

**MOLECULAR ANALYSIS OF MECHANISMS AND IDENTIFICATION OF  
FACTORS OF PYRETHROID RESISTANCE IN *ANOPHELES GAMBIAE SENSU  
LATO* IN SOUTHWESTERN NIGERIA AND SOUTHERN BENIN REPUBLIC**

By

**Jean Rousseau DJOUAKA-FOLEFACK**  
B.Sc. (Cameroon), M.Sc. Zoology (Ibadan),  
S.I. 119677

**A thesis in the Department of Zoology submitted to the Faculty of Science in partial  
fulfilment of the requirements for the degree of**

**DOCTOR OF PHILOSOPHY**

of the  
**UNIVERSITY OF IBADAN**

2011

## ABSTRACT

The development of resistance to insecticides by *Anopheles* mosquitoes continues to threaten the success of malaria control programmes in West Africa. Local data on mechanisms and factors causing resistance in the region are scanty. This study was designed to investigate the environmental factors and mechanisms implicated in resistance to pyrethroids by *Anopheles gambiae* in southwestern Nigeria and southern Benin Republic.

Larvae of *Anopheles* mosquito were collected in 2007 from 19 localities in the six states of southwestern Nigeria and 18 localities in the seven divisions of southern Benin and reared to adults. These were identified morphologically and with Polymerase Chain Reactions (PCR). They were also bioassayed for susceptibility to pyrethroids. Molecular characterisation of pyrethroid resistant phenotypes was carried out using PCR and microarray analyses of the expressed genes. Dissolved Oxygen (DO) and pH were determined using a digital multipurpose meter while physical appearances of breeding sites were assessed visually. Xenobiotic factors such as Spilled Engine Oil (SEO) and agricultural pesticides that might contribute to the emergence of resistance in *Anopheles* populations were examined through bioassay. Associations between pyrethroid resistance with environmental factors and molecular profiles of *Anopheles* were evaluated using Chi square.

*A. gambiae* complex genotyped in Nigeria comprised of 73.6 % *A. arabiensis* and 26.3 % *A. gambiae sensu stricto*; while those genotyped in Benin were 92.9 % *A. gambiae s.s.* and 7.0 % *A. melas*. Pyrethroid resistance in Nigeria and Benin were recorded in 68.4 % and 94.4 % of the localities examined respectively. Breeding sites contaminated with SEO (B-SEO) or Pesticide Residues (B-PR) had low DO (B-SEO =  $13.4 \pm 1.5$  mg/l, B-PR =  $12.2 \pm 1.7$  mg/l), the Non-contaminated Breeding sites (B-NC) had higher levels of DO (B-NC =  $33.1 \pm 2.3$ ) and mainly produced pyrethroid-susceptible *Anopheles* ( $p < 0.05$ ). Significant variations in pH were not recorded. Differences in habitation by resistant-*Anopheles* in breeding sites contaminated with SEO or pesticide residues were observed. *A. gambiae* found around the two agricultural sites (Houeyiho, Benin and Ajibode, Nigeria) exposed to synthetic pesticides showed significant levels of pyrethroid resistance with mortality rates of 70.0 % and 89.7% respectively. *A. gambiae* larvae survived at

SEO concentrations below  $11.8 \times 10^{-3} \mu\text{L}/\text{cm}^2$ . Ninety six percent of larval mortality resulted from direct cuticle contact with SEO whereas only four percentage mortality was from larval suffocation. A cross resistance phenomenon was recorded with SEO and pyrethroids. *A. gambiae* showed the presence of elevated frequencies of knock down resistance West (*kdr-W*) mutations in Benin samples (*kdr-W* ranged from 0.6 to 0.9) and absence of *kdr-W* in Nigeria samples. Two detoxification genes (*CYP6P3* and *CYP6M2*) were up-regulated in resistant-*Anopheles*. Additional detoxification genes specific to agricultural and SEO sites were also over-expressed in the resistant populations.

There was an association between residual synthetic pesticides, spilled engine oil and emergence of pyrethroid-resistance in *A. gambiae* in Nigeria and Benin Republic. The diversified profile of identified metabolic genes reflected the influence of a range of xenobiotics on selection of resistance in mosquitoes.

