PHYTOCHEMICAL SCREENING AND LARVICIDAL EFFECTS OF *BORRERIA VERTICILLATA*(LINNAEUS) AND *STRIGA ASIATICA*(LINNAEUS) AGAINST *CULEX QUINQUEFASCIATUS* (SAY)

BY

FATIMA GARBA KONTAGORA

JANUARY, 2017

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BY

FATIMA GARBA KONTAGORA
(MSC/SCI/2208/11-12)

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DEPARTMENT OF BIOLOGY,
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NIGERIA.

JANUARY, 2017

DECLARATION

I declare that the work in this dissertation entitled "**PHYTOCHEMICAL SCREENING AND LARVICIDAL EFFECTS OF *BORRERIA VERTICILLATA* (LINNAEUS) AND *STRIGA ASIATICA* (LINNAEUS) AGAINST *CULEX QUINQUEFASCIATUS* (SAY)**" has been carried out by me in the Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria. The information derived from the literature has been duly acknowledged in the text and in a list of references provided. No part of this thesis was previously presented for another degree or diploma at this or any Institution.

FATIMA GARBA KONTAGORA _____ _____

Signature Date

CERTIFICATION

This dissertation entitled PHYTOCHEMICAL SCREENING AND LARVICIDAL EFFECTS OF *BORRERIA VERTICILLATA* (LINNAEUS) AND *STRIGA ASIATICA* (LINNAEUS) AGAINST *CULEX QUINQUEFASCIATUS* (SAY)by FATIMA GARBAmeets the regulations governing the award of the degree of Master of Biology of the Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

Prof. A. K. Adamu _____ _____

Chairman, Supervisory Committee Signature Date
Department of Botany,
Ahmadu Bello University, Zaria

Mr. D. A. Adebote _____ _____

Member, Supervisory Committee Signature Date
Department of Zoology,
Ahmadu Bello University, Zaria

Prof.M. L. Balarabe _____ _____

Head, Signature Date
Department of Biology,
Ahmadu Bello University, Zaria

Prof. K. Bala _____ _____

Dean, School of Postgraduate Studies, Signature Date
ABU, Zaria

DEDICATION

I dedicate this work to Almighty Allah for seeing me throughout my studies, my family and siblings.

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I give thanks to Almighty Allah for seeing me through my studies. Allah alone is worthy of praise for giving me good health, strength and ability to complete this research.

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ABSTRACT

The phytochemicals solvent-derived extracts of *Borreriaverticillata*(Linnaeus) and *Strigaasiatica*(Linnaeus) and their larvicidal effects against *Culexquinquefasciatus*(Say) were examined. Each of the two pulverized plant materials were initially subjected to soxhlet extraction method using N-hexane, chloroform, ethyl-acetate and methanol in that order of increasing polarity of the solvents. The various solvent extracts were subjected to preliminary phytochemical screening ofcompounds.Bioassay of plants extracts on late third larval instars of *Culexquinquefasciatus* was tested in the laboratory using2.00 mg/l, 4.00 mg/l, 6.00 mg/l and 8.00 mg/l concentrations.Twenty-five (25) late third instars larvae of *Cx. quinquefasciatus* in triplicates for each concentration and the control devoid of extractswere isolated from the culture media into a test bowl using a dropping pipette. The preliminary phytochemical screeningof the plant extracts revealed the following compounds of insecticidal importance in different proportions: alkaloids, flavonoids, saponins, unsaturated steroid and triterpenes,tannins and cardiac glycosides while anthraquinone was absent in all the plantextracts.Larvicidal effects of theplant extracts of*Borreria verticillata* and *Striga asiatica* were dose-dependent,except the chloroform plant extractof *Borreria verticillata*,which increased and subsequently decreased with further increase in the concentration of the plant extract. The median lethal concentration (LC_{50}) values ofmethanolic, ethyl-acetate, n-hexane and chloroform solvent extracts were 17.49 mg/l, 8.67mg/l, 1.60 mg/l and 1.184×10^{30} mg/l respectively for *Borreriaverticillata*. The LC_{50} values of*Strigaasciatica* for solvent extracts ofmethanol, ethyl-acetate, n-hexane and chloroform were found to be 12.91mg/l, 3.01 mg/l, 1.45 mg/l and 3.03 mg/lrespectively. The results of the phytochemical screening of the solvent extracts of *B.verticillata* and *S.asiatica* have shown that both weeds with the

later being a parasitic weed, have a beneficial side, and part of their benefits was demonstrated in the larval mortality exhibited by the plant extracts. The larval mortality observed wasattributed to the presence of polar compounds such as alkaloids,flavonoids,tannins,saponins,cardiac glycosides and the polar compound,triterpenein the methanolethyl-acetate and n-hexane solvent of *B.verticillata* and *S.asiatica*.It was concluded that,*Borreriaverticillata*and *Strigaasciatica*may be used in the management of noxious mosquito species and serve as alternatives to synthetic insecticides that have many health implications and environmental problems. It isrecommended that, the use of *B.verticillata* and *S.asiatica* extracts need to be tested in the field to ascertain their full potentials.

TABLE OF CONTENTS

Titles Pages

Title Page ……...ii

Declaration Page …….....iii

Certification Page …….....iv

Dedication ……..…….....v

Acknowledgements …….....vi

Abstract …….....vii

Table of Contents …….....ix

List of Tables ……..……xiv

List of Figures……..……xv

CHAPTER ONE

1.0 INTRODUCTION……..……..…..1

1.1 Statement of the Problems…….…….....2

1.2 Justification…….....3

1.3 Aim of the Study……3

1.4 Objectives……...4

1.5 Hypotheses……..4

CHAPTER TWO

2.0 LITERATURE REVIEW……5

2.1 Classification of Mosquito ……..…….5

2.2 Taxonomyof Mosquito ……..……..5

2.3 Life Cycleof Mosquito ……..……...6

2.3.1 Adultof Mosquito……...……7

2.3.2 Eggof Mosquito……..……...7

2.3.3 Larvaeof Mosquito……...……..8

2.4 Habits and Habitatof *Culex* ……..…….8

2.5 Vector and pest status of *Culex* ……..……9

2.6 Geographic distribution of *Culex*……..……..……..10

2.7 Medical Importanceof *Culex*……...……..……12

2.8 The Disease Lymphatic Filariasis……...13

2.9 Mosquito Control…….14

2.9.1 Biological control method directed at the larval stages…….14

2.9.2 Chemical control method directed at the larvae……15

2.9.3 Chemical control directed at the adults…….16

2.9.4 Genetic control method directed at the adult…….17

2.10 Prospects of Botanical Biopesticides in Insect Pest Management……...18

2.11 Traditional Insecticides……..21

2.11.1. Pyrethrum……...21

2.11.2 Pyrethroids…….22

2.11.3 Rotenone……23

2.12 Newer Botanical Insecticides……..23

2.12.1 Neem…….….23

2.12.2 *Borreriaverticillata*……...…….…...23

2.12.3 *Strigaasiatica*……...…….……25

2.13 Plant Phytochemicals and Their Uses……26

2.13.1 Alkaloids……...27

2.13.2 Glycosides……..27

2.13.3 Flavonoids……..29

2.13.4 Phenolics……29

2.13.5 Saponins……30

2.13.6 Tannins……..31

2.13.7 Terpenes……32

2.13.8 Anthraquinones……..33

2.13.9 Essential oils……..33

2.13.10 Steroids……35

CHAPTER THREE

3.0 MATERIALS AND METHODS…….…….45

3.1 Source of Plant Materials……..……..45

3.2 Solvent Extraction of Pulverized Plant Materials……45

3.3 PreliminaryQualitativePhytochemical Screening…….……45

3.3.1 Screening for alkaloids……..46

3.3.2 Screening for flavonoids……46

3.3.3 Screening for saponnins……46

3.3.4 Unsaturated steroid and triterpenes……..……..46

3.3.5 Screening for tannins……47

3.3.6 Screening for anthraquinones……47

3.3.7 Screening for cardiac glycosides……...……..…….47

3.4 Collection of and Maintenance of Mosquito in the Laboratory ……48

3.5 Preparation of Stock Solutions……...48

3.6 Larvicidal Bioassay……..49

3.7 Statistical Analysis……...49

CHAPTER FOUR

4.0 RESULTS…….50

4.1 Quantitative Phytochemical Constituents of *B. verticillata* and *S. asiatica*..50

4.2 LarvicidalEfficacy of *Borreriaverticillata* Against*Culexquinquefasciatus*Mosquito Larvae……....50

4.2.1 The effects of methanolic solvent extracts of *Borreriaverticillata* on *Cx. quinquefasciatus*……...……50

4.2.2 The median lethal concentration (LC_{50}) of methanolic extracts of *Borreriaverticillata*……...……...53

4.2.3 Effects of the ethyl-acetate solvent extract of *Borreriaverticillata* on *Cx.quinquefasciatus*……...56

4.2.4 The median lethal concentration (LC_{50}) of the ethyl-acetate extracts of *Borreriaverticillata*……..…….56

4.2.5. The effects of n-hexane solvent extracts of *Borreriaverticillata* on *Cx. quinquefasciatus*……...……60

4.2.6: The median lethal concentration (LC_{50}) of the n-hexane solvent extracts of *Borreriaverticillata*……...60

4.2.7 The results of the chloroform solvent extract of *Borreriaverticillata* on *Cx.quinquefasciatus*……...……64

4.2.8 The median lethal concentration LC_{50} of the chloroform solvent extracts of *Borreriaverticillata*……..64

4.3 The LarvicidalActivity OfMethanolic Extracts of *StrigaAsiatica*Against*Culexquinquefasciatus* Larvae……68

4.3.1 The effects of the methanolic solvent extracts of *Strigaasiatica* on *Cx. quinquefasciatus*…….……..…..68

4.3.2 The median lethal concentration $LC_{50}$of Themethanolic solvent extracts of *Strigaasiatica*……...….68

4.3.3 The effects of the ethyl-acetate solvent extracts of *Strigaasiatica* on *Cx.quinquefasciatus*……...……72

4.3.4 The median lethal concentration $LC_{50}$of the ethyl-acetate solvent extracts of *Strigaasiatica*……72

4.3.5 Effects of N-hexane solvent extracts of*Strigaasiatica* on *Cx. quinquefasciatus*……...…….76

4.3.6 The median lethal concentration (LC_{50}) of N-hexane solvent extracts of *Strigaasiatica*……76

4.3.7 The effects of the chloroform solvent extracts Of*Strigaasiatica* on *Cx. quinquefasciatus*……..…….80

4.3.8 The median lethal concentrations (LC_{50}) of the chloroform solvent extract of *Strigaasiatica*……..……..80

4.4 The T-test of Solvent Extracts Type between *B. verticillata* and *S. asciatica*….84

CHAPTER FIVE

5.0 DISCUSSION……...88

CHAPTER SIX

6.0 SUMMARY CONCLUSIONS AND RECOMMENDATIONS…….…….95

6.1 Summary ……..95

6.1 Conclusions……...95

6.1 Recommendations……96

` **REFERENCES**……..…….**.....**98

LIST OF TABLES

Tables Pages

4.1:Qualitative phytochemical constituents of the four solvent extracts of *Borreria verticillata* and *Striga aesiatica* (+ = present; - = absent)…51

4.2: Mortality of *Culex quinquefasciatus* larvae(n=75) on exposure for 24 hours to various concentrations of methanol extract of *Borreria verticillata*…….….52

4.3: Median lethal concentration (LC_{50}) of methanol extract of *Borreria verticillata*.....45

4.4: Mortality of *Culex quinquefasciatus* larvae(n=75) on exposure for 24 hours to various concentrations of ethyl-acetate extract of *Borreria verticillata*……..……..57

4.5: Median lethal concentration (LC_{50}) of ethyl-acetate extract of *Borreriaverticillata*against third instar larvae of *Culex quinquefasciatus* by probit analysis …….....58

4.6: Mortality of *Cx.quinquefasciatus*larvae(n = 75) on exposure for 24 hours to various concentrations of N-hexane extract of *B.verticillata*……..…….61

4.7: Median lethal concentration of (LC_{50}) of N-hexane extract of*Borreria verticillata* against third instar larvae of *Culex quinquefasciatus*….53

4.8: Mortality of *Culex quinquefasciatus* l*arvae* (n = 75) on exposure for 24 hours to various concentrations of chloroform extract of *Borreria verticillata*……..……...65

4.9: Median lethal concentration (LC_{50}) of chloroform extract of *Borreria verticillata*against third instar larvae of *Culex quinquefasciatus* by probit analysis…..66

4.10: Mortality of *Culex quinquefasciatus* larvae (n = 75) on exposure for 24 hours to various concentrations of methanol extract of *Striga asiatica* ……..…..69

4.11: Median lethal concentration (LC_{50}) of methanol extract of *Striga asiatica* against third instar larvae of *Culex quinquefasciatus* by probit analysis……..70

4.12: Mortality of *Culex quinquefasciatus* larvae (n=75) on exposure for 24 hours to various concentrations of ethyl acetate extract of *Striga asiatica*……..……...73

4.13: Median lethal concentration (LC_{50}) ofethylacetate extract of*Strigaasiatica*against third instar larvae of *Culex quinquefasciatus* by probit analysis……...……74

4.14: Mortality of *Culex quinquefasciatus*larvae (n=75) on exposure for 24 hours to various concentrations of N-hexane extract of *Striga asiatica* ……..…..77

4.15: Median lethal concentration of (LC_{50})ofN-hexane extract of*Strigaasiatica*against third instar larvae of *Culex quinquefasciatus* by probit analysis……..**.....**78

4.16: Mortality of *Culex quinquefasciatus* larvae (n=75) on exposure for 24 hours to various concentrations of chloroform extract of *Striga asiatica*…..……..81

4.17: Median lethal concentration (LC_{50}) of chloroform extract of *Striga asiatica*……82

4.18: Comparison between the larvicidal efficacies of various solvent extracts of *B. verticillata* and *S. asiatica* against *Cx.quinquefasciatus* (Mean ± SE)……....85

LIST OF FIGURES

Figures Pages

4.1: Empirical probit of mortality of *Culex quinquefasciatus* larvae against the logarithms of the concentrations of methanol extract of *Borreria verticillata*……57

4.2: Empirical probit of mortality of *Culex quinquefasciatus* larvae against the logarithms of the concentrations of ethyl acetate extract of *Borreria verticillata*……59

4.3: Empirical probit of mortality of *Culex quinquefasciatus* larvae against the logarithms of the concentrations of N-haxane extract of *Borreria verticillata*……63

4.4: Empirical probit of mortality of *Culex quinquefasciatus* larvae against the logarithims of the concentrations of chloroform extract of *Borreria verticillata*……67

4.5: Empirical probit of mortality of *Culex quinquefasciatus* larvae against the logarithms of the concentrations of methanol extract of *Striga asiatica*……71

4.6: Empirical probit of mortality of *Culex quinquefasciatus* larvae against the logarithms of the concentrations of ethyl acetate extract of *Striga asiatica*……75

4.7: Empirical probit of mortality of *Culex quinquefasciatus* larvae against the logarithms of the concentrations of N-hexane extract of *Striga asiatica*……79

4.8: Empirical probit of mortality of *Culex quinquefasciatus* larvae against the logarithms of the concentrations of chloroform extract of *Striga asiatica*……83

CHAPTER ONE

1.0 INTRODUCTION

Culex quinquefasciatus is one of the medically important species of mosquitoes occurring in tropical and warm temperate regions of the world (Nikkon *et al.*, 2011). *Culex quinquifasciatus is* a dorminant and important species of the large *Culex* genus, with 768 species. The species is a vector of wuchereria bancrofti the parasitic filarial nematode, responsible for causing human lymphatic filariasis, in endemic parts of the world (WHO, 1987; Service, 1993; Lima *et al*., 2003).The species is the most dominant urban nuisance mosquito in Nigeria known for its nocturnal discomfort and allergies caused as a result of its human biting activities (Adebote *et al.,* 2011). C*ulex quinquefasciatus* has been associated with transmission of the zoonotic filarial nematode,*Dirofilaria immitis*(Ahid *et al*., 2000), avían malaria parasites (VanRiper 111 *et al*., 1986) and several arboviruses (Meyer *et al*., 1983; Sardelis *et al*., *2*001). The use of chemical insecticides are the cornorstone of efforts to manage populations of mosquitoes (Jones, 2012). Pytheroids are the most successful classes of insecticidal chemicals.These insecticides,which are synthetic analogs of natural insecticides (pyrethrins) produced in *chrysanthemum cineraria* folium,were pioneered Micheal Elliot of the Rothamsted experimental research station throughout the 1960's and 70's (Jones, 2012) were used on mosquitoes and other medically important species throughout the 1980's (Jones, 2012).Thus all insecticides regardless of their mode of action, will inevitably select for resistance in target species and therefore novel must be found to replace them. In addition, unintentional damage to non-pest species that results from insecticides application (non-target effects) limits their utility. As a result of this development,there is now considerable international interest in

developing benign natural products as an alternative to harmful synthetic pesticides to control invertebrate pests of medical and economic importance (Sivagnaname and Kalyanasundaram,2004; Dua *et al*.,2009).Although there is no scientific evidence that both *Borreria verticillata* and *Striga asiatica* have been evaluated for their mosquitocidal efficacies, the related *Striga hermonthica* and *Mitracarpus scaber* have been evaluated for mosquitocidal efficacies (Abdullahi *et al*., 2011).

Borreria vertcillata is a woody shrub, belonging to the Rubiaceae family.It is common in humid environments and is known for its antileprotic, antiparalyptic, diuretic and antibilharzia properties (Sofowora, 1993). Also an infusion of the leaves is used to treat various skin diseases (Sofowora, 1993).

Striga asiatica also known as the Indian witch weed is an annual hemi- parasite of several cultivated cereal crops. It belongs to the Scrophulariaceae family and has been used as an appetizer, a stimulant and for treatment of hypertension (Kakpure and Rothe, 2012). It also has antifertility, antibacterial, antifungal and antihelminthic properties (Kakpure and Rothe, 2012). The widespread availability of both plant species and their ethnobotanical importance, make it expedient to investigate their prospects as mosquito larvicides.

1.1 Statement of the Problems

Despite the intensified search forp botanicals with larvicidal efficacy against mosquito species, there is no information on the larvicidal prospects of *Borreria verticillata* and *Striga asiatica* against *Culex quinquefasciatus*. The bioactive chemicals in *Borreria*

verticillata and *Striga asiatica* that could elicit larvicidal effects against *Culex quinquefasciatus* remains largely unknown.

1.2 Justification

The extensive and prolonged use of chemical insecticides to control mosquito vectors, responsible for the transmission of mosquito related diseases, has been associated with the widespread development of physiological resistance in mosquito populations, to these insecticides. These chemical insecticides are also non-target specific and their application may result in the elimination of specific organisms that have specific roles in the ecosystem.This elimination could bring about disruptions of natural biological systems. The continuous applications of non-biodegrable insecticides could accumulate in the environment, resulting in adverse environmental contamination (Brown, 1986). The high cost of the production and delivery of these insecticides, to poor developing communities and nations is another problem associated with insecticides. Long term exposure of humans to chemical insecticides especially, those working in industries where these chemicals are produced have been linked to immune dysfunction, cancers and birth defects (Nigam and Venkatakrishna, 2001; Bounias, 2003). Information derived from this study could expose potent bioactive chemicals inherent in both plant species that could be harnessed to control *Culex quinquefasciatus.*

1.3 Aim of the Study

To evaluate the phytochemicals in solvent-derived extracts of *Borreria verticillata* and *Striga asiatica* and their larvicidal prospects against *Culex quinquefasciatus*.

1.4 **Objectives**

1 To determine the phytochemical constituents in the solvent-derived (N-hexane, chloroform, ethyl-acetate and methanol) extracts of *Borreria verticillata* and *Striga asiatica.*

2 To determine the larvicidal efficacy of the solvent-derived extracts of *Borreria verticillata* against third instar larvae of *Culex quinquefasciatus.*

3 To determine the larvicidal efficacy of the solvent-derived extracts of *Striga asiatica* against third instar larvae of *Culex quinquefasciatus.*

4 To compare the larvicidal efficacies of the four-solvent extracts *Borreria verticillata* and *Striga asistica* against *Cx.quinquefasciatus*

1.4 Hypotheses

1 There is no significant difference in the phytochemical constituents of the solvent extracts of *Borreria verticillata* and *Striga asiatica*.

