

Pyrethroid Resistance Monitoring in *Culex pipiens* Mosquito Populations from Three Egyptian Governorates

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ABSTRACT

Insecticide resistance monitoring of mosquito field populations is a crucial for guide and select the rational application of this insecticide. Both, levels and mechanisms of resistance are important to select efficient insecticides for the control of disease vectors. *Culex pipiens* mosquitoes collected from three different Governorates in Egypt (El-Fayoum, Menofia and Giza), were tested for insecticides susceptibility against Lambda-cyhalothrin and DDT. The results showed high resistance to the tested insecticides among Menofia and Giza collected populations, whereas El-Fayoum population showed low level of resistance. a great precaution should be considered about the type of control measures in these areas, when using the same insecticide. The presented output is alarming to health sectors and vector control decision makers. Mosquito control programs may need considering alternative insecticide classes for control of *C. pipiens* in the monitored areas.

KEYWORDS

*Insecticide
Resistance, Culex
Pipiens, Monitoring,
Pyrethroid.*

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INTRODUCTION

Mosquitoes are among the most important insect vectors that transmit numerous widespread and devastating insect-borne diseases. *Culex* species (Diptera: Culicidae) are the most widely distributed mosquitoes in the world. *Culex pipiens* complex has spread from its origin in Africa to tropical and temperate climate zones in all continents. They transmit numerous serious pathogens of diseases, such as lymphatic filariasis, Rift Valley Fever, West Nile encephalitis, St. Louis encephalitis, Venezuelan equine encephalitis and Japanese encephalitis and eastern equine encephalitis (CDC, 2002; Cui et al., 2006; Kasai et al., 2008 and Zhang et al., 2012). Around 1.4 billion people in 73 countries are presently threatened by lymphatic filariasis. In Egypt, *C. pipiens* is the most widespread mosquito species in urban and rural areas and causes a human health risk (Zahran and Abdelgaleil, 2011).

Chemical control, since the 1950s, has been the main effective method to reduce the vectors disease population (Hemingway et al., 2006). The main groups of chemical insecticides are the most recommended for mosquito vector control programs (organochlorines, organophosphates, carbamates, and pyrethroids). Pyrethroids are the main groups of insecticide used for vector control, represent about 25% of the world insecticide market, because they are fast acting, safe (have low toxicity) to mammals and birds. Also, pyrethroids increasingly deployed in indoor residual spraying (IRS) programs especially in Africa (Zaim et al., 2000; Katsuda et al., 2008; WHO, 2009 b). These insecticides are widely used in both agriculture and public health pesticides (WHO, 2009 a & b). The continuous use of one group of insecticides for long period in the same area may cause, under insecticide selection, development of insecticide resistance in most of species including *C. pipiens*. The World Health Organization described

insecticide resistance as “The development of an ability in a strain of an organism to tolerate doses of toxicants, which would prove lethal to a majority of individuals in a normal (susceptible) population of the same species”. Recently, the insecticide resistance is becoming a major problem in the control of vector-borne diseases and public health concern (Rivero et al., 2010). Lambda-cyhalothrin, is now one of the most important synthetic pyrethroid insecticide that kills insects by stimulating their nervous system (a similar mode of action to DDT), also is commonly used in bed net impregnation and IRS to help control the transmission of insect-borne diseases (Tungu et al., 2010).

Detecting susceptibility changes in vector populations through bioassay are the first step to recognize the potential problem. The aim of this study is to identify the susceptibility status of *C. pipiens* populations from different Governorates of Egypt to pyrethroid and DDT to provide public health sectors crucial information to support designing an effective and successful mosquito control program.

MATERIALS AND METHODS

Test insects

Culex pipiens mosquito populations were reared after the technique mentioned by Chapman and Barr (1969). The reference strain used in this study was *C. pipiens* susceptible strain maintained at Naval Medical Research Unit No. 3 (NAMRU 3) insectary under standard conditions (26-28°C, 12 h:12 h light/dark period, 70-80% relative humidity). This strain has not been exposed to any insecticide or biological control agent for longer than 35 years (Zayed et al., 2006).

