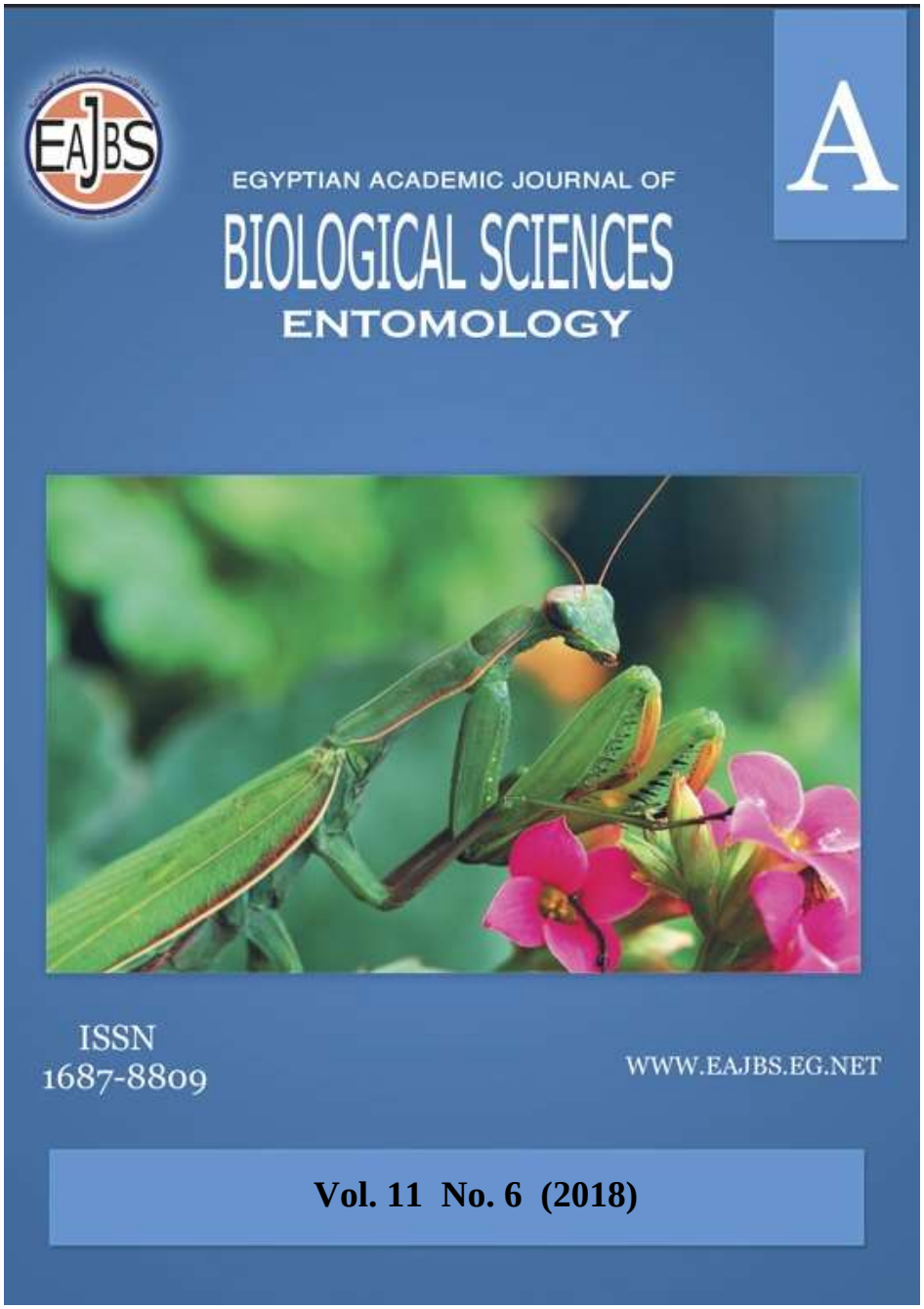

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The Biological Role of *Cymbopogon proximus* Leaf Extracts against the Malaria Vector, *Anopheles pharoensis*