2 Extracts of *Borreria verticillata* have no significant larvicicdal effects on third instar larvae of *Culex quinquefasciatus*

3 Extracts of *Striga asiatica* have no significant larvicidal effects on the third instar larvae of *Culex quinquefasciatus.*

4 There are no significant difference between the larvicidal efficacies of *Borreria verticillata* and *Striga asiatica* against *Culex quinquefasciatus*.

CHAPTER TWO

2.0 LITERATURE REVIEW

The species *Culex quinquifasciatus* is a large and important genus of *Culex*, with 768 species. Apart from its nuisance problem, *Culex quinquefasciatus* is a vector of the parasitic filarial nematode; Wuchereria bancrofti which causes the human lymphatic filariasis, in endemic parts of the world (WHO, 1987; Service, 1993; Lima *et al*., 2003).*Culex quinquefasciatus* has also been associated with transmission of the zoonotic filarial nematode *Dirofilaria immitis*(Ahid *et al*., 2000); avian malaria parasites (VanRiper 111 *et al*., 1986) and several arboviruses (Meyer *et al*., 1983; Sardelis *et al*., 2001).

2.1 Classification of Mosquito

Kingdom-Animalia; Sub kingdom-Metazoa; Phylum-Arthropoda; Class-Insecta; Order-Diptera; Suborder-Nematoda; Infraorder;-Culicomorpha;Super family-Culicoidea; Family-Culicidae. All mosquitoes belong to the family Culicidae; the culicidae family is then further divided into three Sub Families namely; Toxorhynochitinae, Anophelinae and Culicinae (Service,1993).There are 34 genera in these 3 Sub families altogether. In the Sub family Culicinae; there are two tribes which are Sabethini and Culicini. There are althogether 30 genera of mosquitoes in these 2 tribes. The medically important species belong to the following genera *Culex*(*Culex quinquefasciatus* belongs), *Aedes, Mansonia, Sabethes, Haemagogus* and *Psorophora* (Service, 1993).

2.2 Taxonomy and Nomenclature of *Culex*

Culex quinquefasciatus is part of the group of pipens group and belongs to the subgenus *Culex* (Dobrotworky,1965).In 1823 Say named *Culex quinquefasciatus* but in 1828 the name *Culex fatigans* was introduced by Weidmann (Dobrotworsky,1956;Stone,1956).The names were applied to what appears to be the same species and both names have been used as synonyms since they arose (Stone,1956).Until recently this species has been regarded as a subspecies of *Culex pipens* and was called *Culex pipens fatigans* or *Culex pipens quinquefasciatus*. Because of the inherent confusion this variable naming causes, there is a movement towards returning to use the original naming of *Culex quinquefasciatus* (Stone, 1956).A medium, light brown mosquito, the abdominal sternites of females of *Cx.quinquefasciatus* are pale scaled with a few dark scaled patches medially (Belkin, 1968), although in New Zealand specimens, these dark scaled patches are frequently absent Larvae may be differenciated from *Culex pervigilans* in having comb scales in a patch of 30-40, 10-12 teeth in each side of the mental plate and a siphon which is widest about one third from its base (Belkin, 1968).

2.3 Life Cycle of *Culex*

Gravid *Culex quinquefasciatus* females fly during the night to nutrient-rich standing water where they will lay their eggs. They will oviposite in waters ranging from waste water areas to bird baths, old tyres or any container that holds water. If the water evaporates before the eggs hatch or before the larvae completes their life cycle, they die (Hill and Connelly, 2011). The larvae feed on biotic material in the water and require between five to eight days to complete their development at 30°C (Gerberg *et al*., 1994). The larvae progress through four larval instars, and towards the end of the fourth instar they stop eating and moult to the pupal stage. Following 36 hours at 27°C the adults

emerge from the pupal stage (Gerberg *et al*., 1994). The time of development under natural conditions for all stages is variable and dependant on temperature. Both males and females take sugar meals from plants. Following mating, the female seeks a blood meal. *Culex quinquefasciatus* are opportunistic feeders, feeding on mammals and or birds throughout the night. Males survive only on sugar meals, while the female will take multiple blood meals. After a female mosquito digests the blood meal and the eggs develop, she finds a suitable place to lay her eggs, and the cycle begins again. The number of eggs per raft varies with climatic conditions.A single female can lay up to five rafts of eggs in a lifetime (Gerberg *et al*., 1994).

2.3.1 Adult of Mosquito

Adults *Culex quinquefasciatus* vary from 3.96 to 4.25 mm in length (Lima *et al*., 2003). The mosquito is brown, with the proboscis, thorax, wings and tarsi darker than the rest of the body. The head is light brown with the lightest portion in the center. The antennae and the proboscis are about the same length, but in some cases the antennae are slightly shorter than the proboscis. The flagellum has thirteen segments that have few to no scales (Rukhsana *et al*., 2014). The scales of the thorax are narrow and curved. The abdomen has pale, narrow, rounded bands on the basal side of each tergite. The bands barely touch the basolateral spots taking on a half-moon shape (Darsie and Ward, 2005).

2.3.2 Eggof Mosquito

Eggs common to the genus *Culex,* the eggs of *Culex quinquefasciatus* are laid in oval raft loosely cemented together with 100 or more eggs in raft which will normally hatch 24 to 30 hours after being oviposited (Bates, 1949).

2.3.3 Larvae

The larval head is short and stout becoming darker towards the base. The mouth brushes have long yellow filaments that are used for filtering organic materials. The abdomen consists of eight segments, the siphon and the saddle. Each segment has a unique setae pattern (Sirivanakarn and White, 1978).The siphon is on the dorsal side of the abdomen and in *Culex quinquefasciatus* the siphon is four times longer than it is wide with multiple setae tufts (Darsie and Morris, 2000). The saddle is barrel shaped and is located on the vertical side of the abdomen with four anal papillae protruding from the posterior end (Hill and Connelly, 2012).

2.4 Habits and Habitat of *Culex*

Culex quinquefasciatus usually breeds in organically rich and polluted surface waters of artificial containers (Weinstein*et al*., 1997).It has been found breeding in shallow ponds within streams phytotelmata (Derraik, 2005), and in artificial habitats such as drains and drains sumps, wells, oxidation ponds at sewage treatment plants (Derraik and Slaney, 2005), stock drinking troughs, septic tanks, rain water containers types and various other small containers (Lee *et al*., 1989; Laird, 1995). It may also be found utilizing the same container for breeding as other species (Lee *et al*., 1989).

The eggs of *Cx. quinquefasciatus* are not desiccation resistant and are laid as rafts on the water surface (Weinstein *et al*., 1997). Once hatched the larvae are able to hibernate overwinter in the cooler months of July to September, while adult activity ceases (Lee *et*

al., 1989; Weinstein *et al*., 1997). *Culex quinquefasciatus* adults do not usually disperse greater than one kilometre from a release or hatching point and remain close to breeding habitat and host sources (Schreider *et al*., 1988; Reisen *et al*., 1991). Adult females must take blood meal before laying the first batch of eggs (Oda *et al*., 2002).

When breeding, Australian *Cx. quinquefasciatus* has been known to swarm in large number (Lee *et al*., 1989). *Culex quinquefasciatus* is a domesticated species which is often found living in close proximity to humans. Nocturnal biters, the females will readily bite man indoor and outdoor (Weinstein *et al*.,1997), but also bite birds, pigs, horses, cattle, sheep,dogs, rabbits (Holder *et al*., 1999) and even amphibians (Lee *et al*., 1989).

2.5 Vector and Pest Status of *Culex*

Culex quinquefasciatus is an important vector of periodic lymphatic filariasis in some parts of the world (Belkin, 1968) and is known to transmit the causative *Wuchereria bancrofti* to some degree of efficacy in many region of the world. It is an important vector of the West Nile virus in (WNV) in some areas of the world. Studies in the United States have isolated WNV from populations of *Cx. quinquefasciatus* and found some populations to be efficient vector in the laboratory (Goddard *et al.*, 2002). There appear to be regional differences in vector competence of this species which range from some populations which are very poor and improbable vectors in nature, to good vectors with excellent transmitting capabilities (Sardelis *et al*., 2001). There is also some thought that the populations that vector well may have interbred with a related species and known vector of West Nile Virus (WNV) *Culex pipiens* (Goddard *et al*., 2002). *Culex*

quinquefasciatus has been able to transmit Ross River Virus, Alfuy, Alpiwar, Corriparta, Dengue, Sindis and Japanese encephalitis virus (Reuben *et al.*,1994); Reticuloendotheliosis virus (Holder *et al*., 1999) and the protozon ,*Hepatozoon breinli* within the laboratory (Lee *et al*.,1989) and may yet be seen as a vector of these in nature. *Culex quinquefasciatus* is also laboratory host to a wide variety of other arboviruses including Murray Valley Encephalitis (Weintein *et al*., 1997). Reovirus type 3 and chikungunya viruses (Lee *et al*., 1989; Holder *et al*., 1999).

Culex quinquefasciatus is a domestic pest in many urban areas and often comes indoors at night to bite (Holder *et al*., 1999). It is also vector of bird pox and the avian malaria causing protozoa (Derraik, 2004; Derraik and Slaney, 2005), *Plasmodium relictum* (Laird, 1996) and *Plasmodium cathemerium* (Lee *et al*., 1989). The species is also able to transmit *Dirofilaria immitis* (dog heartworm), *Saurofilaria* spp and *Oswadofilaria* spp. which affect two Australian lizards (Holder *et al*., 1999).*Culex quinquefasciatus* is an urban mosquito responsible for transmission of filariasis especially Bancroftian filariasis (Amusan *et al*., 2003).*Culex quinquefasciatus* are not only important transmitters of filariasis but also vectors of severalof the mosquito-borne encephalitides (Gordon and Larvoipierre, 1976; Service, 1980).

2.6 Geographic distribution of *Culex*

Culex quinquefasciatus is one of the most widespread mosquitoes in the world. It is found throughout most of Pan and subtropical America (Barr, 1957; Weinstein *et al*., 1997) the Neotropics, Afro tropics (White, 1975), Indomalayan, Australasian (Lee *et al*., 1989) and Eastern Asian regions of the world (Bram, 1967). It is also present in the

United Kingdom and parts of the Middle East. *Culex quinquefasciatus* is an introduced species to New Zealand and is now one of the most commonly occurring mosquitoes after *Culex pervigilans* and *Aedes notoscriptus.* It was believed to have been first introduced in the 1830's (Sandlant, 2002), and likely carried ports aboard American whaling ships (Weinstein *et al*., 1997) or via Australian populations in open water storage tanks ports at the Bay of Islands and Auckland (Laird,1996).

Originally this species range was restricted to areas around ports of entry through which it was introduced, but this range has expanded inland and also shows a Southward movement (Laird, 1995; Weinstein *et al*., 1997; Holder *et al*., 1999). *Culex quinquefasciatus* is now found throughout much of the North Island and northern parts of the South Island including Marlbrough, Picton and Nelson (Weinstein *et al.,* 1997).It has also been detected in traps in Christchurch and Queenstown; however it does not appear to have established in either area, possibly due to longer colder winters(Weinstein *et al.,* 1997).

In Nigeria, *Cx. quinquefasciatus* is responsible for keeping the Niger Delta region of Nigeria endemic for lymphatic filariasis (Aigbodion *et al*., 2011). Recent studies on filariasis in the Niger Delta area have shown focal distribution of the disease (Aigbodion *et al*., 2011). Due to the rapid growth and development of urban areas in tropical rain forest, mangrove and freshwater swamp zones and the involvement of *Cx. quinquefasciatus* in the transmission of lymphatic filariasis and its potential in the transmission of other arboviruses, this mosquito has become a matter of growing concern in recent years. The development of an effective vector control programme or strategy

against this species will ultimately require knowledge of some aspects of its ecology such as the age structure, time of collection, and spatial and temporal distribution Larval surveys have also shown that *Culex pipiens quinquefasciatus* breeds commonly in numerous polluted streams and open drains of townships like Lagos (mangrove forest and coastal vegetation), Ibadan (lowland rain forest), Bauchi-Jos-Plateau (Guinea savannah), and Sokoto-Birnin Kebbi (Sahel Savannah), (Service, 1963). Okogun *et al*.(2003) from their studies in the Esan region of Edo state suggested that mosquito abundance is related to population and human activities;especially at the university town of Ekpoma occupied predominantly by students and farmers had a higher relative abundance (35.70%) of mosquito larval species.

2.7 Medical Importance of *Culex*

Culex quinquefasciatus is a vector of many pathogens of humans, and both domestic and wild animals. Viruses transmitted by this species include West Nile virus (WNV), Saint Louis encephalitis virus (SLEV) and Western equine encephalitis virus (WEEV). *Culex quinquefasciatus* is the principal vector of SLEV in the southern United States of America.Saint Louis encephalitis virus (SLEV) is maintained in a mosquito-bird-mosquito cycle,with periodic amplication by peridomestic birds and Culex species mosquitoes.Wild birds are the primary vertebrate hosts.Birds sustain inapparent infections but develop viremias (virus in their blood), sufficient to infect the mosquito vectors.Birds that are abundant in the urban-suburban environment such as the house sparrow,pigeoen,blue jay and robin are principally involved. The principal vectors are *Cx. pipiens* and *Culex quinquefasciatus* in the east, *Cx. nigripalpus* in Florida and *Cx. tarsalis* and members of the *Cx. pipiens* complex in western states.Humans and domestic

mammals can acquire SLEV infection, but are dead-end hosts (CDC, 2009). The mosquito may then transmit the virus to humans. St. Louis encephalitis is age-dependent, affecting older humans more than the young. Symptoms of the disease are flu-like and can range from fever and headaches to stiffness and confusion. After a period of several days the brain may begin to swell, accompanied by depression, extreme excitement, sleepiness, or sleeplessness (CDC, 2007). Because humans do not develop high levels of the virus in the blood and therefore are considered dead-end hosts unable to infect mosquitoes (Foster and Walker, 2002). In Florida, major St. Louis encephalitis epidemics occurred in 1959, 1961, 1962, 1977 and 1990 (Day and Stark, 2000; Shroyer, 2004). Other epidemics in the U.S. include Colorado 1985, Arkansas 1991, New York 1999 and Louisiana 2001 (Hill and Connelly, 2012).

2.8 The Disease Lymphatic Filariasis

The disease known as lymphatic filariasis, commonly called elephantiasis, is caused by infection with nematodes (roundworms) of the family Filariodidea. There are three types of these thread-like filarial worms: *Wuchereria bancrofti*, which is responsible for 90% of the case, *Brugia malayi*, which causes most of the remainder of the cases and *B. timori*, which also causes the disease. Lymphatic filariasis is a neglected tropical disease. Infection occurs when filarial parasites are transmitted to humans through mosquitoes.When a mosquito with infective stage larvae bites a person, parasites are deposited on the person's skin from where they enter the body. The larvae then migrate to the lymphatic vessels where they develop into adult worms in the human lymphatic system (WHO, 2013). This disease, although limited in distribution than malaria, is a common disease in the tropics. The disease lymphatic filariasis (elephantiasis) infects

about 120 million people in tropical Africa, India, Southeast Asia, the Pacific Island, South and Central America (WHO, 1987). Recently, this disease has increased as a result of urbanization, which is responsible for the increase in the breeding sites of the *Culex* mosquito, the vector that transmits the nematode *Wuchereria bancrofti*, which causes the disease lymphatic filariasis (Rao *et al.*, 1981).

2.9 Mosquito Control

To prevent the proliferation of diseases transmitted by mosquito and to improve the quality of environment and public health, mosquito control operation is necessary. The control of mosquitoes can be directed at either the immature larval stage or the adult stage or at both stages simultaneously.

2.9.1 Biological control method directed at the larval stages

2.9.1.1 Use of predators

The numbers of mosquito larvae and pupae have been reduced in aquatic environments by the use of predators. The predators commonly used in reducing the mosquito larvae and pupae are fish especially *Gambusia* species. Other predators include tadpoles of frogs and toads and other aquatic insect larvae, but all these have rarely proved effective as control agents. A few mosquitoes have predacious larvae, for example *Toxorhnchites brevipalpis* which have also been used to control larvae of other container breeding mosquitoes (Collins and Blackwell, 2000).

2.9.1.2 Pathogens

Parasitic bacteria may also help fight the spread of mosquito- borne diseases. Infecting mosquitoes with bacterial parasite could help prevent the spread of lymphatic filariasis

one of the major neglected tropical diseases of the developing world. Research conducted previously have shown that infecting mosquitoes with a strain of bacterial parasite *Wolbachia* known as Wmelpop (nicknamed popcorn) can reduce the life-span of the mosquito by half. Mosquito borne parasite, such as the filarial nematode or the malaria parasites require an incubation period between ingestion and transmission so only older mosquitoes can be infective. Skewing the population towards younger individuals reduces the number of infectious insects. Scientists are also looking at the possibility of infecting other species of mosquito such as *Anopheles gambiae*,the mosquito responsible for the majority of malaria infection. Another potential target is the *Aedes polynesiensis* mosquito, which is also responsible for the spread of lymphatic filariasis in the islands of Polynesia, where decades of mass drug administration have failed to eradicate the filarial parasites from the human population (Kambris *et al.,* 2009).

2.9.2 Chemical control method directed at the larvae

Control of mosquitoes directed at the larvae can be achieved by larviciding. Larviciding is a general term for killing immature mosquitoes by applying agents, collectively called larvicides, to control mosquito larvae and/or pupae. A technique known as the Larval Source Management (LSM), involves both the modification of water habitats, often referred to as source reduction and the direct application of larvicides to control mosquito production. Most mosquito species spend much of their life cycle in the larval stage when they are highly susceptible to both predation and control efforts. They often are concentrated within defined water boundaries, immobile with little ability to disperse, and accessible.Adult mosquitoes, in contrast, fly in search of mates, blood meals, or water sources for egg laying and are often inaccessible, not concentrated, and widely

distributed. Therefore, effective larviciding can reduce the number of adult mosquitoes available to disperse, potentially spread disease; create a nuisance, and lay eggs which leads to more mosquitoes (Dean and Nesheim, 1998). Larvicides are used in circumstances where reducing or removing standing water is not feasible. They could be used for West Nile virus control programs to reduce the immature stages of mosquitoes before they develop into adult mosquitoes and disperse. Larvicides are applied as a liquid or as granules/pellets and are consumed by the mosquito larvae. Mosquito larvae reduce their feeding prior to each molt and, therefore, precise timing of larvicide applications is needed with some products for effective control of mosquito larvae **(**Fradin and Day, 2002).

2.9.3 Chemical control directed at the adults

The principal strategy for controlling mosquitoes is application of insecticides, which is aimed at controlling the mosquito vector.It involves indoor residual spraying (IRS) with these insecticides, which is a powerful way of reducing mosquito and diseases transmitted by mosquitoes. These measures target houses, which are sprayed and the indoor spraying is effective for 3-6 months depending on the insecticide used (WHO, 2013). Control of mosquitoes also involves the use of these insecticides within households on a regular basis. Such insecticides as the DDT (Dichloro, diphenyl, trichloro methane were used. Before the advent of resistance to insecticides by mosquitoes, DDT was the best insecticide used.Organochlorine insecticides like Dieldrin (cyclodiene) and HCH which are more toxic to man, are also used for indoor residual spraying under strict safety measures. Organophosphate and carbamate insecticides which are less toxic to man and are not resisted by the mosquitoes replaced the

organochlorine insecticides although more expensive. Organochlorine insecticides are less persistent and spraying may have to be repeated severally (4-6 times) a year. In almost all countries, DDT insecticides have been banned due to its toxic effect on plant and animal tissues, environment and biodiversity (WHO, 1995). Adulticiding, which is the process of spraying low rates (dosages) of pesticides, is also used to kill adult mosquitoes. The process uses special equipment, such as trucks, small aircrafts, and backpack sprayers. Appropriate application allows small droplets of pesticide to drift through areas where adult mosquitoes are flying or resting. Effective adulticide application requires correct equipment settings, specific weather conditions, and timely application, both during the time of day and time of year. Adulticiding is the least effective of all the control methods; however, it does play a small role in any integrated pest management (IPM) programme. Adulticiding may be undertaken to assist in controlling an outbreak of a mosquito-borne diseases (Carney *et al*., 2008).