**Keywords:** *Anopheles*, Pyrethroid resistance, Xenobiotics.

**Word count: 487**

## **DEDICATION**

This project is dedicated to GOD almighty, who has being my source of inspiration and sustenance. To my home institution, colleagues, family members and friends for their constant support in the course of this programme.

UNIVERSITY OF IBADAN

## **CERTIFICATION**

This is to certify that this work was carried out by MR. Jean Rousseau DJOUAKA-FOLEFACK in Cell Biology and Genetics Unit of Department of Zoology, University of Ibadan, Ibadan, Nigeria.

.....

(SUPERVISOR)

Dr. A. A. Bakare

B.Sc., M.Sc., Ph.D (Ibadan)

Cell Biology and Genetics Unit.

Department of Zoology,

University of Ibadan, Ibadan, Nigeria.

UNIVERSITY OF IBADAN

## ACKNOWLEDGEMENT

I would like to take this opportunity to express deep gratitude to my supervisor Dr. A. Bakare and my co-supervisor, Dr. Samson Awolola for their constant encouragement, supervision and guidance during this PhD research. Sincerely, you have given me a lot during this long and very exciting research venture. My profound gratitude to Dr. Ousmane Coulibaly for his guidance and immense support throughout the duration of this programme. I appreciate all the academic and non-academic staffs of the Department of Zoology, University of Ibadan for the prompt and constant willingness to give a helping hand throughout this research study.

The financial support for this PhD research work was partially provided by the LSTM (Prof. Janet Hemingway) and the UNICEF/UNDP/World Bank/WHO Special Programme for Research and training in Tropical Diseases (TDR). I appreciate my laboratory supervisors at the Liverpool School of Tropical Medicine (LSTM) in the United Kingdom for sharing their scientific knowledge with me and providing the necessary training in microarray and other molecular works. Your contribution was of paramount importance in this research. To Prof. Janet Hemingway Director of LSTM, I am very thankful for your scientific and financial contributions and for covering all expenses during my trips to the LSTM and for providing all laboratory consumables needed for this research work. I remember the sleepless night asking myself if I will be able to complete this Ph.D. program; Thanks Prof. Janet, you made this a reality and I sincerely appreciate it.

Huge thanks goes to my family for being incredibly supportive, understanding and patient not only throughout this study, but in everyday of my life. I will like to thank almighty God for giving me a good health and empowering me with more knowledge, the best weapon for survival.

## TABLE OF CONTENT

	Page
Title page.....	i
Abstract.....	ii
Dedication .....	iv
Certification .....	v
Acknowledgement.....	vi
Table of contents .....	vii
List of tables .....	xiv
List of figures .....	xv
List of appendix .....	xix
CHAPTER ONE .....	1
1.0 INTRODUCTION .....	1
1.1 Aim of this study.....	4
1.2 Objectives of this study .....	5
CHAPTER TWO .....	6
2.0 LITERATURE REVIEW .....	6
2.1 Malaria disease and transmission of malaria parasites.....	6
2.2 The biology and the ecology of malaria vectors .....	9
2.2.1 Adults .....	11
2.2.2 Mating and blood-feeding of adult <i>Anopheles</i> .....	11
2.2.3 The ecology of breeding sites .....	12
2.3 Factors involved in malaria transmission .....	12
2.3.1 Preferred sources for blood meals .....	13
2.3.2 Life Span .....	13
2.3.3 Patterns of feeding and resting .....	13
2.4 Major vectors of malaria in sub-Saharan Africa. ....	13
2.4.1 The <i>A. gambiae</i> complex .....	14
2.4.2 <i>A. funestus</i> group .....	14
2.5 Vector Identification .....	15
2.5.1 Application of molecular techniques in the identification of sibling species .....	16