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ABSTRACT

Due to the increased insecticide resistance, control of mosquito vector encounters a number of major difficulties. In this respect, searching for new compounds from nature for their insecticidal activities may offer an effective tool for mosquito vector control. In this study the biological activity, reproductive potential, and repellency effect of methanolic, aqueous, and petroleum ether extracts of *C. proximus* against the malaria mosquito, *An. pharoensis* was evaluated. Based on LC_{50} values, the toxicity may be arranged as the following: petroleum ether extract (LC_{50} = 200.1ppm) > methanol extract (LC_{50} = 216.1ppm) > aqueous extract (LC_{50} = 848.6ppm). The biological activities were significantly ($P<0.01$) affected by different tested extracts, especially at higher concentrations. The female fecundity and the egg-hatchability were remarkably decreased in a concentration-dependent manner. In addition, the repellency percentages were influenced by tested extracts at any concentration used and the highest repellent action was recorded for methanol extract followed by petroleum ether extract. In conclusion, these findings may contribute insight into the possibility of a novel insecticidal development for use in mosquito control programs.

INTRODUCTION

Mosquitoes consider dangerous for humans due to its role in transmitting numerous disease-causing pathogens (Morsy *et al.* 1995; Benelli and Romano 2017). The mosquito *Anopheles pharoensis* is an important disease vector; it transmits protozoan, parasitic, helminthic, and even viral diseases (Benelli and Beier 2017). It is a common mosquito species in Africa. Malaria is a life-threatening disease; *Anopheles* mosquitoes still the most important malaria vectors in many parts of Africa (WHO, 2014). According to the latest World Malaria Report, there were 219 million cases of malaria in 2017, up from 217 million cases in 2016 and the estimated number of malaria deaths stood at 435,000 in 2017 (WHO, 2018).

Mosquito control programs encounter serious problems, mainly the prevalent resistance to chemical insecticides, besides their adverse effects on the environment, health, and food chain (Kumar *et al.* 2013), and the restricted success of Culicidae young instars to the biological control programs (Benelli, 2015). Therefore, a new insecticide from natural origin with a new mechanism of action is essential to replace

chemical insecticides. Consequently, the mosquitocidal and repellent activity of plants may offer an effective and eco-friendly tool in mosquito control.

The potency of plant extract depends on plant species, plant part used, mosquito species targeted, extraction procedure, and the polarity of extraction solvents (Bream *et al.* 2009; Kishore *et al.* 2011; Zewdneh *et al.* 2011; Malik *et al.* 2014). Plant extracts in general, have been recognized as an important natural source of insecticides. Since they possess a rich source of biodegradable bioactive compounds, they may act as alternative sources of mosquito control agents. In addition, plant extracts have been traditionally used in diverse parts of the world against insects due to its own phytochemical derivatives that could be used as larvicides, insect growth regulators, and repellents.

Higher plants, particularly tropical flora, provide a potential source of natural insecticides. *C. proximus* is a well-known weed in Egypt; known as Halfa-bar, it grows in hills, rocky grounds, and the sandy coasts of the Red Sea. *Cymbopogon* is a large genus of family Poaceae that Comprised of about 144 species, it is widely distributed in the tropical and subtropical regions of Africa, Asia, and America (Avoseh *et al.* 2015). *Cymbopogon* species has been reported to possess a wide range of biological activities including antimicrobial, fungicidal, insect-repellant, fumigant, and insecticidal activity that provide a simple, cheap, and environmental safe insecticide alternative to pest control (Dias *et al.* 2017; Yousif, 2017).

No doubt, Insecticides from botanical origin may offer an effective, safe, cheap, and eco-friendly tool for mosquito vector control. The present study aimed to estimate the biological activity, reproductive potential, and repellency effect of methanolic, aqueous, and petroleum ether extracts of *C. proximus* against the mosquito, *An. pharoensis*.

MATERIALS AND METHODS

1- Mosquito Colony:

Mosquito used in this study was *An. pharoensis* Theobald, larvae were collected in January 2018 from El-Fayoum Governorate, Egypt. It was reared for at least four generations, in the insectary of medical entomology, department of zoology, faculty of science, Al-Azhar University, under controlled conditions at a temperature of 27±2 °C, relative humidity RH 70±10%, for photoperiods 14:10 (light: dark) hours. Adult mosquitoes were kept in wooden cages and daily provided with cotton pads soaked in 10% sucrose solution for a period of 3-4 days after emergence. After this period, the females were allowed to take a blood meal from a pigeon host, which is necessary for laying eggs (Haldar *et al.* 2014). Early third larval instar was chosen for subsequent studies.