2.9.4 Genetic control method directed at the adult

Other measures that are used in the control of vectors, involves releasing sterile insects which this has been used as an effective control method, against agricultural pests for over 50 years. However, this measure has not been applied to mosquitoes because of the limitations of conventional sterilization techniques such as radiation. A technique known as RIDL, developed by oxford based company known as oxitec Ltd. uses advanced genetics to mostly male insects to be sterile. These mosquitoes do not bite or spread diseases are then, released to mate with the wild females. No visible off springs can result from these mating and as a result, the mosquito population is reduced below the threshold level that is required to transmit disease. Oxitec has also created RIDL strains of *Aedes*

aeypti, the principal mosquito species responsible for spreading dengue fever. The lead strain – OX313A- has already been both in the laboratory and in contained field conditions (Wellcome Trust, 2010).

2.10 Prospects of Botanical Biopesticides in Insect Pest Management

Pesticides are substances or mixture of substances used to prevent, destroy, repel, attract, sterilize, or mitigate pests. The plant kingdom is recognized as the most efficient producer of chemical compounds, synthesizing many products that are used to defend plants against different pests (Khater, 2012). Botanicals are basically secondary metabolites that serve as a means of defence mechanism of the plants to withstand the continuous selsection pressure from herbivore predators and other environmental factors.Several groups of phytochemicals such as alkaloids, steroids, terpenoids, essential oils and phenolics from different plants have been reported for insecticidal activities (Gosh *et al*., 2012).

Botanicals have been in nature for millions of years without any adversative effects on the ecosystem. The repellency of plant material has been exploited for thousands of years by man by hanging bruised plants in houses, a practice that is still in wide use throughout the developing countries. Moreover, plants have also been used for centuries in the form of crude fumigants where plants were burnt to drive away nuisance mosquitoes and latersoil formulations applied to the skin or clothes which was first recorded in writings by ancient Greek, Roman and Indian scholars (Hanem, 2012).

Pesticidal plants were widely used until the 1940s, when they were replaced by synthetic pesticides that were easier to handle and lasted longer. The consumption of synthetic

pesticides in some of the developed countries is almost 3000 g/hectare. Over enthusiastic use of synthetic insecticides led to problems unforeseen at the time of their introduction. It was reported by the World Resources Institute that more than 500insect and mite species are resistant to one or more insecticides (Hanem, 2012). As pests become tolerant to insecticides, double and triple application rates are needed to maintain control (Hanem, 2012). Synthetic pesticides are generally persistent in nature.The World Health Organization estimates that 200,000 people are killed worldwide, every year, as a direct result of pesticide poisoning (Hanem, 2012).

Furthermore, unlike conventional insecticides which are based on a single active ingredient, plant based insecticides comprises of botanical blends of chemical compounds which act concertedly on both behavioral and physiological processes. Thus there is very little chance of pests developing resistance to such substances (Gosh *et al*., 2012). Identifying bio-insecticides that are efficient, as well as being suitable and adaptive to ecological conditions, is imperative for continued effective vector control management. Botanicals have widespread insecticidal properties and will obviously work as a new weapon in the arsenal of synthetic insecticides and in future may act as suitable alternative products to fight against mosquito-borne diseases (Gosh *et al*., 2012).

Although the use of rotenone and nicotine appears to be waning, pyrethrum and neem are well established commercially, and pesticides based on plant essential oils have recently entered the marketplace. Plant- based insecticides are not only toxic to pests, but can also deter and/or repel pests which may contribute to their overall efficacy against some pests that cause great economic loss at the pre- as well post harvest stages of the crop

production and reduce transmission of diseases to plants and animals including humans. Moreover, the use of synthetic chemicals has been restricted because of their carcinogenicity, teratogenicity, high and acute residual toxicity, ability to create hormonal imbalance, spermatotoxicity, long degradation period and toxic residues in food (Hanem, 2012).

Repetitive use of synthetic pesticides has resulted in pesticide residue hazards, upsetting the balance of nature through disruption of natural enemies, pollinators and other wild life, extensive groundwater contamination, evolution of résistance and resurgence of treated populations, outbreaks ofsecondary pests, i.e. those normally kept under control by their natural enemies (Hanem, 2012). Locally available plants, such as *Derris*, *Nicotiana*, and *Ryania* have been used to control pests during prehistoric times. Botanical pesticides are easily decomposed by a variety of microbes common in most soils as a result, they reduce environmental contamination. They also maintain the biological diversity of predators that are often killed by broad-spectrum synthetic pesticides (Hanem, 2012). Integrated pest management can be defined as the use of all available means to maintain pest populations below levels that would cause economic loss while minimally impacting the environment. Achieving a zero pesticide strategy in tropical agro ecosystems may be easier than in temperate zones, as in many instances tropical farmers have not yet begun the generalized use of pesticides (Hanem, 2012). As a form of allelopathy, some pesitcidal plants serve as control agents forpests and diseases after intercropping or mix-cropping with the main crop. For example *Nicotiana tabaccum* and some other plant species especially grasses have been found to possess this attribute

(Hanem, 2012). *Eucalyptus camaldulensis* and some other pesticidal plants are also used as weed control in farmlands to maximize yields (Khater, 2012).

Farmers in the tropics have included intercropping and mix cropping of main crops withpesticidalplants,forexample, planting *Manihot esculenta* (cassava) with *Ocimum gratissimum* (camphor basil), *Theobromae cacao* (cocoa) with *Carica papaya* (pawpaw) and so on (Khater,2012). Accordingly, crops are protected naturally and simply using pesticidal plants without the carrying out of formulation and application processes, for pest and disease control. Botanical extracts kill and repel pests, affect insect growth and development, have antifeedant and arrestant effects and have antifungal, antiviral, and antibacterial properties against pathogens (Khater, 2012). They can be grouped into five major chemical categories: nitrogen compounds (primarily alkaloids), terpenoids, phenolics, protease inhibitors, and growth regulators (Hanem, 2012). In developing countries, pesticidal plants offer unique and challenging opportunities for exploration and development of botanicals (Khater, 2012).

2.11 Traditional Insecticides

2.11.1. Pyrethrum

Pyrethrum is the most widely and heavily used botanical insecticide worldwide and it is widely used in household aerosols for fast knockdown of pests. The flowers of *Tanacetum cinerariaefolium* (Asteraceae) were ground to powder and then extracted with hexane or a similar nonpolar solvent; removal of the solvent yields an orange-colored liquid that contains the active compounds (Khater, 2012). The majority (> 75%) of the world's supply of pyrethrum was produced in Kenya and Tanzania, but its production

began in Tasmania (Australia) in 1996 and it currently produces almost one-half of the world supply (Hanem, 2012). Technical grade pyrethrum, the resin used in formulating commercial insecticides, typically contains from 20% to 25% pyrethrins (Hanem, 2012). Australia produces a technical grade material comprising 50% pyrethrins by weight. Pyrethrins are common active ingredients in the insecticide products. Without formulation with one of the synergists, most of the paralyzed insects recover. A synergist such as piperonylbutoxide (PBO), derived from sassafras or n-octyl bicycloheptane dicarboximide, increase insect mortality and extends the shelf life of the product (Khater, 2012).

2.11.2 Pyrethroids

Pyrethroids are synthetic materials designed to imitate natural pyrethrum, but they are much more toxic and long lasting (Hanem, 2012). Sunlight does not break them down and they stick to leaf surfaces for weeks, killing any bypassing insect. Even though pyrethroids can be useful insecticides, they induce some deleterious effects as they irritate the human skin; some of them are extremely toxic to natural enemies, honey bees, and fish. They are more harmful to the environment than pyrethrum (Khater, 2012)

2.11.3 Rotenone

Rotenone is an isoflavonoid obtained from the roots or rhizomes of tropical legumes in the genera *Derris*, *Lonchocarpus,* and *Tephrosia*. It is a traditional botanical insecticide in declining use in standard agriculture, but is used for organic food production. In California, about 200 kg are used annually, mostly on lettuce and tomato crops (Hanem, 2012). Rotenone continues to be sold for home and garden use. It has been used for 350

years, in South-east Asia to paralyze fish, causing them to surface and be easily captured. It kills fish at dosages that are relatively nontoxic to other organisms, and is degraded rapidly. Accordingly, it is used to reclaim lakes for game fishing. It commonly sold as a dust containing 1% to 5% active ingredients for home and garden use, but liquid formulations used in organic agriculture can contain as much as 8% rotenone and 15% total rotenoids (Khater, 2012).

2.12 Newer Botanical Insecticides

2.12.1 Neem

Neem (*Azadirachta indica* A. Juss: Meliaceae) is a newer botanical insecticide, native to the Indian sub-continent and well known there as the' Botanical Marvel', 'Village Pharmacy','Wonder Tree', ' all-can-treat-tree', and 'Gift of Nature'(Hanem,2012). Neem is widely grown in other Asian countr ies and in tropical and subtropical areas of Africa, America, and Australia. It grows well even in poor, shallow, degraded, and saline soil. In countries in which it has been introduced, neem is more commonly used as shade trees, windbreaks, or a source of firewood than for its medicinal or insecticidal purposes (Khater, 2012).

2.12.2 *Borreria verticillata*

Borreria verticillata (Linn.) G.F.W.Mey is a woody shrub plant belonging to the Rubiaceae family. It is a perennial, reaching 1m in height, glabrous and erect ramifications. It is very common in humid areas. The stem is obscurely angled, glabrous with stipular sheaths which are glarous or scarid. The leaves are oblanceolate, 3-5cm long, 5-10mm broad, glabrous, with faint lateral nerves (Sofowora, 1993). The terminal

inflorescence is glabose, 1-1.5 cm in diameter, usually with two leafy bracts about 1cm long and reflexed beneath. The flowers are usually bisexual. The calyx is adnate to the ovary with epigynous, more or less tubular corolla. The lobes (4-12) are contorted; stamens are epipetalous and alternate with corolla tubes. The anthers are mostly separate and two celled. The ovary is inferior with two or more cells, an axile and apical or basal placenta. The style is slender. The fruit is a dehiscent drupe containing seeds with ruminate endosperm. The embryo is either straight or curve (Sofowora, 1993). The roots have been used by Senegalese traditional medical practitionres as an antileprotic, antiparalyptic, diuretic and antibilharzia, an abortive and are also used in veterinary medicine (Sofowora, 1993). In Nigeria the root is used to treat various skin diseases. The leaves and inflorescence of *B. verticillata* are also used in Senegal (internally or externally) for treatment of leprosy. An infusion of the leaves is also used in treating various skin diseases. *Borreria verticillata* is also used in treating various skin diseases in Nigeria and in parts eastern Africa (Sofowora,1993).In Senegal, the plant is used as a fumigant from horses suffering from 'Mal ala tate' (Sofowora, 1993).The roots contain 0.1 per cent of the alkaloid emetine, but no cephaeline. However, more recent reports showed that there is no emetine in *B. verticillata* from Senegal or South America (Sofowora, 1993). A study of the Senegalese specimen showed that the roots contain two new indole alkaloids, which represent almost 88% of the total alkaloid fraction-these are borrerine and its apparent dimer borreverine ($C_{12}H_{40}N_4$).The structure of these alkaloids have been determined (Sofowora, 1993).No base was found in the roots but the tops contain a good quality of total alkaloids. Two new alkaloids, dehydroborrecapine and borrecoxine were isolated from a related species, *B. capitata* (Sofowora, 1993).

Sofowora, (1993) Leaves of *B. verticillata* have also been reported to contain volatile oils, which in turn contain sesquiterpene, lactones, phenolic compounds and aromatic polycarboxylic acids (Sofowora, 1993). The oil contains the azulene-guaiaene. The following compounds were isolated from specimens from Madagascar-asperuloside, deacetyl-asperuloside, methylester of deacetyl-asperuloside, asperulosidic acid, deacetyl-asperulosidic acid, feretoside (from the root); campesterol, daphylloside, beta-sitosterol and stigmasterol (stem) (Sofowora, 1993).Volatile oils also obtained from leaves, *B.verticillata* inhibited the growth of Gram-positive and Gram-negative bacteria after prolonged vacuum distillation under a nitrogen atmosphere (Sofowora, 1993). The essential oil of *B. verticillata* has also been reported to show activity against *Schistosoma haematobium* (the organism causing schistosomiasis) at a dose of 100mµg/mL (Sofowora, 1993). A total of eleven alkaloids (borrecapine, borrecoxine, borreline,borrerine,dehydroborrecapine, verticillatine A and verticillatine B),bis-indole (borreverine, isoborreverine and spermacoceine) and tetrahydroisoquinoline {(-)- emetine (7)} skeletons have been isolated from *Borreria capitata*, *Borreriaspp* and *Borreriaverticillata* (Conserva and Ferreira, 2012).

2.12.3 *Striga asiatica*

Striga asiatica (Linn.) O.Kuntze is an herbaceous flowering plant, belonging to the family Orobanchaceae, but older classification placed it under the scrophulariaceae family. It is an annual, hemi parasitic plant of food crops such as rice, (*Oryza sativa* L.), maize (*Zea mays* L.) and sorghum (*Sorghum bicolor* L. Moench) roots. *Striga asiatica* seedlings are not visible above ground, but white succulent shoots can be found attached to host roots. Mature plants have green foliage above ground that is sparsely covered with

coarse, white bulbous-based hairs. *Striga asiatica* are normally 15-30cm tall but have been known to grow to 60cm. Leaves are nearly opposite, narrowly lanceolate, about 1-3cm long, with successive leaf pairs perpendicular to one another *S.asiatica* flowers in summer and fall. Flowers are small (less than 1.5cm in diameter) are sessile and axillary, the corolla is two-lipped, and they occur on loose spikes. Flower colour varies regionally; from red, orange, or yellow in Africa, to pink, white and yellow purple in Asia. The flowers give way to swollen seeds pods, each containing thousands of microscopic seeds. Underground stems are round with scale-like leaves and white but turn blue when exposed to air. The roots are succulent round, without hairs, and found attached to a host species root system (CDFA, 2006). The flowers of *S.asiatica* self-pollinate before opening, when sticky pollen balls cling to the elongating style. The fruit of *S.asiatica* is a seed capsule that is ovoid and five sided with narrow wings at each corner. Capsules contain up to 1400 seeds (550 average). Seeds are brown, oval and 0.2mm long. The seeds surfaces are striated and overlaid with a reticulate pattern that is visible with magnification (CDFA, 2006). *Striga asiatica* has a wide range of medicinal uses; it is used as an appetizer, against hypertension, as a stimulant, to break down fats in the body and to strengthen the body and has pharmacological antifertility effect (Kakpure and Rothe, 2012), antibacterial, antifungal and antihelminthic activities have been approved (Kakpure and Rothe, 2012). The whole plant crude drug has been used for peevishness in unwanted infants and also for icterohepatitis in China (Kakpure and Rothe, 2012).

2.13 Phytochemicals and Their Uses

Compounds as alkaloids, flavonoids,volatile or essential oils, steroidal compounds terpenes,phenols, saponins, tannins and glycosides have been extracted from plants are being used for pharmaceutical preparations.

2.13.1 Alkaloids

These are the largest group of secondary chemical constituents of plants made largely of ammonia compounds comprising basically of nitrogen bases synthesized from amino acid building blocks with various radicals replacing one or more of the hydrogen atoms in the peptide ring, most containing oxygen (Doughari, 2012). The compounds have basic properties and are alkaline in reaction, turning red litmus paper blue. In fact, one or more nitrogen atoms that are present in an alkaloid, typically as primary, secondary, tertiary amines, contribute to the basicity of the alkaloid. The degree of basicity varies considerably, depending on the structure of the molecule, presence and location of the functional groups (Doughari, 2012). They react with acids to form crystalline salts without the production of water (Doughari, 2012). Majority of alkaloids exist as solids, such as atropine; some as liquids containing carbon, hydrogen, and nitrogen. Most alkaloids are readily soluble in alcohol and though they are sparingly soluble in water, their salts are usually soluble. The solutions of alkaloids are intensely bitter. These nitrogenous compounds function in the defence of plants against herbivores and pathogens and are widely exploited as pharmaceuticals, stimulants, narcotics and poisons due to their potent biological activities. In nature, the alkaloids exist in large proportions in the seeds and roots of plants and often in combination with vegetable acids. Alkaloids

have pharmacological applications such as anaesthetics and central nervous system stimulants (Doughari, 2012).

2.13.2 Glycosides

Glycosides in general, are defined as the condensation products of sugars (including Polysaccharides) with a host of different varieties of organic hydroxy (occasionally thiol) compounds (invariably monohydrate in character), in such a manner that the hemiacetalentity of the carbohydrate must essentially take part in the condensation. Glycosides are colourless, crystalline carbon, hydrogen and oxygen-containing (some contain nitrogen andsulfur) water-soluble phytoconstituents, found in the cell sap. Chemically, glycosides contain carbohydrate (glucose) and non-carbohydrate part (aglycone or genin) (Doughari, 2012). Alcohol, glycerol or phenol represents aglycones. Glycosides are neutral inreaction and can be readily hydrolyzed into its components with ferments or mineral acids.Glycosidesis classified on the basis of type of sugar component, chemical nature of aglycone or pharmacological action. The rather older or trivial names of glycosides usually as a suffix ‘in’ and the names essentially included the source of the glycoside. For instance: strophanthidin from *Strophanthus*, digitoxin from *Digitalis*, barbaloin from *Aloe*; salicin from Salix, cantharidin from *Cantharides* and prunasin from *Prunus.* However, the systematic names are invariably coined by replacing the “ose” suffix of the parent sugar with “oside”. These groups of drugs are usually administered in order to promote appetite and aid digestion. Glycosides are purely bitter principles that are commonly found in plants of the Genitiaceae family and though they are chemically unrelated but possess the common property of an intensely bitter taste. The bitters act on gustatory nerves, which results in increased flow of saliva and gastric

juices. Chemically, the bitter principles contain the lactone group that may be diterpene lactones (e.g. andrographolide) or triterpenoids (e.g.amarogentin). Some of the bitter principles are either used as astringents due to the presence of tannic acid, as antiprotozoan, or to reduce thyroxine and metabolism. Examples include cardiac glycosides (acts on the heart), anthracene glycosides (purgative, and for the treatment of skin diseases), chalcone glycoside (anticancer), amarogentin, gentiopicrin, andrographolide, ailanthone and polygalin. Doughari, (2012) reported that extracts of plants that contain cyanogenic glycosides are used as flavouring agents in many pharmaceutical preparations. Amygdalin has been used in the treatment of cancer.Hydrogen cyanide liberated in stomach kills malignant cells), and also as a cough suppressant in various preparations. Excessive ingestion of cyanogenic glycosides can be fatal. Some foodstuffs containing cyanogenic glycosides can cause poisoning (severe gastric irritations and damage) if notproperly handled (Doughari, 2012).

2.13.3 Flavonoids

Flavonoids are important group of polyphenols widely distributed among the flora. Stucturally, they are made of more than one benzene ring (a range of C_{15} aromatic compounds) and numerous reports support their use as antioxidants or free radical scavengers (Doughari, 2012). The compounds are derived from parent compounds knownas flavans. Over four thousand flavonoids are known to exist and some of them are pigments in higher plants. Quercetin, kaempferol and quercitrin are common flavonoids present in nearly 70% of plants. Other groups of flavonoids include flavones, dihydroflavons, flavans, flavonols, anthocyanidins, proanthocyanidins, calchones, catechin and leucoanthocyanidins (Doughari, 2012).

2.13.4 Phenolics

Phenolics, phenols or polyphenolics (or polyphenol extracts) are chemical components tha toccur ubiquitously as natural colour pigments responsible for the colour of fruits of plants .Phenolics in plants are mostly synthesized from phenylalanine via the action of phenylalanine ammonia lyase (PAL). They are very important to plants and have multiple functions. The most important role may be in plant defence against pathogens and herbivore predators, and thus are applied in the control of human pathogenic infections.They are classified into (i) phenolic acids; (ii) flavonoid polyphenolics (flavononones, flavones, xanthones and catechins); and (iii) non-flavonoid polyphenolics (Doughari, 2012). Caffeic acid is regarded as the most common of phenolic compounds distributed in plants followed by chlorogenic acid, known to cause allergic dermatitis among humans (Doughari, 2012). Phenolics essentially represent a host of natural antioxidants, used as nutraceuticals, and found in apples, green-tea, and red-wine for their enormous ability tocombat cancer and are also thought to prevent heart ailments to an appreciable degree and sometimes are anti-inflammatory agents. Other examples include flavones, rutin, naringin hesperidin and chlorogenic.

