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Medicinal Plant Extracts as Green Insecticides and Eco-friendly Approach with Study to Reduce the Use of Insecticides for Controlling *Culex pipiens* larvae

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ABSTRACT

Many environmental problems have arisen from the overuse of insecticides, and their effects on public human health in recent years., organophosphates and pyrethroids insecticides are widely used for *Culex pipiens* mosquito control. Natural products from some medicinal plants are an excellent alternative to synthetic insecticides to reduce harmful impacts on human health and the environmental system. This study investigated the efficiency of six methanolic medicinal plant extracts species: *Curcuma longa, Simondsia chinensis, Zingiber officinale, Cinnamomum zeylanicum, Foeniculum vulgare* and *Ricinus communis* and studied the effects on four commercial insecticides: lambada-cyhalothrin, malathion, dimethoate and mineral oil were tested after 24, 48,72 hrs. under laboratory conditions. From the results, we decide and suggest that *C. pipiens* mosquito develops and increase tolerance and resistance to organophosphates and pyrethroids insecticides with frequent treatment. Also, we can be concluded that medicinal plant extracts have the potential to be used as green insecticides for controlling *C. pipiens* larvae, however, more trials are needed to know more chemicals for plant derivatives used in *C. pipiens* mosquito control.

INTRODUCTION

Culex pipiens mosquitoes are considered one of the most important medicinal insects not only in Egypt but also all over the world for their ability to transmit many vector-borne diseases such as malaria, filariasis, yellow fever, dengue fever and encephalitis, besides allergic responses include local skin and systemic reactions such as angioedema, and urticaria (Benelli *et al.*, 2016; and Aly *et al.*, 2021). *C. pipiens*, therefore is the main target in control programs against these diseases and because insecticides are part of the integrated vector management strategy, and there is an urgent need to develop alternative insecticides to control mosquitoes, and these alternatives must be safer and more effective than the used pesticides (WHO, 2008). The use of natural product chemistry that reduces mosquito populations at the larval stage can provide many associated benefits to vector control. The selection of natural products that limit the environmental impacts of pesticides due to shorter latency may be beneficial in preventing the evolution of resistance (Hardin and Jackson, 2009). Thus, attention was drawn to plants as a source of new chemical compounds, especially since the use of plants as insecticides did not cause damage to the environment (Lundberg, 2002). Therefore, various types of organic and inorganic extracts were used, as well as secondary compounds isolated from plants such as alkaloids, phenols, terpenens and others in controlling insects. Since 1950, it has been recorded that about 247 families possess insecticidal toxins (Silva Aguayo, 2004). The use of insecticides, mostly pyrethroids and organophosphates, has long been the primary means of controlling *Aedes aegypti*, but widespread insecticide resistance has emerged (Smith *et al.*, 2019). The objective of the present investigation is: to evaluate the efficiency of six medicinal plant extracts and four compounds against 4th larval instars of *C. pipiens* under laboratory conditions.

MATERIALS AND METHODS

Mosquitoes: *C. pipiens*. The field (Assiut, Egypt) strain of 4th instar larvae was collected from Al-Azhar University area in Assiut City and then directly transfer to the Central Laboratory at the Faculty of Agriculture, Al-Azhar University, Assiut to conduct the experiment.

Preparation of Plant Extracts:

The obtained plant materials were washed with distilled water and shade dried at room temperature, and then grinded into a fine powder with a grinder. The powdered materials were used for the preparation of methanolic extracts by using 100g powder in 500 ml methyl alcohol 99% for 48 hrs. The mixtures were stirred every 24 hrs. using a shaker apparatus. At the end of extraction, each extract was concentrated at room temperature and stored at 3°C until further use.

Medicinal Plant Extracts:

Six different plants, *Curcuma longa*, *Simondsia chinensis, Zingiber officinale, Cinnamomum zeylanicum, Foeniculum vulgare* and *Ricinus communis* such as in Table (1) for screened toxicity response. Two of the plants were obtained from the farm of the Faculty of Agriculture, Al-Azhar University and the other four plant materials were obtained from the local market at Assiut Governorate, Egypt.

Table 1: Scientific name, Common name and Family to tested plant extracts.

Scientific name	Common name	Family
Curcuma longa L.	Turmeric	Zingiberaceae
Simmondsia chinensis (Link) C. K.	Hohoba	Simmondsiaceae
Zingiber officinale Roscoe	Ginger	Zingiberaceae
Cinnamomum zeylanicum Blume	Ceylon cinnamon	Lauraceae
Foeniculum vulgare Mill.	Sweet fennel	Apiaceae
Ricinus communis L.	Castor plant	Euphorbiaceae

Insecticides

The tested compounds belonging to pyrethroids, organo-phosphate Groups and mineral oil to assess the insecticidal activity of the tested compound were prepared using the commercial formulations in Table (2).