2- Extraction of Plant Materials:

The common Egyptian Lemongrass *Cymbopogon proximus* Stapf (Family: Poaceae), leaves were collected from the roadside weeds, Bilbeis City, Sharqia governorate. The plant part was washed in de-chlorinated water, left to dry in shade conditions at a temperature of 27±2 °C for 15 days and pulverized to powder in a hammer mill. The extraction was performed using methanol, aqueous, and petroleum ether solvents. One hundred grams of powder for each solvent was extracted five times with 300 ml of 70% methanol, aqueous, and petroleum ether at room temperature. After 24 h, the supernatants were decanted, filtrated through Whatman filter paper No. 5 and dried in a rotary evaporator at 40 ºC for 2–3 hours for methanol

and 40–60 minutes for other solvents. The dry extracts were weighed and kept in a deep freezer (- 4ºC) till used.

3- Experimental Bioassay:

In order to study the toxicity of plant extracts tested, the mosquito larvicidal activity was assessed according to the procedure of WHO (1996) with some modifications, tested material of the methanolic extract was dissolved in 0.1ml of 70% methanol, aqueous extract was easily dissolved in water, while petroleum ether extract was dissolved in 2 drops of Tween 80 as emulsifier. Different concentration ranges of each extract were prepared in order to detect their mortalities. All tested materials were performed in 250ml de-chlorinated tap water in plastic cups. Then, twenty-five 3rd instar larvae were put immediately into plastic cups contained different concentrations of extracts. At least three replicates were usually used for each tested concentration. All plastic cups were incubated under controlled conditions at a temperature of 27±2°C, RH 70±10% and 14:10 photoperiods. Mortality was recorded daily.

***Larval mortality*:**

Larval mortality percent was estimated using the following equation: larval mortality% = A-B/A ×100. Where: A = number of tested larvae, B = number of tested pupae (Briggs, 1960).

***Larval duration*:**

Larval duration was calculated as the intervals between the commencement of first instar larvae and the commencement of pupation, it was calculated for each larva and then the mean value was taken.

***Pupation rate*:**

The pupation rate was estimated using the following equation: pupation% = A/B ×100. Where: A = number of pupae, B = number of tested larvae.

***Pupal mortality*:**

The pupal mortality percentage was estimated using the following equation: pupal mortality% =A-B/A ×100. Where: A = number of produced pupae, B = number of observed adults.

Pupal duration:

Pupal duration was calculated as the interval between the commencement of pupation and the commencement of adult emergence.

***Adult emergence*:**

The emerged adult males and females were counted and the adult emergence was calculated using the following equation: adult emergence% = A/B ×100. Where: A = number of emerged adults, B = number of tested pupae.

4- Females Reproductive Potential:

Fecundity:

Successfully emerged adult females from larvae treated with each concentration were collected and transferred with normal adult males obtained from the colony to wooden cages (20×20×20 cm) using an electric aspirator and fed with sucrose solution (cotton pads soaked in 10% sucrose solution) for three days. The adult males and females were left for one day without sugar solution. At the 5th day, starved females were allowed to take a blood meal from a pigeon and allowed to lay eggs on clean water. The eggs were counted using binocular then the mean values were taken.

***Fertility*:**

The egg fertility or hatchability was calculated using the following equation: Egg hatchability% = A/B ×100. Where: A = total no. of hatched eggs, B = total no.

of eggs laid. According to the formula of Toppozada *et al.* (1966), Sterility% = 100 – [a×b /A×B] ×100, Where: a = number of eggs laid/female in treatment, b = percentage of hatched eggs in treatment, A = number of eggs laid/female in control, B = percentage of hatched eggs in control.

5- Repellent Activities:

Different concentrations for each extract were dissolved in 1mL water with a drop of Tween 8. The dissolved concentration was directly applied onto the ventral surface of pigeon after abdominal feathers removal. Positive control tests were carried out using commercial repellent (Deet) that was purchased from Johnson Wax, Egypt, after Uniyal *et al.* (2016). For each experimental treatment and after 10 min, the pigeon was placed for 2 hr in a cage containing twenty-five starved *An. pharoensis* adult females. Alongside with these treatments control test was carried out using water. The number of fed and unfed females were counted and calculated. The repellency percent was calculated by the following formula, Repellency% = [A% − B% /100 - B%] ×100, where A is the percent of unfed females in treatment, and B the percent of unfed females in control.