2.13.5 Saponins

The term saponin is derived from *Saponaria vaccaria* (*Quillaja saponaria*), a plant, which abounds in saponins and was once used as soap. Saponins therefore possess ‘soaplike’behaviour in water, i.e. they produce foam. On hydrolysis, an aglycone is produced, which is called sapogenin (Doughari, 2012). There are two types of sapogenin: steroidal and triterpenoidal. Usually, the sugar is attached at C-3 in saponins, because in

most sapogenins there is a hydroxyl group at C-3. *Quillaja saponaria* is known to contain the toxic glycosides quillajic acid and the sapogenin senegin. Quillajic acid is strenutatory and senegin is toxic. Senegin is also present in *Polygala senega* (Doughari, 2012). Saponins are regarded as high molecular weight compounds in which a sugar molecule is combined with triterpene or steroid aglycone. There are two major groups of saponins and these include: steroid saponins and triterpene saponins. Saponins are soluble in water and insoluble in ether; and like glycosides on hydrolysis, they give aglycones. Saponins are extremely poisonous, as they cause haemolysis of blood and are known to cause cattle poisoning (Doughari, 2012). They possess a bitter and acrid taste, besides causing irritation to mucous membranes. They are mostly amorphous in nature, soluble in alcohol and water, but insoluble in non-polar organic solvents like benzene and n-hexane. Saponins are also important therapeutically as they have been shown to have hypolipidemic and anticancer activities. Saponins are also necessary for activity of cardiac glycosides.The two major types of steroidal sapogenin are diosgenin and hecogenin. Steroidal saponins are used in the commercial production of sex hormones for clinical use. For example, progesterone is derived from diosgenin. The most abundant starting material for the synthesis of progesterone is diosgenin, isolated from *Dioscorea* species, formerly supplied from Mexico, and now from China (Doughari, 2012). Other steroidal hormones, e.g. cortisone and hydrocortisone, can be prepared from the starting material hecogenin, which can be isolated from Sisal leaves, found extensively in East Africa (Doughari, 2012).

2.13.6 Tannins

These are widely distributed in plants. They are phenolic compounds of high molecula rweight. Tannins are soluble in water and alcohol and are found in the root, bark, stem and outer layers of plant tissues. Tannins have a characteristic feature of being used tan; i.e. to convert skin into leather. They are acidic in reaction due to the presence of phenolics or carboxylic group (Doughari, 2012). They form complexes with proteins, carbohydrates, gelatin and alkaloids. Tannins are divided into hydrolysable tannins and condensed tannins. Hydrolysable tannins, upon hydrolysis, produce gallic acid and ellagic acid and depending on the type of acid produced, the hydrolysable tannins are called gallotannins or egallitannins. On heating, they form pyrogallic acid. Tannins are used as antiseptic and this activity is due to presence of the phenolic group. Common examples of hydrolysable tannins include theaflavins (from tea), daidezein, genistein and glycitein. Tannin-rich medicinal plants are used as healing agents in a number of diseases. In Ayurveda, formulations based on tannin-rich plants have been used for the treatment of diseases like leucorrhoea, rhinnorhoea and diarrhea (Doughari, 2012).

2.13.7 Terpenes

Terpenes are among the most widespread and chemically diverse groups of natural products (Doughari, 2012). They are flammable unsaturated hydrocarbons, existing in liquid form commonly found in essential oils, resins or oleoresins (Doughari, 2012). Terpenoids includes hydrocarbons ofplant origin of general formula $(C_5H_8)_n$ and are classified as mono-, di-, and tri- and sesqui-terpenoids depending on the number of carbon atoms. Examples of commonly important monoterpenes include terpinen-4-ol, thujone, camphor, eugenol and menthol. Diterpenes (C_{20}) are classically considered to be resins and taxol, the anticancer agent, is the common example. The triterpenes (C_{30})

include steroids, sterols, and cardiac glycosides with anti-inflammatory, sedative, insecticidal or cytotoxic activity (Doughari, 2012). Common triterpenes: amyrins, ursolic acid and oleanic acid sesquiterpene (C_{15}) like monoterpenes, are major components of many essential oils (Doughari, 2012). The sesquiterpene acts as irritants when applied externally and when consumed internally their action resembles that of gastrointestinal tract irritant. A number of sesquiterpene lactones have been isolated and broadly they have antimicrobial (particularly antiprotozoal) and neurotoxic actions (Doughari, 2012). The sesquiterpene lactone, palasonin, isolated from *Butea monosperma* has anthelminthic activity, inhibits glucose uptake and depletes the glycogen content in *Ascaridia galli* (Doughari, 2012).Terpenoids are classified according to the number of isoprene units involved in their formation.

2.13.8 Anthraquinones

These are derivatives of phenolic and glycosidic compounds. They are solely derived from anthracene giving variable oxidized derivatives such as anthrones and anthranols s (Doughari, 2012). Other derivatives such as chrysophanol, aloe-emodin, rhein, salinosporamide, luteolin and emodin have in common a double hydroxylation at positions C-1 and C-8. To test for free anthraquinones, powdered plant material is mixed with organic solvent and filtered, and an aqueous base, e.g. NaOH or NH_4OH solution, is added to it. A pink or violet colour in the base layer indicates the presence of anthraquinones in the plant ample (Doughari, 2012).

2.13.9 Essential oils

Essential oils are the odorous and volatile products of various plant and animal species. Essential oils have a tendency to evaporate on exposure to air even at ambient conditions and are therefore also referred to as volatile oils or ethereal oils**.** They mostly contribute to the odoriferous constituents or 'essences'of the aromatic plants that are used abundantly in enhancing the aroma of some spices (Doughari, 2012). Essential oils are either secreted directly by the plant protoplasm or by the hydrolysis of some glycosides and structures. Plant structures associated with the secretion of essential oils include: glandular hairs (Lamiaceae e.g. *Lavandula angustifolia*), oil tubes (or vittae) (Apiaceae eg. *Foeniculum vulgare* and *Pimpinella anisum* (Aniseed), modified parenchymal cells (Piperaceae e.g. *Piper nigrum* - Black pepper), schizogenous or lysigenum passages (Rutaceae e.g. *Pinus palustris* - Pine oil). Essential oils have been associated with different plant parts including leaves, stems, flowers, roots or rhizomes. Chemically, a single volatile oil comprises of more than 200 different chemical components, and mostly the trace constituents are solely responsible for its characteristic flavour and odour (Doughari, 2012). Essential oils can be prepared from various plant sources either by direct steam distillation, expression, extraction or by enzymatic hydrolysis. Direct steam distillation involves the boiling of plant part in a distillation flask and passing the generated steam and volatile oil through a water condenser and subsequently collecting the oil in florentine flasks (Doughari, 2012). Depending on the nature of the plant source the distillation process can be either water distillation, water and steam distillation or direct distillation. Expression or extrusion of volatile oils is accomplished by either by sponge method, scarification, rasping or by a mechanical process. In the sponge method, the washed plant part e.g. citrus fruit (orange, lemon, grape fruit, bergamot) is cut into

halves to remove the juice completely, rind turned inside out by hand and squeezed when the secretary glands rupture. The oozed volatile oil is collected by means of the sponge and subsequently squeezed in a vessel. The oil floating on the surface is separated (Doughari, 2012). For the the scarification process, the apparatus, Ecuelle a Piquer (a large bowl meant for pricking the outer surface of citrus fruits) is used. It is a large funnel made of copper having its inner layer tinned properly. The inner layer has numerous pointed metal needles just long enough to penetrate the epidermis. The lowerstem of the apparatus serves two purposes; first, as a receiver for the oil; and secondly as a handle. Now, the freshly washed citrus fruits (lemons) are placed in the bowl and rotated repeatedly causing the oil glands are punctured (scarified) thereby discharging the oil right into the handle. The liquid, thus collected, is transferred to another vessel, where on keeping theclear oil may be decanted and filtered (Doughari, 2012). For the rasping process, the outer surface of the peel of citrus fruits containing the oil gland is skillfully removed by a grater. The 'raspings' are now placed in horsehair bags and pressed strongly so as to ooze out the oil stored in the oil glands. Initially, the liquid has a turbid appearance but on allowing it to stand the oil separates out which may be decanted and filtered subsequently (Doughari, 2012). The mechanical process involves the use of heavy duty centrifugal devices so as to ease the separation of oil/water emulsions invariably formed and with the advent of modern mechanical devices the oil output has increased impressively. The extraction processes can be carried out with either volatile solvents (e.g hexane, petroleum ether or benzene) resulting into the production of'floral concretes'- oils with solid consistency and partly soluble in 95% alcohol, or non-volatile solvents (tallow, lard or olive oil) which results in the production of perfumes. Examples

of volatile oils include amygdaline (volatile oil of bitter almond), sinigrin (volatile oil of blackmustard), and eugenol occurring as gein (volatile oil of *Geum urbanum*) (Doughari, 2012).

2.13.10 Steroids

Plant steroids (or steroid glycosides) also referred to as 'cardiac glycosides', are one of the most naturally occurring plant phytoconstituents that have found therapeutic applications as arrow poisons or cardiac drugs (Doughari, 2012). The cardiac glycosides are basically steroids with an inherent ability to inflict a very specific and powerful action mainly on the cardiac muscle when administered through injection into man or animal. Steroids (anabolicsteroids) have been observed to promote nitrogen retention in osteoporosis and in animals with wasting illness (Doughari, 2012). Caution should be taken when using steroidal glycosides as small amounts would exhibit the much needed stimulation on a diseased heart; where as excessive dose may cause even death. Diosgenin and cevadine (from *Veratrum veride*) are examples of plant steroids (Doughari, 2012).

The major option for the reduction of adult *Culex quinquefasciatus* in large cities is the insecticidial space spraying. In Khartoum city fumigation with pyrethroids by using fogging machines is adopted for the treatment of mosquito resting places in culverts and drains. This practice is very effective when breeding sites are limited but it is generally adopted when the adult density becomes very high. Chloropyrifos (Durs-ban) and Feintrothion are the two main larvicides used in Sudan against the urban mosquito *Culex quinquefasciatus* (Ali and El-rabaa, 2010). There have been efforts towards the

development of an integrated and coordinated mosquito control in the Sudan. Attempts were being made to study the effects of pesticides used in mosquitoe, snail and agricultural pests control, against natural predators such as fish and other non-target organisms (Ali and El-rabaa, 2010).DDT-resistance was reported in *Anopheles gambiae* earlier in Sudan.The continued wide-spread use of Malathion in *Aedes aegypti* control programs in Latin America has generated insecti-cide resistance to this chemical in *Culex quinque-fasciatus* insecticide resistance was alsoreported in *Culex quinquefasciatus, Aedes aegypti and Aedes albopictus larvae* against Malathion, permethrin and temephos in Malaysia (Ali and El-rabaa, 2010). However, temephosis still effectiveagainst *Culex quinque-fasciatus* (Ali and El-rabaa, 2010). Insecticide resistance was also reported in *Culex quinquefasciatus, Aedes aegypti* and *Aedes albopictus* larvae against Malathion, permethrin and temephos in Malaysia (Ali and El-rabba, 2010). However, temephos is still effective against *Culex quinquefasciatus* (Ali and El-rabaa, 2010).

Ali and El-rabaa (2010) considered larval resistance, when it larvae Survived concentrations of Malathion just below 0.625 mg/l. Malathion, Fentrithion and Fenthion have been used in Khartoum since 1985. Ali and El-rabba (2010), reported that in many parts of the world the LC_{50} of Malathion to various mosquito larvae extended between 0.029 ppm and 0.232 ppm. The LC_{50} of Malathion against Culex larvae was 2.2693 mg/l and the calculated LC_{99} was equal to 15.6 mg/l. These figures showed the measure of resistance offered by the mosquito larvae (Ali and El-rabaa, 2010).*Culex quinquefasciatus* is highly resistant to Malathion; this probably explains the complaints of Khartoum citizens despite door to door spraying with Malathion. Latex of Uschar (*Calotropis procera*) and Labakh (*Ficus benghalensis*) plant species was found to contain

noticeable toxic activity against mosquito larvae. (Ali and El-rabba, 2010).The latex of *Calotropis procera* was reported to be poisonous, acidic and coagulates on standing and retains its toxicity even in the solid state for a very long time (Ali and El-rabaa, 2010). The latex also contains trypsin in addition to cardio active substances which comprise active glycosides. The only work conducted on *Ficus benghalensis* as larvicidal agent was reported most recently, when the larvicidal efficacy of the crude leaf extracts of *Ficusbenghalensis* with three different solvents (methanol, benzene and acetone) was tested against the larvae of *Culex quinquefasciatus*, maximum efficacy was observed in methanol. The LC_{50} values of *Ficus benghalensis* against early second, third and fourth larvae of *Culex quinquefasciatus*were 41.43, 58.21 and 74.32 ppm (Ali and El-rabaa, 2010). The excessive wriggling movements made by the larvae while in the toxicant may be due to high chemo kinesis which in normal situations may lead the larvae to more favourable places. Colour change as a sign of toxicities has been recorded by many investigators (Ali and El-rabaa, 2010). The failure of mosquito larvae to hang down from the air/water interface in case of *Ficus benghalensis* latex may be due to the presence in the latex of a substance that lowers the surface tension of water to the extent that the adhesion force between water and the hydrophilic hair at the end of the larval abdomen no longer support the mosquito in its normal position (Ali and El-rabaa, 2010). This principle may also explain the success of mosquito to keep normal hanging position when they get in touch with the wall of the container where the adhesive force between the solid wall and the hair of mosquito is much greater (Ali and El-rabaa, 2010). The random movements of mosquito larvae clearly involve a type of reaction involving chemo

kinesis. Once the larvae become in touch with the wall of the container low thermo kinesis override chemo kinesis and the larvae stop wriggling.

Calotropis procerais a common revirain semi desert weed widely distributed in the Sudan.Its effectiveness in low concentrations against *Culex* larvae and probably against other species of mosquitoes and medically important aquatic insect, encourage its application in dirty bodies of water not accessible to and not used by man or his animals, such as pit latrines, sewage ponds and the ditch (Ali and El-rabaa, 2010).In Sudan *Ficusbenghalensis* is used mainly as a shading plant and no medical value has been claimed by the people. However, the figs are claimed by parents, as being poisonous and warn the children not to eat it.The figs of this tree are also described locally as "the poison of rats" and the latex is also claimed to cause blindness. According to Ali and El-rabaa, (2010), the latex of this tree yields no poison and is used in West Africa as bird-lime and that the fruit is edible and is used in beer-making (Ali and El-rabaa, 2010).The powdered bark is used on wounds and for colds and throat affection in West Africa. In Tanganyika the bark is used as an influenza remedy, while the root bark is used for stimulating lactation and also for making bark-cloth24 and that the latex in the"Belgian Congo" used for the treatment of burns.There was no available nformation on the to the chemical nature of the latex of *Ficus benghalensis* (Ali and El-rabaa, 2010).

Tridax procumbens is commonly used in Indian traditional medicine as anticoagulant, antifungal and insect repellent, in bronchial catarrh, diarrhoea and dysentery (Kamaraj *et al*., 2011). The entire plant is used for the treatment of malaria, leishmaniasis, vaginitis, dysentery and gastrointestinal disorders (Kamaraj *et al*., 2011). Essential oils of the

leaves *Tridax procumbens* showed a more effective repellent activity, at 6 per cent concentration against *Anopheles stephens* and methanol leaf extracts and purified compound (35)-16,17-didehydrofalcerinol (1) showed effective antileishmanial activity against promastigotes of *Leishmania Mexicana* (Kamaraj *et al*.,2011). *Tridax procumbens* L have earlier been tested by several investigators, where larvicidal and antifeedent activities were exhibited against *Anopheles subpictus* and *Culex tritaeniorhynchus* and also *Leishmania maxicone* (Kamaraj *et al*., 2011).

Chrysanthemum indicum is a traditional herb used to treat various disorders, hypertension symptoms and several infectious diseases in Korean and Chinese medicine (Kamaraj *et al*., 2011). Kamaraj *et al*. (2011), reported that the leaves of *C.morifolium* extracted sequentially with hexane, ethyl acetate, and methanol were found to reduce the growth of *Trichoplusia* larvae (Kamaraj *et al*., 2011).Powders from the flowers of *Chrysanthemum fuscatum* and *Chrysanthemum myconis* have been tested for antifeeding and growth regulatory activity against *Spodoptera littoralis* (Kamaraj *et al*., 2011).

Annona squamosa, commonly known as custard apple is a native of West Indies and is cultivated through out India, mainly for its edible fruit (Kamaraj *et al* ., 2011). Kamaraj *et al*. (2011) reported that the ethanol leaf extract of *Anona squamosa* was found to have the most promising larvicidal activity against *Culex quinquefasciatus* larvae. The larvicidal and mosquitocidal activities of ethanolic water mixture extract of *Anonasquamosa* and *Centella asiatica* were effective against larvae of *AnophelesStephensi.* The leaves methanolic extract and the seeds petroleum ether extract

of *Anona squamosa* showed larvicidal activity when tested against *Anophelesstephensi*, *Culexquinquefasciatus* and *Aedes aegypti*(Kamaraj *et al*., 2011).

Gosh *et al*. (2012), described approximately 1,200 plant species having potential insecticidal value Only about 344 plant plant species, listed and discussed exhibited mosquitocidal activity (Gosh *et al*., 2012). Gosh *et al*.(2012) reviewed the current state of knowledge of plant species.The larvicidal potentials, extraction processes, growth and reproduction inhibiting phytochemicals,botanical ovicides, synergistic, additive and antagonistic joint action effects of mixtures residual capacity. They also reviewed effects on non-target organisms, resistance and screening methodologies, and discussed some promising advances made in phytochemical research. The reviewed mechanism of action of plant secondary metabolites on insect body and documented several physiological disruptions, such as inhibition of acetylecholinesterase (by essentialoils), GABA-gated chloride channel (by thymol), sodium and potassium ion exchange disruption (by pyrethrin) and inhibition of cellular respiration (by rotenone) (Gosh *et al*., 2012).Such disruption also includes the blockage of calcium channels (by nodine), of nerve cell membrane action (by sabadilla), of octopamine receptors (thymol), hormonal balance disruption, mitotic poisioning (by azadirachtin), disruption of the molecular events of morphogenesis and alteration in the behavior and memory of cholinergic system (by essential oil), etc. of these, the most important activity is the inhibition acetylcholinerase activity (AChE) as it is a key enzyme responsible for terminating the nerve impulse transmission through synaptic pathway; AChE has been observed to be organophosphorus and carbamate resistant and it is well-known that the alteration in AChE is one of main resistance mechanisms in insect pests(Gosh *et al*., 2012).

Tennyson *et al*. (2013), screened essential oils of the Brazilian plants, Ocimum gratissimum(60ppm),Lippa sidoides (63ppm), Ocimum americanum (67ppm), Cymbopo gon citrates (69ppm), Hypissuaveolens (261ppm), Alpinia limonia (519ppm) and Citrus sinensis (538ppm) and found their LC50 values to be effective against the larvae of *Aedes aegypti*.Essential oils of eleven plants were evaluated for larvicidal activity against *Aedes aegypti* early fourth instar larvae and their LC_{50} and their LC_{90} values ranged from 63.7 to 162.9 ppm with *Ocimum lamifolium* and *Chenopodium ambrosoides* exhibiting highest larvicidal effects with LC_{50} of 47.3 and 97.9ppm(Tennyson *et al* ., 2013).

Essential oil from *Mentha piperita* showed larvicidal action against *Aedes aegypti* with LC_{50} and LC_{90} values of 111.9 and 395.1ppm after 24 hour exposure (Tennyson *et al* ., 2013). Tennyson *et al*.,(2013), studied also the larvicidal activity of essential oils from four plants and the highest activity was observed in essential oil from *Zingiber officinale* against *Culex tritaeniorhynchus* and *Anopheles Subpictus* with LC_{50} and LC_{90} values as 98.83, 57.98 and 186.55, 104.23 ppm, respectively.