Larvae of mosquito populations were collected from three different sites across, Menofia (Gazour, Quesna district), Giza (Mansouria canal) and El-Fayoum (Sinnuris) Governorates (Fig. 1). The collection was carried out during November 2013- till

November 2014. Collected mosquito larvae were morphologically identified to species level using the keyset by **Harbach (1985)**. *Culex pipiens* larvae were separated in plastic trays (27 × 16 × 6.5 cm) filled with tap water and fed with fish food until adult emergence. Adults were released in screened net cages (12 × 12 × 12 cm), supplied with 10% sucrose solution and fed on adult pigeon's blood. All tests were conducted using the progeny of the F1 generation.

Animal used for blood feeding process was conducted according to the guidelines of Institutional Animal Care and Use Committee IACUC (protocol No. 14-01).

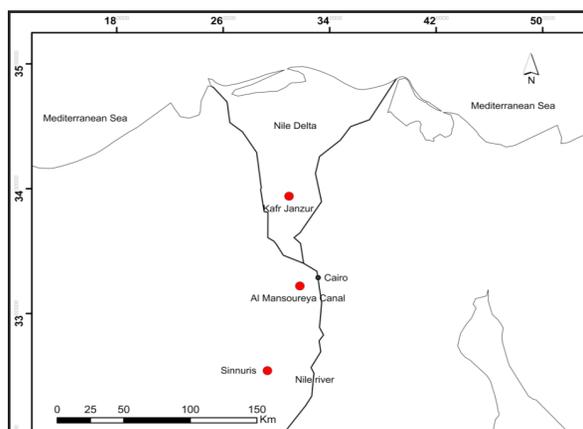


Fig. (1): Map of Egypt showing the sites where populations of *Culex pipiens* were collected.

Insecticide susceptibility tests

The susceptibility status of adult mosquitoes was determined according to WHO standard procedure (**WHO, 1981a, 1998 and 2013**). Insecticides used, 0.05% Lambda-cyhalothrin and 4% DDT, were obtained from WHO Collaborating Centre, Malaysia in the form of insecticide impregnated papers. Batches of 20–25 healthy unfed adult female, aged 3–5 days were exposed to insecticide impregnated papers for 1 h (diagnostic concentrations) of 0.05% lambda-cyhalothrin and 4% DDT. During exposure, the knocked down individuals were counted every 15 minutes. After the exposure time, the tested mosqui-

toes were transferred to the holding tubes, provided with cotton pads soaked in 10% sugar solution and mortality was recorded 24 hours post-exposure. The laboratory susceptible colony strain of *C. pipiens* was used as a reference to ensure the reliability of impregnated papers. Four replicates were conducted for each insecticide and control. All susceptibility tests were carried out at 26–28°C and 70–80% relative humidity.

Statistical analyses

Susceptibility test data were analyzed according to WHO criteria (**Davidson and Zahar, 1973 & WHO, 2013**) as follows; mortality in the range between 98–100% indicates susceptibility; while 80–98% mortality indicated possibility of resistance that needed further confirmation, and <80% mortality indicated resistance.

Mortality data were subjected to one-way analysis of variance, and the Tukey honestly significant difference test ($P < 0.05$) multiple comparison procedure was used to separate significantly different means (**SPSS Statistics, 2008**).

RESULTS

Insecticide resistance status

A field population of mosquito larvae were collected and morphologically identified as *Culex pipiens*. An average of 150 *C. pipiens* females mosquitoes from NAMRU 3 colony were assayed for each of the insecticides tested and control. *C. pipiens* strain was found to be fully susceptible to all of them by yielding 100 % mortality after 24 h of observation and therefore confirmed the effectiveness of the insecticide-impregnated papers.

Two hundred females from each study area (Menoufia, El Fayoum and Giza) were exposed to 0.05% lambda-cyhalothrin and 4% DDT for 1 hour, and mortality were counted after 24 hours. The percentages of knocked-down mosquitoes after exposure

to lambda-cyhalothrin for one hour were increased from 7% to 25%, from 9% to 35% and from 13% to 55% at time intervals 15 and 60 min. for Menofia, Giza and EL-Fayoum populations, respectively (Fig. 2). The percentages of knocked-down mosquitoes after exposure to DDT for one hour increased from 7% to 24%, from 8% to 33% and from 12% to 53%

