

**DETERMINATION OF INSECTICIDAL EFFECTS OF
RICINUS COMMUNIS CRUDE EXTRACTS AND BLOOD
BAITED TRAPS WITH CASTOR OIL ADHESIVE ON
PHLEBOTOMINE SAND FLIES (DIPTERA:
PSYCHODIDAE)**

SAMUEL MONG'ARE

DOCTOR OF PHILOSOPHY

(Medical Parasitology and Entomology)

**JOMO KENYATTA UNIVERSITY OF
AGRICULTURE AND TECHNOLOGY**

2017

**Determination of insecticidal effects of *Ricinus communis* crude
extracts and blood baited traps with castor oil adhesive on
phlebotomine sand flies (Diptera: Psychodidae)**

Samuel Mong'are

**A thesis submitted in fulfilment of the requirement for the degree of
Doctor of Philosophy in Medical Parasitology and Entomology in the
Jomo Kenyatta University of Agriculture and Technology**

2017

DECLARATION

This thesis is my original work and has not been presented for a degree in any other University.

Signature----- Date-----

Samuel Mong'are

This thesis has been submitted for examination with our approval as the University Supervisors:

Signature..... Date.....

Prof. Zipporah Ng'ang'a, PhD

JKUAT, Kenya

Signature..... Date.....

Prof. Peter Ngure, PhD

TUK, Kenya

Signature..... Date.....

Dr. Philip Ngumbi, PhD

KEMRI, Kenya

DEDICATION

I dedicate this thesis to my beloved wife Elizabeth Regina and sons Steve and Enoch for your unequivocal support.

ACKNOWLEDGEMENT

I am indebted to my God who has been so gracious to me. He gave me good health, intellect, wisdom, perseverance and strength; necessary for completion of this work. I will give praise and always glorify your name for you are the corner stone of my life.

Sincere thanks to my supervisors; Prof. Zipporah Ng'ang'a of Jomo Kenyatta University of Agriculture and Technology, Prof. Peter Ngiro of Technical University of Kenya and African population and health research center and Dr. Philip Ngumbi of Kenya Medical Research Institute for their professional guidance and encouragement throughout the study period. I gratefully acknowledge your continuous scientific and professional support and critical evaluation of my research work. You saw hope where I saw despair, light where I saw darkness and gain where I saw pain. You have really made a change in my life.

I sincerely thank all my fellow students who in one way or the other gave an input towards my project work. My special thanks to my colleagues at work place for their support during the study period. Specifically, I want to acknowledge them for their criticism and valuable suggestions which eventually led to the successful completion of this work.

I wish to express my sincere appreciation to Kenya Medical Research Institute Scientists and technical staff for their enormous support. They facilitated my work at the center and gave me a step by step guidance throughout my stay at KEMRI. I honestly appreciate the assistance of Mr. Moindi Enock of the Center for Traditional Medicine and Drug Research for supervising and aiding in extraction of the plants. Special thanks to members of the leishmaniasis research programme for their support throughout the study period. I specifically acknowledge the support that I received from Ms. Chebet Alphine, Mr. Ingonga Johnstone, Ms. Milkah Mwangi, Mr. Osero Bernard, Ms. Cirindi Josyline, Dr. Karanja Robert and Dr. Anjili Christopher. God bless you all as you continue mentoring other students.

TABLE OF CONTENTS

DECLARATION.....	ii
DEDICATION.....	iii
ACKNOWLEDGEMENT.....	iv
TABLE OF CONTENTS.....	v
LIST OF TABLES.....	xi
LIST OF FIGURES.....	xiii
LIST OF PLATES.....	xv
LIST OF APPENDICES.....	xvii
ABBREVIATIONS AND ACRONYMS.....	xviii
ABSTRACT.....	xix
CHAPTER ONE.....	1
INTRODUCTION.....	1
1.1 Background information	1
1.2 Statement of the problem.....	4
1.3 Justification.....	5
1.4 Research questions	6
1.5 Null hypotheses.....	6
1.6 Objectives	6
1.6.1 General objective	6
1.6.2 Specific objectives	6
CHAPTER TWO.....	8

REVIEW OF LITERATURE.....	8
2.1 Impact of leishmaniasis.....	8
2.2 Forms and manifestation of leishmaniasis	11
2.2.1 Cutaneous leishmaniasis	11
2.2.2 Mucocutaneous leishmaniasis.....	12
2.2.3 Visceral leishmaniasis.....	13
2.2.4 Post kala-azar dermal leishmaniasis	14
2.3 Global distribution of leishmaniasis.....	15
2.4 Leishmaniasis in Kenya	18
2.5 Vectors of leishmaniasis	20
2.6 Identification of sand fly species	23
2.7 Reservoir hosts for <i>Leishmania</i>	24
2.8 Sand fly surveillance	25
2.9 The biology and life cycle of <i>Leishmania</i>	29
2.10 Biology and life cycle of sand flies.....	31
2.11 Control and treatment of leishmaniasis	33
2.11.1 Chemotherapy.....	33
2.11.2 Use of immunomodulators in leishmaniasis treatment.....	35
2.11.3 Natural products in treating leishmaniasis.....	35
2.12 Control of phlebotomine sand flies	37

2.12.1 Environmental management	38
2.12.2 Residual insecticide application.....	39
2.12.3 Prophylactic methods.....	40
2.12.4 Bioinsecticides	40
2.12.5 Role of pheromones	41
2.12.6 Plant crude extracts in controlling vectors.....	41
2.13 <i>Ricinus communis</i> morphology.....	43
2.13.1 Morphology and classification of <i>R. communis</i>	43
2.13.2 Uses of <i>R. communis</i>	45
2.13.3 <i>Ricinus. communis</i> crude extracts and insecticidal properties	46
2.13.4 Bioactive compounds in <i>R. communis</i>	48
2.13.5 Toxicity of ricin and ricinine	48
CHAPTER THREE.....	50
MATERIALS AND METHODS.....	50
3.1 Study site.....	50
3.2 Study design	50
3.3 Experimental animals	51
3.4. Sand Fly Colony	51
3.5 Preparation of <i>Ricinus communis</i>	52
3.5.1. Collection of the plant materials.....	52

3.5.2 Preparation of aqueous extracts of castor bean leaves and bark.....	52
3.5.3 <i>R. communis</i> toxicity test	53
3.6 Effects of <i>R. communis</i> extracts on <i>Phlebotomus duboscqi</i> pre-emergent stages..	53
3.6.1 Ovicidal effects of <i>Ricinus communis</i> crude extracts	53
3.6.2 Larvicidal effects of <i>Ricinus communis</i> crude extracts	54
3.7 Adulticidal effects of <i>Ricinus communis</i> extract	55
3.8 Design of the trap.....	55
3.9 Mounting and identification of trapped sand flies.....	58
3.10 Ethical and Biosafety considerations	58
3.11 Statistical analysis	58
CHAPTER FOUR.....	60
RESULTS.....	60
4.1 Ovicidal effects of <i>R. communis</i> extract	60
4.1.1 Effect of methanol <i>R. communis</i> leaf extract on the egg	60
4.1.2 Effect of extract on egg hatchability.....	64
4.2 Larvicidal effects of <i>R. communis</i> bark and leaf extracts.....	70
4.3. Larvicidal effects of <i>R. communis</i> extract	71
4.3.1 Larval morphology and mortality after extract ingestion/contact	71
4.3.2 <i>Phlebotomus duboscqi</i> larval duration after treatment with <i>R. communis</i> bark extracts	75

4.3.3 <i>Phlebotomus duboscqi</i> pupal duration after treatment with <i>R. communis</i> bark extracts	79
4.3.4 <i>Phlebotomus duboscqi</i> larval duration after treatment with <i>R. communis</i> leaf extracts	81
4.4 <i>Phlebotomus duboscqi</i> adult emergence after <i>R. communis</i> larval treatment.....	87
4.5 Comparing larvicidal effects of <i>R. communis</i> bark and leaf extracts from Kisii, Narok and Juja	88
4.6 Adulticidal effects of <i>R. communis</i> bark and leaf extracts on <i>P. duboscqi</i>	90
4.6.1 Mortality of sand flies by ingestion of methanol leaf extracts of <i>R. communis</i> from Narok County	90
4.6.2 Mean survival time after feeding on <i>R. communis</i> leaf and bark extracts	94
4.7 Potential of a modified trap for sand fly surveillance	95
4.7.1 Cattle, sheep, goat and chicken blood baited traps	96
4.7.2 Potential of cattle, sheep and goat urine Baited traps	97
4.8 Ripe banana, apple, grapes and mango fruit baited traps	99
4.9 Identification of collected sand flies	100
CHAPTER FIVE.....	102
DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS.....	102
5.1 Effect of <i>R. communis</i> on egg shell membranes	102
5.2 Effect of <i>R. communis</i> extract on hatchability of eggs	103
5.3 Effect of <i>R. communis</i> extract on larvae	104

5.4 Adulticidal effects of <i>Ricinus communis</i> extract	107
5.5 Potential of blood baited traps in attracting phlebotomine sand flies	109
5.6 Potential of urine baited traps in attracting phlebotomine sand flies	110
5.7 Potential of fruit baited traps in attracting hlebotomine sand flies.....	111
5.8 Identification of sand flies	113
5.9 Conclusions	115
5.10 Recommendations	115
REFERENCES.....	116
APPENDICES.....	136

LIST OF TABLES

Table 2.1:	Human pathogenic species of <i>Leishmania</i> and their vectors in Old World.....	21
Table 4.1:	Mean (\pm SE) <i>P. duboscqi</i> larval duration after treatment with aqueous <i>R. communis</i> bark extracts obtained from Kisii, Juja and Narok regions	76
Table 4.2:	Mean (\pm SE) <i>P. duboscqi</i> larval duration after treatment with methanol <i>R. communis</i> bark extracts obtained from Kisii, Narok and Juja	77
Table 4.3:	Mean (\pm SE) <i>P. duboscqi</i> larval duration after treatment with ethyl acetate <i>R. communis</i> extracts from three regions	78
Table 4.4:	<i>P. duboscqi</i> pupal duration after treatment with <i>R. communis</i> bark aqueous extracts from three different regions.....	79
Table 4.5:	<i>P. duboscqi</i> pupal duration after treatment with <i>R. communis</i> extracts from three different regions	80
Table 4.6:	<i>P. duboscqi</i> pupal duration after treatment with <i>R. communis</i> bark methanol extracts from three different regions.....	81
Table 4.7:	<i>P. duboscqi</i> larval duration after treatment with <i>R. communis</i> aqueous leaf extracts	82
Table 4.8:	<i>P. duboscqi</i> larval duration after treatment with methanol <i>R. communis</i> leaf extracts	83
Table 4.9:	<i>P. duboscqi</i> larval duration after <i>R. communis</i> ethyl acetate extract treatment	84
Table 4.10:	<i>P. duboscqi</i> pupal duration after aqueous <i>R. communis</i> leaf extract treatment	85

Table 4.11: *P. duboscqi* pupal duration after treatment with methanol *R. communis* leaf extracts 86

Table 4.12: *P. duboscqi* pupal duration after larval treatment with ethyl acetate *R. communis* leaf extracts..... 87

Table 4.13: Cumulative mortality of female *P. duboscqi* after feeding on *R. communis* extracts 90

LIST OF FIGURES

Figure 2.1:	Global distribution of reported visceral leishmaniasis areas	16
Figure 2.2:	Reported cases of visceral leishmaniasis in Kenya	19
Figure 2.3:	Life cycle of <i>Leishmania</i> species	30
Figure 2.4:	Life cycle of a phlebotomine sand fly	33
Figure 4.1:	Per cent hatchability of eggs after treatment with <i>R. communis</i> aqueous extracts from 3 sites: Narok, Juja and Kisii	65
Figure 4.2:	Per cent egg hatchability after treatment with <i>R. communis</i> methanol extracts from 3 sites; Narok, Juja and Kisii	66
Figure 4.3:	Per cent hatchability of <i>P. duboscqi</i> eggs treated with <i>R. communis</i> ethyl acetate leaf extracts from 3 sites: Narok, Juja and Kisii	67
Figure 4.4:	Per cent hatchability of eggs after treatment with <i>R. communis</i> bark (methanol) extracts from Narok, Juja and Kisii	68
Figure 4.5:	Per cent hatchability of eggs after treatment with <i>R. communis</i> bark (ethyl acetate) extracts from Narok, Juja and Kisii.....	69
Figure 4.6:	Per cent hatchability of <i>P. duboscqi</i> eggs treated with <i>R. communis</i> bark extracts (aqueous) from 3 sites: Narok, Juja and Kisii	70
Figure 4.7:	Comparing larval mortality after contact with extracts from Juja, Kisii and Narok Counties	75
Figure 4.8:	Comparing male and female mortality rates of <i>P. duboscqi</i> after feeding on methanol <i>Ricinus communis</i> extracts.....	92
Figure 4.9:	Mortality rates of <i>P. duboscqi</i> sand flies after feeding on aqueous <i>R. communis</i> extract	93

Figure 4.10:	Comparison of male and female <i>P. duboscqi</i> mortalities after feeding on <i>R. communis</i> ethyl acetate extract	94
Figure 4.11:	Mean survival time of <i>P. duboscqi</i> sand flies after feeding on different <i>R. communis</i> extracts.....	95
Figure 4.12:	Mean number of sand flies caught by cattle, goat, sheep and chicken blood baited traps compared to positive and negative controls.....	97
Figure 4.13:	Mean number of sand flies caught by cattle, sheep and goat urine baited traps	98
Figure 4.14:	Comparing number of sand flies caught by blood baited traps and urine baited traps	99
Figure 4.15:	Comparison of number of sand flies caught by ripe banana, mango, grapes and apple fruit baited traps.....	100