6- Statistical analysis

One way analysis of variance (ANOVA) using SPSS (statistical package for social sciences, ver.15.0) was used in analyzing experimental data and the significance among the samples was compared at ($P \leq 0.05$). Abbott's formula was used to correct control mortality which was less than 3% (Abbott, 1925). The larval mortality data were also subjected to probit analysis for calculating LC_{50}, using (SPSS). Results were represented as mean ± SD.

RESULTS

The LC_{50} values of *C. proximus* different solvent extracts were presented in table (1). On the Basis of LC_{50} values, the toxicity of *C. proximus* different solvent extracts maybe arranged as the following: petroleum ether extract (LC_{50} = 200.1ppm) > methanol extract (LC_{50} = 216.1ppm) > aqueous extract (LC_{50} = 848.6ppm).

Table 1: LC_{50} of *C. proximus* different solvent extracts against *An. pharoensis*

Extract	$LC_{50\ (ppm)}$	Slope (b)	Correlation coefficient (R^2)
Methanol	216.1	0.1598	0.93844
Aqueous	848.6	0.0514	0.94804
Petroleum ether	200.1	0.1843	0.93458

1- Methanol extract:

The biological and reproductive aspects of *C. proximus* methanol extract against *An. pharoensis* besides its repellency effect are given in (Tables 2, 3, 4). The larval mortality percent, mean larval duration, pupal mortality percent, and the mean pupal duration were positively correlated with the applied concentrations. Meanwhile, the pupation percent and the adult emergence were highly reduced particularly at higher concentrations. Highly significant ($P<0.01$) effects of methanolic extract were recorded especially at concentrations 400, 500ppm (table 2).

Table 2: Effect of *C. proximus* methanol extract on different biological parameters of *An. pharoensis.*

Concentrations (ppm)	Larval mortality %	Mean larval duration (days)±SD	Pupation %	Pupal Mortality %	Mean pupal duration (Hours)±SD	Adult Emergence %
600	100.0	0.0	0.0	0	0.0	0.0
500	97.3	11.4±0.61**	٤.0	50.0	57.9±0.40**	50.0
400	86.7	11.2±0.25**	16.0	50.0	56.8±0.76**	50.0
300	70.7	10.4±0.14*	32.0	37.5	55.6±0.53*	62.5
200	54.7	10.2±0.25*	44.0	27.3	54.4±0.51*	72.7
100	30.7	9.4 ± 0.09^{ns}	72.0	5.6	52.4±0.45*	94.4
50	12.0	9.1 ± 0.06^{ns}	96.0	0.0	51.5 ± 0.46^{ns}	100.0
Control	1.3	9.2±0.20	92.0	0.0	51.0±1.0	100.0

(*) Significant, P < 0.05, (**) highly Significant, P < 0.01, ns=non-significant, P > 0.05. N=3

The fecundity of females resulted from 3rd instar treated larvae was significantly (P<0.01) decreased from 2433.3±2.1 eggs/25♀♀ at concentration 200ppm to 350.0±2.0 at concentration 500ppm compared to 3483.3±1.2 eggs/25♀♀ for the control (Table 3). There was a remarkable concentration-dependent reduction in the egg hatchability of females resulted from treated larvae, the egg hatchability was significantly (P<0.01) decreased from 87.7±3.1 eggs/♀ at concentration 200ppm to 16.7±2.9 eggs/♀ at 500ppm compared to 124.0±3.6 eggs/♀ for the control (Table 3).

Table 3: Effect of *C. proximus* methanol extract on fecundity and egg-hatchability of *An. pharoensis*.

Concentrations (ppm)	Fecundity/25♀♀	Egg-hatchability/♀
	Mean±SD	
600	0.0	0.0
500	350.0±2.0**	16.7±2.9**
400	783.3±2.3**	27.0±2.6**
300	1541.7±2.1**	46.7±2.9**
200	2433.3±2.1**	87.7±3.1**
100	3033.3±4.2*	110.3±0.6*
50	3275.0 ± 1.0^{ns}	117.0 ± 2.6^{ns}
Control	3483.3±1.2	124.0±3.6

(*) Significant, P < 0.05, (**) highly Significant, P < 0.01, ns=non-significant N=3.