2.5.1.1 Restriction fragment length polymorphism .....	18
2.6 Malaria vector control strategies .....	19
2.6.1 Insecticide-treated bed nets .....	19
2.6.1.1 Bednets ownership patterns in Nigeria .....	22
2.6.1.2 Bednets ownership patterns in Benin Republic .....	23
2.6.2 Indoor residual spraying .....	24
2.6.3 Breeding sites reduction .....	25
2.6.4 Less implemented vector control strategies.....	25
2.6.5 The development of genetically modified <i>Anopheles</i> (GMA) for malaria control ..	26
2.7 The use of insecticides in Public Health .....	26
2.7.1 Insecticides and mode of action .....	27
2.7.1.1 Organochlorine .....	27
2.7.1.2 Organophosphate .....	30
2.7.1.3 Carbamates .....	30
2.7.1.4 Pyrethroids .....	30
2.7.2 Protective mechanisms of <i>Anopheles</i> and development of resistance to insecticides... ..	31
2.7.2.1 Resistance of <i>Anopheles</i> to insecticides .....	31
2.7.2.2 Behavioral mechanisms of resistance .....	34
2.7.2.3 Metabolic mechanisms of resistance .....	34
2.7.2.3.1 Cytochrome P450s (monooxygenase or oxidase mechanisms) .....	34
2.7.2.3.2 Glutathione-S-transferase .....	34
2.7.2.3.3 Target site modification (mutations) .....	34
2.8 Detection of insecticide resistance in malaria vectors .....	36
2.8.1 Bio-assay for phenotyping insecticide resistance in vectors .....	36
2.8.2 The bottle tests with synergists .....	38
2.8.3 Polymerase chain reaction (PCR) for target modification .....	38
2.8.4 Microarray technique for detection of detoxification genes .....	38
2.8.5 Reported cases of resistance of <i>A. gambiae</i> to insecticides in Nigeria .....	39
2.8.6 Reported cases of resistance of <i>A. gambiae</i> to insecticides in Benin .....	40



2.8.7 Reported cases of resistance of <i>A. gambiae</i> to insecticides in other African countries .....	40
2.9 Factors favouring the emergence of resistance in mosquito populations .....	41
2.9.1 The selection of insecticide resistance by ITNs and IRS .....	41
2.9.2 Agricultural pesticide residues and other xenobiotics .....	42
2.9.3 Types of mechanisms of resistance selected by ITNs, IRS, agricultural pesticide residues and xenobiotics .....	43
CHAPTER THREE .....	44
3.0 MATERIALS AND METHODS .....	44
3.1 Susceptibility of <i>Anopheles</i> populations to pyrethroid in studied sites .....	44
3.1.1 Description of sampling sites .....	44
3.1.2 Collection of <i>Anopheles</i> larvae .....	44
3.1.3 Rearing of larvae to adult stage in the insectary .....	44
3.1.4 WHO susceptibility tests on adult <i>Anopheles</i> from surveyed localities .....	48
3.1.5 Mapping of permethrin susceptibility in <i>Anopheles</i> populations from studied localities .....	48
3.2. Molecular characterization of <i>Anopheles</i> populations from studied sites: PCR-species, PCR-forms, PCR- <i>kdr</i> .....	50
3.2.1 DNA extraction .....	50
3.2.2 Polymerase chain reaction for <i>A. gambiae</i> speciation .....	54
3.2.3 Polymerase chain reaction for detection of the knock down ( <i>kdr</i> ) mutation in <i>A. gambiae</i> populations .....	54
3.2.4 Specific primers used for PCR-species and PCR- <i>kdr</i> .....	54
3.2.5 Electrophoresis of PCR products .....	55
3.3 Evaluation of potential contributions of agricultural pesticide residues and spilled petroleum products in the selection of pyrethroid resistance in <i>Anopheles</i> populations .....	55
3.3.1 Screening of pesticide residues in water and soil samples from vegetable sites .....	55
3.3.1.1 Screening technique .....	55
3.3.1.2 Collection of water and soil samples used for the bio-assay .....	55
3.3.1.3 History of synthetic pesticides utilization by farmers in target agricultural sites...	56