LIST OF PLATES

Plate 2.1:	<i>Ricinus communis</i>	45
Plate 3.1:	Design of the trap	56
Plate 3.2:	Baiting and setting the traps: A shows experimental set up with traps around the termite mound, B shows defibrinated blood in petri dishes, C shows blood and urine in petri dishes ready for transfer into the traps, D shows a CDC light trap set at the entrance of a termite mound	57
Plate 4.1:	(a) <i>P. duboscqi</i> egg treated with distilled water (control), (b) egg treated with 125µg/ml methanol <i>R. communis</i> leaf extract, (c) <i>P. duboscqi</i> egg at 250 µg/ml methanol <i>R. communis</i> leaf extract (d) <i>P. duboscqi</i> egg at 500 µg/ml methanol <i>R. communis</i> leaf extract.....	61
Plate 4.2:	(a) <i>P. duboscqi</i> egg treated with 125µg/ml aqueous <i>R. communis</i> extract (b) <i>P. duboscqi</i> egg treated with 250µg/ml aqueous <i>R. communis</i> extract (c) <i>P. duboscqi</i> egg treated with 500 µg/ml aqueous <i>R. communis</i> extract (d) Untreated egg (control)	62
Plate 4.3:	Egg at 500µg/ml aqueous <i>R. communis</i> bark extract (14 days post treatment) from Narok	63
Plate 4.4:	(A) Egg treated with methanol <i>R. communis</i> leaf extract from Kisii (B) Egg treated with <i>R. communis</i> from Juja (C) Untreated egg (control)	63
Plate 4.5:	Effect of <i>R. communis</i> on larvae: (A) first larval instar under 125µg/ml extract concentration with almost empty gut: CB-caudal bristle, G-gut, H-head (B) First larval instar (control) which is fed.....	71
Plate 4.6:	Second instar larvae treated with 250µg/ml of <i>R. communis</i> leaf extract. Arrows indicate; A-gut detaching from head, B-gut fully	

	detached from the head, C-split gut, D- rotting larvae; E-untreated larvae (control).....	72
Plate 4.7:	Third instar larvae treated with methanol extract of <i>R. communis</i> ; (A) Dead larvae (B) Rotting larvae (C) rotting larvae (D) Viable larvae from the control group	73
Plate 4.8:	Third instar larvae treated with ethyl acetate leaf extract of <i>R. communis</i> ; A-larvae with deterred feeding, B-Gut starting to detach from head, C- gut splitting D-gut splitting, E-dead larvae, F-Untreated larvae (control).....	74
Plate 4.9:	Comparing larvae that had fed on aqueous <i>R. communis</i> Powder obtained from Kisii, Narok and Juja. A: Larvae that had fed on <i>R. communis</i> leaf powder from Narok, B: Larvae that had fed on <i>R. communis</i> bark powder from Kisii, C: Larvae that had fed on <i>R. communis</i> bark powder from Juja, D: Larvae fed on larval food only (control)	89

LIST OF APPENDICES

Appendix 1: KEMRI scientific steering committee approval.....	136
Appendix 2: KEMRI ethics review committee approval.....	137
Appendix 3: Standard operating procedures.....	138
Appendix 4: Publications.....	143

ABREVIATIONS AND ACRONYMS

ANOVA:	Analysis of variance
CDC:	Center for disease control and prevention
CL:	Cutaneous leishmaniasis
DALYS:	Disability adjusted life years
DC:	Direct current
DCL:	Diffuse Cutaneous Leishmaniasis
DDT:	Dichlorodiphenyltrichloroethane
DEET:	N, N-diethylmethyletoluamide
HIV:	Human immunodeficiency virus
ITNs:	Insecticide treated nets
KEMRI:	Kenya medical research institute
LD:	Lethal dosage
MCL:	Mucocutaneous leishmaniasis
MST:	Mean survival time
NTD:	Neglected Tropical Disease
PKDL:	Post Kala-Azar Dermal Leishmaniasis
PBS:	Phosphate buffered solution
SPSS:	Statistical package for social scientists
SSG:	Sodium stibogluconate
VL:	Visceral leishmaniasis
WHO:	World health organization

ABSTRACT

Sand flies are small haematophagous insects that transmit *Leishmania* parasites. Infection with *Leishmania* results in diseases known as leishmaniasis which infects 14 million people worldwide with 2 million new cases occurring annually. The drawbacks for pentamionals and synthetic chemical insecticides have necessitated the search for new therapies against leishmaniasis and *Phlebotomus duboscqi* (Diptera: Psychodidae, Phlebotominae). This study aimed at assessing the effects of *Ricinus communis* (castor oil plant) extracts on *P. duboscqi* pre-emergent stages and adults in the laboratory. The study also sought to assess the potential of an improved sand fly trap incorporated with castor oil and baited with animal blood, urine and fruit attractants in the field. This trap could help solve the problems of the center for disease control (CDC) light trap since it is a simple and inexpensive trap which is light independent. Aqueous, methanol and ethyl acetate extracts were prepared from *R. communis* plant collected from Suswa (Narok County), Juja (Kiambu) and Nyanchwa (Kisii County). Freshly laid eggs were moistened with 1 ml of each extract separately during the incubation period. Larvae were also fed on larval food mixed with the powdered crude extract and adults were fed on the extracts through cotton wool pads which were soaked in the extracts and placed on the screen tops. Further, defibrinated blood (from cattle, goat, sheep and chicken); urine (from cattle, sheep and goat) and ripe fruits (bananas, apples, grapes and mango) were placed inside each trap to act as sand fly attractants. The study was carried out using a completely randomized design with three replicates. Median survival times were calculated using SPSS version 17.0. The efficacy of the different treatments was compared using the final cumulative mortalities. Differences in mortality rates, rate of pupation, and adult longevity were analyzed by analysis of variance and probability values of <0.05 were considered significant. 250 $\mu\text{g/ml}$ and 500 $\mu\text{g/ml}$ of methanol *R. communis* leaf extracts from Narok eroded all the chorionic membranes of the egg shell while egg hatchability was significantly inhibited with 9% (13.33 ± 1.67 , $P<0.001$) of eggs hatching at 500 $\mu\text{g/ml}$ of methanol *R. communis* extract. This was in comparison to 84% (96.67 ± 7.26) of eggs hatching in the untreated control group ($P<0.001$). Larval

mortality was 100% at 500 µg/ml methanol *R. communis* extract (both leaf and bark) that was obtained from Narok. The larval period was prolonged to 87 days with the life cycle lasting for 101 days, a significantly long duration ($P=0.002$). *R. communis* extracts from Narok showed insecticidal effects against adults. At 48 hours post treatment, the LC_{50} was 121.15 µg/ml and 126.21 µg/ml for methanol bark and leaf extracts respectively. *P. duboscqi* adults were found to be highly susceptible to methanol extracts. The modified trap collected 1302 sand flies within three trapping nights. Goat blood baited trap yielded more sand flies (202.33 ± 2.85) while chicken blood baited traps caught the least (65.00 ± 1.53). Among the fruit baited traps, bananas attracted the highest number of sand flies (94.33 ± 4.63) followed by mango baited trap (89.67 ± 2.33) although the difference was not significant ($P=0.682$). 64.3% of the collected sand flies belonged to the genus *Sergentomyia* and 35.7% to *Phlebotomus* with *Phlebotomus martini* (35.7%), *Sergentomyia schwetzi* (34.1%) and *S. antennata* (15.7%) being the dominant species. Volatiles from plant and animal hosts may be used instead of light or carbon dioxide to improve the efficiency of traps for haematophagous insects. Further, *R. communis* extracts have deleterious effects on hatching of eggs, larval and pupal development and adult emergence of *P. duboscqi*; hence *R. communis* should be used against sand flies and *Leishmania* in situ. The knowledge of phlebotomine ecology gained in this study will increase the ability to develop effective integrated vector control programs in visceral leishmaniasis (VL) endemic areas in Kenya.

CHAPTER ONE

INTRODUCTION

1.1 Background information

Phlebotomine sand flies (Diptera: Psychodidae) are found in the tropics and subtropics. They transmit *Leishmania*, protozoan parasites which cause visceral leishmaniasis (VL) and other various forms of cutaneous leishmaniasis (CL) infecting more than 350 million people in more than 80 countries worldwide (Marziel *et al.*, 2014). With few exceptions, phlebotomine sand flies are the unique haematophagous insects proven to transmit leishmaniases through the bite of infected females (Maroli *et al.*, 2012). Leishmaniasis is largely ignored in discussions of tropical disease priorities making it a neglected disease. This neglect is because of its complex epidemiology and ecology, lack of simple and easily applied tools for case management (Jorge *et al.*, 2012).

Leishmaniasis consists of four main clinical syndromes: cutaneous leishmaniasis (CL), muco-cutaneous leishmaniasis (MCL), visceral leishmaniasis (VL) and post-kala-azar dermal leishmaniasis (PKDL) (Chappuis *et al.*, 2007). Cutaneous leishmaniasis is the most common form of leishmaniasis (Desjeux, 2004) and it produces skin lesions mainly on the face, arms and legs. Depending on the species of *Leishmania*, the infection may be characterized by ulcers, smooth nodules, flat plaques or hyperkeratotic wart-like lesions. Although this form is often self-healing, it can create serious disability and permanent scars (WHO, 2000).

In mucocutaneous leishmaniasis patients suffer from progressively destructive ulcerations of the mucosa, extending from the nose and mouth to the pharynx and larynx. If untreated, the disease can progress to ulcerative destruction of the nasopharyngeal mucosa. Visceral leishmaniasis is the second largest parasitic killer in the world after malaria (Picado, *et al.*, 2012). The infection involves internal organs and is characterized by irregular fever, weight loss, swelling of the liver and spleen and anaemia. It is the most severe form of leishmaniasis, and is usually fatal if left untreated with a mortality rate of almost 100% (Umakant & Sarman, 2008). Post-

kala-azar dermal leishmaniasis is characterized by a macular, maculo-papular or nodular rash around the mouth and is a complication of VL that is frequently observed after VL treatment (Chappuis *et al.*, 2007). It usually occurs within 6 months of VL, and typically disappears within a year without treatment.

Other than leishmaniasis, phlebotomine sand flies are vectors of other pathogens such as Bartonella and viruses belonging to three different genera: (i) the Phlebovirus (family Bunyaviridae) including sand fly fever, Silician virus, sand fly fever Naples virus, Toscana virus and Punta Toro virus; (ii) the Vesiculovirus (family Rhabdoviridae) including Chandipura virus and (iii) the Orbivirus (family Reoviridae) including Changuinola virus (Mong'are *et al.*, 2013).

The sand fly *Phlebotomus duboscqi* (Diptera: Psychodidae) is the vector for zoonotic cutaneous leishmaniasis caused by *Leishmania major* (Kinetoplastida: Trypanosomatidae) in Baringo County, Kenya (Anjili *et al.*, 2014). This sand fly is found in animal burrows where it rests and feeds on rodents. *P. longipes* and *P. pedifer* also transmits *L. aethiopica* which causes CL and diffuse cutaneous leishmaniasis (DCL) in Kenya and Ethiopia. *P. argentipes*, *P. orientalis* and *P. martini* are the proven vectors of *L. donovani* which causes kala-azar and PKDL (Umakant & Sarman, 2008).

Currently, there are limited leishmaniasis treatment options because most of them are expensive and problematic due to resistance, toxicity and side-effects (Malaria consortium, 2010). Pentavalent antimonials (sodium stibogluconate (SSG) or meglumine antimonate) are traditionally used as the first line drugs for VL and CL. However, antimonials have limitations including potentially toxic effects, high cost, long treatment periods and increasing reports of treatment non-responsiveness (Nisha *et al.*, 2012). Amphotericin B is also a drug of choice in some areas because of its high efficacy. However, this drug is expensive, toxic and its side effect has been reported.

Other drugs include Miltefosine, the first oral drug for the treatment of VL; however, it has a median long half-life of approximately 152 hours which could encourage development of resistance. Further, its teratogenic and abortifacient nature limits its

use in pregnancy (Nisha *et al.*, 2014). Paromomycin and Sitamaquine cures both VL and CL more effectively but have side effects like vomiting, dyspepsia, cyanosis, nephritic syndrome and glomerulonephritis.

Further, there is no potent vaccine against leishmaniasis hence control is based on case management and vector control. The main sand fly vector control methods are: chemical control, environmental management, and biological control. However, several factors like cost of the insecticides, high summer temperatures, strong radiation, accumulation of dust, low acceptance by the community, low community participation and the emergence of resistance affect the long-term use of these interventions (Claborn, 2010). Another promising chemical control method is the use of synthetic pheromones to attract adult sand flies into traps, but evidence is not yet available (Umakant & Sarman, 2008). On the other hand, case management is difficult to be conducted since it is restricted by lack of access to affordable, active drugs, incorrect prescribing and poor compliance (Mong'are *et al.*, 2015).

Since anti-leishmanial vaccines are still being developed, the current control strategies for leishmaniasis rely on reducing man-vector contact through application of vector control measures. Synthetic insecticides pose adverse effects to the user and the environment. The effectiveness of these spraying programmes is not the only issue for concern but their side effects are also important on health and environment, and their potential for sustainability, which depends on the cost of the insecticides and their application. Sand flies have also developed resistance to the chemicals, mainly to DDT and in some cases to Malathion and pyrethroids (Umakant & Sarman, 2008).