Larviciding is a successful method of reducing mosquito population in their breeding places before they emerge into adults (Tennyson *et al*., 2013). The advantage of targeting larvae is that they cannot escape from their breeding sites until the adult stage. The control of mosquito at the larval stage is necessary and efficient in integrated mosquito management since during the immature stage, mosquitoes are relatively immobile and mosquitoes in the larval stage are attractive targets for insecticides because mosquitoes breed in water and thus, it is easy to deal with them in this habitat (Tennyson *etal* ., 2013). Essential oils of many plants were observed to have mosquito larvicidal property

and have received attention as potentially controlling vectors of mosquito-borne disease (Tennyson *etal*., 2013). Therefore, the use of plant oils in insect/mosquito control is an alternative pest control method for minimizing the noxious effects of some pesticidal compounds on the environment (Tennyson *etal*., 2013).

The acetone, chloroform and methanol leaf extracts of *Solanum trilobatum* exhibited larvicidal activity against *Aedes aegypti*,*Culex quinquefasciatus* and *Anopheles stephensi* with LC_{50} values of 125.67,125.87 and 125.43; 123.50, 121.06 and 122.77; 121.99. Further, the plant is reported for its mosquitocidal property which includes ovicidal activity against *Culex quinquefasciatus* and *Culex tritaeniorhynchus* larvicidal activity oviposition deterrent and skin repellent activity against *Anopheles stephensi* (Sakthivadivel *etal*., 2014). The acetone, chloroform and methanol leaf extracts of *Solanum trilobatum* was reported larvicidal, pupicidal and adult emergence inhibition properties against *Aedes aegypti*, *Culex quinquefasciatus* and *Anopheles stephens* (Sakthivadivel *etal*., 2014).

Plant bioactive components may serve as a suitable alternative to chemical insecticide as they are relatively safe and available everywhere in the world.The efficacy of botanicals however, generally depends on the plant part extract concentration, age of plant or location found, solvent used and species of larvae tested (Nganjiwa *et al*., 2015).Most studies report active compounds as steroidal saponins. Saponins are freely soluble in both organic solvents and water, and they work by interacting with the cuticle membrane of the larvae, ultimately disarranging the membrane, which is the most probable reason for larval death (Nganjiwa *et al*., 2015).The hexane, chloroform, ethyl acetate and methanol

extracts of the leaf, flower, and whole plant of *Jatropha curcas, Hyptis suaveolens,Abutilon indicum* and*Leucas aspera* were tested against third instar larvae of filarial vector, *Cx. quinquefasciatus* and larval mortality was recorded from the four plant extracts (Kovendan *et al*., 2015).The observed highest mortality with methanolic extract of *Jatropha Curcas* was 48–92.2%; *Hyptis suaveolens* was 54–93%; *Abutilon indicum* was 49–95.4%; and *Leucas aspera* was 52–98.2% (Kovendan *et al*., 2015). All plant extracts showed moderate larvicidal effects of after 24 h; maximum larvicidal activity was observed in the methanol extract followed by ethyl acetate, chloroform, and hexane extract. The hexane, chloroform,ethyl acetate, and methanol extract of *J. curcas* have LC_{50} values of 230.32, 212.85, 192.07, and 113.23 ppm;*H. suaveolens* have LC_{50} values of 213.09, 217.64, 167.59, and 86.93 ppm; *A. indicum* have LC_{50}values of 204.18, 155.53,166.32, and 111.58 ppm; and *L. Aspera* have LC_{50}values of 152.18, 118.29, 111.43 and 107.73 ppm, respectively (Kovendan *et al*.,2015). The crude petroleum ether leaf extract of *J. curcas* was shown to have high larvicidal potency on the early fourth instar larvae of *A. aegypti* and *Cx.quinquefasciatus* with the LC_{50} values of8.79 and 11.34 ppm, respectively. The leaf extract of methanol *J. Curcas* was also tested against the first to fourth instar larvae with LC_{50} values ; 01.200%, 1.290%, 1.358%, and1.448% and LC_{90} values ;02.094%, 2.323%, 2.444%, and 2.544%, respectively (Kovendan *et al*.,2015). Crude and column chromatographic fractions of methanol leaf extract of *J. curcas*were tested for their larvicidal activities against laboratory-reared late third instar larvae of *A. arabiensis* (Kovendan *et al*., 2015).Petroleum ether leaf extract of *J. curcas* was shown to larvicidal effects on vector mosquitoes, including *Cx. quinquefasciatus, An. Stephensis* and *Aedes aegypti* (Kovendan *et al*., 2012). Kovendan *et al*. (2012) reported the larvicidal effects

of *J. curcas* leaf extracts and their column chromatographic fractions against *A. stephensis* in Ethiopia and other parts of Africa.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Source of Plant Materials

The whole plants of *B.verticillata* and *S.asiatica* were collected from farmlands in Kundu village, located in Giwa Local Government area of Kaduna State. The plants were then taken to the herbarium Unit, of the Department Biological Sciences, Ahmadu Bello University, Zaria Nigeria for proper identification and authentication. The plant materials were assigned voucher numbers of 670 for *B.verticillata* and 24389 for *S.asiatica* respectively. The plant materials werespread out to dry on wooden tables at room temperature in the laboratory, until they became brittle.The plants were then pulverized separately using a mortar and pestle and stored in labeled polythene bags.

3.2 Solvent Extraction of Pulverized Plant Materials

Each of the two pulverized plant materials were initially subjected to soxhlet extraction method with methanol, ethylacetate, n-hexane and chloroform respectively, with N-hexane,chloroform,ethyl-acetate and methanol in the order of increasing polarity of the solvents. The condensed extracts were screened for phytochemicals (Kokate, 1988; Anonymous, 2008). Rotary evaporator was used to remove excess solvents from the extracts until solidification. Solidified extracts were stored in labelled vials at room temperature until used for larvicidal bioassay.

3.3 Preliminary Qualitative Phytochemical Screening

The various solvent extracts were subjected to preliminary phytochemical screening according to the procedures and methods described by Harborne(1973) andTrease and Evans(2002**).**

3.3.1 Screening for alkaloids

(a) Mayer's test

To a portion of the extract, a few drops of Mayer's reagent was added.A creamy white precipate indicated the presence of alkaloids (Trease and Evans, 2002).

(b) Dragendoff's Test

To a portion of the extract, a few drops of Dragendoff reagent was added.A reddish brown precipitate indicated the presence of alkaloids (Trease and Evans, 2002).

3.3.2 Screening for flavonoids

Sodium hydroxide Test

A few drops of 10% sodium hydroxide were added to a portion of the extract.A yellow coloration indicated the presence of flavonoids (Trease and Evans, 2002).

3.3.3 Screening for saponnins

Frothing Test

About 10ml of distilled was added to a portion of the extract and was shaken vigorously for 30 seconds. A honeycomb froth that persisted for 10-15 minutes indicated the presence of saponins (Trease and Evans, 2002).

3.3.4 Unsaturated steroid and triterpenes

Liebermann-Buccard Test

To a portion of the extract, equal volume of acetic acid anhydride was added and mixed gently. 1ml of concentrated sulphuric acid was added down the side of the test tube to form a layer. Colour changes were observed immediately and over a period of one hour.

A blue to blue-green in the upper layer and reddish, pink or purple colour indicated the presence of triterpene (Trease and Evans, 2002).

3.3.5 Screening for tannins

Ferric chloride Test

To a portion of the extract, 3-5 drops of ferric chloride solution was added. A blue or brownish precipitate indicated the presence of hydrolysable tannins (Trease and Evans, 2002).

3.3.6 Screening for anthraquinones

Test for Anthraquinone family of quinine

Bontrager's Test To a portion of the extract in a dry test tube, 5ml of chloroform was added and shaken for at least for 5 minutes.This was filtered and the filterate shaken with equal volume of 10% ammonia solution,there was no color change observed which indicated the absence of anthraquinone (Trease and Evans, 2002).

3.3.7 Screening for cardiac glycosides

Keller-kiliani Test A portion of the extract was dissolved in 1ml of glacial acetic acid containing traces of ferric chloride solution.This was then transferred into a dry test tube and 1ml of concentrated sulphuric acid was added down the side of the test tube to form a lower layer at the bottom.A purple-brown ring was observed at the interphase,which indicated the presence of desoxy sugars and a pale green color in the upper acetic layer which indicated the presence of cardiac glycosides (Trease and Evans,2002).

3.4 Collection and Maintenance of Mosquitoes in the Laboratory

Indoor resting adult blood-feed female *Culex quinquefasciatus* mosquitoes were obtained with test-tubes from various indoor resting sites within the Biological Sciences Department, Faculty of Sciences, Ahmadu Bello University, A.B.U, Zaria, Nigeria. Trapped mosquitoes were covered with cotton wool and taken to the Parasitology and Entomology Research Laboratory Department of Biological Sciences, A.B.U, Zaria and were then released into entomological cages containing bowls of distilled water. The mosquitoes then oviposited on water in the bowls (Adebote *et al*., 2011). The laid egg rafts hatched into first instar larvae and were fed with baker's yeast and dog biscuit dor 5-6 days until they developed into third instar larvae. Room temperature of 25 (°C) was maintained. The third instar larvae were confirmed to belong to *Cx. quinquefasciatus* using the identification keys by Hopkins(1952).

3.5 Preparation of Stock Solutions

Stock solutions of each of the eight solvent based extracts of both plants were made with distilled water in separate labeled flasks.Serial dilutions of the stock solutions were done to obtain diagnostic concentrations to which the mosquito larvae were exposed. Tentatively, concentrations of 2.00, 4.00, 6.00 and 8.00mg/l were used (these were reduced or increased as preliminary results dictated**).** To obtain miscible solutions of the solidified extracts, the solid residues were first homogenized with tween 8 before diluting to the desired concentrations with distilled water.

3.6 Larvicidal Bioassay

Twenty-five (25) third instar larvae of *Cx. quinquefasciatus* were isolated from the culture into a test tube using a dropping pipette. Larvae were transferred into 250mL capacity plastic bowls containing 100ml each of the test plant extracts concentrates. Experimental plastic bowls were replicated thrice; each bowl labeled with extract type, extract concentration and time of larval inoculation. Mosquito larvae were exposed in the test concentrations and control bowls at room temperature 26±2 (°C) for 24 hours after which larval mortality in each bowl was determined. Larvae were recorded dead when they fail to respond to touch of a pin in their cephalic region.

3.7 Statistical Analyses

Larval mortality data of the third instar of *the Culex quinquefasciatus* mosquito was subjected to probit analysis to determine the median lethal concentration (LC_{50}) of each of the test extracts against *Cx. quinquefasciatus* larvae.Fiducial limits of upper and lower confidence of LC_{50} were determined. Regression equation and coefficient of determination (R^2) were also determined. One way Analysis of Variance (ANOVA) was used to test for significance differences in mean larval mortality in control concentration and amongst the various experimental concentrations. Duncan Multiple Range Test was used to separate differences in means.All data were tested at $P \leq 0.05$ level of significant.

CHAPTER FOUR

4.0 RESULTS

4.1 Qualitative phytochemical constituents of the four solvent extracts of *Borreria verticillata*and *Striga asiatica*

Table 4.1 shows the phytochemical constituents in the various solvent extracts of *B.verticillata* and *S.asiatica*. In all 17 and 19 classes of phytochemicals were detected in *B.verticillata* and *S.asiatica*.Alkaloids were detected in all four extracts of both *B.verticillata* and *S.asiatica* respectively. While anthraquinone was not detected in any of the extracts of both plant species.Tannins, cardiac glycosides and triterpeneswere all detected in the four extracts of *B.verticillata*and *S.asiatica*, respectively.Steroids was also not detected in any of the extracts of both plant species. Flavonoids and saponins were jointly detected only in the methanol extracts of *B.verticillata* but jointly detected in both ethyl acetate and methanol extracts of S.asiatica (Table 4.1).

4.2 Larvicidal Efficacy of *Borreria verticillata* Extracts Against*Culexquinquefasciatu* Larvae

4.2.1 Effects of methanolic extract of *Borreria verticillata* on *Cx. quinquefasciatus*

The mortality of late third instar larvae of *Culex quinquefasciatus* on exposure for 24 hours to various concentrations of *Borreria verticillata*methanolic extract as shown on Table 4.2.Mortality of *Cx. quinquefasciatus* larvae ranged from 0/75 (0%) in the controlto 21/75(28%) at concentrations of 6mg/L and 8mg/L.Larval mortality increased in a concentration-dependent fashion. Mean larval mortalities at the various experimental concentrations were significantly ($P<0.05$) higher than the control;but increase in concentration from 2mg/L to 4mg/L and from 6mg/L to 8mg/L did not produce significant ($P>0.05$) increase in mean larval mortality. Increase in concentration from

Table 4.1: Qualitative phytochemical constituents of the four solvent extracts of *Borreria verticillata* and *Striga aesiatica* (+ = present; - = absent)

Plant species	Extract type	Phytochemicals							
		Alkaloids	Anthraquinones	Cardiac glycosides	Flavonoids	Saponins	Steroids	Tannins	Triterpenes
Borreria verticillata	N-hexane	+	-	+	-	-	-	+	+
	Chloroform	+	-	+	-	-	-	+	+
	Ethyl acetate	+	-	+	-	-	-	+	+
	Methanol	+	-	+	+	+	-	+	+
Striga aesiatica	N-hexane	+	-	+	-	-	-	-	+
	Chloroform	+	-	+	-	-	-	-	+
	Ethyl acetate	+	-	+	+	+	-	+	+
	Methanol	+	-	+	+	+	-	+	+

Table 4.2: Mortality of *Culex quinquefasciatus* larvae(n=75) on exposure for 24 hoursto various concentrations of methanol extract of *Borreria verticillata*

Conc. (mg/L)	Number (Mean; 95% CI±SE) of dead larvae at 24h post exposure	% Mortality	F-value	ANOVA probability (p-value)
0 (Control)	0 (0.00^{c}; 0.00-0.00±0.00)	0	11.8951	8.11×10^{-4}
2.0	5 (1.67^{b}; -3.50-6.84± 1.20)	6.67		
4.0	6 (2.00^{b}; -4.58-8.58± 1.48)	8.00		
6.0	21 (7.00^{a}; 4.52-9.48± 0.58)	28.00		
8.0	21 (7.00^{a}; 4.52-9.48 ± 0.58)	28.00		

Means followed by the same superscript letter are not significantly different ($p>0.05$)

4mg/L to 6mg/L produced significant ($P< 0.05$) increase in mean larval mortality (Table 4.2).

4.2.2 The median lethal concentration (LC_{50}) of methanol extractof *Borreriaverticillata*against*Culex quinquefasciatus* larvae

Table 4.3 shows the result of the probit analysis of mortalities of *Cx. quinquefasciatus* larvae on exposure to various concentrations of methanol extract of *B.verticillata*.On conversions of the extract concentrations to natural logarithims,and percentage mortalities to empirical probits of kill,a plot of probits of kill against logarithim of concentrations (Figure 4.1) gave a regression equation of $Y=1.6971x+2.891$ and a coefficient of determination (R^2)of 0.7847; and a median lethal concentration (LC_{50}) of 17.49mg/L for the methanol extract of *B.verticillata* against *Cx.quinquefasciatus* larvae.The LC_{50} value was obtained by substituting Y for 5 in the regression equation and solving for X , which yielded 1.2427. Antilogarithim of 1.2427 gave 17.49as the LC_{50} value.

Table 4.3:Median lethal concentration (LC_{50}) of methanol extract of *Borreria verticillata*

Conc. (mg/L)	Log-conc.	% Mortality	Empirical probit of mortality	Regression equation (R^2-value)	LC_{50} (mg/L)
8.0	0.9031	28	4.42	y = 1.6971x + 2.891	17.49
6.0	0.7782	28	4.42	(R^2 = 0.7847)	
4.0	0.6021	8	3.59		
2.0	0.3010	7	3.52		
Control	-	0	-		

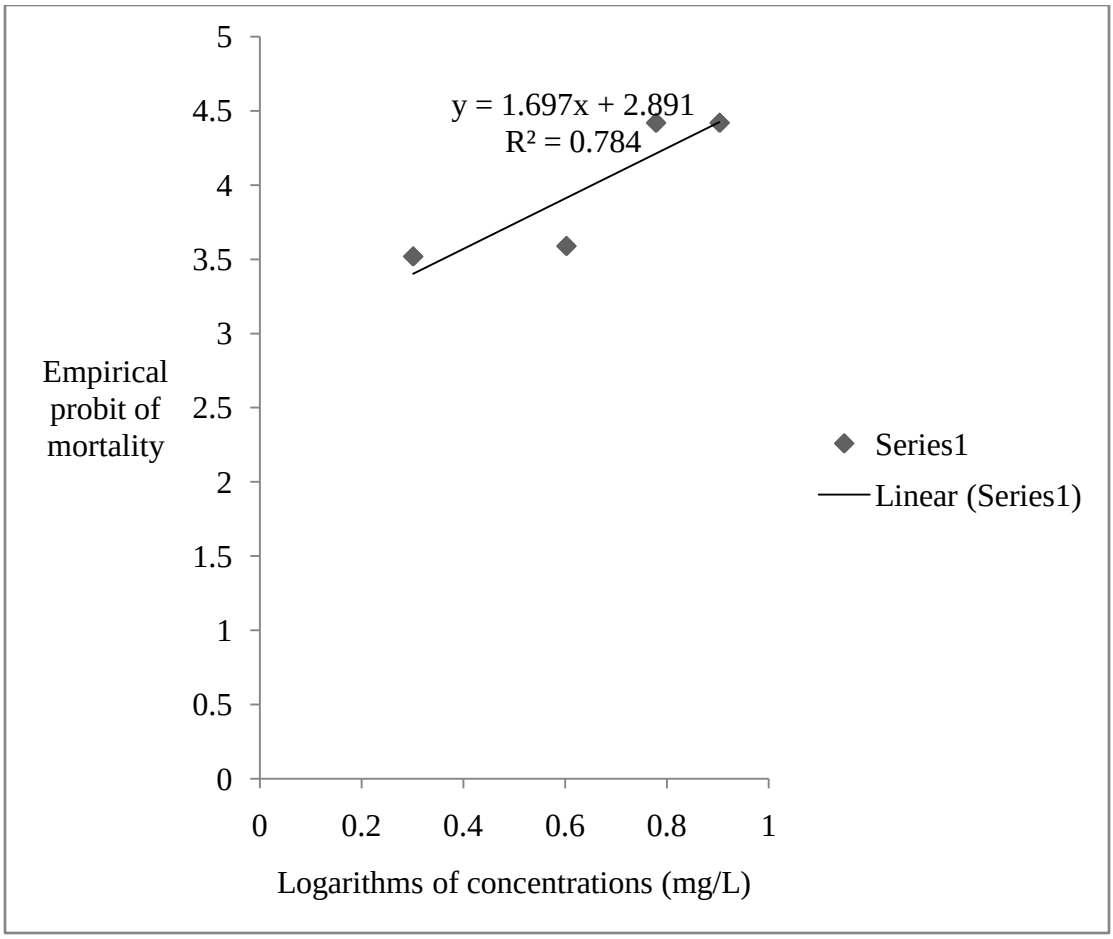


Figure 4.1: Empirical probit of mortality of *Culex quinquefasciatus* larvae against the logarithms of the concentrations of methanol extract of *Borreria verticillata*

4.2.3. Effect of the ethyl-acetate extract of *Borreria verticillata* on *Cx. quinquefasciatus* larvae

Table 4.4 shows the mortality of *Cx. quinquefasciatus* larvae after 24 hours exposure to various concentrations of the ethyl-acetate extract of *B. verticillata*.Mortality of *Cx.quinquefasciatus*larvae varied between 0/75 (0%) in the control to 49/75 (65.33%) at the highest concentration of 8mg/L.Larval mortality was concentrationdependent.Mean larval mortalities at the various experimental concentrations were significantly ($P= 6.50 \times 10^{-5}$; $P< 0.05$) higher than the control; but increase in concentration from 4mg/L to 8mg/L did not produce significant ($P>0.05$) increase in mean larval mortality (Table 4.4).

4.2.4 The median lethal concentration (LC_{50}) of the ethyl-acetateextract of *Borreria verticillata* against *Culex quinquefasciatus* larvae

By probit analysis, extract concentrations of *B.verticillata*were converted to natural logarithims as shown while percentage mortalities of *Cx.quinquefasciatus*were converted to empirical probits of kill (Table 4.5). A plot of the probit of kill against the logarithim of concentrations (Figure 4.2) gave a regression equation of $Y= 1.4434X + 4.0599$, a coefficient of determination (R^2) of 0.9246 and a median lethal concentration (LC_{50}) of 4.48mg/L for the ethyl-acetate extract of *B.verticillata* against *Cx.quinquefasciatus* larvae.The LC_{50} value was obtained by substituting Y for 5 in the regression equation and solving for X, which yielded 0.6513.Antilogarithm of 0.6513gave 4.48 as the LC_{50} value.