Table 2: Common name, Commercial formulations of tested insecticides.

Common name	Trade name (Formulation and % Active ingredient)	Chemical Group	Importers and distributors company
***Lambda-Cyhalothrin**	Axon 5% EC	Pyrethroids	Commercial Agricultural Materials (Cam)
****Malathion**	Malason Cheminova 57%EC	Organophosphate	Kafr El-Zayat Pesticides and Chemicals
*****Dimethoate**	Saydon/Cheminova 40% EC		
Mineral oil	KZ oil 95% EC	Distillate of petroleum	

IUPAC Name OF Insecticides Used:

*Lambda-Cyhalothrin**:** [(R)-cyano-(3-phenoxyphenyl) methyl] (1S,3S)-3-[(Z)-2-chloro-3,3,3-trifluoroprop-1-enyl]-2,2-dimethylcyclopropane-1-carboxylate

**Malathion: diethyl 2-dimethoxyphosphinothioylsulfanylbutanedioate

***Dimethoate**:** 2-dimethoxyphosphinothioylsulfanyl-N-methylacetamide

Toxicological studies:

The toxicity test method was conducted on 20 fourth instar larvae. However, the larvae were placed in glass cups each containing 100 ml of distilled water. Each test was conducted in three replicates, per concentration (n= 60). Each container with 20 larvae was treated with the test dose concentration supplied with food (yeast). Control received only distilled water, and was run concurrently with each series of tests. At least five concentrations were used for each test. Every test was held at 25°C (~ 60%RH). Mortality counts were made after 24, 48, and 72-hrs of treatment. When the control mortality was between 5% - 20% it was corrected by Abbot's formula (Abbot, 1925). Larvae were considered dead if they were unresponsive to touching with a probe or if they could not reach the surface of the water.

Statistical analysis

The toxicity test data were pooled and analyzed (LC_{50}, LC_{90} and 95% CL values) by using Proban Probit analysis programme version 1.1. Computations performed by this programme are based on Finney (1971). In addition, the confidence intervals were done to obtain the significant difference among means using lower and upper values at 0.05 levels.

RESULTS AND DISCUSSION

Toxicity Effects of Six Tested Methanolic Plant Extracts against Fourth Instar Larvae of *C.pipiens* Mosquitoes:

The toxicity of six methanol plant extracts belonging to six plant species of *Curcuma longa*, *Simmondsia chinensis, Zingiber officinale, Cinnamomum zeylanicum, Foeniculum vulgare* and *Ricinus communis* were evaluated on the fourth instar larvae of *Culex pipiens* (Tables: 3, 4 and 5) after 24, 48h and 72 hrs. respectively, post-treatment in laboratory conditions, based on LC_{50} and LC_{90} values, results showed that all tested plant extracts exhibited toxicity against the fourth instar larvae of *C. pipiens*, and the toxicity of all extracts increased appreciably as treatment time increased especially after 72-hrs. exposure. For all plant extracts, data in (Table 3) *C. zylanicum* plant extract showed the highest toxic effect against 4th instar larvae. The LC_{50} and LC_{90} values were 940.24 and 11394.59 ppm, respectively. The relative potency was 2.91. The lowest toxic plant extract was *Z. officinalis* having a high LC_{50} and LC_{90} values (2744.20 and 11394.59 ppm) and a low relative potency value (1.00). Hence, from the relative potency values, it is suggested that *C. zylanicum* plant extract was 2.91 times more toxic than *Z. officinalis* plant extract. Comparing the slope values, the fourth instar larvae of *Culex pipiens* showed homogeneity response to *C. zylanicum* extract (8.75) whereas, the fourth instar showed heterogeneity response to *Z. officinalis* extract (2.07) after 24-h of treatment. From the mortality data it was also observed that *Z. officinalis* and *S. chinensis* plant extracts showed dramatically increased toxicity after 48 and 72-h exposure LC_{50} 623.92, 231.54 and 632.63 and 207.27 by 2.23,5.47 and 2.19, 6.12 folds, respectively compared to 24-h data, this is may be due to the accumulation the effective material for plant extract inside the insect's body or that materials in plant extracts may need a period of time degradable inside the digestive tract of insect hence the events its effect on the insect. The basis of LC50 and the toxic effect of the plant extracts tested varied depending on the plant species, part, solvent used in the extraction and the extract concentrations (Hasaballah, 2015).