Recent researches focused on some synthetic agents like chalcones and natural products have shown a wise way to get a true and potentially rich source of drug candidates against leishmaniasis (Rejeshree *et al.*, 2012). *Ricinus communis* (castor bean or castor oil plant) is the most studied plant species with insecticidal properties across the world but its activities against *Phlebotomus duboscqi* sand flies is unclear. Products of this plant have shown strong larvicidal effect on *Plutella xylostella*, with 100% mortality recorded on 3rd instar larvae treated with 10% oil emulsion in both

ingestion and contact toxicity tests (Tounou, *et al.*, 2011). Further studies have shown that castor oil and ricinine are the active ingredients of *R. communis* that act against *Spodoptera frugiperda* (Ramos-López *et al.*, 2010).

Toxicity, inconsistent efficacy and resistance between *Leishmania* species or strains and long lasting parenteral administration of pentamoniols hinder leishmaniasis treatment (Khan & Umar, 2015). Further, sand flies have developed resistance to synthetic insecticides while vector surveillance is hampered by their high cost, regular maintenance and can stay in the field for a limited time. These factors led to the design of this study which sought to determine the insecticidal effects of *R. communis* as a potential insecticide for sand flies in the laboratory and at the same time to modify an existing trap so that it can attract sand flies by using scent which is normally produced by host animals and plants.

1.2 Statement of the problem

Sand flies are vectors of leishmaniasis and sand fly fever, which pose a significant health threat to 14 million people worldwide (WHO, 2010). Effective and consistent control strategies have been difficult to develop and implement. Control measures include chemotherapy using the pentavalent antimonials and vector control programs. Pentavalent antimonials are expensive, toxic, and have variable efficacy with resistant strains being reported. Limited drug options and the possibility of resistance development is a major and serious hurdle in the elimination of leishmaniasis in disease endemic countries (Nisha *et al.*, 2014).

Repeated pesticide applications can reduce sand fly numbers, but as the reduction is short lived, this method is used only in epidemics (Ryan *et al.*, 2006). Vector control programs are limited by the high cost of synthetic insecticides, toxicity, vector tolerance due to rampant use and the high cost of application equipment. The current insecticides have non-target effect killing even the beneficial insects and end up causing soil and water contamination. Traps like sticky traps, CDC light traps and animal baited traps used for surveillance are hampered by their high cost and logistical problems in the field. Sand fly attractants from hosts and volatiles from plants can be used to improve the efficiency of sticky and animal baited traps.

Insecticides can also be incorporated into this trap so that after trapping, the sand flies can also be killed. This will overcome the challenges of CDC light trap and other traps in remote and leishmaniasis endemic areas. Consequently, this will lower the high cost of treatment, reduce resistance of drugs and insecticides and also interrupt the life cycle of *Leishmania*.

1.3 Justification

Most of the drugs for leishmaniasis treatment are expensive and problematic due to issues of resistance, toxicity and side-effects (Malaria consortium, 2010). The increased cost, toxicity, long treatment periods, parenteral route of administration and resistance of pentavalent antimonials indicates the need for new therapeutic agents against leishmaniasis.

Immunomodulators against *Leishmania* parasites are also effective in activating macrophages to produce toxic nitrogen and oxygen metabolites that kill the intracellular amastigotes. However, their use in leishmaniasis treatment is very costly. Further, the use of natural products against leishmaniasis has received little attention and is under-funded because leishmaniasis is a neglected disease. Vector control programs are challenged by insecticide resistance and high cost of insecticides hence the need for alternative control methods, including new insecticides.

Vector surveillance is also facing many challenges since the traps available for surveillance are very costly. CDC traps, motorcycle batteries, CO₂ tanks and propane tanks for combustion traps are expensive, need regular maintenance, are heavy and can stay in the field for only a limited amount of time. In remote areas, logistics can be a serious problem. A CDC light trap requires 0.320 Amps per hour to operate at 6.0-6.3 volts DC. Hence, four D-size flashlight batteries in series will provide power for 1 night's operation. This is not affordable in remote areas where sand flies are prevalent. Simple un-baited or light baited sticky traps, on the other hand, often yield only small catches. Phytochemicals and attractants from hosts have untapped potentials for sand flies and could be in combination with simple glue traps an

alternative for existing vector surveillance and control measures. Such traps would be extremely cheap, easy to maintain and they could stay for long periods in the field.

Because of the limitations of current treatment options for leishmaniasis and vector control, there is need to explore natural substances as drug and insecticide candidates for leishmaniasis and sand flies respectively. These products are likely to lower the high cost of treatment, reduce resistance of drugs and insecticides and also reduce environmental pollution.

1.4 Research questions

1. What are the effects of *R. communis* extracts on *P. duboscqi* eggs, larvae and pupae?
2. What are the effects of *R. communis* extracts on *P. duboscqi* adults?
3. What are the consequences of incorporating animal blood, urine and ripe fruits in sand fly traps without light?

1.5 Null hypotheses

1. Extracts from *R. communis* do not have effect on *P. duboscqi* pre-emergent stages
2. Extracts from *R. communis* do not have insecticidal activities against *P. duboscqi*.
3. Animal blood, urine and ripe fruits do not attract sand flies.

1.6 Objectives

1.6.1 General objective

To determine the insecticidal effects of *R. communis* crude extracts and blood and fruit baited traps with castor oil adhesive on *P. duboscqi* in the laboratory and field.

1.6.2 Specific objectives

1. To assess the effects of *R. communis* extracts on *P. duboscqi* pre-emergent stages.
2. To determine the adulticidal effects of *R. communis* crude extracts on *P. duboscqi*.

3. To assess the effectiveness of sand fly traps baited with animal blood, urine and ripe fruits with castor oil adhesive in the traps.

CHAPTER TWO

REVIEW OF LITERATURE

2.1 Impact of leishmaniasis

The increase in leishmaniasis' incidence worldwide is mainly attributed to the increase of several risk factors that are clearly man-made and include massive migration, deforestation, urbanization, immunosuppression, malnutrition and treatment failure (Assimina *et al.*, 2008). These factors may increase human exposure to infected sand flies. Leishmaniasis is a zoonotic infection caused by protozoans of the genus *Leishmania*. The infection is transmitted to humans by infected sand flies of the genus *Phlebotomus* and *Lutzomyia* (Piscopo and Mallia, 2006). *Leishmania* parasites enter the immune system cells following the bite of an infected sand fly and spread either to the skin, causing disfiguring lesions, or to internal organs, causing lethal infections (Getti *et al.*, 2009).

The majority of human infections by *Leishmania* parasites remain asymptomatic (Assimina *et al.*, 2008). These asymptomatic human subjects are able to clear the infection or they remain asymptomatic carriers for years. Thus, pathogenesis of leishmaniasis depends on several risk factors such as malnutrition, immunosuppression, age, immunological status and genetic factors. As a neglected tropical disease, leishmaniasis shares the characteristics of a typical poverty-related disease, that is, a lack of recognition, political prioritization, visibility of its burden, national strategies for its control and accurate information on its extent and distribution (WHO, 2014).

Leishmaniasis is endemic in 98 countries in the world with 350 million people being at risk. An estimated 14 million people are infected, and each year about two million new cases occur (WHO, 2007). The global burden of leishmaniasis has remained stable for some years, causing morbidity and mortality loss of 2.4 million

disability adjusted life-years (DALYs) and approximately 70,000 deaths, a significantly high rank among communicable diseases (Ngure *et al.*, 2009).

This significant rise in the cases of leishmaniasis is attributed to international travel and tourism, military operations, and influx of immigrants from the endemic countries to other parts of the world (Subathra *et al.*, 2014). Further, human immunodeficiency virus (HIV) epidemic, lack of effective vaccines, difficulties in controlling vectors, international conflicts and the development of resistance to chemotherapy could increase the cases of leishmaniasis (Rajeshree *et al.*, 2012).

The wild endemic pattern has been replaced by the spread of the disease associated with environmental modifications, disordered human occupation and substandard living conditions. Therefore, the disease is spreading in both rural and urban areas, exceeding old defined geographic limits and becoming a serious public health problem (Sthenia *et al.*, 2009).

Leishmaniasis is not only associated with poverty but also propagates poverty, because treatment is expensive and either unaffordable or imposes a substantial economic burden, including loss of wages (WHO, 2007). The global prevalence of leishmaniasis has risen in recent times because of an increase in international travel and human alteration of both vector and host habitats. Recent international conflicts have also contributed to an increase in and spread of leishmaniasis in previously unaffected countries (Rosypal *et al.*, 2003).

Leishmaniasis occurs in tropical and sub-tropical areas, mainly in Asia, Europe, Africa, and the Americas where it is considered a growing public health concern (Camargo & Langoni, 2006). In the European region, leishmaniasis is a neglected and poorly reported disease with an underestimated or undetermined burden in most countries with incidence of VL and CL being estimated at less than 2% of the global burden of leishmaniasis (WHO, 2014).

Co-infection with other infectious diseases is an increasing concern with human immunodeficiency virus-visceral leishmaniasis (HIV-VL) co-infection being reported in 35 out of 98 countries in which leishmaniases are endemic (Ter Horst *et*

al., 2008). In Africa, particularly Ethiopia, Sudan and Southern Europe, HIV-*Leishmania* co- infection is regarded as an emerging disease and as many as 70% adults with VL also have HIV infection (Dawit & Shishay, 2014). As a result, it is likely that leishmaniasis burden will increase, due to: increasing migration, climate change and impaired immunity resulting from malnutrition and/or HIV.

There is scanty information on the economic impact of leishmaniasis. However, leishmaniasis aggravates poverty because the economic impact of the disease includes direct non-medical costs like transport and income loss for patients and their families due to absence from work (WHO 2010). However, the economic impact of the disease in most countries is due to high cost of treatment and also time lost during hospitalization (Dawit & Shishay, 2014) and the disease affects the rural poor community causing significant morbidity and mortality.

In most countries, it is probable that the disease promotes poverty because patients and their families are significantly impacted by the disease through productivity losses due to hospitalization (Kolaczinski *et al.*, 2008). In India, villagers feel powerless in the face of dire emotional and economic consequences, and many equate a kala azar (visceral leishmaniasis) diagnosis with a death sentence because the economic burden of kala azar in a family is heavy (Ahluwalia *et al.*, 2003).

In East Africa, VL has a high case-fatality rate and its epidemics are frequent (WHO, 2010). Further, the disease disfigures the nose and other body parts leading to a psychosocial burden associated with the deformities (Camargo & Langoni, 2006). Therefore, leishmaniasis and MCL can lead to exclusion from society because of the mistaken belief that the disease is directly contagious. Mothers with cutaneous disease may refrain or be prohibited from touching their children; young women with scars are unable to marry and the disease may provide the pretext for a husband to abandon a wife (WHO, 2012).

In Kenya, children are forced to drop out of school due to stigma and alienation by the local community as evidenced by a seven-year-old child whose social life had been disrupted by the raised lesions on his cheeks, nose, around the eye socket and

on the forehead (Daily nation, May 6, 2014). When Kibet's abdomen began to swell his family was convinced that he had been bewitched by their enemies because of his academic progression (Daily nation, May 19, 2016). VL is a grave public health problem in this area that imposes an additional strain on the local health authorities and is unlikely to be resolved by the current strategies. Alternative methods of vector control, other than the conventional indoor spraying of houses with residual insecticide should be considered (Ryan *et al.*, 2006).

All these issues arise because a cost effective cure for the disease does not exist. The chemotherapeutic agents most commonly used for treating leishmaniasis, that is, sodium stibogluconate, *N*-methylglucamine antimoniate, pentamidine, and amphotericin B, are not effective when administered orally. Moreover, they often require long periods of treatment and cause serious side effects, including cardiac and renal toxicity (Akendengue *et al.*, 2002). This has prompted the World Health Organization to emphasize the need for development of new drugs in the treatment of leishmaniasis (WHO, 2005).

2.2 Forms and manifestation of leishmaniasis

Leishmaniasis is a major tropical disease with a wide clinical spectrum of cutaneous, mucocutaneous and visceral involvement whose presentation is often varied and diagnosis can be challenging. Clinical manifestation depends on the parasite species and the host's specific immune responses to *Leishmania* antigens (Roberts, 2006).

2.2.1 Cutaneous leishmaniasis

Globally, cutaneous leishmaniasis (CL) is the most common form of leishmaniasis with an annual incidence of 1-1.5 million cases (Desjeux, 2004). Multiple species produce CL in children and adults, primarily *L. major*, *L. tropica*, and *L. aethiopica*, *L. infantuma*, *L. chagasi*, *L. mexicana*, *L. amazonensis*, *L. braziliensis*, *L. Viannia panamensis*, *L.(V) peruviana* and *L.(V) guyanensis* (Murray *et al.*, 2005). Confirmed vectors include *P. duboscqi*, *P. guggisbergi* and *P. pedifer*. The disease produces skin lesions mainly on the face, arms and legs (Akilov *et al.*, 2007).

Cutaneous leishmaniasis is found in South America, Asia, Europe and Africa. Latin America is the most important endemic area particularly the Amazon (Igor, 2015). 90% of infections are concentrated in Afghanistan, Algeria, Brazil, Iran, Peru, Saudi Arabia, and Syria. In Brazil, it has been identified in almost all states where the number of cases has gradually increased in the last 20 years, and the country showed the highest occurrence, with more than 30,000 annual cases (Carmago & Langoni, 2006).