3.3.2 Evaluation of the contribution of petroleum products in the selection for insecticide resistance in <i>A. gambiae</i> .....	58
3.3.2.1 KAP studies on the empirical utilisation of petroleum products (PP) in rural communities .....	58
3.3.2.2 <i>Anopheles</i> populations used for analysing the lethal activities of PP. ....	58
3.3.2.3 Determination of lethal concentrations of 4 PP on larvae of <i>A. gambiae</i> .....	58
3.3.2.4 Identification of the mode of action of PP on <i>Anopheles</i> larvae.....	58
3.3.3 Analysis of associations between the presence of petroleum products in breeding sites and the emergence of pyrethroid resistant populations of <i>Anopheles</i> .....	61
3.3.3.1 Physico-chemical properties of breeding sites of resistant and susceptible <i>Anopheles</i> .....	61
3.3.3.2 Selection of oviposition spots by gravid females of <i>Anopheles</i> in localities where some breeding sites are contaminated with spilled petroleum products .....	61
3.3.3.3 Monitoring of the development of <i>Anopheles</i> larvae (resistant and susceptible strains) in breeding sites with petroleum products residues .....	62
3.4 Screening of candidate metabolic genes overexpressed in pyrethroid resistant populations of <i>A. gambiae</i> from Benin and Nigeria .....	62
3.4.1 Selection of permethrin resistant <i>A. gambiae</i> for micro-array analysis .....	62
3.4.1.1 Target preparation and microarray hybridizations .....	63
3.4.1.2 cDNA synthesis, labelling and hybridization .....	63
3.4.1.3 Array scanning and visualization .....	67
3.5. Data analysis .....	67
3.5.1 Analysis of data on the susceptibility level of <i>Anopheles</i> to permtehrin: .....	67
3.5.2 Questionnaires, focuss group discussions and indepth-interviews with farmers and petroleum products users .....	67
3.5.3 Lethal activities of petroleum products on <i>Anopheles</i> larvae .....	69
3.5.4 Cross analysis of the physico-chemical properties of breeding sites and the susceptibility status of emerging <i>Anopheles</i> populations .....	69
3.5.5 Analysis of data on the oviposition preference of gravid <i>Anopheles</i> , eggs hatching rates and larval developments in simulated breeding sites .....	69

3.5.6 Allelic frequencies of the <i>kdr</i> mutation in permethrin resistant and susceptible phenotypes of <i>Anopheles</i> mosquitoes analysed .....	71
3.5.7 Analysis of micro-array spots for expressed metabolic genes identified in studied <i>Anopheles</i> populations .....	71
CHAPTER FOUR .....	73
4.0 RESULTS .....	73
4.1 Screening of the susceptibility of <i>Anopheles</i> populations to pyrethroid in southwestern Nigeria and southern Benin .....	73
4.1.1 Susceptibility to permethrin of <i>A. gambiae</i> populations in southwestern Nigeria ...	73
4.1.2 Susceptibility to permethrin of <i>A. gambiae</i> collected in southern Benin .....	75
4.2 Molecular characterization of <i>Anopheles</i> populations from surveyed sites in Nigeria and Benin (PCR-species, PCR-forms and PCR- <i>kdr</i> ) .....	78
4.2.1 Molecular characterisation of mosquito populations from the southwestern Nigeria.....	79
4.2.2 Molecular characterisation of mosquito populations from the southern Benin .....	79
4.3 Evaluation of potential contributions of agricultural pesticides in the selection of pyrethroid resistance in <i>Anopheles</i> populations breeding around vegetable farms ....	88
4.3.1 The use of synthetic pesticides in the vegetable farm of Houeyiho in Benin .....	88
4.3.2 The use of synthetic pesticides in the vegetable farm of Ajibode in Nigeria .....	88
4.3.3 Susceptibility to permethrin of <i>A. gambiae</i> collected around vegetable farms of Houeyiho and Ajibode .....	89
4.3.4 Assessment of the presence of pesticides residues in breeding sites found around vegetable sites through monitoring of the hatching rates of <i>Anopheles</i> eggs .....	90
4.3.5 Assessment of the presence of pesticides residues in breeding sites found around vegetable sites through monitoring of larval development rates.....	93
4.4 Evaluation of the potential contributions of spilled petroleum products in the selection of pyrethroid resistance in <i>A. gambiae</i> populations .....	99
4.4.1 The empirical utilisation of petroleum products by rural communities in the southern Benin for mosquito control. ....	99
4.4.2 Analysis of lethal effect of petrol on permethrin resistant larvae of <i>A. gambiae</i> from Ladji and from Ojoo .....	100