Table 4.4: Mortality of *Culex quinquefasciatus* larvae(n=75) on exposure for 24 hours to various concentrations of ethyl-acetate extract of *Borreria verticillata*

Conc. (mg/L)	Number (Mean; 95% CI±SE)of dead larvae at 24h post exposure	% Mortality	F-value	ANOVA probability (p-value)
0 (Control)	0 (0.00^{c}; 0.00-0.00±0.00)	0	21.6669	6.50×10^{-5}
2.0	25 (8.33^{b}; 0.33-16.33±1.86)	33.33		
4.0	31 (10.33^{a}; 4.07-16.59±1.46)	41.33		
6.0	45 (15.00^{a}; 10.03-19.97±1.16)	60		
8.0	49 (16.33^{a}; 9.15-23.51±1.67)	65.33		

Means followed by the same superscript letter are not significantly different (P>0.05)

Table 4.5: Median lethal concentration (LC_{50}) of ethyl-acetate extract of *Borreriaverticillata*against third instar larvae of *Culex quinquefasciatus* by probit analysis

Conc. (mg/L)	Log-conc.	% Mortality	Empirical prprobit of mortality	Regression quation (R^2-value)	LC_{50} (mg/L)
8.0	0.9031	65	5.39	Y = ⊦44444444.0599	8.67
6.0	0.7782	60	5.25	(R^2 = 0.9246)	
4.0	0.6021	41	4.77		
2.0	0.3010	33	4.56		
(0) Control	-	0	-		

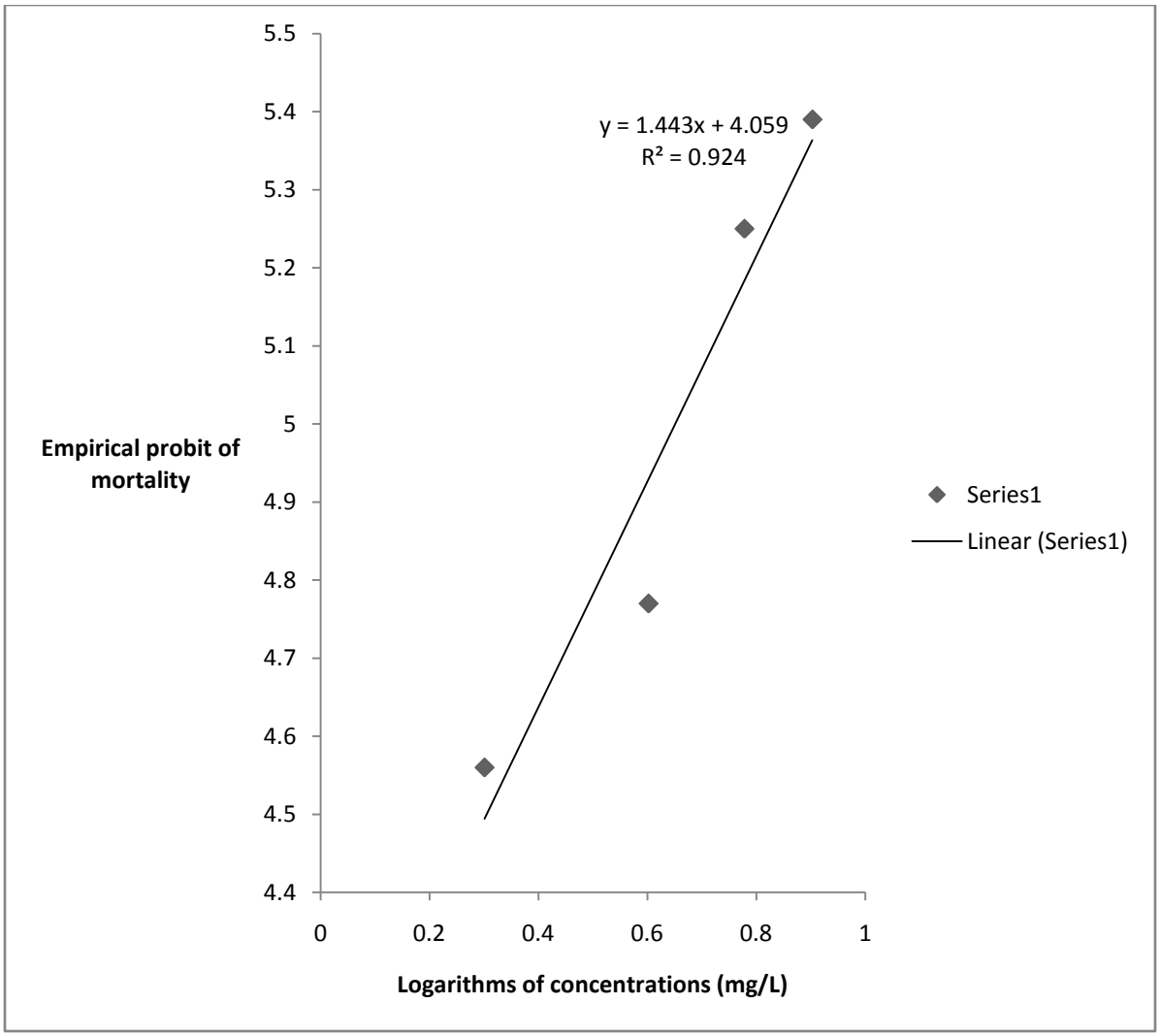


Figure 4.2: Empirical probit of mortality of *Culex quinquefasciatus* larvae against the logarithms of the concentrations of ethyl acetate extract of *Borreria verticillata*

4.2.5. Effects of N-hexane extract of *Borreria verticillata* on *Cx. quinquefasciatus* larvae

Results of the mortality of *Cx.quinquefasciatus* larvae after 24 hours exposure to various concentrations of N-hexane extract of *B.verticillata* are shown on Table 4.6. Larval .mortality was recorded in all the experimental concentrations except the control.The mean larval mortality increased with increase in concentration from 14 larvae at 2.0mg/L to 23.33 larvae at 8.0mg/L. Increase in extract concentrations from 2.0mg/L to 8.0mg/L did not produce significant ($P>0.05$) increase in mean larval mortality.Mean larval mortalities in the experimental were significantly $P<0.05$) higher than in the control.Mortality of *Cx.quinquefasciatus* larvae varied between 0/75 (0%) in the control and 70/75 (93.33 %) at the highest concentration of 8.0mg/L (Table 4.6).

4.2.6 The median lethal concentration (LC_{50}) of the N-hexane extract of*B.verticillata* against *Cx.quinquefasciatus*larvae

By probit analysis, extract concentrations were converted to natural logarithims as shown, while percentage mortalities were converted to empirical probits of kill (Table 4.7).A plot of the probit of kill against the logarithm ofconcentrations (Figure 4.3) gave a regression equation of $Y= 2.1557X + 4.5572$ and a coefficient of determination (R^2) of 0.9745.Subsititution of Y for 5 in the regression equation yielded an X value of 0.2054.Antilogarithm of 0.2054 gave 1.6mg/L as the median lethal concentration (LC_{50}) of the N-hexane extract of *B.verticillata* against *Cx.quinquefasciatus* larvae (Table 4.7).

Table 4.6: Mortality of *Cx.quinquefasciatus*larvae(n = 75) on exposure for 24 hours to various concentrations of N-hexane extract of *B.verticillata*

Conc.(mg/L)	Number (Mean; 95% CI±SE) of dead larvae at 24h post exposure	% Mortality	F-value	ANOVA probability (p-value)
0 (Control)	0 (0.00^{b}; 0.00-0.00 ±0.00)	0	13.5017	4.86×10^{-4}
2.0	42 (14.00^{a}; 7.42-20.58±1.53)	56.00		
4.0	63 (21.00^{a}; 3.78-38.22±4.00)	84.00		
6.0	66 (22.00^{a}; 19.52-24.48±0.58)	88.00		
8.0	70 (23.33^{a}; 6.12-40.55±4.00)	93.33		

Means followed by the same superscript letter are not significantly different (P>0.05)

Table 4.7: Median lethal concentration of (LC_{50}) of N-hexane extract of*Borreria verticillata* against third instar larvae of *Culex quinquefasciatus*

Conc. (mg/L)	Log-conc.	% Mortality	Empirical Pprobit of mortality	Regression equation (R^2-value)	LC_{50} (mg/mL)
8.0	0.9031	93	6.48	Y = 2.1557X+ 4.5572	1.60
6.0	0.7782	88	6.18	(R^2 = 0.9745)	
4.0	0.6021	84	5.99		
2.0	0.3010	56	5.15		
0(Control)	-	0	-		

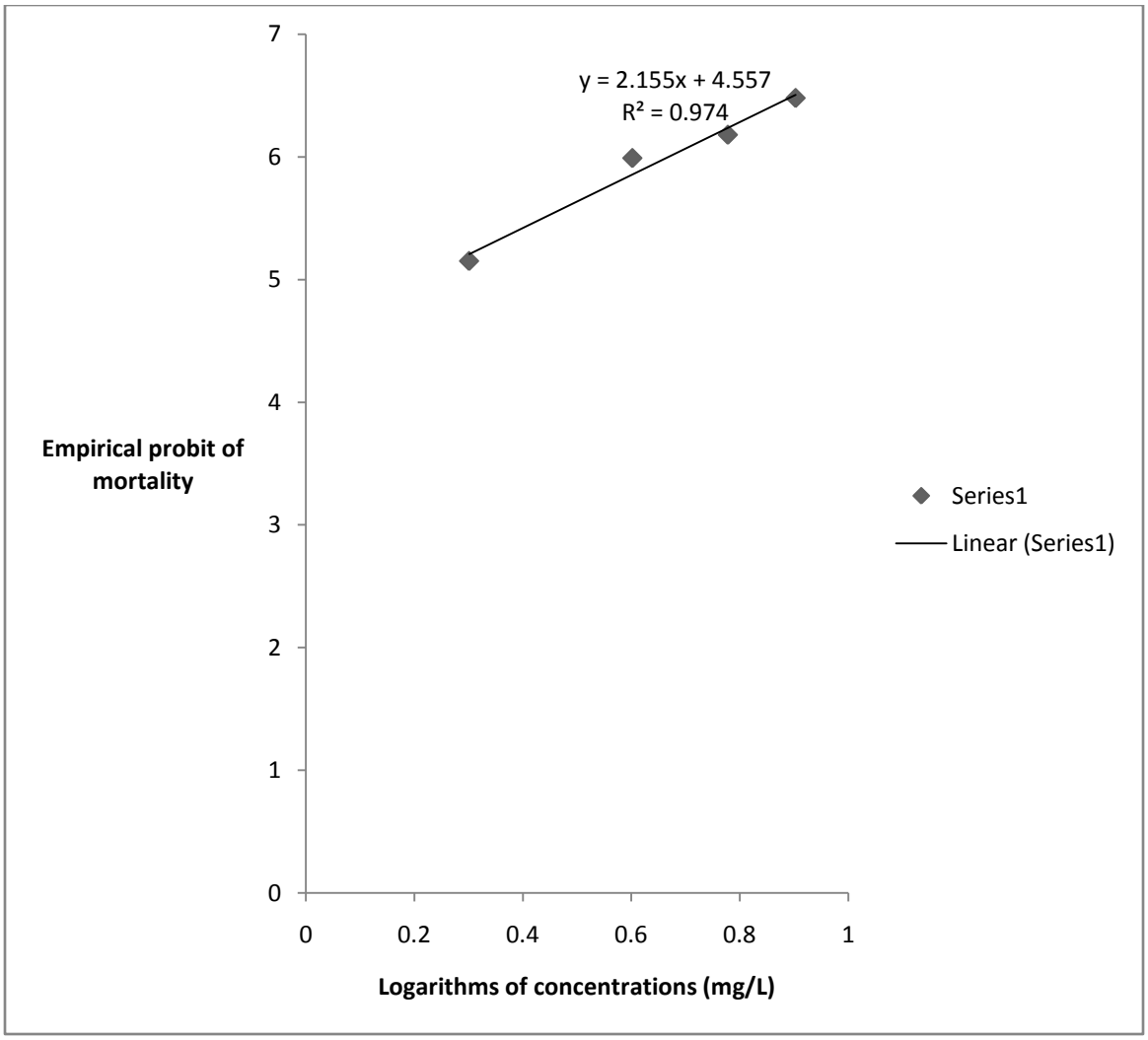


Figure 4.3: Empirical probit of mortality of *Culex quinquefasciatus* larvae against the logarithms of the concentrations of N-haxane extract of *Borreria verticillata*

4.2.7 Effects of chloroform extract of *Borreria verticillata* on *Cx.quinquefasciatus*larvae

The mortality of the third instar larvae of *Culex quinquefasciatus* on exposure for 24 hours to various concentrations of *Borreria verticillata*chloroform extract are shown on Table 4.8. Mortality of *Culex quinquefasciatus* was 0/75 (0%) in the control, then the mortality increased to8/75 (10.67%) at 2.0mg/L, then decreased to 4/75 (5.33%) with increase in concentration to 4.0mg/L, then again increased to 6/75 (8.0%) and8/75 (12%) with further increase in the concentrations from 6.0mg/L to 8.0mg/L.Although the mean larval mortalities at the various experimental concentrations were significantly higher than the control,increase in concentration from 2.0 mg/L to 8.0mg/Ldid not produce significant ($P>0.05$) increase in mean larval mortality.

4.2.8 The median lethal concentration (LC_{50}) of chloroform extract of *B.verticillata* against *Cx.quinquefasciatus* larvae

By probit analysis, the concentrationsof chloroform extract of *B.verticillata* were converted to natural logarithms as shown while percentage mortalities were converted to empirical probits of kill (Table 4.9). A plot of the probit of kill against the logarthms of concentrations (Figure 4.4), gave a regression equation of $Y= 0.0463X + 3.6076$ and a coefficient of determination (R^2) of 0.0033.Substitution of Y for 5 in the regression equation, yielded an X value of 30.0734. Antilogarithm of 30.0734 gave 1.184×10^{30}mg/L as the median lethal concentration (LC_{50}) of the chloroform extract of *B.verticillata* against *Cx.quinquefasciatus* larvae (Table 4.9).

Table 4.8: Mortality of *Culex quinquefasciatus* l*arvae* (n = 75) on exposure for 24 hours to various concentrations of chloroform extract of *Borreria verticillata*

Conc. (mg/L)	Number (Mean; 95% CI±SE) of dead larvae at 24h post exposure	% Mortality	F-value	ANOVA probability (p-value)
0 (Control)	0 (0.00^{b}; 0.00-0.00 ± 0.00)	0	5.83966	1.09×10^{-2}
2.0	8 (2.67^{a}; -0.19-5.53 ±0.66)	10.67		
4.0	4 (1.33^{a}; -0.11-2.78 ±0.34)	5.33		
6.0	6(2.00^{a}; -0.48-4.48±0.58)	8.00		
8.0	9 (3.00^{a}; 0.52-5.48± 0.58)	12 .00		

Means followed by the same superscript letter are not significantly different (P>0.05)

Table 4.9: Median lethal concentration (LC_{50}) ofchloroform extract of *Borreria verticillata*against third instar larvae of *Culex quinquefasciatus* by probit analysis

Conc. (mg/L)	Log-conc.	% Mortality	Empirical probit of mortality	Regression equation (R^2-value)	LC_{50} (mg/L)
8.0	0.9031	12	3.66	y = 0.0463X + 3.6076	1.184×10^{30}
6.0	0.7782	8	3.44	(R^2 = 0.0033)	
4.0	0.6021	5	3.25		
2.0	0.3010	11	3.59		
0(control)	-	0	-		

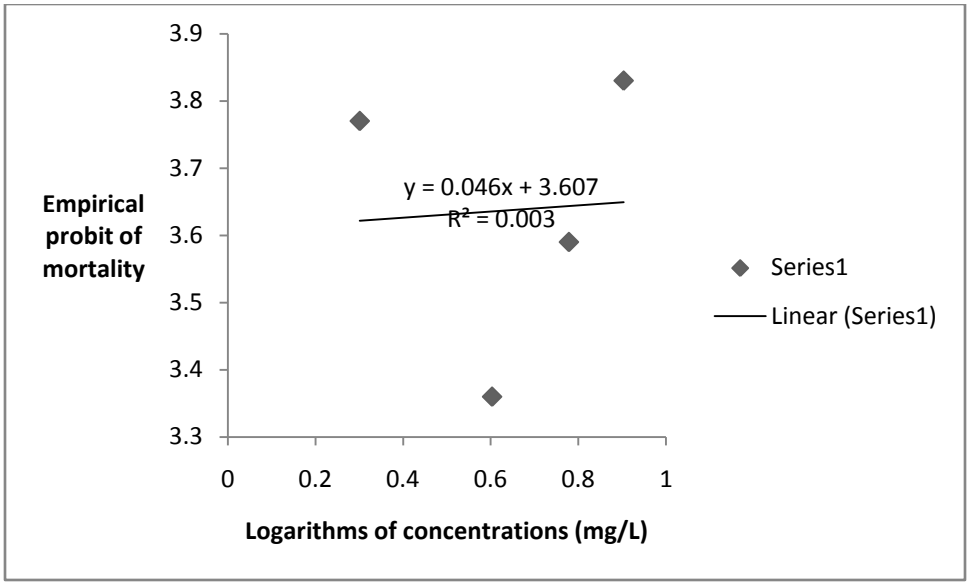


Figure 4.4: Empirical probit of mortality of *Culex quinquefasciatus* larvae against the logarithims of the concentrations of chloroform extract of *Borreria verticillata*

4.3 **Larvicidal Efficacy of *Striga asiatica* Extracts Against*Culexquinquefasciatus* Larvae**

4.3.1 The Effect of methanolic extract of*Striga asiatica* on *Culex quinquefasciatus* larvae

The mortality of third instar larvae of *Culex quinquefasciatus* on exposure for 24 hours to various concentrations of *Striga asiatica* methanolic extract as shown shown on (Table 4.10).Mortality of *Culex quinquefasciatus* larvae varied between 0/75 in the control and 37/75 (49%) at the highest concentration of 8.0mg/L.Mean larval mortalities increased significantly ($P<0.05$) with increase in concentrationfrom 2.0mg/L to 6.0mg/L, but did not produce significant increase, withincrease in concentration from 6.0mg/L to 8.0mg/L.Mean larval mortalities at the various experiment al concentrations weresignificantly ($P<0.05$) higher than the control.

4.3.2 The median lethal concentration $LC_{50}$of the methanolic extract of *Striga asiatica*against *Culex quinquefasciatus* larvae

By probit analysis, extract concentrations were converted to natural logarithms as shown, while percentage mortalities were converted to empirical probits of kill (Table 4.11).A plot of killagainst the logarithims of concentrations (Figure 4.5) gave a regression equation of $Y=2.6065X+2.4609$ and coefficient of determination (R^2) of 0.9442 .Substitution of Y for 5 in the regression equation yieled an X value of 0.9741.Antilogarithm of 0.9741 gave 9.42mg/L as the median lethal concentration (LC_{50}) of the methanol extract of *S.asiatica* against *Cx.quinquefasciatus* larvae (Table 4.11).