In West Africa, CL is proposed to be endemic in a belt running from Mauritania, Gambia and Senegal in the west to Nigeria and Cameroon in the east (Boakye *et al.*, 2005). Cutaneous leishmaniasis also occurs in the highlands of Ethiopia where sporadic cases of CL have been diagnosed from many localities in the northern, central and southern highlands (Dawit & Shishay, 2014). The disease is of limited importance in Eastern Africa where it occurs in small foci in North Sudan, Kenya, and Ethiopia (Malaria consortium, 2010). However, in Kenya it is prevalent in Rift valley, Eastern, Central and Western provinces in more than 20 districts (Malaria consortium, 2010).

Cutaneous leishmaniasis starts as a papule at the site of a sand fly bite, which then increases in size, crusts and eventually ulcerates (Piscopo and Mallia, 2006). This disease produces skin lesions mainly on the face, arms and legs. The patient generally presents with one or several ulcers or nodules in the skin. These ulcers heal spontaneously although slowly in immunocompetent individuals, but cause disfiguring scars (Chappuis *et al.*, 2007). Although this form is often self-healing, it can create serious disability and permanent scars (WHO, 2000). Therefore, prevention strategies should be considered for CL, which is also a major burden for certain areas, with serious psychosocial effects.

2.2.2 Mucocutaneous leishmaniasis

Mucocutaneous leishmaniasis (MCL) also called espundia, produces disfiguring lesions to the face, destroying the mucous membranes of the nose, mouth and throat (WHO, 2007). MCL may occur many years after the initial cutaneous ulcer has healed. After an initial skin lesion, that slowly but spontaneously heals, chronic

ulcers appear after months or years on the skin, mouth and nose, with destruction of underlying tissues like the nasal cartilage.

Mucocutaneous leishmaniasis is mostly related to *Leishmania* species of the New world such as *L. braziliensis*, *L. panamensis* and *L. guyanensis*. The mucosal lesions have also been reported in Old-world leishmaniasis caused by *L. donovani*, *L. major* and *L. infantum* in immunosuppressed patients (Desjeux, 2004). Ninety percent of all cases of MCL occur in Bolivia, Brazil and Peru. The salient feature of the species that cause MCL is that they cause metastasis to the mucosal tissues of the mouth and upper respiratory tract by lymphatic or haematogenous dissemination (WHO, 2010).

Mucocutaneous leishmaniasis produces lesions, which can lead to extensive and disfiguring destruction of mucous membranes of the nose, mouth, throat cavities and surrounding tissues (Umakant & Sarman, 2008). Malnourished young adults and male migrants are at special risk. Other risk factors include the site of the primary lesion above the waist, multiple or large primary lesions or delayed healing of the primary cutaneous leishmaniasis (WHO, 2010).

2.2.3 Visceral leishmaniasis

Visceral leishmaniasis (VL) is the most severe form of leishmaniasis and is potentially fatal if left untreated (Garg & Dube, 2006). VL is largely considered a rural disease, often correlated with malnutrition, poor sanitary conditions, and other factors associated with poverty (Claborn *et al.*, 2011). Visceral leishmaniasis coexists with malaria and other debilitating parasitic infections (Hailu *et al.*, 2005).

Parasites of the *L. donovani* complex (in East Africa and India) and *L. infantum* (in Europe, Latin America and North Africa) are the typical etiological agents of VL (Assimina *et al.*, 2008; Mukhopadhyay & Mandal, 2006). The main sand fly vector is *P. martini*, which in East Africa, breeds in termite hills, animal burrows, tree holes and house walls (Ngumbi *et al.*, 1998). *L. tropica* has been reported to produce visceral disease in immunocompromised persons while visceralization by *L. amazonensis* has also been reported (Herwaldt, 1999).

In Europe, VL is endemic in all southern countries where most of the reported cases are due to zoonotic visceral leishmaniasis, which is the most dangerous form with a high fatality rate when untreated (Assimina *et al.*, 2008). In the Asian continent, VL is restricted to northeast India, Bangladesh and Nepal, where *P. argentipes* is the sole vector. In Ethiopia, both forms of leishmaniasis such as CL and VL are endemic and becoming a major public health concern (Edessa *et al.*, 2008). Ethiopia has the highest known VL/HIV co-infection rate in the world with sporadic cases of VL being diagnosed from Wolkayit Tsegede, Gibdo, Raya, Kobo, Kijawa (Gambella) and Gelana (Sidamo) and Genale (Bale) river basins (Dawit & Shishay, 2014).

In East Africa (Kenya, Ethiopia, Somalia, Sudan and Uganda), the distribution of *L. donovani* is associated with those of *P. orientalis* and/or *P. martini* (Maroli *et al.*, 2012). However, in Kenya, VL is endemic in semi-arid and arid areas of Rift Valley, Eastern and North Eastern provinces with important foci being Baringo, Pokot, Turkana, Wajir, Isiolo, Samburu, and Marakwet districts (WHO, 2012). Even though VL is curable, it still causes high morbidity and sometimes death due to its low index of suspicion by health providers, late diagnosis and case management.

Migration, lack of control measures, malnutrition, immune suppression and HIV/VL co-infection are the main factors driving the increased incidence of VL (Chappuis *et al.*, 2007). The clinical symptoms of visceral leishmaniasis include splenomegaly, recurring and irregular fever, anemia, pancytopenia, weight loss and weakness. VL symptoms often persist for several weeks to months before patients either seek medical care or die from bacterial co-infections, massive bleeding or severe anemia (Chappuis *et al.*, 2007). The disease kills almost all untreated patients (Hailu *et al.*, 2005).

2.2.4 Post kala-azar dermal leishmaniasis

Post kala-azar dermal leishmaniasis (PKDL) appears after treatment of visceral leishmaniasis (Ghalib & Modabber, 2007), and it requires lengthy and costly treatment (WHO, 2006). The condition usually appears 6 months to 1 or more years

after apparent cure of visceral leishmaniasis but may occur earlier or even concurrently with visceral leishmaniasis in Sudan (WHO, 2012).

Post kala-azar dermal leishmaniasis is a dermatropic form of leishmaniasis characterized by skin lesions that are macular, maculopapular or nodular, and usually spread from the peri-oral area to other areas of the body. The symptoms first appear around the mouth; those which do not heal spontaneously become dense and spread over the entire body (Berman, 1997). The lesions can become nodular and spread to the limbs and trunk. However, the lesions are non-itchy and are symmetrical (WHO, 2012). PKDL cases are highly infectious because the nodular lesions contain many parasites, and such cases are the putative reservoir for anthroponotic VL between epidemic cycles (Chappuis *et al.*, 2007).

2.3 Global distribution of leishmaniasis

Leishmaniasis causes substantial clinical, public health and socioeconomic problems in endemic regions in more than 98 countries in the Indian sub-continent, South Western Asia, Southern Europe, Africa, and Central and South America (Desjeux, 2004) (Fig. 2.1). There is a remarkable increase in risk factors for leishmaniasis worldwide and the disease burden is increasing (Reithinger *et al.*, 2007). Socio-economic conditions like poverty, malnutrition and HIV, environmental changes such as atmospheric temperature and humidity, ecological conditions affecting the vector, parasite and its reservoir, and migration of people and tourism are the main risk factors which interfere with global distribution of leishmaniasis (Igor *et al.*, 2015).

Visceral leishmaniasis is endemic in the tropical and sub-tropical regions of Africa, Asia, the Mediterranean, Southern Europe, South and Central America. The distribution of VL in these areas however is not uniform; it is patchy and often associated with areas of drought, famine and densely populated villages with little or no sanitation.

Status of endemicity of visceral leishmaniasis, worldwide, 2013

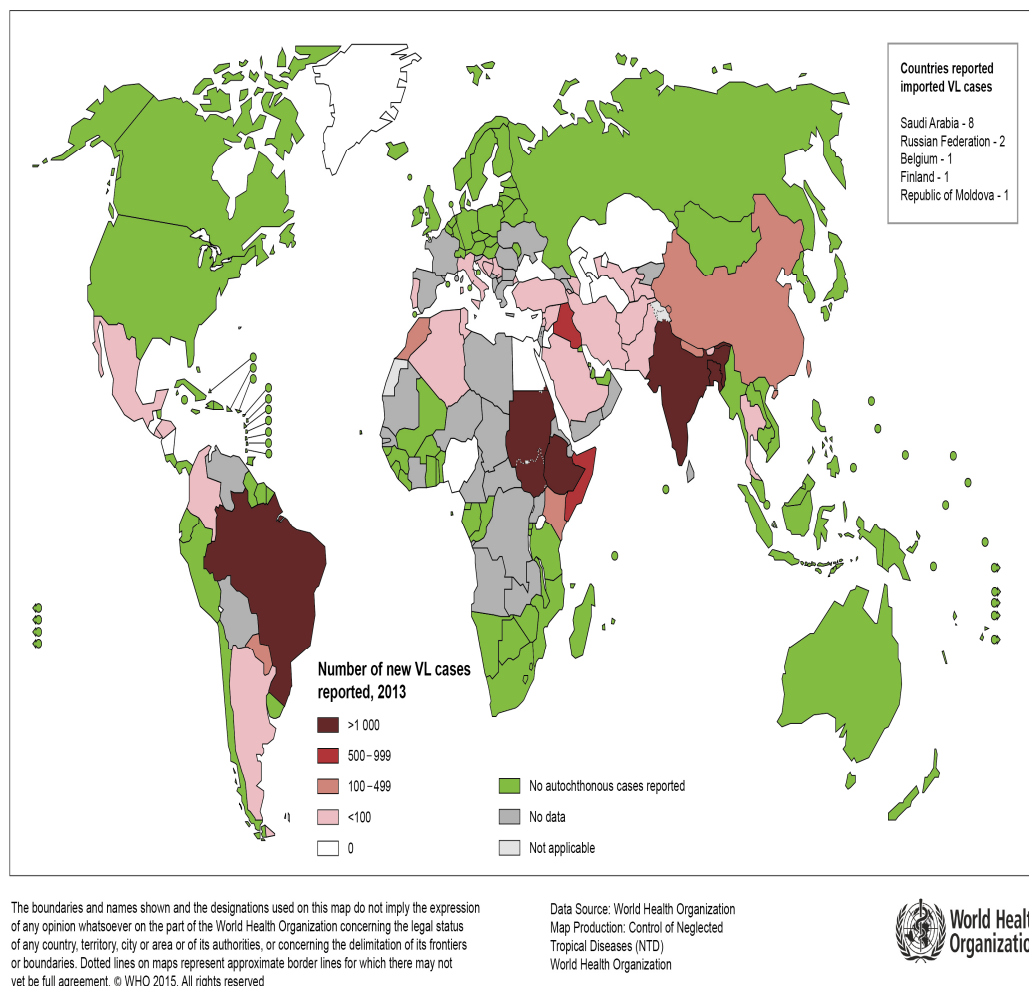


Figure 2.1: Global distribution of visceral leishmaniasis areas (Source: WHO, 2013)

The global estimate for new cases of VL is 500 000 cases per year out of which 90% arise in just five countries. These include Bangladesh, Brazil, India, Nepal and Sudan (Desjeux, 2004). Each year, there are 1.5 million new cases of CL in more than 70 countries worldwide with 90% of the cases reported in Afghanistan, Algeria, Brazil, Islamic Republic of Iran, Peru, Saudi Arabia and Syria (Ghalib & Modabber, 2007). Ninety per cent of MCL cases occur in Bolivia, Brazil and Peru (Desjeux, 2004). In

India, visceral leishmaniasis is endemic in the districts of Bihar, Uttar Pradesh, Orissa, Tamil, Nadu and Gujarat. The leishmaniasis rank as the leading neglected tropical disease (NTD) in terms of mortality and morbidity with an estimated 50,000 deaths in 2010 and 3.3 million disability adjusted life years (Piggot *et al.*, 2014).

In Africa, much of leishmaniasis is concentrated in Eastern Africa. VL is a particular problem in Kenya, Sudan, Ethiopia and Eritrea (Wasunna *et al.*, 2005). Sudan is the most affected country, being one of the five countries that constitute 90% of all global cases of VL (Guerin, *et al.*, 2002). Sudan also has the highest incidence of Post Kala-azar Dermal Leishmaniasis (PKDL) in the world (Ghalib & Modabber, 2007).

Visceral leishmaniasis has been known in Sudan since 1904 to be endemic along the Blue Nile where it enters Ethiopia and its tributaries (Berman, 2006). Since the first reported case of VL in Sudan, the disease has become wide spread and is endemic in south and eastern parts of the White Nile and Upper Nile states. Other areas affected include the provinces of Kasala, Jonglei and Kapoeta in the south, El Fasher and El Nahud in the west and north of Khartoum. Doctors without Borders have been active in the area since 1985, having treated 51 000 cases of VL, relapsed VL, and PKDL with a cure rate of 90.8% (Subathra *et al.*, 2014).

