بسم الله الرحمن الرحيم

MONA MAGHRABY

شبكة المعلومات الجامعية

التوثيق الالكتروني والميكروفيلم

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شبكة المعلومات الجامعية

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جامعة عين شمس

التوثيق الإلكتروني والميكروفيلم

قسم

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Evaluation of the role of microRNAs as regulators of Cytochrome P450 genes in pyrethroid-resistant *Culex pipiens* mosquito (Diptera: Culicidae)

Thesis

Submitted for the Ph.D. Degree of Science in Biochemistry

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I declare that this thesis has been composed by myself and the work there in has not been submitted for a degree at this or any other University.

Nermeen Talaat Fahmy

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ABSTRACT

Over the past decades, the extensive use of pyrethroids insecticides for vector control has resulted in the development of insecticide resistance. Recently, studying the relative expression of miRNAs and their putative corresponding cytochrome oxidase P450s targets in response to insecticides resistance in mosquitoes has caught a great attention. In the current study, *Culex pipiens* mosquitoes were collected from Giza Governorate in Egypt and tested for insecticide susceptibility against deltamethrin. First detection for Knockdown resistance gene (*Kdr*) mutations in field collected mosquitoes was performed. Activities of P450 detoxification enzyme that synchronized with the resistance development, was monitored. Cloning of P450 family gene followed by sequencing was performed. Primers were designed for the P450 gene generated from sequencing results. Expression profile of miRNAs previously reported in *Cx. pipiens pallens* and their putative cytochrome P450s targets associated with pyrethroid resistance were evaluated in pyrethroid resistant field-collected *Cx. pipiens*. Colorimetric miRNA microarray and stem loop RT-qPCR were used for miRNA expression profile, while RT-qPCR was used for P450s expression profile. This expression profile has not been previously reported in *Cx. pipiens* mosquito in Egypt. Our results elucidated the pyrethroid resistance development and its relation to the metabolic and target site modification mechanisms with a first report of *L1014F-kdr* mutation detection. Our sequences revealed the presence of a new variant of P450 gene named *CYP4J19v* in field-resistant population. RT-qPCR results have revealed the down-regulation of miR-278-3P, miR-2, miR-71, and miR-285

and the upregulation of miR-13, miR-2951, miR-309 and the studied P450s targets. Negative expression patterns between miRNAs and their corresponding P450s targets have been detected, except for miR-13, miR-2951, miR-309, which showed positive expression pattern with their corresponding P450s targets. Interestingly, our results were the first to detect negative expression pattern between miR-285 and its potential *CYP6Cp1* target gene. These findings have highlighted the importance of directing more research efforts towards understanding the miRNA expression pattern in regulating pyrethroid-resistant through P450s in *Culex* vector, to provide a better understanding of their role in the development of insecticide resistance, that would help in shaping strategies to combat such vectors.

Key word: *Culex pipiens*, *kdr*, cytochrome P450, microRNA, pyrethroid resistance, expression profiling

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LIST OF CONTENTS

LIST OF ABBERIVIATIONS

Ae.	Aedes
An.	Anopheles
Asn	Asparagine
AS-PCR	Allelic specific polymerase chain reaction
BLAST	Basic Local Alignment Search Tool
β-ME	Beta-mercaptoethanol
bp	Base pair
CCD	Charge-coupled device digital camera
cDNA	Complementary DNA
CPR	Cytochrome P450 reductase
Ct	Threshold cycle
Cys	Cysteine
Cx.	Culex
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
dNTP	Dinucleotide triphosphate
EDTA	Ethylenediaminetetraacetic acid
FAD	Flavin adenine dinucleotide
FMN	Flavin mononucleotide
FRET	Fluorescence Resonance Energy Transfer
Gly	Glycine
HOLA	Heated Oligonucleotide Ligation Assay
IRAC	Insecticide Resistance Action Committee
Kdr	Knockdown resistance
KPO4	Potassium phosphate
Leu	Leucine
LC_{50}	Lethal median concentration
MEGA	Molecular Evolutionary Genetics Analysis
mRNA	messenger RNA

miRNA	MicroRNA
NADPH	Nicotinamide adenine dinucleotide phosphate
NaOAc	Sodium acetate
ncRNA	Non-coding RNA
NCBI	National Center for Biotechnology Information
NGS	Next Generation Sequence
nt	Nucleotides
PBS	Phosphate-buffered saline
PCR	Polymerase Chain Reaction
P450	Cytochrome P450
Phe	Phenyl alanine
RQ	Relative Quantification
rpm	Revolutions per minute
RNA	RNA ribose nucleic acid
RT	Reverse transcriptase
RT-PCR	Reverse Transcriptase polymerase chain reaction
RVF	Rift Valley Fever
SE	Standard Error
Ser	Serine
SDS	Sodium dodecyl sulfate
TAE	Tris-acetate EDTA
TMB	3,3',5,5'-Tetramethylbenzidine
Tyr	Tyrosine
UTR	Untranslated regions
UV	Ultra violet
Va	Valine
WHO	World Health Organization
WNV	West Nile Virus
X-Gal	Bromochloroindolylgalactopyranoside