Table 4.10: Mortality of *Culex quinquefasciatus* larvae (n = 75) on exposure for 24 hours to various concentrations of methanol extract of *Striga asiatica*

Conc.(mg/L)	Number (Mean; 95% CI±SE) of dead larvae at 24h post expepost xposure	% Mortality	F-value	ANOVA probability (p-value)
0 (Control)	0 (0.00^{d}; 0.00-0.00±0.00)	0	33.0259	9.75×10^{-6}
2.0	4 (1.33^{c}; -0.11-2.77±0.34)	5.33		
4.0	9 (3.00^{b}; 0.52-5.48±0.58)	12.00		
6.0	21 (7.00^{a}; 4.52-9.48±0.58)	28.00		
8.0	37 (12.33^{a}; 4.88-19.78±2.03)	49.33		

Means followed by the same superscript letter are not significantly different (P>0.05)

Table 4.11: Median lethal concentration (LC_{50}) of methanol extract of *Striga asiatica* against third instar larvae of *Culex quinquefasciatus* by probit analysis

Conc. (mg/L)	Log-conc.	% Mortality	Empiricalofmortality	Regression equation (R^2-value)	$LC_{50.}$(mg/L)
8.0	0.9031	49	4.97	y = 2.3239x	12.91
6.0	0.7782	28	4.42	(R^2 =	
4.0	0.6021	12	3.83		
2.0	0.3010	5	3.36		
0 (control)	-	0			

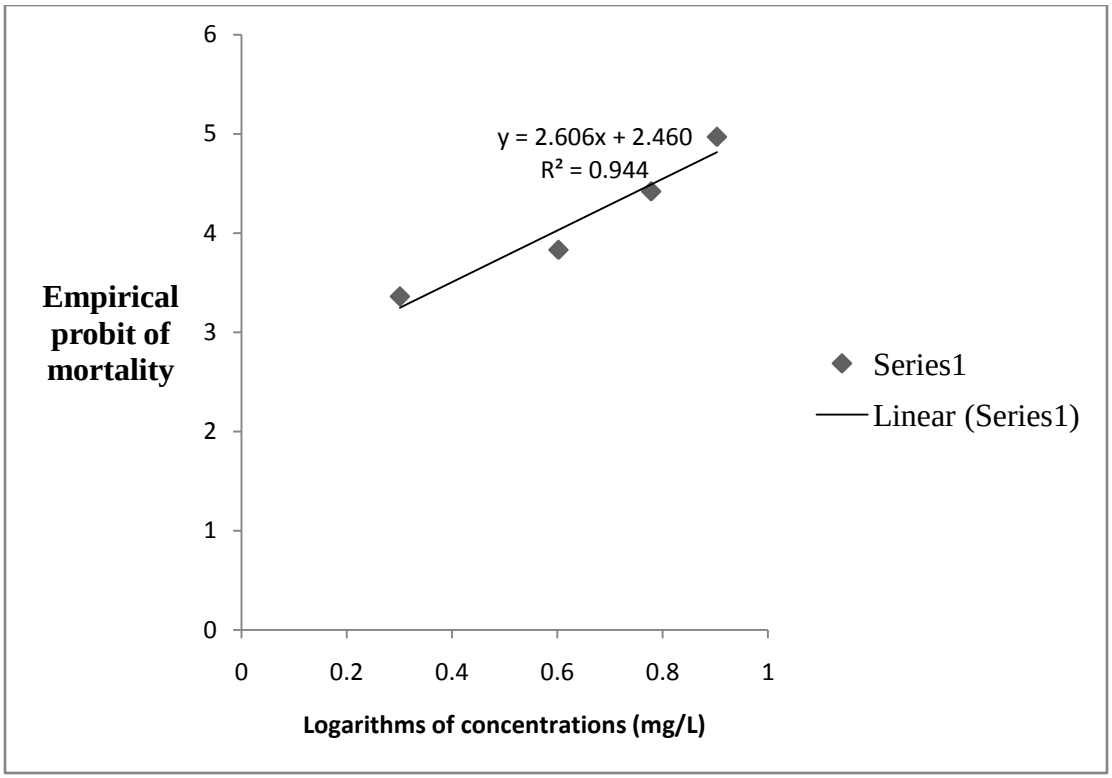


Figure 4.5: Empirical probit of mortality of *Culex quinquefasciatus* larvae against the logarithms of the concentrations of methanol extract of *Striga asiatica*

4.3.3 Effects of the ethyl-acetate extract of*Striga asiatica* on *Culex quinquefasciatus*larvae

Mortality of the third instar larvae of *Cx. quinquefasciatus* on exposure for 24 hours to various concentrations of *Striga asiatica*ethyl-acetate extract as shown on Table 4.12.Mortality of *Culex quinquefasciatus*varied between 0/75 (0%) in the control, to 75/75 (100%) at the highest concentration of 8.0mg/L.Larval mortality was concentration dependent.Mean larval mortalities at the various experimental concentrations were significantly (P>0.05) higher than in the control.Increase in concentration from 6.0mg/L to 8.0mg/Ldid not however, produce a significant (P< 0.05) increase in mean larval mortality.

4.3.4. The median lethal concentration (LC_{50}) of the ethyl-acetate extractof *Striga asiatica*against *Cx. quinquefasciatus* larvae

By probit analysis, extract concentrations were converted to natural logarithim as shown, while percentage mortalities were converted to empirical probits of kill (Table 4.13).A plot of kill against the logarithims of concentration (Figure 4.6) gave a regression equation Y= 6.811X+1.7419 and a coefficient of determination (R^2) of 0.8383.Subsititution of Y for 5 in the regression equation yielded an X value of 0.4783.Antilogarithmof 0.4783 gave 3.01mg/L as the median lethal concentration (LC_{50}) of the ethyl-acetate extract of *Striga asiatica* against *Culex quinquefasciatus* larvae (Table 4.13).

Table 4.12: Mortality of *Culex quinquefasciatus* larvae (n=75) on exposure for 24 hours to various concentrations of ethyl acetate extract of *Striga asiatica*

Conc. (mg/L)	Number (Mean; 95% CI±SE) of dead larvae at24h post exposure	% Mortality	F-value	ANOVA probability (p-value)
0 (Control)	0 (0.00^{d}; 0.00-0.00±0.00)	0	150.011	7.00×10^{-9}
2.0	18 (6.00^{c}; 1.70-10.30±1.00)	24.00		
4.0	40 (13.33^{b}; 7.07-19.59±1.46)	53.33		
6.0	70 (23.33^{a}; 19.53-27.13±0.88)	93.33		
8.0	75 (25.00^{a}; 25.00-25.00±0.00)	100.00		

Means followed by the same superscript letter are not significantly different (P>0.05)

Table 4.13: Median lethal concentration (LC_{50}) ofethylacetate extract of*Strigaasiatica*against third instar larvae of *Culex quinquefasciatus* by probit analysis

Conc. (mmg/L)	Log-conc.	% Mortality	Empirical probit of mortality	Regression equation	LC_{50} (mg/L)
8.0	0.9031	100	∞	y = 6.811X + 1.7419	3.01
6.0	0.7782	93	6.48	(R^2 = 0.8383)	
4.0	0.6021	53	5.08		
2.0	0.3010	24	4.29		
0 (Control)	-	0	-		

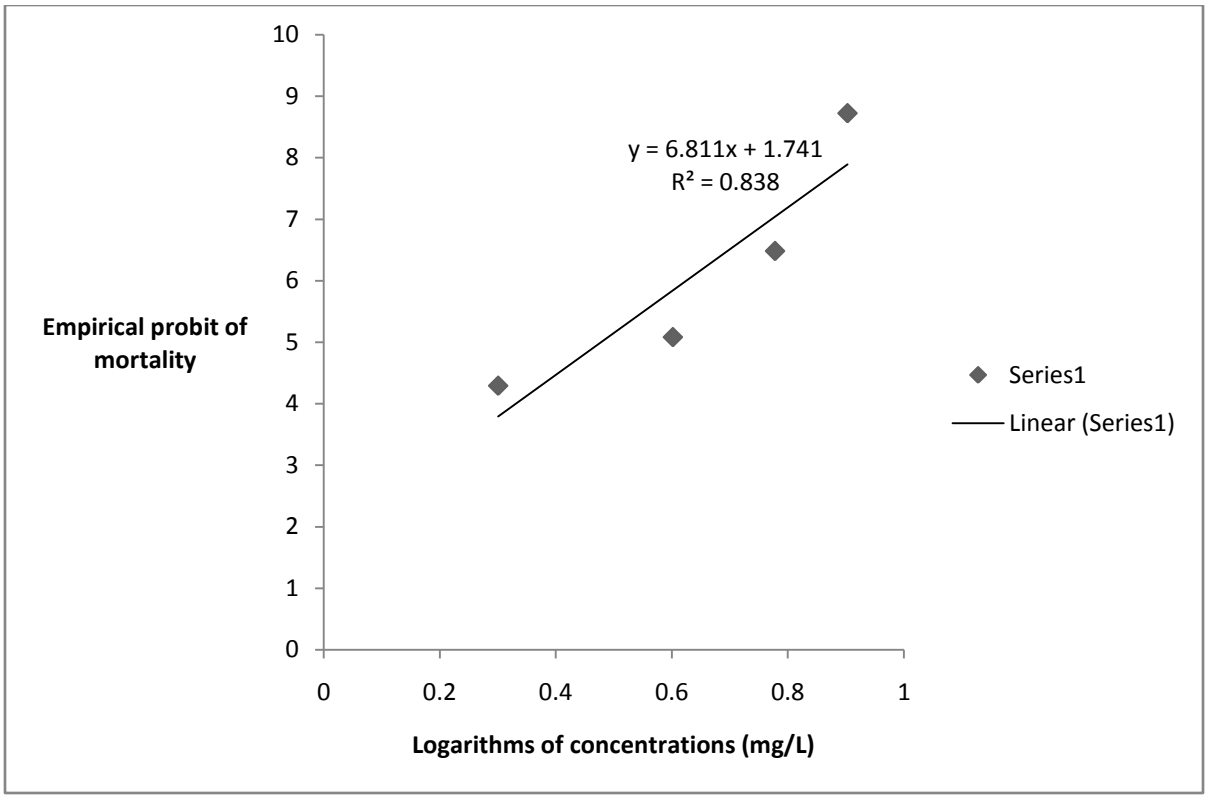


Figure 4.6: Empirical probit of mortality of *Culex quinquefasciatus* larvae against the logarithms of the concentrations of ethyl acetate extract of *Strigaasiatica*

4.3.5 Effects of N-hexane extract of *Striga asiatica* on *Cx. quinquefasciatus*larvae

Table 4.14 shows themortality of*Culex quinquefasciatus* mosquito larvae after 24 hours exposure to various concentrations of N-hexane extract of *Striga asiatica*.Larval mortality was recorded in all experimental concentration except the control.The mean larval mortality increased with increase in concentrations from 64 larvae at 2.0mg/L to 75 larvae at 8.0mg/L.Increase in concentration from 2.0mg/L to 8.0mg/L did not produce significant ($P>0.05$) increase in mean larval mortality.Mean larval mortalities in the experimentals were significantly higher ($P<0.05$) higher than the control.Mortality of *Cx.quinquefasciatus* larvae varied between 0/75(0%) in the control and 75/75 (100%) at the highest concentration of 8.0mg/L.

4.3.6. The median lethal concentration (LC_{50}) of N-hexane extract of *Striga asiatica*against *Cx. quinquefasciatus* larvae

By probit analysis, extract concentrations were converted to natural logarithimsas shown, while percentage mortalities were converted to empirical probits of kill (Table 4.15). A plot of kill against the logarithim of concentrations (Figure 4.7) gave a regression equation of Y= 3.9377+4.3609 and a coefficient of determination (R^2)of 0.6475. Substitution of Y for 5 in the regression equation yielded an X value 0.1623.Antilogarithm 0f 0.1623 gave 1.45mg/L as the median lethal concentration (LC_{50}) of the N-hexane extract of *Striga asiatica*against*Cx. quinquefasciatus* larvae (Table 4.14).

Table 4.14: Mortality of *Culex quinquefasciatus*larvae (n=75) on exposure for 24 hours to various concentrations of N-hexane extract of *Striga asiatica*

Conc. (mg/L)	Number (Mean; 95% CI±SE) of dead larvae at 24h post exposure	% Mortality	F-value	ANOVA probability (p-value)
0 (Control)	0 (0.00^{b}; 0.00-0.00 ±0.00)	∞	78.8063	1.61×10^{-7}
2.0	64 (21.33^{a}; 13.38-29.28 ±1.86)	85.33		
4.0	63 (21.00^{a}; 13.55-28.45±1.73)	84.00		
6.0	73 (24.33^{a}; 21.5-27.21±0.67)	97.33		
8.0	75 (25.00^{a}; 25.00-25.00 ±0.00)	100.00		

Means followed by the same superscript letter are not significantly different (p>0.05)

Table 4.15: Median lethal concentration of (LC_{50})ofN-hexane extract of*Strigaasiatica*against third instar larvae of *Culex quinquefasciatus* by probit analysis

Conc. (mg/L)	Log-conc.	% Mortality	Empirical probit of mortality	Regression equation (R^2-value)	LC_{50} (mg/L)
8.0	0.9031	100	∞	Y = 3.9377X + 4.3609	1.45
6.0	0.7782	97	6.88	(R^2 = 0.6475)	
4.0	0.6021	84	5.99		
2.0	0.3010	85	6.03		
Control	-	0	-		

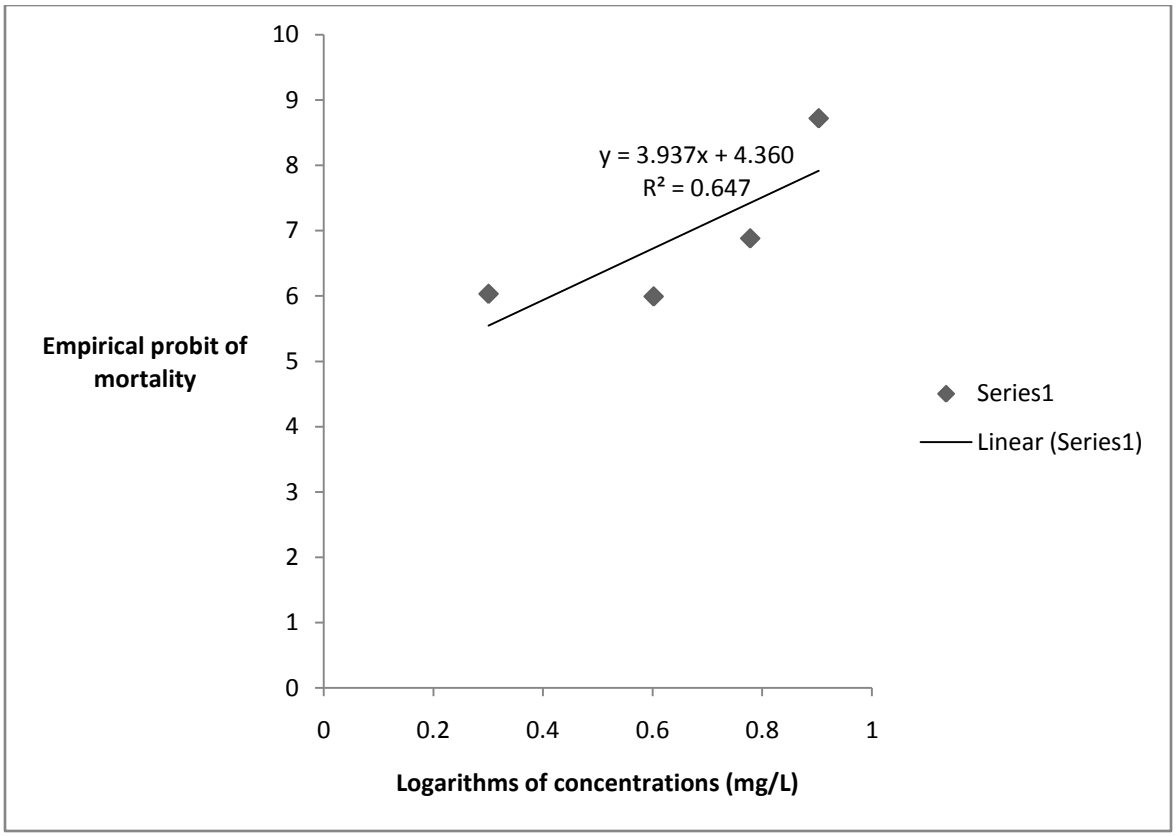


Figure 4.7: Empirical probit of mortality of *Culex quinquefasciatus* larvae against the logarithms of the concentrations ofN-hexane extract of *Striga asiatica*

4.3.7 The effects of the chloroform extract of *Striga asiatica* on *Cx. quinquefasciatus*larvae

The results of the mortality of the *Cx. quinquefasciatus* larvae after 24 hours exposure to various concentrations of chloroform extract of *Striga asiatica* as shown on Table 4.16.Larval mortality was recorded in all the experimental concentrations except the control.The mean larval mortality increased with increase in concentration from 14 larvae at 2.0mg/L to 75 larvae at 8.0mg/L.Increase in extract concentrations from 2.0mg/L to 8.0mg/L produced a significant ($P<0.05$) increase in mean larval mortality. Larval mortality was dose-dependent. Mean larval mortalities at various experimental concentrations were significantly ($P<0.05$) higher than the control.

4.3.8: The median lethal concentrations (LC_{50}) of the chloroform extract of *Striga asiatica*against *Cx.quinquefasciatus* larvae

By probit analysis, extract concentrations were converted to natural logarithims as shown, while percentage mortalities were converted to empirical probits of kill (Table 4.17). A plot of the probit of kill against the logarithims of concentration (Figure 4.8) gave a regression equation of $Y= 6.8589X + 1.696$ and a coefficient of determination (R^2) of 0.8565. Substitution of Y for 5 in the regression equation yielded an X value of 0.4817.Antilogarithim of 0.4817 gave 3.03mg/L as the median lethal concentration (LC_{50}) of the Chloroform extract of *Striga asiatica* against *Cx. quinquefasciatus* larvae (Table 4.17)

Table 4.16: Mortality of *Culex quinquefasciatus* larvae (n=75) on exposure for 24 hours to various concentrations of chloroform extract of *Striga asiatica*

Conc. (mg/L)	Number (Mean; 95% CI±SE) of dead larvae at 24h post exposure	% Mortality	F-value	ANOVA probability (p-value)
0 (Control)	0 (0.00^{e}; 0.00-0.00±0.00)	0	112.196	2.90×10^{-8}
2.0	14 (4.67^{d}; -1.59-10.93±1.46)	18.67		
4.0	50 (16.67^{c}; 9.07-24.27±1.77)	66.67		
6.0	67 (22.33^{b}; 20.89-23.77±0.34)	89.33		
8.0	75 (25.00^{a}; 25.00-25.00 ±0.00)	100.00		

Means followed by the same superscript letter are not significantly different ($p>0.05$)

Table 4.17: Median lethal concentration (LC_{50}) of chloroform extract of *Striga asiatica*

Conc. (mg/L)	Log-conc.	% Mortality	Empirical probit of mortality	Regression equation (R^2)	LC_{50} (mg/L)
8.0	0.9031	100	∞	Y = 6.8589X + 1.696	3.03
6.0	0.7782	89	6.23	(R^2 = 0.8565)	
4.0	0.6021	67	5.44		
2.0	0.3010	19	4.12		
0(Control)	-	0	-		

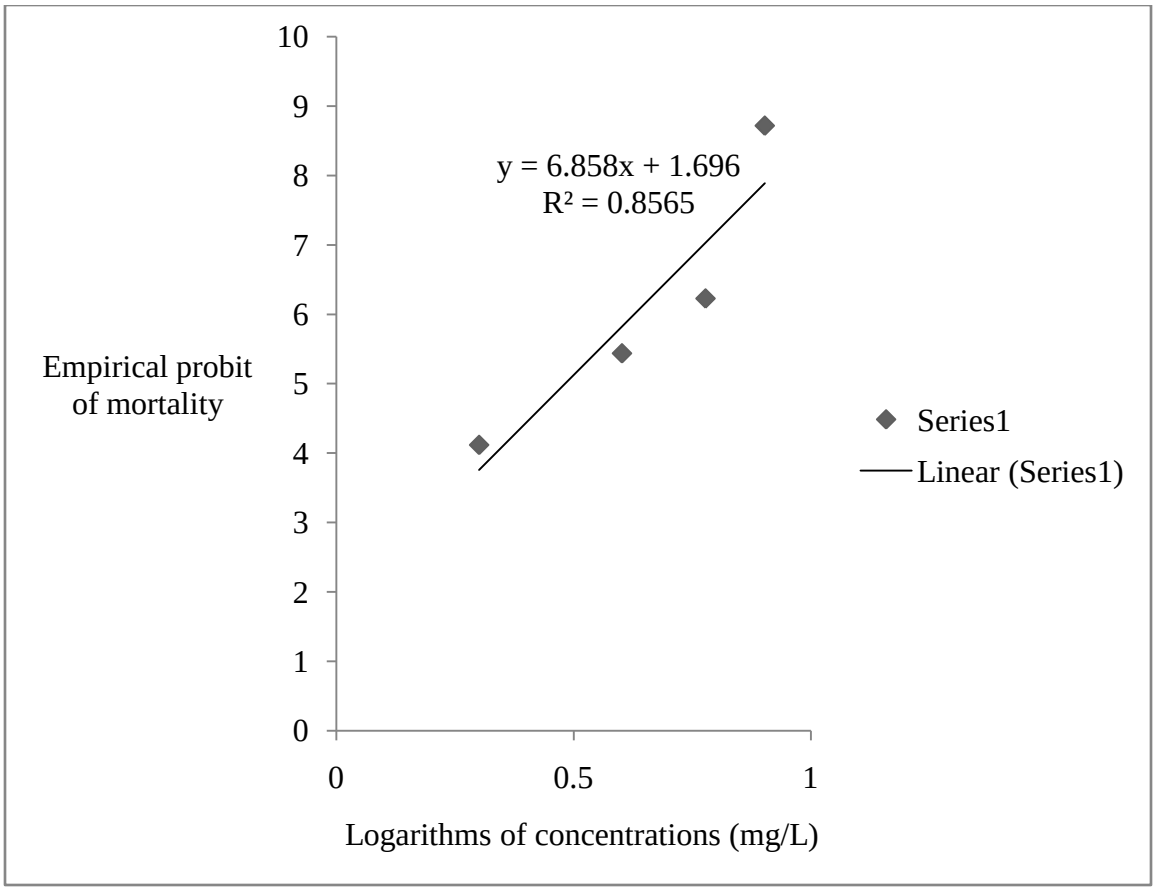


Figure 4.8: Empirical probit of mortality of *Culex quinquefasciatus* larvae against the logarithms of the concentrations of chloroform extract of *Striga asiatica*