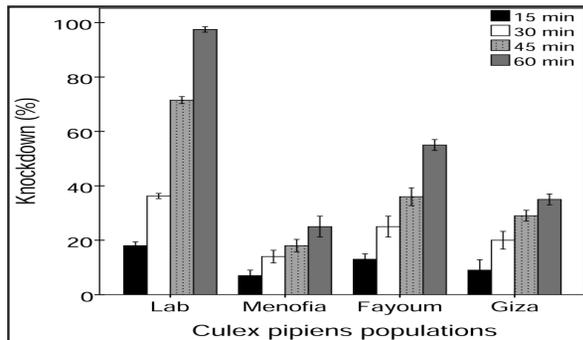


Fig. (2): Percentage Knockdown at four-time intervals of lab and three wild populations of *Culex pipiens* adults exposed to 0.05% Lambda-cyhalothrin.

at time intervals 15 and 60 min. for Menofia, Giza and EL-Fayoum populations, respectively (Fig. 3). Twenty-four-hour percentage mortalities were 43%, 50% and 89% for lambda-cyhalothrin and 40%, 48% and 81% for DDT (Table, 1 and Fig. 4) for Menofia, Giza and EL-Fayoum populations, respectively.

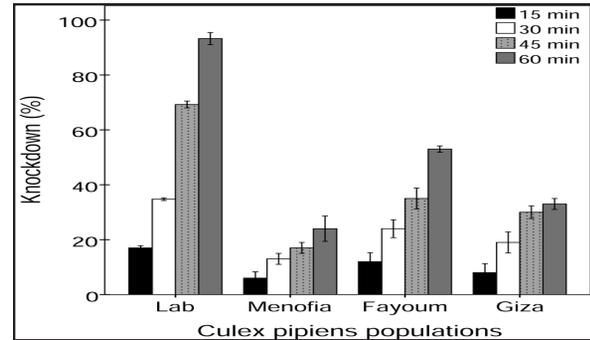


Fig. (3): Percentage Knockdown at four-time intervals of lab and three wild populations of *Culex pipiens* adults exposed to 4% DDT.

Table (1) : Percentage mortalities of lab and three wild populations of *Culex pipiens* adults under laboratory conditions after one-hour exposure to 0.05% Lambda-cyhalothrin and 4% DDT.

Population	Insecticide	
	0.05% Lambda-cyhalothrin	4% DDT
Lab	100.0 ± 0.0 ^a	100.0 ± 0.0 ^a
Menofia	43.0 ± 1.9 ^b	40.0 ± 1.6 ^b
Fayoum	89.0 ± 1.0 ^c	81.0 ± 34 ^c
Giza	50.0 ± 2.0 ^d	48.0 ± 1.6 ^b

- Data are presented as mean ± SE of four replicates.
- Means followed by the same letter within each column are not significantly different (analysis of variance [ANOVA]; Tukey test; $P > 0.05$).

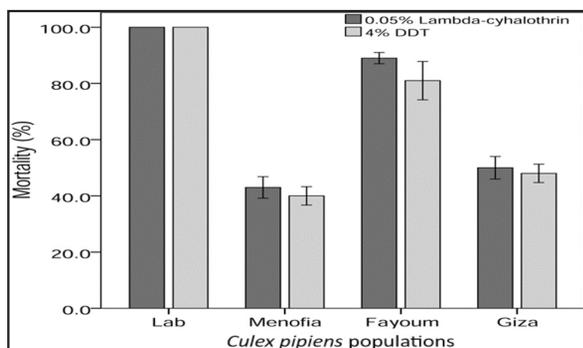


Fig. (4): Percentage mortalities of lab and three wild populations of *Culex pipiens* adults under laboratory conditions after one-hour exposure to 0.05% Lambda-cyhalothrin and 4% DDT.

DISCUSSION

Insecticide application is the most efficient component in the global mosquito vector control effort (Zaim et al., 2000; Najera & Zaim, 2001 and McCarroll & Hemingway, 2002). Pyrethroids are currently the most widely worldwide used insecticides for insect pest control and human disease vectors. Insecticide resistance is a major concern in controlling vector borne diseases, especially in the tropical regions. Accurate monitoring of the resistance status is crucial to guide the rational application of insecti-

cides and resistance management.

According to the WHO criteria (WHO, 2013), the present study demonstrated that *C. pipiens* populations from El-Fayoum, Menofia and Giza are resistant to Lambda-cyhalothrin (pyrethroids) and DDT (organochlorine) with variation in the resistance levels, ranging from the highest resistance and lowest mortality in Menofia to the lowest resistance and highest mortality in El-Fayoum.