The repellency effect of *C. proximus* methanol extract against starved *An. pharoensis* females was shown in (Table 4). Data obtained revealed that the repellency percent was highly affected with tested extract, and the highest activity (91.97% and 94.64%) was obtained at concentrations (500 and 600ppm), respectively as compared to 100% repellency for Deet at a concentration 10ppm.

Table 4: Effect of *C. proximus* methanol extract on the repellency percent of *An. pharoensis.*

Concentrations (ppm)	Fed%	Unfed %	Repellency %
600	2.7±1.2	97.3±1.2	94.64
500	5.3±0.6	94.7±0.6	91.97
400	10.7±0.6	89.3±0.6	86.64
300	29.3±1.2	70.7±1.2	67.97
200	44.0±0.0	56.0±0.0	53.31
100	66.7±0.6	33.3±0.6	30.64
50	81.3±0.6	18.7±0.6	15.97
Deet (10 ppm)	0.0	0.0	100
Control	97.3±0.6	2.7±0.6	2.7

Repellency effect was applied on 25 starved females

2- Aqueous extract:

The larval mortality percent of *C. proximus* aqueous extract was remarkably reduced at concentrations 1500 and 2000ppm. The mean larval duration and the mean pupal duration was significantly (P<0.01) prolonged at 750, 1000 and 1500ppm. The pupal mortality percent was positively correlated with applied concentrations. Contrarily, the pupation and the adult emergence percentages were highly reduced particularly at concentration 1500ppm (Table 5).

Table 5: Effect of *C. proximus* aqueous extract on different biological parameters of *An. pharoensis.*

Concentrations (ppm)	**Larval mortality %**	**Mean larval duration (days)±SD**	**Pupation %**	**Pupal Mortality %**	**Mean pupal duration (Hours)±SD**	**Adult Emergence %**
2000	100.0	0.0	0.0	0.0	0.0	0.0
1500	85.3	10.2±0.15**	1◦.0	50.0	53.7±0.76**	50.0
1000	66.7	10.0±0.15**	32.0	25.0	55.2±0.76**	75.0
750	56.0	9.7±0.25**	44.0	18.2	52.4±0.55**	81.8
500	34.7	9.6±0.38*	64.0	12.5	51.6±0.36*	87.5
250	13.3	9.2±0.17*	84.0	9.5	50.4±0.53*	90.5
100	2.7	9.1 ± 0.12^{ns}	96.0	4.2	50.3 ± 0.42^{ns}	95.8
Control	2.7	9.3±0.20	96.0	0.0	49.7±0.58	100.0

(*) Significant, P < 0.05, (**) highly Significant, P < 0.01, ns=non-significant, P > 0.05. N=3

The fecundity of females resulted from 3rd instar larvae treated with *C. proximus* aqueous extract was significantly (P<0.01) reduced at all tested concentrations except at 100ppm. In addition, a remarkable reduction in the egg hatchability was recorded. The egg hatchability was significantly (P<0.01) decreased from 96.7±3.5 eggs/♀ at concentration 500ppm to 19.3±2.3 eggs/♀ at 1500ppm compared to 124.0±3.6 eggs/♀ for the control (Table 6).

Table 6: Effect of *C. proximus* aqueous extract on fecundity and egg-hatchability of *An. pharoensis*.

Concentrations (ppm)	Fecundity/25♀♀	Egg-hatchability/♀
	Mean±SD	
2000	0.0	0.0
1500	750.0±3.0**	19.3±2.3**
1000	1683.3±2.5**	38.3±1.5**
750	2233.3±1.2**	61.0±2.6**
500	2833.3±2.9**	96.7±3.5**
250	3200.0±2.0**	112.3±2.5*
100	3333.3±1.2^{ns}	118.3±2.9^{ns}
Control	3550.0±2.0	124.0±3.6

(*) Significant, P < 0.05, (**) highly Significant, P < 0.01, ns=non-significant N=3.

The repellency effect of *C. proximus* aqueous extract against starved *An. pharoensis* females was presented in (Table 7). Data obtained revealed that the repellency percent was concentration-dependent; the highest repellency effect (93.3%) was obtained at the highest concentration 2000ppm as compared to 100% repellency for Deet at a concentration 10ppm.