Data in Tables (3, and 4), showed that *F. vulgare* plant extract was the least effective extract (LC_{50} values = 1392.20 and 1268.72 ppm for 48, and 72-hrs. exposure, respectively). The relative position of the six methanolic plant extracts on the basis of their LC_{50} and relative potency values was in the order: *C. zylanicum* > *Curcurma* sp. > *R. communis* > *F. vulgare* > *S. chinensis* > *Z. officinalis* after 24-h exposure, *Z. officinalis* > *S. chinensis* >

Curcurma sp. > *C. zylanicum* > *R. cummunis* > *F. vulgare* after 48-h exposure and *S. chinensis* > *Z. officinalis* > *Curcurma* sp. > *C. zylanicum* > *R. communis* > *F. vulgare* after 72-h exposure, respectively. Many studies showed that derivatives from the different families of evaluated plants have high insecticide toxicity on larvae of different species of mosquitoes and are derived especially from Zingiberacea, Buxaceae, Lauraceae, Apiaceae and Euphorpiaceae, which are potential candidates for control alternatives in these species of insects. For example, these findings (Aguilera *et al*., 2003; Leyva *et al*., 2010). Several groups of phytochemicals have been previously reported to have an insecticidal activity such as alkaloids, stimulants, terpenoids, essential oils and phenols. More than 2000 plant species have been known to produce chemical factors and metabolites of value in pest control programs, members of plant families: Solanaceae, Asteraceae, Cladophoraceae, Labiatae, Miliaceae, Oocystaceae and Rutaceae have various ideal phytochemicals that should possess a combination of toxic effects and residual capacity. Acute toxicity is required at doses comparable to some commercial synthetic insecticides to be highly competitive and effective (Shaalan *et al.,* 2005 and Ghosh *et al.,* 2012).

Table 3: Toxicity response of tested plant extracts against 4th larval instar of *C. pipiens* after 24 h exposure.

Extracts	LC_{50} (ppm) (95% CL)[a]	LC_{90} (ppm) (95% CL)	Relative potency[b]	Slope±SE
Turmeric	1072.61 (979.75-1192.47)	1793.90 (1527.19-2396.17	2.55	5.73 (0.92)
Hohoba	2414.87 (2156.63-2789.18)	4995.52 (3932.02-8066.10)	1.13	4.06 (0.75)
Ginger	2744.20 (2117.08-5567.47)	11394.59 (5597.65-340925)	1.00	2.07 (0.70)
Cinnamon	940.24 (876.94-1010.70)	1316.92 (1198.28-1516.20)	2.91	8.75 (1.10)
Sweet fennel	1276.07 (1040.37-1486.23)	2316.95 (1942.40-1335.74)	2.15	4.94 (0.90
Castor plant	1246.46 (1082.20-1409.76)	2511.26 (2111.41-3326.70)	2.20	4.21 (0.61

[a] LC_{50} and 95% confidence limits (CLs) are given in ppm.
[b] Relative potency is calculated as LC_{50} of the tested plant Ex./LC_{50} of the most effective plant Ex.

Table 4: Toxicity response of tested plant extracts against 4th larval instar of *C. pipiens* after 48 h exposure.

Extracts	LC_{50} (ppm) (95% CL)[a]	LC_{90} (ppm) (95% CL)	Relative potency[b]	Slope±SE
Turmeric	755.96 (618.98-858.70)	1585.32 (1303.20-2422.73)	1.84	3.98 (0.83)
Hohoba	632.63 (110.21-986.31)	2106.11 (1524.05-4361.52)	2.19	2.45 (0.80)
Ginger	623.92 (144.11-932.37)	1699.36 (1243.90-2893.03)	2.23	2.94 (0.93)
Cinnamon	799.58 (735.06-864.37)	1197.44 (1076.91-1416.81)	1.74	7.30 (1.04)
Sweet fennel	1392.20	2674.26	1.00	4.52 (3.11)
Castor plant	938.96 (761.34-1087.05)	2033.33 (1707.25-2716.16)	1.48	3.81 (0.62)

[a] LC_{50} and 95% confidence limits (CLs) are given in ppm.
[b] Relative potency is calculated as LC_{50} of the tested plant Extracts. /LC_{50} of the most effective plant Extracts.

Table 5: Toxicity response of tested plant extracts against 4th larval instar of *C. pipiens* after 72 h exposure.