4.4.3 Analysis of lethal activity of kerosene on permethrin resistant larvae of <i>A. gambiae</i> from Ladji and from Ojoo .....	104
4.4.4 Analysis of lethal effect of new engine oil on permethrin resistant larvae of <i>A. gambiae</i> from Ladji and from Ojoo .....	106
4.4.5 Analysis of lethal activity of used engine oil on permethrin resistant larvae of <i>A. gambiae</i> from Ladji and from Ojoo .....	106
4.4.6 Identification of the mode of action of PP on <i>A. gambiae</i> larvae .....	107
4.4.7 Analysis of physico-chemical properties of breeding sites producing pyrethroids resistant and susceptible populations of <i>A. gambiae</i> in southwestern Nigeria and southern Benin.....	110
4.4.8 Identification of the preferred types of breeding sites selected by pyrethroids susceptible and resistant strains of <i>Anopheles</i> for ovipositions .....	115
4.4.9 Hatching rate of eggs laid by pyrethroids susceptible and resistant strains in oily breeding sites from Nigeria and Benin .....	118
4.4.10 Development of larvae of pyrethroid susceptible and resistant strains in oily breeding sites (rate of larvae getting to pupae stage) .....	118
4.5 Identification of detoxifying genes up-regulated in pyrethroids resistant <i>Anopheles</i> from sites of spilled petroleum products and agricultural sites under pesticides utilisation .....	120
4.5.1 Genotyping and bioassay of <i>Anopheles</i> populations prior to micro-array analysis .....	120
4.5.2 Identification of metabolic genes over transcribed on <i>Anopheles</i> samples from agricultural setting .....	122
4.5.3 Identification of metabolic genes over transcribed on <i>Anopheles</i> samples from oil spillage site .....	122
4.5.4 Comparative expression profiles of metabolic genes in permethrin resistant <i>Anopheles</i> from Akron in southern Benin and Ojoo in the southwestern Nigeria.....	122
CHAPTER FIVE .....	130
5.0 Discussion .....	130
5.1 The susceptibility pattern of <i>Anopheles</i> populations to pyrethroid in	

southwestern Nigeria and southern Benin .....	130
5.2 Genotyping of permethrin resistant phenotypes .....	131
5.3 The hatching rate of <i>Anopheles</i> strains in breeding sites simulated with soil and water samples from vegetable farms .....	132
5.4 Water and soil samples from vegetable farms contain compounds that inhibit <i>Anopheles</i> larval development. ....	133
5.5 The implication of pesticides residues in the emergence of pyrethroid resistance in malaria vectors .....	134
5.6 The treatment of mosquito breeding sites with petroleum products and the selection of pyrethroid resistance in malaria vectors .....	135
5.7 Identification of the mode of action of PP on <i>A. gambiae</i> larvae .....	136
5.8 Inhibitory effects of petroleum on <i>Anopheles</i> oviposition and larval development ..	136
5.9 Existence of a cross resistance between petroleum products and permethrin in sampled <i>Anopheles</i> .....	137
5.10 Identification of detoxifying genes up-regulated in pyrethroids resistant <i>Anopheles</i> from sites of spilled petroleum products and from agricultural areas under pesticides utilisation .....	138
5.11 Differential expression of metabolic genes by <i>A. gambiae</i> populations collected around vegetable farms and those from localities of spilled petroleum products .....	140
CHAPTER SIX .....	142
6.0 CONCLUSION AND RECOMMENDATIONS .....	142
References.....	145
Appendix .....	165

## LIST OF TABLES

	<b>Page</b>
4.1 Susceptibility to permethrin of <i>A. gambiae</i> in southwestern Nigeria .....	74
4.2 Susceptibility of <i>A. gambiae</i> populations to permethrin in southern Benin.....	77
4.3 Distribution of members of <i>A. gambiae</i> complex in studied localities in Nigeria.....	82
4.4 Allelic frequencies of the <i>kdr</i> mutation in <i>Anopheles</i> species from the studied localities in Nigeria.....	83
4.5 Distribution of members of <i>A. gambiae</i> complex in studied localities in Benin.....	86
4.6 Distribution of the <i>kdr</i> alleles in <i>Anopheles</i> species from the studied localities in Benin.....	87
4.7 The use of pesticides in the vegetable farm of Houeyiho in Benin and Ajibode in Nigeria.....	91
4.8 Comparison of the mean mortality rates to permethrin of <i>A. gambiae</i> populations produced by oily and non-oily sites in Nigeria.....	112
4.9 Physico chemical parameters (pH and DO) of breeding sites producing susceptible and resistant populations of <i>Anopheles</i> in southwestern Nigeria.....	113
4.10 Comparison of the mean mortality rates to permethrin of <i>A. gambiae</i> populations produced by turbid and non-turbid sites in southern Benin.....	114
4.11 Physico chemical parameters (pH and DO) of breeding sites producing susceptible and resistant populations of <i>Anopheles</i> in southern Benin.....	116
4.12 Molecular form, percentage mortality and <i>kdr</i> frequency of <i>A. gambiae</i> from southern Benin and southwestern Nigeria.....	123
4.13 Candidate metabolic genes from micro-array analysis of resistant and susceptible field samples from Akron and Orogun.....	125
4.14 Candidate metabolic genes up-regulated in resistant populations of <i>Anopheles</i> from Ojoo .....	127