In Ethiopia, there is the presence of a significant number of patients co-infected with HIV and VL (Ngure *et al.*, 2009). There are about 1000-2000 VL cases reported annually in Ethiopia, and 20-40% is co-infected with HIV (Subathra *et al.*, 2014). The incidence has increased due to agricultural development programmes causing influx of working migrants in the Tigray region. The etiologic agent associated with the epidemic is *L. donovani* and the sand fly vector is *P. orientalis* (Seaman *et al.*, 1996). The first case of VL in Ethiopia was documented in 1942 in the southern parts of the country. Since then the disease has spread to become endemic in the Segen, Woito and Gelana river valleys. The highest incidence has been recorded in the Aba Roba area (WHO, 2010). Visceral leishmaniasis cases have also been reported in the villages close to the Segen river valley.

In Somalia, sporadic cases of VL first appeared in 1934, mainly in the Middle Shabelle and Lower Juba areas. Children below the age of 15 years are at the highest risk of infection and males are over three times more susceptible than females (Shiddo *et al*, 1995). In Israel VL is rare and the few cases that have been reported are largely confined to the run down Arab villages in western Galilee, indicating that the disease is linked to poverty, poor sanitation and sub-standard housing.

In West Africa, leishmaniasis is endemic although it is one of the less recognized or under-reported parasitic infections in this region (Boakye, 2005). Cases of leishmaniasis have been reported in Niger, Mali, Nigeria, Senegal, Cameroon, Burkina Faso, Mauritania, Gambia, and Guinea. There is high prevalence of both HIV and leishmaniasis co-infection in Burkina Faso (Niamba *et al.*, 2007). Algeria is among the eight countries that contribute 90% of worldwide cases of CL (Reithinger *et al.*, 2007).

2.4 Leishmaniasis in Kenya

Leishmaniasis has been endemic in Kenya for a long time. The most prevalent forms are cutaneous leishmaniasis, visceral leishmaniasis and post kala-azar dermal leishmaniasis (WHO 2010). The occurrence of CL is rare compared to VL, and the two diseases do not tend to overlap geographically. VL is found predominantly in the arid, low-lying areas of the Rift Valley, Eastern and North Eastern provinces, whereas CL occurs over a wide range of environmental conditions, from semi-arid lowlands to high plateaus in the Eastern, Rift Valley, Central and Western provinces (malaria consortium, 2010).

Visceral leishmaniasis is endemic in Baringo, Koibatek, Turkana, West Pokot, Kitui, Meru, Keiyo, Marakwet, Mwingi, Tana River and Machakos districts (Wasunna *et al.*, 2005). In 2001, there was an outbreak of VL in Wajir and Mandera districts of North Eastern Kenya with 904 patients diagnosed between May 2000 and August 2001 (Marlet *et al.*, 2003) (fig 2.2). Visceral leishmaniasis outbreaks are reported frequently in Kenya; 303 cases in 1952, 2142 cases in 1953 (Kitui), 1500 cases in

1966 (Meru), and in 2001, there was a VL outbreak in the previously non-endemic regions of Wajir and Mandera, with 904 patients (Subathra *et al.*, 2014).

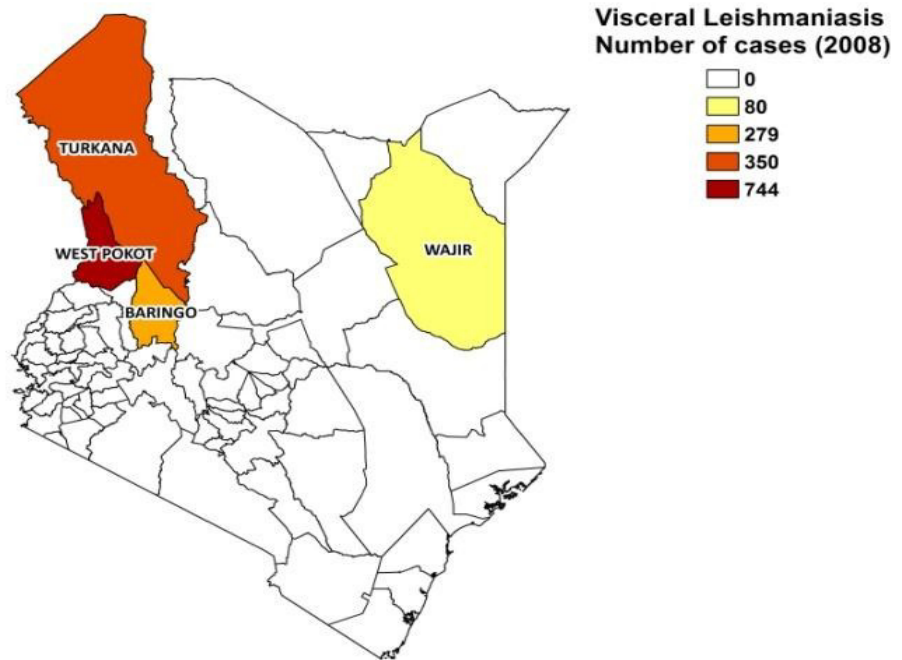


Figure 1.2: Reported cases of visceral leishmaniasis in Kenya, 2010

(Source: <http://www.who.int/leishmaniasis/resources/KENYA.pdf>)

Visceral leishmaniasis is caused by *L. donovani* and transmitted by *P. martini* in Kenya though other vectors including *P. orientalis* have been reported (Ngure *et al.*, 2008). *P. orientalis* is a dry season species; associated with the *Acacia seyal-Balanites aegyptica* woodlands and black cotton soil whereas *P. martini* appears to favor more humid habitats, for example termite mounds (Mueller *et al.*, 2014).

In Kenya, cutaneous leishmaniasis is caused by *L. major*, *L. aethiopica* and *L. tropica* (Ngure *et al.*, 2009). Cutaneous leishmaniasis due to *L. major* is transmitted by *P. duboscqi* and is mainly found in animal burrows where it feeds on rodents that are frequently infected. Diffuse cutaneous leishmaniasis (DCL) was first reported in Kenya in 1969 in Bungoma district and Mount Elgon area, *L. aethiopica* has been identified as the etiological agent while hyraxes and rodents are the animal reservoirs and *P. pedifer* Lewis as the vector of DCL in Mt. Elgon region (Ashford, 2000).

In Kenya, PKDL was first described in 1959 by Manson-Bahr (Ngure *et al.*, 2009). Although various aspects of the transmission and control of leishmaniasis have been studied in Kenya, the impact of the disease and particularly VL is still enormous (Tonui, 2006).

2.5 Vectors of leishmaniasis

Phlebotomine sand flies (Diptera: Psychodidae, Phlebotominae) are the subject of far more research than might be expected based on their relatively small size. Natural transmission of leishmaniasis may be zoonotic or anthroponotic, and it is usually by the bite of a phlebotomine sand fly species of the genera *Phlebotomus* (Old World) and *Lutzomyia* (New World) (Umakant & Sarman, 2008). Morphologically these species resemble very closely with each other.

These species are principally present in the warm zones of Asia, Africa, Australia, southern Europe and the Americas (Maroli *et al.*, 2012). In most cases, the vector adapts easily to the peridomestic conditions of impoverished areas, exploiting the accumulation of organic matter produced by domestic animals and poor sanitary conditions (Sthenia *et al.*, 2009). They are the subject of far more research than might be expected because females are the only proven natural vectors of *Leishmania* species (Kinetoplastida: Trypanosomatidae), the parasitic protozoans that are the causative agents of the neglected tropical disease leishmaniasis (Bates *et al.*, 2015).

About 30 species of sand flies are proven vectors of at least 20 *Leishmania* species. Sand flies in the genus *Phlebotomus* are vectors of a bacterium (*Bartonella bacilliformis*) that causes Carrion's disease (orocho fever) in South America. In parts of Asia and North Africa, they spread a viral agent pappataci virus that causes sand fly fever (pappataci fever) as well as protozoan pathogens (*Leishmania* spp.) that causes leishmaniasis (Umakant and Sarman, 2008). Additionally, sand flies have been reported to be involved in the transmission of arboviral infection and suspected in the transmission of Chandipura virus (Kumar *et al.*, 2012).

The sand fly characteristically feeds at dusk, and, being a weak flier, tends to remain close to its breeding area, not too high from the ground. Different species have different feeding and resting patterns of behaviour. These different characteristics are important in formulating control strategies (Piscopo & Mallia, 2006).

Only 30 of the over 500 species of phlebotomine sand flies are known to transmit *Leishmania* parasites (Table 2.1). These include *P. argentipes* on the Indian sub-continent, *P. duboscqi*, *P. martini* and *P. orientalis* in Africa and the Mediterranean basin, *P. chinensis* and *P. alexandri* in china. In the new world *L. longipalpis* is the only known vector of *L. chagasi* (Murray *et al.*, 2005).

Table 2.1: Human pathogenic species of *Leishmania* and their vectors in Old World (Umakant and Sarman, 2008)

Sand fly vector	Geographical distribution	<i>Leishmania</i> transmitted	Disease form
<i>P. papatasi</i> , <i>P. duboscqi</i> , <i>P. salehi</i>	North Africa, central and West Asia	<i>L. major</i>	Rural, zoonotic, cutaneous leishmaniasis, or oriental sore
<i>P. sergenti</i>	Central & west Asia and western India	<i>L. tropica</i>	Urban, anthroponotic cutaneous oriental sore
<i>P. longipes</i> , <i>P. pedifer</i>	Ethiopia and Kenya	<i>L. aethiopica</i>	Cutaneous leishmaniasis, diffuse
<i>P. argentipes</i> , <i>P. orientalis</i> , <i>P. martini</i>	India, Nepal, Bangladesh and east Africa	<i>L. donovani</i>	Visceral leishmaniasis, kala-azar, post-kala-azar dermal leishmaniasis
<i>P. ariasi</i> , <i>P. perniciosus</i>	Mediterranean basin, central and west Asia	<i>L. infantum</i>	Infantile visceral leishmaniasis
<i>Lu. flaviscutellata</i> , <i>Lu. longipalpis</i>	Brazil	<i>L. (L.) amazonensis</i> , <i>L. (V.) braziliensis</i>	CL, ADCL, MCL & VL CL, MCL

Phlebotomus martini and *P. celiae* are associated with the presence of termite mounds, soil moisture and a prolonged wet season while *P. orientalis* prefers drier habitats and is the main man-biter in Acacia-Balanites forests in Sudan and Ethiopia (Sadlova *et al.*, 2013). Sand flies live in rodent burrows, crevices, holes in river banks, trees and houses in the Old World while in the New World sand flies also dwell in the tree canopies and forest litter (Arfan & Simeen, 2008).

In the European region, the main proven and suspected vectors for VL and CL due to *L. infantum* include *Ph. alexandri*, *Ph. kandelakii*, *Ph. balcanicus*, *Ph. turanicus*, *Ph. halepensis*, *Ph. syriacus*, *Ph. longiductus*, *Ph. perfiliewi*, *Ph. perniciosus*, *Ph. ariasi*, *Ph. tobbi*, *Ph. transcaucasicus* and *Ph. neglectus* (WHO, 2014). Most of them are proven vectors of leishmaniasis and several phleboviruses. In Italy, eight sand fly species have been described; seven of which belong to the genus *Phlebotomus* and one to the genus *Sergentomyia* (Veronesi *et al.*, 2007).

Phlebotomus papatasi Scopoli occurs in a broad swath from France across most of the Mediterranean Basin, and eastward to India, including the Arabian Peninsula and Ethiopia. In Panama and Colombia, *Lutzomyia trapidoi* and *Lu. panamensis* are the proven vectors of *Le. (V) Panamensis*. Other suspected vectors such as *Lu. gomezi*, *Lu. ylephiletor*, *Lu. sanguinaria* and *Lu. carrerai thula* are very abundant and highly aggressive anthropophilic species (Dutari & Loaiza, 2014).

In Iraq, vectors for leishmaniasis belong to three species of *Phlebotomus*: *P. alexandri*, *P. papatasi*, and *P. sergenti* (Stoops *et al.*, 2013). These sand flies are most active on warm, clear nights with little wind. *P. sergenti* Parrot is one of the most widely distributed sand fly species in the Old World, ranging from Portugal and the Mediterranean Basin eastward to Ethiopia, the Arabian Peninsula, and India.

Fauna of Indian sub-zone is represented by 46 species, of these; 11 belong to Phlebotomine species and 35 to *sergentomyia* species. *P. argentipes* is the proved vector of kala-azar in India (Kishore *et al.*, 2006). *P. argentipes* occurs in Nepal,

India, Sri Lanka and Pakistan eastward to Myanmar, Thailand, Vietnam, Laos and Indonesia.

In Africa, much of leishmaniasis is concentrated in Eastern Africa where *L. donovani* is endemic in parts of Sudan, Somalia, Ethiopia, Kenya and Uganda, and causes 4000 deaths annually, with 385 000 DALYs lost ((Subathra *et al.*, 2014).). *P. martini* Parrot is found in Ethiopia, Kenya, Sudan, Somalia, and Uganda. *P. orientalis* Parrot is distributed in Sahel region of Africa from Niger to Egypt; Ethiopia, Kenya, Rwanda, Uganda; Saudi Arabia and Yemen on the Arabian Peninsula. The sand fly *Phlebotomus (Larroussius) orientalis* Parrot is the main vector of VL in Sudan and in Northern Ethiopia where it is frequently associated with *Acacia seyal* and *Balanites aegyptiaca* woodlands growing in black cotton soils (Oscar *et al.*, 2013).