Extracts	LC_{50} (ppm) (95% CL)[a]	LC_{90} (ppm) (95% CL)	Relative potency[b]	Slope±SE
Turmeric	576.13 (358.15-697.84)	1366.35 (1120.89-2252.88)	2.20	3.41 (0.87)
Hohoba	207.27	1093.47	6.12	1.77 (1.02)
Ginger	231.54	1062.56	5.47	1.93 (1.28)
Cinnamon	630.89 (506.72-717.38)	1048.51 (922.56-1302.93)	2.01	5.80 (1.14)
Sweet fennel	1268.72	2648.73	1.00	4.00 (2.41
Castor plant	634.14 (389.46-787.17)	1431.36 (1186.56-2074.22)	2.00	3.62 (0.86)

[a] LC_{50} and 95% confidence limits (CLs) are given in ppm.
[b] Relative potency is calculated as LC_{50} of the tested plant Ex./LC_{50} of the most effective plant Ex.

Toxicological Studies of Insecticides on Fourth Instar Larvae of *C. pipiens* Mosquitoes*:*

The effectiveness of Lambda-Cyhalothrin 5% EG, Malathion 57% EC, Dimethoate 40% EC and Mineral oil 95% EC insecticides were bio-assayed upon 4th instar larvae of *C. pipiens* at various concentrations. Records in terms of mortality were taken at an interval of 24, 48 and 72-hrs. of exposure to the test insecticides. No control mortality was seen to occur. Toxicity of different insecticides on fourth instar larvae of *C. pipiens* after 24, 48, 72-hrs. is shown in Tables (6, 7, and 8). After 24 h is shown in Table 6. Pyrethroid (Lambda-Cyhalothrin 5% EC) was the most toxic insecticide (LC_{50} was 0. 72 ppm) among all tested insecticides whereas mineral oil (95% EC) was the most potent (LC_{50} 4.172 ppm). However, two organophosphates Malathion (57% EC) and Dimethoate (40% EC) are shown modest toxicity.

All the LC_{50} values decreased after 48 and 72-hrs. when compared with 24-h data (Tables 7 and 8). The toxicity of all insecticides was increased after 72-h, for example, Lambda-Cyhalothrin (5% EC) increased in toxicity from 100-fold after 48 h to 590.12 fold after 72 h compared to the toxicity after 24 h. Furthermore, this trend was shown strongly in pyrethroid compound toxicity. Moreover, the relative potency of the most toxic insecticides after 24 h which are Lambda-Cyhalothrin (5% EG) and mineral oil (95% EC) was (105.83 fold and 1.82 fold, respectively) and Malathion (57% EC) and Dimethoate (40% EC) which is moderate in toxicity the relative potency was (1.00 fold and 1.007 fold, respectively) whereas the lowest relative potency was observed noticeably in Malathion (57% EC) 1.00 fold after all three periods of treatment. In *C.quinquefasciatus* after 24-hour LC50 of malathion to the larvae was 2.2693 mg /L. The most potent plant extract had killed 50% of the larval population at a concentration of 0.0062% (V/V) (Ali and El-Rabaa, 2010) also a previous study reported that may be alkaline phosphatase and non-specific esterases were probably responsible for the detoxification of chlorpyrifos in field populations (Emtithal and Thanaa, 2012). sensitivity to the tested compounds and variable values of slope indicated the homogenous response of the treated *C.pipiens* to different concentrations of the tested compounds and difference between these compounds due to variation in liability toxicant penetration to inside larva or touching it, these results agree with (Jansen and warnier, 2011 and Ibrahim *et al*., 2018). In general, bioassay studies Laboratory that closely simulates the field position help us to establish a correlation between the laboratory bioassay and the field conditions care, to determine tolerance and resistance ratio by establishing consistent log concentration and probit mortality relationship (Ball, 1981; Roush and Miller, 1986; Schouest and Miller, 1988).

Table 6: Toxicity of four tested insecticides against 4th instar larvae of C.pipiens after 24 h exposure.

Insecticides	LC values with 95% confidence limits						Relative potency[(b)]	Slope±SE
	LC_{50}	(95% CL)[(a)]		LC_{90}	(95% CL)			
		Lower	Upper		Lower	Upper		
Lambda-Cyhalothrin 5% EC	0.072	0.010	0.143	0.639	0.403	1.524	105.83	1.35 (0.36)
Malathion 57% EC	7.620	3.466	11.216	33.343	23.831	60.459	1.00	1.99 (0.43)
Dimethoate 40% EC	7.564	3.113	11.514	36.758	25.835	68.950	1.007	1.86 (0.41)
Mineral oil 95% EC	4.172	1.544	6.989	52.581	26.718	263.426	1.82	1.16 (0.29)

[a] LC_{50} and 95% confidence limits (CLs) are given in ppm.
[b] Relative potency is calculated as LC_{50} of the tested insecticide /LC_{50} of the most effective insecticide.