## LIST OF FIGURES

	<b>Page</b>
1.1 Trends of malaria in the world.....	2
2.1 The life Cycle of malaria parasite.....	8
2.2 The biological Cycle of <i>Anopheles species</i> .....	10
2.3 The exponential amplification of the gene in Polymerase Chain Reaction (PCR); (A) represents the denaturation phase, (B) the annealing or hybridization and (C) the amplification or extension phase.....	17
2.4 Chromosomal inversions in <i>A. gambiae</i> females; this mosquito is heterokaryotypic for inversions 2Rb and 2Rc (A) as well as the 2La inversion (B) .....	20
2.5 The use of insecticide-treated bed nets (ITNs) for malaria vector control .....	21
2.6 The different targets of insecticides in <i>Anopheles</i> mosquitoes.....	28
2.7 Chemical structure of some insecticides used in public health.....	29
2.8 Chemical equations of enzymatic activities developed by mosquitoes to withstand insecticide lethal doses.....	32
2.9 Series of mechanisms of resistance to insecticides developed by <i>Anopheles</i> .....	33
2.10 Mutation on the sodium channel in <i>kdr</i> resistant <i>Anopheles</i> from West and East Africa.....	37
3.1 Map of study areas (Divisions and States) in Benin and Nigeria.....	45
3.2 Identification of breeding sites and field collection of <i>Anopheles</i> larvae.....	46
3.3 The horizontal position of <i>Anopheles</i> larvae at the surface of water.....	47
3.4 WHO bioassay steps for analysing the susceptibility status of <i>Anopheles</i> to insecticides.....	49
3.5 DNA extraction for PCR analysis of collected <i>Anopheles</i> samples.....	51
3.6 DNA amplification (PCR reactions) of collected <i>Anopheles</i> samples.....	52
3.7 Agarose gel migration of PCR products of amplified <i>Anopheles</i> DNA.....	53
3.8 Map of sites selected for screening of pesticides residues in vegetable farm (Akpakpa-CREC, Houeyiho, Ajibode and UI- Ibadan).....	57
3.9 Quantification of the lethal activity of 4 petroleum products on <i>Anopheles</i> larvae introduced in plates containing different concentrations of PP.....	60

3.10 Map showing the 3 study sites selected for candidate metabolic gene search and their locations in relation to the control site (Orogun).....	64
3.11 Steps for producing and labeling cDNA of <i>Anopheles</i> samples from target sites (Ojoo, Akron and Orogun) for micro-array hybridization.....	65
3.12 Gel Analysis of migrated total RNA prior to cDNA production.....	66
3.13 Steps for synthesis and printing of detoxifying gene probes of <i>A. gambiae</i> on the “Detox” chip array.....	68
3.14 Visualisation and analysis of scanned microarray slide; the red spots correspond to upregulation of genes from resistant <i>Anopheles</i> on a given probe while the green dots correspond to up regulation of genes from susceptible populations of <i>Anopheles</i> .....	72
4.1 Map of permethrin susceptibility status of <i>Anopheles</i> populations in the surveyed localities of southwestern Nigeria and southern Benin.....	76
4.2a Members of the <i>A. gambiae</i> complex identified in southwestern Nigeria.....	80
4.2b Samples from the locality of Challenge: the banding pattern shows <i>Anopheles</i> <i>gambiae</i> ss (coded “g”) and <i>A. arabiensis</i> (coded “Ar”) living together in similar site.....	81
4.3a Mechanisms of permethrin resistance identified in southwestern Nigeria and southern Benin.....	84
4.4 Members of the <i>A. gambiae</i> complex identified in the southern Benin.....	85
4.3b The <i>kdr</i> Banding pattern recorded with samples from Benin.....	89
4.5 Hatching rate of resistant and susceptible strains of <i>Anopheles</i> in simulated water and soil samples from vegetable farms of Houeyiho .....	92
4.6 Hatching rate of resistant and susceptible strains of <i>Anopheles</i> in simulated water and soil samples from vegetable farms of Ajibode.....	94
4.7 Larval development of resistant and susceptible strains of <i>Anopheles</i> in simulated water and soil samples from vegetable farms of Houeyiho.....	95
4.8 Larval development of resistant and susceptible strains of <i>Anopheles</i> in simulated water and soil samples from vegetable farms of Ajibode.....	97
4.9 The yield of rearing resistant and susceptible strains of <i>Anopheles</i> in simulated water and soil samples from vegetable farms of Houeyiho.....	98