Table 7: Effect of *C. proximus* aqueous extract on the repellency percent of *An. pharoensis.*

Concentrations (ppm)	Fed%	Unfed %	Repellency %
2000	5.3±1.2	94.7±1.2	93.3
1500	17.3±0.6	82.7±0.6	81.3
1000	28.0±0.0	72.0±0.0	70.7
750	40.0±1.7	60.0±1.7	58.7
500	54.7±1.5	45.3±1.5	44.0
250	77.3±1.5	22.7±1.5	21.3
100	92.0±1.0	8.0±1.0	6.7
Deet (10 ppm)	0.0	0.0	100.0
Control	98.7±0.6	1.3±0.6	1.3

Repellency effect was applied on 25 starved females

3- Petroleum ether extract:

Data given in tables (8, 9, 10) showed the effect of *C. proximus* petroleum ether extract on the biological, reproductive aspects and repellency effect against *An. pharoensis*. At concentration 400ppm the larval mortality percent, mean larval duration, pupation percent, pupal mortality percent and adult emergence were extremely affected. The mean pupal duration was significantly (P<0.01) prolonged and this effect was positively correlated with applied concentrations (Table 8).

Table 8: Effect of *C. proximus* petroleum ether extract on different biological parameters of *An. pharoensis.*

Concentrations (ppm)	Larval mortality %	Mean larval duration (days)±SD	Pupation %	Pupal Mortality %	Mean pupal duration (Hours)±SD	Adult Emergence %
500	100.0	0.0	0.0	0.0	0.0	0.0
400	86.7	11.4±0.66**	20.0	60.0	57.5±0.50**	40.0
300	69.3	10.8±0.14*	32.0	50.0	56.5±0.87**	50.0
200	58.7	10.9±0.38*	44.0	27.3	56.5±0.50**	72.7
100	45.3	10.1±0.52*	56.0	21.4	55.5±0.50**	78.6
50	17.3	9.6 ± 0.33^{ns}	76.0	10.5	53.0±1.00*	89.5
25	5.3	8.9 ± 0.12^{ns}	92.0	4.3	51.3 ± 0.58^{ns}	95.7
Control	2.7	9.3±0.30	92.0	0.0	50.3±0.58	100.0

(*) Significant, P < 0.05, (**) highly Significant, P < 0.01, ns=non-significant, P > 0.05. N=3

The fecundity of females resulted from 3rd instar larvae treated with *C. proximus* petroleum ether extract was significantly reduced at all tested concentrations except at the lowest concentration 25ppm. A remarkable reduction of females' egg hatchability was recorded, the egg hatchability decreased significantly (P<0.01) from 44.3±1.2 eggs/♀ at concentration 200ppm to 12.3±1.5 eggs/♀ at 400ppm compared to 123.3±2.9 eggs/♀ for the control (Table 9).

Table 9: Effect of *C. proximus* petroleum ether extract on fecundity and egg-hatchability of *An. pharoensis*.

Concentrations (ppm)	Fecundity/25♀♀	Egg-hatchability/♀
	Mean±SD	
500	0.0	0.0
400	325.0±2.6**	12.3±1.5**
300	841.7±3.5**	24.0±1.0**
200	1591.0±2.5*	44.3±1.2**
100	2491.3±1.2*	85.3±1.5*
50	3066.7±3.1*	110.0±2.0*
25	3300.0 ± 2.0^{ns}	116.0 ± 1.7^{ns}
Control	3533.0±2.3	123.3±2.9

(*) Significant, P < 0.05, (**) highly Significant, P < 0.01, ns=non-significant N=3.

The repellency effect of *C. proximus* petroleum ether extract against starved *An. pharoensis* females was presented in (Table 10). Data obtained showed that the repellency percent was highly affected with tested extract, and the highest repellent activity (94.6%) was obtained at 500ppm as compared to 100% repellency for Deet at a concentration 10ppm.

Table 10: Effect of *C. proximus* petroleum ether extract on the repellency percent of *An. pharoensis.*

Concentrations (ppm)	Fed%	Unfed %	Repellency %
500	2.7±0.6	97.3±0.6	94.6
400	13.3±1.2	86.7±1.2	84.0
300	20.0±0.0	80.0±0.0	77.3
200	40.0±1.0	60.0±1.0	57.3
100	49.3±0.6	50.7±0.6	48.0
50	74.7±1.2	25.3±1.2	22.6
25	90.7±0.6	9.3±0.6	6.6
Deet (10 ppm)	0.0	0.0	100.0
Control	97.3±0.6	2.7±0.6	2.7

Repellency effect was applied on 25 starved females

DISCUSSION

Egypt is rich with so many plants, weeds, and herbs that may serve as a potential source for biologically active constituents. It is also a valuable source of potential insecticides and active derivatives that may be due to phenolics, terpenoids, flavonoids, and alkaloids included (Ravikumar *et al.* 2011; Elango *et al.* 2012). In this study, *C. proximus* leaf extracts of different solvents were tested for their biological, reproductive, and repellent activity against the mosquito vector, *An. pharoensis.* It was observed that different tested parameters were highly affected with the applied extracts. Such results may offer an opportunity to minimize the application of synthetic/chemical insecticides, which in turn lead to the development of safe, cheap, and biodegradable botanical chemicals.