In Kenya, *P. martini* and *P. orientalis* which transmit *L. donovani* are the main vectors for visceral leishmaniasis while *P. duboscqi* transmitting *L. major* is the vector for cutaneous leishmaniasis (Ngure *et al.*, 2009). *P. duboscqi* is mainly found in animal burrows where it feeds on rodents which are frequently infected with *Leishmania* parasites. *P. guggisbergi* has been identified as the vector for *L. aethiopia* which causes diffuse cutaneous leishmaniasis. DCL (diffuse cutaneous Leishmaniasis) has also been reported in Kenya, caused by *L. aethiopia*, with rodents as animal reservoirs and *P. pedifer* as the vector (Subathra *et al.*, 2014). *P. guggisbergi*, *P. aculeatus*, *P. celiae*, and *P. vansomeranae* are also vectors in Kenya.

2.6 Identification of sand fly species

Epidemiological studies on leishmaniasis often begin with vector identification, though taxonomic identification of adult insects is difficult. It has been estimated that there are currently 988 valid phlebotomine species and subspecies from all continents except Antarctica, including 29 fossils, with 512 extant and 17 fossil taxa found in the Americas (Bates *et al.*, 2015). They are grouped in the suborder Nematocera of the order Diptera, family Psychodidae, and sub-family Phlebotominae. Sand fly taxonomy is mainly based on the morphological and

anatomical characteristic features, which require dissection and mounting of freshly collected or preserved sand fly specimens (Kumar *et al.*, 2012).

Based on these features, some taxonomists have identified three genera of sand flies: *Phlebotomus*, *Sergentomyia* and *Chinius* that are widely accepted by modern Old World taxonomists. In the genus *Phlebotomus*, 11 subgenera, 96 species and 17 subspecies have been recognized by Lewis (Maroli *et al.*, 2012). Still, some authors place sand flies in the family Phlebotomidae while others retain them in the sub-family Phlebotominae of Psychodidae. However, there is no general agreement on the number of genera but five have been identified: *Phlebotomus*, *Sergentomyia*, *Lutzomyia*, *Warileyia* and *Brumptomyia*.

There are several methods of vector identification but experienced scientists can reliably identify sand fly specimens on the basis of external features and behaviour like colour, size and other characteristics (WHO, 2010). However, it is necessary to confirm identities with the aid of a compound microscope during dissections by observing the cibarial armatures, spermatheca and the pharynx (Abonnenc & Minter, 1965). Currently, the classification of phlebotomine sand flies remains controversial and cumbersome. At the same time, taxonomy of sand flies remain an integral component for understanding biodiversity, biology and behaviour of vector species of sand flies in order to plan appropriate intervention measures.

2.7 Reservoir hosts for *Leishmania*

Vertebrate animals, other than man, act as animal reservoirs. The infection is, therefore, mostly zoonotic, where man is an accidental host. There are, however, situations where man may be the reservoir and the cycle then is anthroponotic. Each species of *Leishmania* favors one or more animal reservoirs, except *L. donovani* and *L. tropica*, which are thought to be mainly, if not exclusively, anthropomorphic (Arfan and Simeen, 2008).

Leishmania is hosted by a large number of animals called the wild reservoir or the animal hosts, the vector sand fly is the intermediate host and domestic dogs and man

are domestic or accidental hosts. In anthroponotic (urban or dry type) cutaneous leishmaniasis, caused by *L. tropica*, the transmission is generally man-sand fly-man, although in some countries dogs, hyraxes and rats have been found to be infected with this parasite and these may be serving as the reservoir host. Therefore, dogs are the principle reservoir of these parasites and play a central role in the transmission cycle to man by phlebotomine sand flies (Mohammad & Mahbobeh, 2008).

In zoonotic cutaneous leishmaniasis (rural, wet type) caused by *L. major*, the rodent-sand fly-rodent cycle is maintained in wild rodent/gerbil colonies as sand flies breed in abundance in the cool and shady burrows (Rab *et al.*, 1986). *Rhombomys opimus*, *Meriones* spp. and *Psammomys obesus* are the three major reservoir species of the rodents that maintain infection in most of Central Asia, Middle East and North Africa. Others that are implicated in various parts of the world include dogs, cats, jackals, carnivores, fox, wolves, rats etc.

A number of rodent species have been implicated as the animal reservoir hosts. In Pakistan, these include *Meriones hurriane* or other species of gerbils, *Rhombomys opimus* and *Tatera indica* (Rab *et al.*, 1986). Other rodents that might be important in this respect include, *Meriones persicus*, *M. crassus*, *M. lybicus erythrorus* and *Mus musculus* (Arfan and Simeen, 2008). Dogs, cats, jackals, carnivores, fox, wolves and rats have been identified as reservoirs in other parts of the world. Identification and control of reservoir host has an immense influence on the epidemiology of the disease.

2.8 Sand fly surveillance

Sand fly surveillance is important because sand flies have been, and will continue to be, a major preventive medicine issue during military exercises and operations conducted within their geographic range (Armed Forces Pest Management Board, 2015). Vector surveillance also aims at detecting the presence of a vector in a given population or to determine vector abundance for estimating the risk of disease transmission (Alten *et al.*, 2015). Therefore, the presence of potential vector species illustrates the importance of maintaining and eventually extending the

surveillance to other regions in order to study the distribution and define areas at high risk of transmission (Veronesi *et al.*, 2007).

Suitable, accurate and cheaper epidemic prediction tools for predicting outbreaks of leishmaniasis and warning residents are needed. The three standard surveillance techniques for adult phlebotomine sand flies are human landing collections, sticky papers, and CDC light trap collections (Muller *et al.*, 2011). Currently, with increasing accessibility to new technologies like remote sensing and GIS, it has become possible to monitor land-use features on earth's surface over various time intervals to develop methods for rapid stratification of high susceptible areas and for the design of remedial measures (Kishore *et al.*, 2006).

Traps are mainly used in determining the presence of sand flies in an area, collecting specimens for identification, establishing baseline population density and determining effectiveness of control measures in surveillance. Studies of sand fly-parasite relationships, vector behaviour, ecology and taxonomy require appropriate sampling methods that can maintain the viability of the captured insects before processing them in the laboratory (Alexander, 2000). The commonly used traps include sticky traps, CDC light-traps, emergence traps, Shannon traps, human bait landing collection, human mouth aspirators on resting sites, household insecticide knockdown collection, and malaise traps (Yusuf, 2011).

Sampling methods exist for both the adult and the immature stages of sand flies; however, the ones for immature stages are rarely used because the habitat for the immature stages is unknown. Malaise traps are designed to catch flying insects, and consist of a tent-like nylon structure hung across trees or bushes in likely insect flight paths (Alten *et al.*, 2015). Sand flies and other insects that encounter it are forced to fly upwards then diverted outwards along conical extensions then funneled into transparent containers with killing agents like carbon tetrachloride or ethyl acetate (Alexander, 2000). This trap collects sand flies of both sexes. This method also allows for the detection of all sand fly species present within a given habitat, while

reducing sampling bias (Alten *et al.*, 2015). However, this trap catches insects of all sizes hence, the larger insects damages the smaller and fragile insects like sand flies.

Sticky traps are the most commonly used tools in the Old World for sampling sand flies by interception. The sticky trap is constructed from A4 white, plastic coated paper painted with castor oil as the adhesive (Gunter *et al.*, 2011). The trap is then placed in an area where sand flies are thought to be active, including man-made structures, fields, rock crevices, or at the openings of animal burrows and nests (Alexander 2000). Sticky traps have no known attractiveness to sand flies. However, they are generally used for determining species composition of an area as they randomly sample the species where they are set (Kasap *et al.*, 2009). They are also inexpensive and easy to manufacture in large numbers.

Further, castor-oil impregnated sticky traps can be used in productively dry areas like deserts and dry mountain valleys (Alexander, 2000). Several sticky traps can be hung at floor or ceiling level and used to sample intradomicilliary activities of sand flies. Sticky traps can also be rolled up and placed in holes within walls surrounding chicken coops, animal shelters, houses, or gardens (Asli & Bulent, 2011) to intercept exiting sand flies.

The Centre for Disease Control (CDC) light-trap is the most widely used trap in sand fly surveillance. It is a battery-operated light suction trap which has a photocell for activating it at night. Hence, this trap can stay overnight unattended (Volf & Volfova, 2011). Each CDC light trap can be baited with either carbon dioxide (CO₂) from combustion of butane gas (MMP), dry ice (CDC and BGS traps), light (MCU and S360), or dry ice and light (CDC) (Hoel *et al.*, 2010). CDC light traps are hung on a pole and placed 50cm above the ground (Gunter *et al.*, 2011). Although live sand flies can be recovered from light traps, they are likely to be stressed, hence it is advisable to kill and preserve them as soon as possible (Alexander, 2000). These traps are hampered by the fact that they attract only specific fly species, range of attraction is limited and specimens are often damaged and dead (Aten *et al.*, 2015). CDC light traps are also very expensive.

Mouth aspirators are used to sample sand flies from resting sites, like walls of houses, animal dwellings, caves, and tree holes. It consists of a tube sealed at one end by rubber, except for a narrow tube through which the sand flies enter. It is possible to collect live sand flies. This technique also helps in revealing where sand flies rest during the day, to aid control measures (Aten *et al.*, 2015). The problem with aspirators is that sand flies shed large numbers of hairs which are inhaled by the collector and can cause respiratory problems (Volf & Volfova, 2011).

Animal baited traps include Disney trap, the cone trap and animal-baited box trap. The trap consists of a metal tray in the centre of which is placed a small cage containing the animal which could either be cattle, goat, rodent, chicken, duck or dog (Alten *et al.*, 2015). The tray is then coated with castor oil and the whole apparatus is covered by a canvas or plastic roof to keep off the rain. This trap operates on the principle that sand flies usually do not land on the host directly but alight on a nearby surface and approach the animal in a series of short hops (Alexander, 2000). Therefore, sand flies approaching the bait animal are trapped in the sticky castor oil. Their advantage is that there is large collection of live sand flies; however, the trap is logistically heavy.

The cone trap uses large animals like horses or donkeys as baits which are tethered inside a large tent. This allows the animal to be seen as well as permitting the passage of stimuli like body heat, odour and CO₂. Sand flies are attracted to the bait animal and after blood feeding; they can be aspirated from the walls of the tent.

During the study that investigated the efficacy of different sampling methods of sand flies in Iran, Hesam-Mohammadi *et al.*, (2014) found out that Disney trap and sticky traps exhibited the most productivity than other traps. Further, a previous study conducted in Jordan using four different sampling methods showed that sticky traps with chemical light caught even more individuals and species than the CDC traps with light (Veronesi *et al.*, 2007). Further research has shown that traps baited with chicken and goat attracts more phlebotomine sand flies than other baits (Kasili *et al.*, 2009). Both sticky traps and animal baited traps use castor oil as the component which sticks attracted sand flies. Due to this, it was found necessary to

combine the principles of animal baited traps and sticky traps to come up with a modified trap using animal attractants as the only baits in trapping sand flies in the field.

2.9 The biology and life cycle of *Leishmania*

Leishmania is a protozoon that is capable of infecting animals, humans and rodents. There are at least 20 species of *Leishmania* each causing a disease specific to the species and the host response (Piscopo & Mallia, 2006). *Leishmania* parasites have a digenetic life cycle with an extracellular developmental stage in the female phlebotomine sand fly and a developmental stage in mammals, which is mostly intracellular (Roberts, 2006). In 1903, Leishman and Donovan separately described the protozoan now called *Leishmania donovani* in splenic tissue from VL patients in India.

Amastigotes are small, round to oval, bodies which measure about 2-5 μ m and found only in the macrophages of infected vertebrate hosts. The promastigote forms are seen in the culture media and in the gut of sand flies, mosquitoes and bugs but it is only in the sand fly that the parasite reaches the buccal cavity which becomes the insect vector of the parasite (Arfan & Simeen, 2008).

Leishmania are obligate intracellular parasites existing in two morphologic forms: promastigote and amastigote. Promastigotes are found in digestive tract of sand fly and are long spindle-shaped with a single delicate flagellum (15-28 μ M long). The flagellum is attached to cytoplasmic organelle called, kinetoplast containing intertwined circular DNA (kDNA) molecules known as maxicircles and minicircles, which make up 5-10% of total DNA (Mishra *et al.*, 2011).

The small, round to oval bodies called amastigotes (2 - 3 μ M in length) are the non-infective *Leishmania* parasites occurring in monocytes, polymorphonuclear leucocytes or endothelial cells of vertebrates (hosts) while promastigotes represent the infective stage in sand fly (Rajeshree *et al.*, 2012). Promastigotes are transmitted by the sand fly to vertebrate hosts e.g. canines, marsupials, edentates and

rodents. The promastigotes are phagocytosed by host macrophages where they transform into non-motile, spherical, non-flagellated amastigotes (Nisha *et al.*, 2012).

Infection starts when female sand flies (*Phlebotomus* and *Lutzomyia* species) acquire *Leishmania* parasites when they feed on an infected mammalian host like canines, marsupials, edentates, and rodents. Development in the vector is initiated when female sand flies ingest blood containing macrophages infected with amastigotes (Dostálova & Volf, 2012), and their uptake by the blood feeding sand fly is assisted by the cutting action of the mouthparts. This tissue damage associated with the creation of the wound releases the skin macrophages and/or freed amastigotes into the pool of blood, and enables their subsequent uptake into the abdomen of the sand fly (Fig. 2.3).