4.10 The yield of rearing resistant and susceptible strains of <i>Anopheles</i> in simulated water and soil samples from vegetable farms of Ajibode, Ibadan.....	100
4.11 Utilisation of petroleum products for mosquito control in rural communities in southern Benin.....	102
4.12 Larvicidal effect of petroleum products on larvae of <i>A. gambiae</i> from Ladji; a similar mortality trend is recorded with most PP except petrol which shows a lower larvicidal effect .....	103
4.13 Larvicidal effect of petroleum products on larvae of <i>A. gambiae</i> from Ojoo.....	105
4.14 Comparative analysis of recorded HIC of petroleum products on larvae of <i>Anopheles gambiae</i> from Ladji and from Ojoo larvae.....	108
4.15 Comparative analysis of recorded LoC <sub>100</sub> of petroleum products on larvae of <i>A. gambiae</i> from Ladji and Ojoo larvae.....	109
4.16 Mode of action of petroleum products on <i>Anopheles</i> larvae (Mortality rates recorded with sieved and crude or raw petroleum products from field contaminated breeding sites) .....	111
4.17 Number of eggs laid by resistant ( <i>A. gambiae</i> from Ojoo) and susceptible ( <i>A. gambiae</i> from UI) strains of <i>Anopheles</i> in oily and non-oily breeding sites from southwestern Nigeria and southern Benin.....	117
4.18 Hatching rate of eggs laid by resistant ( <i>A. gambiae</i> from Ojoo) and susceptible ( <i>A. gambiae</i> from UI) strains of <i>Anopheles</i> in oily and non-oily breeding sites from Nigeria and Benin.....	119
4.19 Rate of larvae (larvae from hatched eggs) of <i>A. gambiae</i> from Ojoo and <i>A. gambiae</i> from UI developing to pupae stage in oily and non-oily breeding sites from southwestern Nigeria and southern Benin.....	121
4.20 Candidate metabolic genes over transcribed on resistant <i>Anopheles</i> mosquitoes from the agricultural site of Akron when co-hybridized with the susceptible strain <i>A. gambiae</i> from Orogun.....	124
4.21 Candidate metabolic genes over transcribed on resistant <i>Anopheles</i> mosquitoes from the oil spillage locality of Ojoo when co-hybridized with the susceptible strain <i>A. gambiae</i> from Orogun.....	128

4.22 Cohort of expressed genes in resistant population of *A. gambiae*  
from the Agricultural site of Akron in Benin and the site of spilled  
oil of Ojoo in Nigeria.....129

UNIVERSITY OF IBADAN

## LIST OF APPENDIX

	<b>Page</b>
1. Indepth interview guide on knowledge, attitudes and practices (KAP) of communities on synthetic pesticides use in Agriculture .....	165
2. Indepth interview guide on knowledge, attitudes and practices (KAP) of communities on the use of petroleum products (PP) for mosquito control .....	166
3. WHO bioassay for insecticide susceptibility using adult mosquitoes .....	167
4. Laboratory protocols and preparation of solutions .....	169
5. Characteristics and toxicity of petroleum products on insects.....	171

UNIVERSITY OF IBADAN