The crude leaf extracts of *C. proximus* with different solvents, namely; methanol, aqueous, and petroleum ether when tested for their larvicidal activity against *An. pharoensis* showed remarkable larvicidal activities. The LC_{50} values recorded were 216.1ppm, 848.6ppm, and 200.1ppm, respectively. These results agreed with those previously demonstrated by Pushpanathan *et al.* (2006) using *C. citratus* against *Culex quinquefasciatus*; Mohan and Ramaswamy (2007) against *Aedes aegypti* and *Cx. quinquefasciatus*; Govindarajan (2010) against *Cx. quinquefasciatus*, *Ae. aegypti*, and *An. Stephensi;* Haldar *et al.* (2014) using *Ficus krishnae* extract against *An. stephensi* and *Cx. vishnui*.

The results obtained showed that the larval mortality percent of different solvents was significantly affected particularly at higher concentrations, which confirm their potentiality as mosquito control agents. In the same context, Cymbopogon-derived extracts showed remarkable larvicidal activity against *Ae. aegypti* (Sosan *et al.* 2001; Cavalcanti *et al.* 2004), *Cx. quinquefasciatus* (Pushpanathan *et al.* 2006), and *An. Stephensi* (Amer and Mehlhorn, 2006). The prolonged effect of tested extracts on the mean larval and mean pupal durations were found to be concentration-dependent, the same results were obtained by Murugan *et al.* (1996) and Nathan *et al.* (2005) using sub-lethal doses of *Azadirachta indica* against *An. stephensi.*

The pupal stage might exhibit a much higher tolerance to chemicals agents than active larval stages (Papachristos and Stamopoulos, 2002), Elimam *et al.* (2009) reported that the pupal stage of *Cx. quinquefasciatus* was not affected with concentrations less than 5000ppm. In contrast, a remarkable reduction in pupation percentages and increased pupal mortality percentages by almost all tested concentrations was exhibited, which prove and support the advantageous use of natural-origin derivatives (EL-Sheikh *et al.* 2012; Panneerselvam *et al.* 2013; Hasaballah, 2015).

The adult emergence percentages from treated larvae were concentration-dependently reduced. The complete adult emergence inhibition (0.0%) was recorded at 500ppm, 2000ppm, and 500ppm for methanol, aqueous, and petroleum ether extracts respectively. Similar results were obtained by Jeyabalan *et al.* (2003) using *Pelargonium citrosa* leaf extracts against *An. stephensi*; Elimam *et al.* (2009) using *Ricinus communis* aqueous leaf extracts against 3rd instar larvae of *An. arabiensis* and *Cx. quinquefasciatus*; Howard *et al.* (2009) who reported 50% inhibition of adult emergence using less than 0.4g of neem bark chippings of *A. indica*; Elango *et al.* (2012) using leaf crude extracts against *Cx. tritaeniorhynchus*.

The female fecundity of the 3rd instar treated larvae was significantly reduced in a concentration-dependent manner with almost all concentrations tested. The most effective solvent extract was petroleum ether (fecundity= 308.3 eggs/♀♀, at 400ppm), followed by methanol extract (fecundity= 541.7 eggs/♀♀, at 500ppm), and aqueous extract (fecundity= 441.7 eggs/♀♀, at 1500ppm). These results in harmony with earlier reports of Nathan *et al.* (2005), who tested the biological effects of neem seed against *An. stephensi* and found that at higher doses, the female fecundity was significantly decreased; Mong'are *et al.* (2013) who tested different crude leaf extracts against *Phlebotomus duboscqi* mosquito and found that the fecundity was reduced by 53%. In addition, the eggs hatchability of females resulted from treated larvae was remarkably decreased especially for methanolic and petroleum ether extracts and this effect was concentration-dependent. Many authors recorded similar results using different plant extracts against mosquito species (Jeyabalan *et al.* 2003; Nathan *et al.* 2006; Pavela, 2009; Cheah *et al.* 2013; Veni *et al.* 2017).