4.5 Comparison Between and Among the Larvicidal Efficacies of Solvent Extracts of *B.verticillata* and *S. asciatica*Against *Culex quinquefasciatus*

Table 4.19 compares thelarvicidal efficacies (mean larval mortality) of each of the solvent extract of *B.verticillata* with the corresponding extract type of *S.asciatica* against *Culex quinquefasciatus*.There was no significant (P>0.05) difference between the mean larval mortality of *Cx.quinquefasciatus* due to exposure to methanol extract of *B.verticillata* (4.42 larvae) and Exposure to methanol extract of *S.asciatica* (5.92 larvae).The mean larval mortality of *Cx.quinquefasciatus* on exposure to ethyl acetate extract of *B.verticillata* (12.50) did not differ significantly (P>0.05) from the 16.92 obtained from exposure to ethyl acetate extract of*S.asciatica*.Similarly,there was no significant (P>0.05) difference between the mean larval mortality (20.08 larvae) of *Cx.quinquefasciatus* on exposure to n-hexane extract of *B.verticillata* and the 22.92 larvae obtained when exposed n-hexane extract of *S.asciatica*. However, the mean larval mortality of *Cx.quinquefasciatus*on exposure to the chloroform extract of *S.asciatica* (17.17 larvae) wassignificantly (P =0.05) higher than the 2.25 larvae obtained as a result of exposure to the chloroform extract of *B.verticillata* (Table 4.19).

Table 4.20 shows comparison of the larvicidal efficacies among the various solvent extracts of *B.verticillata*and among the various solvent extracts of *S.asiatica*.The larvicidal efficacies of the various solvent extracts of *B.verticillata*differ significantly (P<0.05), N-hexane extract of *B.verticillata* produced significantly (P<0.05) highest mean larval mortality of 20.08 larvae while the chloroform extract of *B.verticillata* produced the least mean larval mortality (2.25 larvae) of *Cx.quinquefasciatus*.

Table 4.18: Comparison between thelarvicidal efficacies of various solvent extracts of *B. verticillata*and *S. asiatica* against *Cx.quinquefasciatus* (Mean ± SE)

Plants	Methanol (%)	Ethyl acetate (%)	n-hexane (%)	Chloroform (%)
Borreria verticillata	4.42±1.49 (17.68)	12.50±1.89 (50.00)	20.08±2.08 (80.32)	2.25±0.37 (9.00)
Striga asiatica	5.92±2.45 (23.68)	16.92±4.46 (67.68)	22.92±1.02 (91.68)	17.17±4.51 (68.68)
P-value	0.34	0.19	0.17	0.05

Table 4.20: Comparison among the larvicidal efficacies of various solvent extracts of *B. verticillata* and *S. asiatica* against *Cx.quinquefasciatus* larvae

	Mean±SE larval mortality per solvent type				
Plants	Methanol	Ethyl acetate	n-hexane	Chloroform	P-value
Borreria verticillata	4.42^{c}±1.49 (17.68)	12.50^{b}±1.89 (50.00)	20.08^{a}±2.08 (80.32)	2.25^{c}±0.37 (9.00)	0.00
Striga asiatica	5.92^{b}±2.45 (23.68)	16.92^{a}±4.46 (67.68)	22.92^{a}±1.02 (91.68)	17.17^{a}±4.51 (68.68)	0.03

Means followed by the same superscript letter per row are not significantly different ($p>0.05$).

The mean larval mortality of *Cx.quinquefasciatus*obtained with the ethyl acetate extract of *B.verticillata* (12.50 larvae) was significantly (P<0.05) lower than those for n-hexane extract,but significantly (P<0.05) higher than those of methanol (4.42 larvae) and chloroform extract (2.25 larvae).There was no significant (P>0.05) difference between the mean larval mortality of *Cx.quinquefasciatus* exposed to the methanol and chloroform extracts of *B.verticillata*(Table 4.20). The larvicidal efficacies of the various solvents extracts of *S. asiatica* against *Cx.quinquefasciatus* differ significantly (P<0.05). However, there were no differences (P>0.05) among the mean larval mortalities of *Cx.quinquefasciatus* exposed to ethyl acetate, n-hexane and chloroform extracts.The methanol extract of *S.asiatica* produced significantly (P<0.05) least mean larval mortality of *Cx.quinquefasciatus*(Table 4.20).

CHAPTER FIVE

5.0 DISCUSSION

Borreriaverticillata and *Striga asiatica* are rich in alkaloids, flavonoids, tannins, steroids/triterpenes, saponins and cardiac glycosides.These classes of phytochemicals could have accounted for their overt larvicidal efficacies against *Cx.quinquefasciatus*.Plants have been known to serve as huge storage of compounds that have biological action (Gutierrez*et al*.,2014).Ushie *et al*. (2013) have also opined that the most important bioactive constituents of *B.verticillata*are alkaloids, tannins, flavonoids and phenolic compounds. Anthraquinone and steroids were absent in all the extracts of *B.verticillata*. The presenceofalkaloids in all the four solvent extracts of *B.verticillata*in this study agrees with the findings of Sofowora (1993) who isolated two alkaloids, dehydroborrecapine and borrecoxine from the related species of*B. capitata*. Furthermore, the findings of Karou *et al*. (2011)stated the universal occurrence of alkaloidsin plants belonging to theRubiaceae family;althoughLeal and Elisabetsky (1996) demonstrated the absence of alkaloids in *Psychotria carthagenesis*. Compounds such as flavonoids, alkaloids, tannins and saponins in plants are known to be responsible for the insecticidal effects and toxicity to other animals (Gutierrez*et al*., 2014).

The presence of saponins, flavonoids and tannins in the methanol extract of *Borreria verticillata*could have been responsible for its mosquito larvicidal activity. Gutierrez*et al*. (2014) observed that the presence of carbohydrates, saponins, phytosterols, phenols, flavonoids and tannins in the plant extract,mosquito larvicidal activities.

Alkaloids, flavonoids, saponins, tannins, triterpenes, and cardiac glycosides were revealed in the extracts of*S.asiatica*.The presence of flavonoids and saponins in the

methanol extract of *S.asiatica*could have been responsible for its larvicidal activity. This agrees with the findings of Gutierrez*et al*.(2014) who stated that compounds such as, flavonoids, alkaloids, tannins and saponins in plants are responsible fortheir insecticidal activity and toxicity to other animals.Saponins isolated from *Achyranthes aspera* through bioassay-guided fractionation possessed a larvicidal efficacy against *A.aegypti* and*Cx.quinquefasciatus*. Moreover flavonoids isolated from water extracts of *Annona squamosa* was effective as insecticides against mosquito killing 80% of*Cx.chinensis* (Gutierrez*et al*.,2014).The N.-hexane extract of *S. asiatica* however, revealed the presence of alkaloids and triterpenes; steroids was however absent in the extract which may be due to the extraction method. This study is in alignment with the work of Edriss *et al*.(2012) whoexplained that generally, petroleum ether and hexane solvents are involved in the extraction of non polar compounds (such as triterpenes and sterols), while alcohol (ethanol) solvents are involved in the extraction of polar components like alkaloids and polar flavonoids.

Although the larvicidal prospects of*B.verticillata* and *S.asiatica* against *Cx.quinquefasciatus* are not welldocumented, this study has however demonstrated the susceptibility of *Cx.quinquefasciatus* mosquitolarvae to the different dose concentrations of the methanol, ethyl-acetate, chloroform and N-hexane solvent extracts of *B. verticillata* and *S.asiatica*. Thus, the extracts of *B. verticillata* and *S. asiatica*could be employed, in the population reduction management of *Cx.quinquefasciatus* vector mosquito and indirectly to control the diseases vectored by thespecies.

The results of the larvicidal bioassay of the methanol extract of *B.verticillata* showed the larvicidal prospects against *Cx.quinquefasciatus* andwas dose-dependent due to the increase in the percentage larval mortality with increase in the methanol extract;the low larval mortality despite the use of a polar solvent (methanol) could be attributed to the presence of substances with low insecticidal activity such as tannins. de Oliveira *et al*. (2012), suggested that polar solvents extract substances with low insecticidal activity like sugars and tannins. The lack of significant differences between, the mean larval mortalities of the some concentrations of the methanol extract of*B.verticillata* against *Cx.quinquefasciatus* could be attributed to the presence of similar or related substances with larvicidal efficacy present in nearly equal proportions.

The results of the larvicidal bioassay of the ethyl-acetate solvent extract of *B.verticillata* against *Cx.quinquefasciatus* showed that the percentage larval mortality increased in a dose-dependent manner with increase in the dose concentration of the ethyl- acetate extract of *B.verticillata*. The result also indicated that there was a significant difference between someof the mean larval mortalities of the dose concentrations of ethyl-acetate extract of *B.verticillata*.This significant difference could be attributed to the heterogenous distribution, of the active compounds with larvicidal activity, against *Cx.quinquefasciatus* larvae.Gosh *et al*.(2012)reported that moderately polar solvents such as chloroform or ethyl-acetate extracted alkaloids, steroids and these biochemicals, extracted usingmoderately polar solventswere also seen to give good results as recorded in a few bioassay.

The decrease and then increaseof the percentage larval mortality with increase in the dose concentration of the chloroform extract of *B.verticillata*could be attributed to the fact that, there were a few voracious larvae thatmay have reached the third instar stage ahead of the many non-voracious ones and were probably more susceptible, to the increase in the dose concentration of the chloroform extract, while the many non-voracious reached the third instar stage later and were probably less susceptible to the increase, in the dose concentration of the chloroform extract, the increase in dose concentration had an effect on the larvae.Ezechi *et al*. (2015) observed that mean mortality decrease, with increase in developmental stages of *Cx.quinquefasciatus*larvae when a comparative analysis, of the petroleum ether and aqueous extracts of neem Leaf was carried out on the different developmental stages of *Anopheles gambiae*.There was also no significant difference statistically between the mean larval mortalities of the differentconcentrations of the chloroform extract.

The increase in the concentration of the N-hexane extract of *B.verticillata*which resulted in very high increase in percentage larval mortality,indicating, not only a dose-dependent response but a high larvicidal activity of the extract, this very high larvicidal activitycould be attributed to the presence of apolar constituents such as triterpenes this findings agrees with the findings of Edriss *et al*.(2012) who indicated superior mosquitocidal effects of apolar constituents of some plants extracted with petroleum ether or hexane which have been attributed mainly to triterpenoids. There was no significant difference statistically between the mean larval mortalities of the n-hexane solvent extracts of *B.verticillata* against the third instar larvae of *Cx.quinquefasciatus* mosquito, this uniform activity observed in the mean larval mortalities of the different

dose concentrations could be attributed tothe presence of similar or related phytoconstituents having larvicidal effects in nearly equal proportions.

The results of the larvicidal prospects of the methanol solvent extract of *S.asiatica* against the third instar mosquito larvae of *Cx.quinquefasciatus* showed that there was no significant difference between the different doses concentrations of the extract, thisweak larvicidal activity of the methanol extract of *S.asiatica* could be attributed to the presence of certain compounds with low insecticidal activity. These findings are in consonance with the observations ofde Oliveira *et al*. (2012), who reported that polar solvents extract substances with low insecticidal activity like sugars and tannins. ButMohapatra *et al*. (1995) had a different view that the parts of plants determine the larvicidal activitiesand not the solvents used for the extraction.

The result of the larvicidal prospect of the ethyl-acetate solvent extract of *S. asiatica* against third instar larvae of *Cx.quinquefasciatus* has demonstrated that *Cx.quinquefasciatus* mosquito larvae were susceptible to the ethyl-acetate solvent extract. The increase in the dose concentration of the solvent extract resulted in high percentage larval mortality, these dose dependent response could be linked to the homogenous distribution of bioactive compounds such as steroids, alkaloids etc in the solvent extract. These findings are in consonance with the results of Abdulrahuman*et al*. (2008) that linked dose dependent mortality to the homogeneous distribution of active compound in the extractof *Abutilon indicum* against larvae of *Aedes* species.The larvicidal prospects of the ethyl-acetate extract could also be attributed to the presence of phytochemicals such as alkaloids flavonoids, tannins, saponins etc this is in consonance with the findings of

Kumar and Maneemegalai(2008) who observed, the presence of carbohydrates, saponins, phytosterols, phenols, flavonoids and tannins in plant extracts having mosquito larvicidal activity. Gutierrez*et al*. (2014) reported that saponins are plant's bioactive compounds with significant insecticidal action and other biological properties.In addition, saponins are responsible for amplifying pest insects' death intensity, lowering food intake, retardation in development, instability in development and declining reproduction.Moreover, saponins are freely soluble and can beextracted in both aqueous and organic solvents and perform their action by attacking the cuticle membrane of the larvae, eventually disrupting the membrane, which is the main cause of larval death

The result of the larvicidal activity of the chloroform extract of *S.asiatica*has shown thatincrease in the dose concentration of the extract resulted in the high increase of the percentage larval mortality of the *Cx.quinquefasciatus* larvae thus indicating a dose-dependent response of the extracts against the mosquito larvae. .Similar study conducted by Adebote *et al*. (2011) reported a dose dependent mortality response of *Cx. quinquefasciatus* larvae to treatments of methanol, N-hexane, chloroform and aqueous extracts of the stem bark of *Bobgunia madagascariensis*. The result also indicated that the meanlarval mortalities differed significantly with increase, in some of the dose concentrations of the ethyl-acetate extract of *B.verticillata*.The increase in the percentage mortality of the treated mosquito larvae could be supported by the presence of phytochemicals in the plant extracts which have pesticidal activities.

The presence of alkaloids, observed in the chloroform solvent extract could also be attributed to the larvicidal prospects of the solvent extract.Theresult has also shown that

there was a significant difference between the mean larval mortalities of the different dose concentrations of the chloroform solvent extrac. Theses findings also agrees with the findings of Adebote *et al*. (2011) who reported that the mean larval mortalities of the different dose concentrations of the chloroform extracts of the stem bark of *Bobgunnia madagascariensis* differed significantly with increase in the dose concentration of the chloroform extract against the *Cx.quinquefasciatus* larvae.

The result of N-hexaneextract of *S.asiatica* against *Cx.quinquefasciatus* larvae exhibiteda highpercentage larval mortality at the high and low dose concentrations of the N-hexane extract, indicating a high larval mortality,dose-dependent response.The high larval mortality exhibited by the N-hexane solvent extract, at the high and low dose concentrations of the extract is in line with the findings of Kovendan*et al*. (2012) that most plant extracts showed high larvicidal potential against mosquitoes at relatively high and low concentrations. Results from this larvicidal bioassay has also shown that mean larval mortalities did not differsignificantly with increase in the dose concentrations of the N-hexane extract, this shows that there was no significant difference in the larvicidal activities of the different dose concentrations of the N-hexane solvent extract.This could be attributed to the presence of similar or related chemicals having larvicidal efficacy in nearly equivalent proportions in the N-hexane extract of *S.asiatica*.

CHAPTER SIX

6.0 SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

6.1 Summary

Larvicidal activity of *Borreria verticillata* and *Striga asiatica* was tested on the third instarlarvaof *Cx. quinquefasciatus* in laboratory condition. The study was conducted in the Department of Biological Sciences, ABU, Zaria. Soxhlet extraction method was employed using methanol, ethylacetate, n-hexane and chloroform as solvents were used to extract *B. verticillata* and *S. asiatica*. Blood fed *Culex* species were collected from class rooms and induced to lay egg in the Entomological cages containing water in the Entomology and Parasitology Laboratory. The larvalspecies of the mosquito wereidentified using Hopkins (1952) method to be *Cx. quinquefasciatus*. Late third instar larvae were subjected to treatment using extracts of *B. verticillata* and *S. asiatica*. Mortality was recorded after 24 h exposure. There was activity in all the tested extracts on the larvae of *Cx. quinquefasciatus*.

6.2 Conclusions

i. Alkaloids, flavonoids, saponins, tannins, steroids and cardiac glycosides except anthraquinones were detected in some solvent extracts of *S. asiatica* and *B. verticillata*.

ii. Variuos solvent-derived extracts of *B. verticillata*produced significant larvicidal effects against *Cx.quinquefasciatus,*at mean larval mortality ranging from 2.25 (for chloroform extract) to 20.08 (for n-hexane) and LC_{50} ranged from 2.65mg/ml (for n-hexane) to 3.21×10^{26}mg/ml (for chloroform).

iii. Various solvent-derived extracts of *S. asiatica* produced significant larvicidal effects against *Cx.quinquefasciatus* with mean larval mortality ranging from 5.92 (for methanol extract) to 22.92 (for n-hexane) and LC_{50} ranging from 0.59mg/ml (n-hexane) to 12.91mg/ml (for methanol).

iv. N-hexane extracts of *B. verticillata* and *S. asiatica* produced significantly ($p<0.05$) highest larvicidal efficacies against *Cx. quinquefasciatus* compared to the other three extracts.

6.3 Recommendations

i. Extracts of *B.verticillata* and *S.asiatica*need to be evaluated in the field as larvicides in mosquito control programmes to reduce the population of *Cx. quinquefasciatus*.

ii. Further work is needed to identify the potent active component in the extracts responsible for observed larvicidal effects.

iii. Investigation of the effects of the solvent extracts on other life stages (eggs, pupae and adults) and species ofmosquito,on non-targetaquatic organisms thatco-inhabit with mosquitos is recommended.

CONTRIBUTION TO KNOWLEDGE

Title: Phytochemical Screening and Larvicidal Effects of *Borreria Verticillata* (Linnaeus) and *Striga Asiatica* (Linnaeus) against *Culex Quinquefasciatus* (Say)

Name: Fatima Garba Kontagora

Registration Number: M.Sc./SCIE/2208/2011-2012

Programme: MSc. Biology

The study has contributed to the scientific knowledge as follows:

i. Compounds of insecticidal importance in different proportions found were: alkaloids, flavonoids, saponins, unsaturated steroid and triterpenes, tannins and cardiac glycosides while anthraquinone was absent in all the plant extracts.

ii. The median lethal concentration (LC_{50}) values of methanolic, ethyl-acetate, n-hexane and chloroform solvent extracts were 17.49 mg/l, 8.67 mg/l, 1.60 mg/l and 1.184×10^{30} mg/l respectively for *Borreria verticillata*.

iii. The LC_{50} values of *Striga asciatica* for solvent extracts of methanol, ethyl-acetate, n-hexane and chloroform were found to be 12.91 mg/l, 3.01 mg/l, 1.45 mg/l and 3.03 mg/l respectively.

iv. N-hexane had the highest larval mortality compared other solvents used with 22.92/25 (91.68%) mortality caused by *Striga asiatica*,while *Borreria verticillata* had 20.08/25 (80.32%).

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