

TABLE OF CONTENTS

	Page
ABSTRACT	i
ABSTRAK	iv
ACKNOWLEDGEMENTS	viii
LIST OF PUBLICATIONS FROM THESIS	x
TABLE OF CONTENTS	xiii
LIST OF TABLES	xxi
LIST OF FIGURES	xxvii
LIST OF PLATES	xxxiii
LIST OF APPENDICES	xxxix
LIST OF ABBREVIATIONS & SYMBOLS	xliii
CHAPTER 1: GENERAL INTRODUCTION	
1.1 Scope of study	1
1.2 Objective of study	6
CHAPTER 2: LITERATURE REVIEW	
2.1 Mosquitoes	8
2.2 Biology and life cycle	8
2.2.1 Egg	8
2.2.2 Larvae	9
2.2.3 Pupae	10
2.2.4 Adult	10
2.3 Insecticide resistance	13
2.3.1 Insecticide resistance in vector mosquitoes	13
	xiii

2.4	Insecticide resistance mechanism and causes of resistance	14
2.4.1	Genetic basis of resistance	15
2.4.2	Biochemical basis of resistance	15
2.4.2.1	Increased detoxication	16
2.4.2.2	Target-Site Mechanisms	17
2.4.3	Reduced penetration	18
2.4.4	Cross-resistance	18
2.4.5	Life cycle dependent resistance	20
2.5	Resistance of vector mosquitoes to insecticides	20
2.5.1	Malathion	20
2.5.2	Permethrin	21
2.5.3	Temephos	21
2.6	Mode of action of insecticides	24
2.7	Biochemistry of esterases enzyme	24
2.7.1	Esterases	25
2.7.1.1	Non-specific esterases	26
2.7.1.2	Biochemical enzyme determination microassay	26
2.7.1.3	Microplate assay	27
2.8	Native Sodium Dodecyl Sulphate – Polyacrylamide Gel Electrophoresis (Native SDS)	29
2.9	Vector control	30
CHAPTER 3: LARVAL BIOASSAY AND SELECTION PRESSURE FOR DETERMINATION OF INSECTICIDE SUSCEPTIBILITY AND RESISTANCE DEVELOPMENT IN VECTOR MOSQUITOES		
3.1	INTRODUCTION	32

3.2	MATERIALS AND METHODS	35
3.2.1	Test mosquitoes	35
3.2.2	Field strain mosquitoes	35
3.3	Rearing method (<i>Aedes albopictus</i> , <i>Aedes aegypti</i> and <i>Culex quinquefasciatus</i>)	36
3.3.1	Egg hatching and mosquito rearing	36
3.4	Insecticides	40
3.5	Larval bioassay test procedures	43
3.6	Larval selection pressure with insecticide	45
3.7	DATA ANALYSIS AND INTERPRETATION	46
3.8	RESULTS	47
3.8.1	Susceptibility and resistance development in <i>Culex quinquefasciatus</i>	47
3.8.2	Susceptibility and resistance development in <i>Aedes aegypti</i>	49
3.8.3	Susceptibility and resistance development in <i>Aedes albopictus</i>	52
3.9	DISCUSSION	60
CHAPTER 4: RESISTANCE DEVELOPMENT MONITORING IN INSECTICIDE SELECTED VECTOR MOSQUITOES USING ADULT BIOASSAY		
4.1	INTRODUCTION	63
4.2	MATERIALS AND METHODs	65
4.2.1	Mosquitoes	65
4.2.2	WHO Test Kits	65
4.2.3	WHO Adult Bioassay Test Procedures	67
4.3	DATA ANALYSIS AND INTERPRETATION	71
4.4	RESULTS	73
		xv

4.4.1	Adult bioassay for <i>Cx. quinquefasciatus</i>	73
4.4.1.1	Susceptibility and resistance development in adult <i>Cx. quinquefasciatus</i> malathion selected strain	73
4.4.1.2	Susceptibility and resistance development in adult <i>Cx. quinquefasciatus</i> permethrin selected strain	73
4.4.1.3	Susceptibility and cross-resistance development in adult <i>Cx. quinquefasciatus</i>	74
4.4.1.4	24 hours post-exposure treatment for <i>Cx. quinquefasciatus</i> malathion	75
4.4.1.5	24 hours post-exposure treatment for <i>Cx. quinquefasciatus</i> permethrin selected strain	76
4.4.1.6	24 hours cross-resistance post-exposure treatment for <i>Cx. quinquefasciatus</i> malathion selected strain	76
4.4.1.7	24 hours cross-resistance post-exposure treatment for <i>Cx. quinquefasciatus</i> permethrin selected strain	90
4.4.2	Adult bioassay for <i>Aedes aegypti</i>	90
4.4.2.1	Susceptibility and resistance development in adult <i>Ae. aegypti</i> malathion selected strain	90
4.4.2.2	Susceptibility and resistance development in adult <i>Ae. aegypti</i> permethrin selected strain	90
4.4.2.3	Susceptibility and cross-resistance development in adult <i>Ae. aegypti</i>	91
4.4.2.4	24 hours post-exposure treatment for <i>Ae. aegypti</i> malathion selected strain	92
4.4.2.5	24 hours post-exposure treatment for <i>Ae. aegypti</i> permethrin selected strain	93
4.4.2.6	24 hours cross-resistance post-exposure treatment for <i>Ae. aegypti</i> malathion selected strain	93
4.4.2.7	24 hours cross-resistance post-exposure treatment for <i>Ae. aegypti</i> permethrin selected strain	93