Different solvent extracts used in this study displayed repellency effects against starved *An. pharoensis* adult females. The repellent action was varied according to the solvent and the concentration used. The highest repellency percent was (92.0%) and it was recorded for methanol extract (500ppm) followed by petroleum ether extract (87.9%) at the same concentration. These results are in accordance with such results obtained by Govere *et al.* (2000) using *C. excavatus* extracts against *An. arabiensis*, Mullai *et al.* (2008) using leaf extracts of *Citrullus vulgaris* against *An. stephensi*, Bream *et al.* (2009) using *Phragmites australis* extracts against *Cx. pipiens*, Haldar *et al.* (2014) using *F. krishnae* extract against *An. stephensi* and *Cx. vishnui*, Karunamoorthi *et al.* (2014) using extracts of some medicinal plants against *An. arabiensis*, Kimutai *et al.* (2017) using the essential oils of Cymbopogon against the sand fly, *Ph. duboscqi*. Interestingly, the topical application of Cymbopogon 1% v/v solution exhibited ≥50% repellency against the starved culture of *Ae. aegypti* lasting for 2–3 h (Yousif, 2017).

Conclusion:

On the basis of LC_{50} values, petroleum ether extract exhibited high larvicidal activity than methanol or aqueous extract. Complete larval mortality was achieved at concentrations, 600ppm, 2000ppm, and 500ppm for methanol, aqueous, and petroleum ether extract, respectively. Different tested extracts significantly ($P<0.01$)

enhanced the biological activities of *An. pharoensis* especially at higher concentrations. The female fecundity of the 3rd instar *An. pharoensis* treated larvae besides the egg-hatchability were remarkably decreased in a concentration-dependent manner with preference to petroleum ether extract followed by methanol extract. The repellent action was also enhanced by tested extracts at any concentration used and the highest repellency effect was recorded by methanol extract followed by petroleum ether extract. In general, these findings may contribute insight into the possibility of a novel insecticidal development for use in mosquito control programs.

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ARABIC SUMMERY

"الدور البيولوجي لمستخلصات أوراق نبات *سيمبوبجن بروكسيمس* ضد البعوضة الناقلة لداء الملاريا *أنوفيلس فرعوني* "

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تواجه مكافحة البعوض الناقل للأمراض العديد من الصعوبات نتيجة المقاومة المستمرة والمتزايدة للمبيدات الحشرية المخلقة كيميائيا والتي يمكن تجنبها بالبحث عن بدائل أخري من الطبيعة مثل النباتات التي بدورها يمكن ان يكون لها دورا فعالا في برامج مكافحة الحشرات وذلك لرخص تكلفة إنتاجها وفعاليتها وأيضا لكونها أكثر أمانا علي الإنسان والبيئة المحيطة به. في هذه الدراسة تم تقييم النشاط البيولوجي، القدرة الإنتاجية والتأثير الطارد لكل من (مستخلص الميثانول، مستخلص مائي، ومستخلص إثير البترول) لأوراق نبات *سيمبوبجن بروكسيمس* ضد البعوضة الناقلة لداء الملاريا *أنوفيلس فرعوني*.

أظهرت نتائج الدراسة أن المستخلصات المستخدمة قد تسببت وبإختلاف تركيزاتها فى زيادة وفيات الطور اليرقي بنسب ملحوظه للبعوضة محل الدراسة حيث سجل مستخلص إثير البترول أعلي تأثير يليه الميثانول ثم المستخلص المائي. أيضا سجلت الدراسة نقصا معنويا ملحوظا في نسب إنتاجية البيض وخصوبته وكان هذا النقص يعتمد علي التركيز - يزداد بزيادة التركيز المستخدم- وذلك لجميع المستخلصات المستخدمة. أيضا تأثرت نسب التأثير الطارد بكل التركيزات المستخدمه لجميع المستخلصات حيث سجلت أعلي نسبة لمستخلص إثير البترول. هذه الإستنتاجات من الممكن أن تساهم في تخليق مبيدات حشرية جديدة من مصدر امن وفعال تساهم بدورها في برامج مكافحة الحشرات.