Resistance of *C. pipiens* mosquitoes to pyrethroids has been reported in many countries such as China, (Liu *et al.*, 2013), Egypt (Zayed *et al.*, 1997, Mostafa and Allam, 2001, Zayed *et al.*, 2006, El-Sheikh, 2011 and El-Sheikhet *et al.*, 2014), Greece (Kioulos *et al.*, 2014), Japan (Kasai *et al.*, 2009), Korea (Shin *et al.*, 2012), La Reunion (Tantely *et al.*, 2010), Mauritius (Pocquet *et al.*, 2013), Mayotte (Pocquet *et al.*, 2014), Saudi Arabia (Al-Sarar, 2010), and the United States (Ahmed *et al.*, 2012).

The field population collected from EL-Fayoum displayed low level of resistance to lambda-cyhalothrin. In a similar study, *C. pipiens* field strain from Saudi Arabia, Riyadh. Two populations from Wadi Namar (WN1 and WN2) were highly resistant to deltamethrin, while the field population from Al-Wadi district showed low resistance to lambda-cyhalothrin (Al-Sarar, 2010). This was also reported in *C. quinquefasciatus* field strain from Brazil (Gonza' lez *et al.*, 1999) and Malaysia (Nazni *et al.*, 2005). In addition, resistance was detected in *Ae. aegypti* populations to Lambda-cyhalothrin in India (Sharma *et al.*, 2004) and in Saudi Arabia (Al-sheikh *et al.*, 2016).

The present results are in consistence with Zayed *et al.* (2006) who found that adults *C. pipiens* collected from Qalubiya, Sharkiya and Assiut governorates, Egypt were resistant to DDT, but not to Lambda-cyhalothrin. The present findings regarding the low resistance of El-Fayoum population to lambda-cyhalothrin (pyrethroids), is contradicting with that of Mostafa and Allam (2001), who found that

C. pipiens adults from El-Fayoum were susceptible to deltamethrin (pyrethroids).

During this study, DDT resistance was observed in all sites. DDT has not been used in Egypt since the 1970s (Zayed *et al.*, 2006) because high levels of resistance to DDT is not clear. This might be due to that both Pyrethroids and DDT act on the nervous system, and pyrethroid resistance might be associated with cross-resistance of DDT (Hemingway and Ranson, 2000). Cross-resistance between DDT and Pyrethroids has been reported previously in several locations throughout the world. Fonseca-González, *et al.* (2009) reported resistance to lambda-cyhalothrin and DDT among the same population in Colombia despite that fact that DDT was not used recently but 17 years before being replaced by lambda-cyhalothrin for malaria control.

The present results showed that populations from El-Fayoum, Menofia and Giza Governorates (agricultural areas) were resistance to pyrethroids. This might be due to the intensive use of pyrethroids to control agricultural pests in those areas, as previously suggested by Zayed *et al.* (2006). Coordination between health and agricultural sectors in terms of insecticide judicial use is highly recommended to avoid unpredictable development of insecticide resistance in vector populations.

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رصد مقاومة Pyrethroid في تجمعات البعوض *Culex pipiens* من ثلاث محافظات مصرية

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مراقبة مقاومة المبيدات الحشرية المستخدمة في مكافحة البعوض أمر بالغ الأهمية على حد سواء تعد مستويات وآليات المقاومة أمر هام لتحديد كفاءة المبيدات لمكافحة ناقلات الأمراض. وقد تم اختبار المبيد Lambda-cyhalothrin و DDT على بعوض *Culex pipiens* التي تم جمعها من ثلاث محافظات مختلفة في مصر (الفيوم والمنوفية والجيزة). وأظهرت النتائج مقاومة ملحوظة لهذه المبيدات الحشرية التي تم اختبارها بين البعوض المجمع من المنوفية والجيزة ، في حين أظهر البعوض الذي تم تجميعه من الفيوم العكس. وهذا قد يكون جرس انذار لعدم استخدام نفس المبيدات للمكافحة في هذه المناطق . على الجانب الآخر تشيد بالقطاعات الصحية ومتخذي القرار لمكافحة ناقلات الأمراض ان برامج مكافحة البعوض قد تحتاج إلى النظر في مبيدات حشرات بديلة ل *Culex pipiens* في المناطق التي يتم رصدها.

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