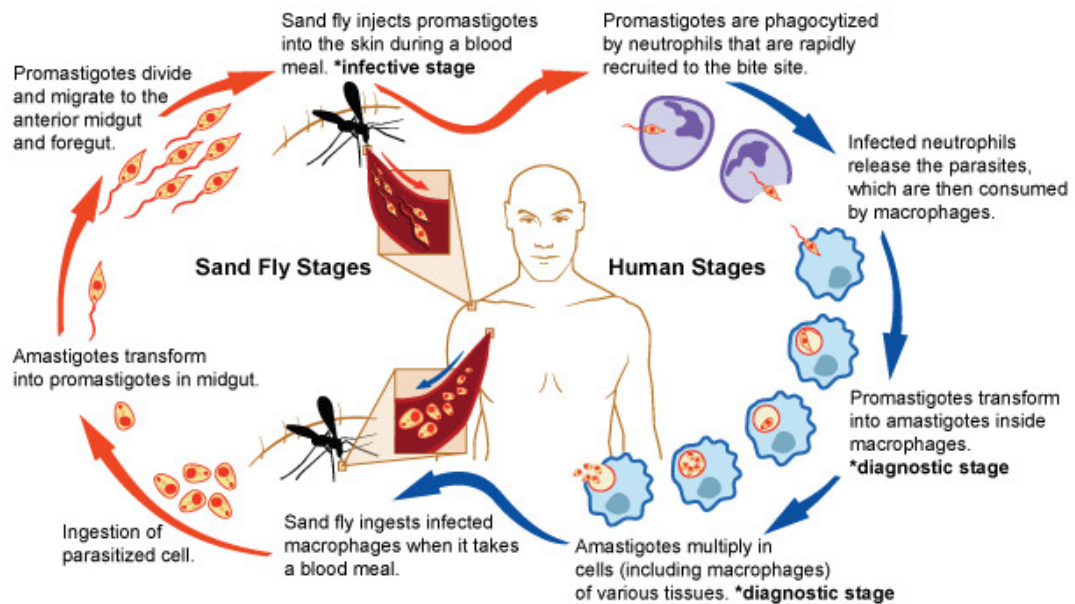


Figure 2.3: Life cycle of *Leishmania* species (Source: <http://www.niaid.nih.gov>)

The amastigotes transform into motile promastigotes with flagella beating at the anterior end. This first stage in the vector is called a procyclic promastigote which is a weakly motile, replicative form that multiplies in the blood meal. This initial “blood meal phase” is confined by the peritrophic matrix, a chitin and protein mesh secreted by the midgut epithelium that encloses the blood being digested within (Secundino *et al.*, 2005).

After a few days, the parasites begin to slow their replication and differentiate into elongate, strongly motile nectomonad promastigotes. These are migratory forms that accumulate at the anterior end of the peritrophic matrix and break out of the blood meal. They move towards the anterior midgut, some of them attaching to the microvilli of the midgut epithelium, until they reach the stomodeal valve (cardia) that guards the junction between foregut and midgut (Ramalho-Ortigao *et al.*, 2005).

Once they reach the stomodeal valve the nectomonad promastigotes transform into haptomonad promastigotes, shorter forms that resume replication (Gossage *et al.*, 2003). Finally, haptomonads differentiate into metacyclic promastigotes (Rogers *et al.*, 2002), which are the mammal-infective stages. These are deposited in the skin of a new mammalian host when the fly takes another blood meal, leading to the transmission of disease.

2.10 Biology and life cycle of sand flies

Leishmania parasites are transmitted by phlebotomine sand flies which are widely distributed in the tropics and other warm mainland areas and extend northwards to latitudes in the region of 50⁰ N (Arfan & Simeen, 2008). They are small, fragile, nocturnally active insects with weak direct flight capabilities (Alexander, 2000). Male and female sand flies feed on nectar and sap from plants including honeydew from aphids and other homopterans, nectar from flowers and fruits, and other plant juices. However, females are blood feeders generally active at dusk and throughout the night and in their search for blood they cover a radius of a few to several hundred meters around its habitat (Sharma & Singh, 2008).

Feeding and resting patterns may differ between sand fly species, but typically they feed close to their breeding grounds and mostly between dusk and dawn (Malaria consortium, 2010). They are considered “pool” feeders, and their bite is relatively painful, irritative and often causes red spots that may blister. There are some 600 species in five genera within the subfamily *Phlebotominae*. Species in three genera, *Phlebotomus*, *Lutzomyia* and *Sergentomyia*, suck blood from vertebrates; only the former two transmit disease to man (Arfan & Simeen, 2008).

Adult phlebotomine sand flies are 2-5 mm long. The body and the small wings are very hairy in appearance. They have relatively large eyes and relatively long and stilt like legs. At rest, the wings are held nearly erect and in a characteristic upright V-formation (Umakant & Sarman, 2008). They show nocturnal activity and have a characteristic hopping type of flights. Adults are weak fliers and do not usually disperse more than a few hundred meters from their breeding places.

Sand flies live in termite mounds, rodent burrows, rock crevices, holes in river banks, trees, tree holes and houses in the Old World while in the New World sand flies also dwell in the tree canopies and forest litter (Adebayo *et al.*, 2013). Only females bite since they need several blood meals before they can lay eggs. They bite especially at night and dusk; there are exceptions to this such as *Lutzomyia wellcomei*, which bites mainly during day time. They have short mouthparts and are pool feeders (Umkant & Sarman, 2008). They can suck blood both from animals (cats, dogs, various rodents, cattle, birds and lizards) and human.

Adult sand flies shelter during the day in dark, humid places like termite mounds, tree holes, animal burrows or under rocks. Phlebotomine sand flies undergo complete metamorphosis through four developmental stages: egg; larva (four instars); pupa and adult (Maroli *et al.*, 2012). Eggs are laid in terrestrial microhabitats rich in organic matter that provides food for larvae (Alexander, 2000). Eggs will hatch between 7-11 days emerging as larva which feed on dead organic matter present in the breeding site. Larvae are small and caterpillar-like with a well developed head capsule and numerous brush-like setae on the body, and long caudal setae that are nearly as long as the body (Armed Forces Pest Management Board, 2015).

Larvae molt and undergo development to second, third and fourth instar stages. Larvae are caterpillar-shaped with head capsules and small leaf-like antennae. They have long caudal setae that can help in their identification as sand fly larvae (Maroli *et al.*, 2012). In the 4th instar, larvae bear a darker sclerotized plate on the dorsum of the last abdominal segment.

The fourth instar larvae pupate and adults emerge and immediately look for a good blood meal (Fig. 2.4). Mating occurs at the site of emergence. The larval duration

may take up to 18 days while the pupal stage usually lasts 7-12 days; males usually emerge before females (Armed Forces Pest Management Board, 2015). This life cycle could take 30-45 days depending on climatic conditions.

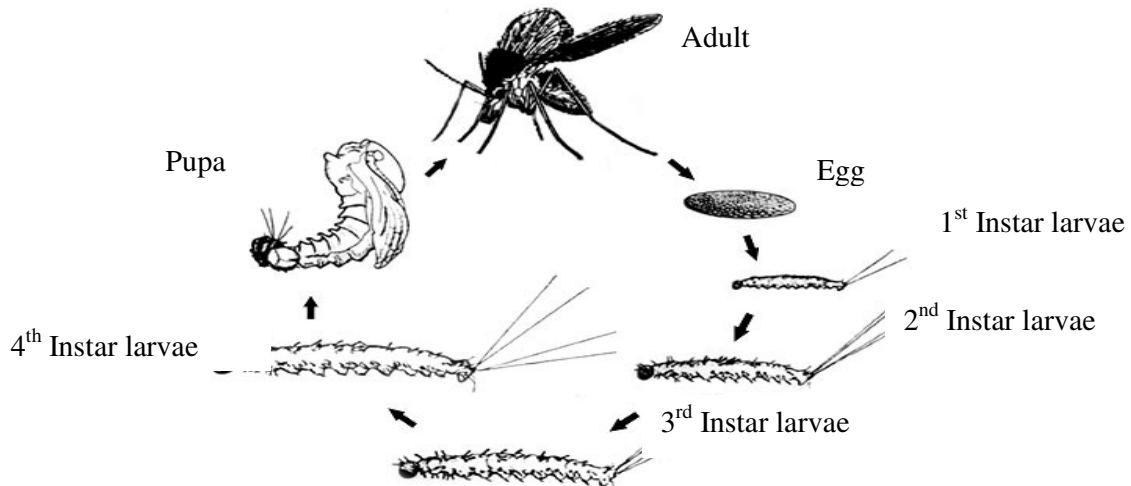


Figure 2.4: Life cycle of a phlebotomine sand fly

(Source: http://pcwww.liv.ac.uk/leishmania/life_cycle__habitats.htm)

2.11 Control and treatment of leishmaniasis

2.11.1 Chemotherapy

Current treatment options are very limited; most are expensive and problematic due to the issues of resistance, toxicity and side-effects. Since anti-leishmanial vaccines are still being developed, the current control strategies for leishmaniasis rely on case management. However, case management is difficult to be conducted since it is restricted by factors like lack of access to affordable, active drugs, incorrect prescribing and poor compliance (Assimina *et al.*, 2008).

Pentavalent antimonials are the first-choice drugs for leishmaniasis worldwide. The recommended therapeutic dose is 20 mg per kg of body weight for 20–30 days (Nisha *et al.*, 2012). Sitamaquine is an oral drug which is administered at a rate of 2

mg/kg daily for 28 days in Brazil, 1.7 mg/kg daily for 28 days in Kenya and 1.75 mg/kg daily for 28 days in India (Croft *et al.*, 2006).

Miltefosine, Paromomycin and liposomal Amphotericin B are gradually replacing antimonials and conventional Amphotericin B in some regions, especially where there is drug resistance or the need to develop combination therapy to prevent the emergence of resistance to new drugs (Ready, 2010).

However these drugs have drawbacks such as serious side effects, long courses of treatment and rampant drug resistance especially of the antimonials (WHO, 2007). Toxicity and the cost of the drugs is often prohibitively high (Malaria Consortium 2010). Pentostam® (sodium stibogluconate) and Glucantime® (meglumine antimoniate), are equally costly with treatment costing approx 200 USD per patients (Mishra *et al.*, 2011). Some compounds belonging to the imidazole group and triazoles like fluconazole, itraconazole and ketoconazole have been shown to possess anti-leishmanial activity. However, despite the numerous experimental pharmaceuticals available in the market, a prevalent cure for the disease is difficult to establish due to the variability of the pathogen with region (Subathra *et al.*, 2014).

Leishmania-HIV co-infection has been globally controlled in Southern Europe since 1997 by highly active anti retroviral therapy (HAART), but it appears to be an increasing problem in other countries such as Ethiopia, Sudan, Brazil or India where both infections are becoming more and more prevalent (Mishra *et al.*, 2011) and antiretroviral drugs are proving to be ineffective.

Currently, several limitations have decreased the use of antimonials: the variable efficacy against CL and VL, the emergence of significant resistance and the long course treatment which allows anti-leishmanial levels of the drug to accumulate in tissues, particularly in liver and spleen (Monzote, 2009). Therefore, the current chemotherapy scenario urges for more efficient and secure anti-leishmanial treatments, encouraging the search for bioactive compounds such as those from

natural origin. Due to this, plant based natural products are currently representing a promising class of drug candidates against leishmaniasis and sand flies (Igor *et al.*, 2015).

2.11.2 Use of immunomodulators in leishmaniasis treatment

Immunomodulators have also been used against *Leishmania* parasites. This is because the cure of leishmaniasis appears to be dependent upon the development of an effective immune response that activates macrophages to produce toxic nitrogen and oxygen metabolites to kill the intracellular amastigotes. Use of human IFN as adjunct antimony therapies for VL and Amphotericin B in conjunction of IL-12 or IL-10 have shown promising results against *Leishmania* parasites (Rajeshree *et al.*, 2012).

Recently, tucaresol (a Schiff base forming compound which enhances TH1 response and the production of IL-12 and IFN) has been shown to have activity against infection caused by *L. donovani* in BALB/c mice and C57BL/6 at a dose of 5 mg/Kg (Monzote, 2009). Imiquimod is an immunomodulator which stimulates a local immune response at the site of application and this in turn resolves the infection. This drug also induces the production of cytokines and nitric oxide in macrophages and has been shown to have an effect in experimental infections of cutaneous leishmaniasis (Croft *et al.*, 2006). However, the price of immunomodulators is exorbitantly high for the poor population.

2.11.3 Natural products in treating leishmaniasis

The leishmanial research has made significant progress during the recent years, however; a safe, effective, inexpensive, and true anti-leishmanial drug is still missing (Nisha *et al.*, 2012). Due to the limitations of first and second line drugs for leishmaniasis, natural products have shown a safe way to get a true and potentially rich source of drug candidates against leishmaniasis (Rajeshree *et al.*, 2012). Natural products are potential sources of new and selective agents for the treatment of neglected tropical diseases especially protozoan parasites.

The design of specific inhibitors from natural products has been explored as a possible means for controlling the parasites' growth without damaging the host. As a

result, several synthetic products have demonstrated their anti-leishmanial potentialities. For example: azasterols are inhibitors of 24-methyltransferase, have showed activity against promastigotes of *L. donovani* and axenic amastigotes of *L. amazonensis*; edelfosine and ilmofosine, new alkyllysophospholipid derivatives, have demonstrated high *In vitro* activity against *L. donovani* promastigotes and amastigotes (Rajeshree *et al.*, 2012). The synthetic products have been considered successful with many benefits like cost, time of abstention, novelty and scale-up and low intellectual property complications being realized.