Table 7: Toxicity of four tested insecticides against 4th instar larvae of C. pipiens after 48 h exposure.

Insecticides	LC values with 95% confidence limits						Relative potency[(b)]	Slope±SE
	LC_{50}	(95% CL)[(a)]		LC_{90}	(95% CL)			
		Lower	Upper		Lower	Upper		
Lambda-Cyhalothrin 5% EC	0.044	-	-	0.155	-	-	100.54	2.35 (1.26)
Malathion 57% EC	4.424	0.527	7.911	21.155	14.202	44.441	1.00	1.88 (0.59)
Dimethoate 40% EC	1.177	$1.38X10^{-5}$	4.799	9.393	1.172	18.856	3.75	1.42 (0.70)
Mineral oil 95% EC	2.172	0.445	4.085	25.251	14.121	99.019	2.03	1.20(0.31)

[a] LC_{50} and 95% confidence limits (CLs) are given in ppm.
[b] Relative potency is calculated as LC_{50} of the tested insecticide /LC_{50} of the most effective insecticide

Table 8: Toxicity of four tested insecticides against 4th instar larvae of C.pipiens after 27 h exposure.

Insecticides	LC values with 95% confidence limits						Relative potency[(b)]	Slope±SE
	LC_{50}	(95% CL)[(a)]		LC_{90}	(95% CL)			
		Lower	Upper		Lower	Upper		
Lambda-Cyhalothrin 5% EC	0.008	-	-	0.074	-	-	590.12	1.38 (1.14)
Malathion 57% EC	4.721	0.507	$8.5x10^{-3}$	17.108	11.673	32.303	1.00	2.29 (0.76)
Dimethoate 40% EC	0.086	-	-	3.105	-	-	54.89	0.82 (0.66)
Mineral oil 95% EC	1.574	0.191	3.237	17.958	10.244	65.825	2.99	1.21 (0.34)

[a] LC_{50} and 95% confidence limits (CLs) are given in ppm.
[b] Relative potency is calculated as LC_{50} of the tested insecticide /LC_{50} of the most effective insecticide

Conclusion

Effect of six methanolic plant extracts and four insecticides against 4^{th} instar larvae of *C. pipiens* under laboratory conditions indicated that *C. zylanicum* plant extract was the highest toxic plant followed by *Z. officinalis* but *F. vulgare* methanolic plant extract was the lowest toxic one after the three periods. For insecticides, both tested insecticides exhibited efficiency against the larvae but the pyrethroid (Lambda-Cyhalothrin 50% EC) was more efficient than organophosphate (Malathion57% EC). Indicate that *C.pipiens* mosquitoes in Egypt still increase resistance to organophosphates compared with the results of previous studies and further studies are needed to know chemicals for plant derivatives used in mosquito control.

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ARABIC SUMMARY

مستخلصات النباتات الطبية كمبيدات حشرية خضراء واتجاه صديق للبيئة مع دراسة لتقليل استخدام المبيدات الحشرية لمكافحة يرقات بعوض كيوليكس بيبينز

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نشأت في السنوات الأخيرة العديد من المشاكل البيئية نتيجة الإفراط في استخدام المبيدات الحشرية وأيضا تأثيراتها على الصحة العامة للإنسان. تستخدم المبيدات الحشرية الفوسفورية العضوية والبيريثرويد ات على نطاق واسع لمكافحة بعوض كيوليكس بيبينز. تعتبر المنتجات الطبيعية من بعض النباتات الطبية بديلاً جيدا للمبيدات الحشرية ولتقليل الآثار الضارة على صحة الإنسان والنظام البيئي. بحثت هذه الدراسة كفاءة ستة أنواع من مستخلصات النباتية الطبية: الكركم- الجوجوبا - الزنجبيل - القرفة – الشمر- الخروع لكحول الميثانول ودراسة تأثير أربعة مبيدات حشرية: لامبادا سيهالوثرين، ملاثيون، دايميثوات والزيت معدني كمستحضرات تجارية تم اختبارها بعد 24 ، 48 ، 72 ساعة تحت ظروف المعمل. أظهرت النتائج أن بعوض كيوليكس بيبينز يطور ويزيد من التحمل وكذلك المقاومة للمبيدات الحشرية الفوسفاتية العضوية والبيريثرويدات مع المعاملة المتكررة. أيضًا، يمكن أن نستنتج أن مستخلصات النباتات الطبية يمكن استخدامها كمبيدات حشرية خضراء آمنة وصديقة للبيئة للسيطرة على يرقات بعوض كيوليكس بيبينز.