4.4.2.8	24 hours cross-resistance post-exposure treatment for <i>Ae. aegypti</i> temephos selected strain	94
4.4.3	Adult bioassay for <i>Aedes albopictus</i>	110
4.4.3.1	Susceptibility and resistance development in adult <i>Ae. albopictus</i> malathion selected strain	110
4.4.3.2	Susceptibility and resistance development in adult <i>Ae. albopictus</i> permethrin selected strain	110
4.4.3.3	Susceptibility and cross-resistance development in adult <i>Ae. albopictus</i>	111
4.4.3.4	24 hours post-exposure treatment for <i>Ae. albopictus</i> malathion selected strain	112
4.4.3.5	24 hours post-exposure treatment for <i>Ae. albopictus</i> permethrin selected strain	112
4.4.3.6	24 hours cross-resistance post-exposure treatment for <i>Ae. albopictus</i> malathion selected strain	113
4.4.3.7	24 hours cross-resistance post-exposure treatment for <i>Ae. albopictus</i> permethrin selected strain	113
4.4.3.8	24 hours cross-resistance post-exposure treatment for <i>Ae. albopictus</i> temephos selected strain	114
4.5	DISCUSSION	130
CHAPTER 5: BIOCHEMICAL ASSAY FOR RESISTANCE CHARACTERIZATION AND VARIATION OF ESTERASE ACTIVITY WITH THE LIFE STAGES OF INSECTICIDE SELECTED MOSQUITOES		
5.1	INTRODUCTION	133
	MATERIALS AND METHODS	136
5.2.1	Preparation of enzyme homogenates	136
5.2.2.2	Non-specific esterases testing procedures	138
	DATA ANALYSIS AND INTERPRETATION	140
5.4	RESULTS	142

5.4.1	Biochemical enzyme microassay in <i>Culex quinquefasciatus</i>	142
5.4.2	Biochemical enzyme microassay in <i>Aedes aegypti</i>	150
5.4.3	Biochemical enzyme microassay in <i>Aedes albopictus</i>	159
5.5	DISCUSSION	167
CHAPTER 6: ELECTROPHORETIC PATTERNS OF NON-SPECIFIC ESTERASES IN LIFE STAGES AND SEXES OF RESISTANT AND SUSCEPTIBLE STRAINS OF VECTOR MOSQUITOES		
6.1	INTRODUCTION	172
6.2	MATERIALS AND METHODS	175
6.2.2	Preparation of reagents for Native SDS-PAGE	175
6.2.2.1	Chemicals	175
6.2.2.1.1	Preparation of stock solutions: Electrode Buffer (pH 8.0)	176
6.2.2.1.2	Preparation of stock solutions: Phosphate Buffer Solution (PBS) – pH 7.4	176
6.2.2.1.3	Preparation of stock solutions: Stacking Buffer (pH 6.8)	176
6.2.2.1.4	Preparation of stock solutions: Ammonium persulfate 0 %	176
6.2.2.1.5	Preparation of stock solutions: 5% xylene cyanole marker	177
6.2.2.1.6	Preparation of stock solutions: (α & β - naphthylacetate) – Substrate	177
6.2.2.1.7	Preparation of stock solutions: Fast Blue	177
6.2.3	Gel cassette preparation	179
6.2.4	Separating Gel Preparation	182
6.2.4.1	Preparation of separating (Resolving, Lower) Gel	182

6.2.5	Stacking Gel Preparation	183
6.2.5.1	Preparation of Stacking (upper) Gel	183
6.2.6	Sample preparation	185
6.2.7	Electrophoresis	185
6.2.8	Removal of Gel	189
6.2.9	Gel Incubation	190
6.3	ANALYSIS OF BAND PATTERNS	193
6.4	RESULTS AND DISCUSSION	194
6.4.1	Electrophoretic patterns of non-specific esterases in <i>Cx. quinquefasciatus</i>	194
6.4.2	Electrophoretic patterns of non-specific esterases in <i>Ae. aegypti</i>	202
6.4.3	Electrophoretic patterns of non-specific esterases in <i>Ae. albopictus</i>	211
	CHAPTER 7: GENERAL DISCUSSION	220
	CHAPTER 8: CONCLUSION	233
	REFERENCES	236
	APPENDICES	262
	PUBLICATIONS	