Herbal remedies have been used for long period of time in both traditional and modern medicine around the world, where different plants of medical value are traditionally used worldwide for treatment of leishmaniasis (Sharquie *et al.*, 2016). Historically, many medicinal plants i.e. *Cinchona calisaya* (bark), *Strychnos pseudoquina* (bark), *Deianira erubescens* (roots and leaves) and *Remijia ferruginea* (bark) have been used against different parasitic diseases (Mishra *et al.*, 2011). For example, in 1970s, artemisinin, an important antimalarial drug was identified from traditional Chinese medicine *Artemisia annua* and since then many artemisinin derivatives were prepared and evaluated in various pre-clinical and clinical trials to use for the treatment of malaria.

Loranthus europaeus is one species of Loranthaceae, and many studies showed that it contains many biological compounds for example alkaloids whose *in vitro* studies have shown anti-leishmanial activity. Specifically, the plant was found to play a role in interference of the parasite's iron metabolism hence showing leishmanicidal action (Sen *et al.*, 2008). Quercetin also induces the production of reactive oxygen species (ROS), that lead to mitochondrial dysfunction and ultimately causing leishmanial parasite's death (Sharquie *et al.*, 2016).

Methanol extracts of *Tridax procumbens* L. (the whole plant) have been reported to exhibit significant leishmanicidal activity. *In vitro* studies on the activity of *Tridax procumbens* extracts against promastigotes of *L. mexicana* revealed significant activity (Adebayo *et al.*, 2013) with 50% inhibitory concentration (IC₅₀) of 3µg/ml.

In Bolivia, the plant evanta (*Angostura longiflora*) is being used for the treatment of leishmaniasis and other parasitic diseases. On top of having direct activity against *L. braziliensis*, its extracts also interfere with the activation of both mouse and human T cells (Igor *et al.*, 2015).

Many *in vitro* studies about natural products for the treatment of leishmaniasis have been conducted with excellent activity against *Leishmania* parasite. The most common bioactive natural compounds are: Alkaloids, quinones, terpenes (monoterpenes and triterpenes), saponins, flavonoids especially quercetin and phenolic compounds; all with anti-leishmanial activity (Sharquie *et al.*, 2016). However, due to lack of serious interest, none of them has undergone clinical evaluation (because leishmaniasis is a NTD).

2.12 Control of phlebotomine sand flies

Vector targeted strategies are particularly attractive, since the vectorial capacity to transmit infectious diseases to humans is related to vector density and in an exponential way, to vector survival (Umkant & Sarman, 2008). The main control measures for phlebotomine sand flies include environmental management, residual insecticide application, insecticidal plants and bioinsecticides. In the zoonotic foci where carriers are involved and dogs are the main vertebrate hosts, the effective methods of controlling sand flies will be the destruction of dogs and environmental management (Kishore *et al.*, 2006).

An effective strategy for reducing human leishmaniasis is to control sand fly vectors, especially in domestic and peridomestic transmission habitats (WHO, 2014). The growing incidence of resistance for the generic pentavalent antimony complex for treatment of leishmaniasis in endemic and non-endemic regions has seriously hampered their use. As a result, vector control remains a key component of many anti-leishmaniasis programs and probably will remain so until an effective vaccine becomes available (Claborn, 2010). However, sand fly control programmes in most VL foci have advanced slowly when compared to that of other haematophagous

arthropods like mosquitoes, ticks and black flies. Leishmaniasis experts advocate for vector control especially for areas of anthroponotic transmission (Hailu *et al.*, 2005).

Measures employed include spraying houses with insecticides where sand flies are endophilic and using treated and untreated bed nets where sand flies are endophagic (Piscopo & Mallia, 2006). Personal protection using repellents and nets is an important aspect. In endemic areas, spraying with dichlorodiphenyltrichloroethane (DDT) and other residual insecticides is effective in sand fly control (Conjivaram & Ruchir, 2007).

In the zoonotic foci where carriers are involved and dogs are the main vertebrate host, the effective methods include destruction of dogs and elimination of sand flies by environmental and chemical control (WHO, 2005). Control measures are aimed at reducing sand fly populations and man-vector contact by the use of residual spraying in houses and animal shelters, insecticide treated nets for human use, repellents applied on people's skin exposed to sand fly bites, and topical application of insecticides on dogs for prevention of canine leishmaniasis (Maroli, 2012).

Insecticides pose adverse effects to the user and the environment. The effectiveness of spraying programmes is not the only issue of concern but their side effects are also important on health and environment, and their potential for sustainability, which depends on the cost of the insecticides and their application. Sand flies have also developed resistance to the chemicals, mainly to DDT and in some cases to Malathion and pyrethroids (Umkant & Sarman, 2008). The use of pheromones is another strategy of controlling insect vectors. This property of pheromones is yet to be exploited in the control of vectors of leishmaniasis.

2.12.1 Environmental management

The principle of environmental management is to make it unsuitable for the vector to live and reproduce (Kishore *et al* 2006). Measures used include pruning trees in order to increase sunshine, thereby decreasing soil shading and preventing favorable conditions (temperature and humidity) for the development of sand fly larvae (Ministerio, 2007) and modification of their ideal developmental habitats. In technological control of sand flies, the walls of the resting sites can be plastered

filling all the cracks and crevices by mud and lime, and the breeding of sand flies can be stopped. This reduced sand fly density, but cracks and crevices reappeared within seven months (Umkant & Sarman, 2008). Environmental management also involves relocation of human settlements away from sand fly habitats and physical modification of the habitats hence disrupting their daily activities.

2.12.2 Residual insecticide application

The level of insecticide effectiveness depends on the class of insecticide used, the susceptibility of sand flies to the insecticide, the type of surface treated, the dosage and method of application and overall coverage (WHO, 2010). Insecticides that have been used in controlling sand flies include products like organochlorines, organophosphates, carbamates and synthetic pyrethroids (Umkant & Sarman, 2008).

Dichlorodiphenyltrichloroethane (DDT) still remains the insecticide of choice because of its low cost, high efficacy, long residual action and relative safety when used for indoor residual spray (Kishore *et al.*, 2006). However, resistance to the organochlorine DDT has been reported (WHO, 2010). The newer pyrethroid insecticides have provided several new potential chemicals for use as residual sprays. For example, a 90% reduction in sand fly populations was achieved following treatment of the termite mounds and animal burrows with pyrethroids. Unfortunately, the reduction lasted for 2 weeks only (Claborn, 2010).

Residual sprays are also limited by several environmental factors including high summer temperatures, strong radiation and the accumulation of dust. These conditions can reduce the toxicity of insecticides (Claborn, 2010). It has also been shown that residual spraying is much more effective in urban situations when every house and animal shelter is treated than in rural areas where relatively few dispersed houses are sprayed and the sand flies represent a small proportion of the vector population (Assimina *et al.*, 2008). Because the breeding sites of sand flies are generally unknown, control measures that act specifically against sand fly larvae are not feasible.

2.12.3 Prophylactic methods

These include self protection by use of mosquito nets and repellents. The use of insect repellents like DEET (Diethyl-toluamide) or protective clothing has been used as a prophylactic measure against leishmaniasis. Insecticide-treated nets are considered to be effective, relatively cheap and a sustainable method for sand fly control (WHO, 2010).

However, studies have revealed that impregnated clothing to protect humans from sand fly vectors may be impractical because the use of ITNs is not effective in preventing VL transmission in Kenya (Tonui, 2006). The use of dog collars impregnated with pyrethroid insecticides in Iran reduced the incidence of visceral leishmaniasis in children although this technique led to many operational problems, such as loss of collars (WHO, 2010). People living in highly endemic foci should use personal protective measures to avoid bites by sand fly vectors of leishmaniasis.

Application of mustard oil alone exposed to the uncovered areas acts as repellent against *P. argentipes*. It has also been found that 0.1 per cent allethrin (in coil) and 1.6 per cent prallethrin in liquid form can give maximum protection against the bite of *P. argentipes* (Kishore *et al.*, 2006).

2.12.4 Bioinsecticides

The indiscriminate use of insecticides induces insect resistance and disrupts food chains, affecting the population density of non-target species. These factors, associated with the many diseases transmitted by dipterans, calls for the development of new control strategies one of them being biological control. However very scanty information is available on the use of biological agents in controlling sand flies.

Bacillus thuringiensis var. *israelensis* or *Bacillus sphaericus* have been used against sand flies. They are currently being used to control mosquito larvae and black flies (Sthenia *et al.*, 2009). *Bacillus sphaericus* was successfully used in the control of *P. martini* in Kenya where inhibitory effect of *B. sphaericus* on hatching of eggs of *P.*

duboscqi was observed (Umkant & Sarman, 2008). However, the application of biolarvicides in the field is difficult due to diverse breeding habitat of sand fly (Kishore *et al.*, 2006).

2.12.5 Role of pheromones

Pheromones are chemical substances which help in attracting the insects at a particular site for mating. This property of pheromones is yet to be exploited in the control of vector of leishmaniasis and scanty information on their use is available. Some workers have started efforts to explore the role of synthetic pheromones as a potential sand fly control strategy (Umkant & Sarman, 2008). Oviposition and sex pheromones and stimulants may offer potential for exploitation through monitoring or control traps however little interest has been shown in their development (Hamilton, 2008). Such pheromones could offer the possibility for the development of monitoring and control traps for sand flies.

Sex pheromones and oviposition pheromones in combination with host odour can attract females to mating aggregations formed on or above animals at dusk (Bray *et al.*, 2014). Therefore, if the synthetic sex pheromones could attract blood-feeding females away from feeding on people towards insecticide-treated areas where they can be killed then, the effectiveness of insecticide spraying in animal houses as a means of managing local sand fly populations can be increased. In the long run, the use of such pheromone lures may exert a negative pressure on population fecundity for a long period.

2.12.6 Plant crude extracts in controlling vectors

Synthetic chemical pesticides have been the most widely adopted method for controlling insect pests. Dichlorodiphenyltrichloroethane and other synthetic pyrethroids have been the insecticides of choice in controlling many insect vectors for a long time. However, there are many problems associated with the extensive use of these compounds, such as build-up of pesticide resistance, negative impact on natural enemies, in addition to negative environmental and health impacts (Ramos-López *et al.*, 2010). Therefore, there is need for the development of new and safer products against sand flies.

The need for more efficient and safer methods to control insects has also stimulated the search for new insecticides in plants. Plants have been important sources of active compounds against insects, such as pyrethroids (pyrethrin and allethrin) and rotenoids. Crude plant extracts and inorganic larvicides were largely used as natural insecticides before the organic laboratory-synthesized insecticides became available in the 1940s (Diwakar *et al.*, 2014).

Currently, the environmental safety of an insecticide is of paramount importance, and botanical insecticides have been considered as suitable alternatives to synthetic insecticides, because they are generally pest specific and are relatively harmless to non-target organisms (Shyamapada, 2010). Plants are rich sources of natural substances that can be utilized in the development of environmentally safe methods for insect pest control (Kandagal & Khetagoudar, 2011).

Many of these plant extracts have been shown to affect insect growth and behaviour, acting as insect growth regulators, antifeedants and toxicants (Peta & Pathipati, 2011). Further, crude plant extracts often consist of complex mixtures of active compounds hence, they may show greater overall bioactivity compared to the individual constituents.

Plants have been an important source of active compounds against a wide range of insects. Some plant phytochemicals have a toxic effect on insect adults and larvae by interfering in their growth, development or reproduction (Rania *et al.*, 2014). The aerial parts of *R. communis* extract have been shown to possess insecticidal activity against *Callosobruchus chinensis*, *Cosmopolites sordidus*, *Culex pipiens*, *Aedes caspius*, *Culiseta longiareolata* and *Anopheles maculipennis* (Ramos-Lopez *et al.*, 2010). Other researchers have shown that *R. communis* seed extracts have larvicidal effects with 100% killing activities for *Culex quinquefasciatus*, *Anopheles stephensi* and *Aedes albopictus* larvae (Manpreet *et al.*, 2012).

Studies have shown that some phytochemicals have a toxic effect against insects (larvae and adults) by interfering with their growth, development and/or reproduction, or by producing attractive or repellent scents (IMCR Bulletin, 2003).

Luitgards-Moura *et al* (2002) tested the effects of *Antonia ovata* and *Derris amazonica* on adults of *Lu. Longipalpis*, observing death rates of 80% and 100%, respectively, 72 hours after exposure. Other findings show that *Solanum jasminoides*, *R. communis* and *Bougainvillea glabra*, are toxic for adult sand flies. These plants are used as sources of sugar by sand flies (Umkant & Sarman, 2008).

In many instances, natural products are the alternative for accessible treatments against parasitic diseases. Plant-based insecticides tend to have a broad-spectrum activity and comprise of botanical blends of chemical compounds which impact on both behavioural and physiological processes of insects (Diwakar *et al.*, 2015). Thus, there is little chance of pests developing resistance to such products. Unfortunately, most of them are hardly explored and their mechanism of action is unknown. Plants have a wide range of compounds with pharmacological effects which if explored extensively could provide safe insecticides for controlling sand flies hence prevention of leishmaniasis.

2.13 *Ricinus communis* morphology

2.13.1 Morphology and classification of *R. communis*

R. communis Linn. (Family: Euphorbiaceae) was selected because of its reported bioactive components which interfere with the life cycle of most insect pests (Amandeep & Jasneet, 2016; Sabina *et al.*, 2014; Tounou *et al.*, 2011). Studies of aerial parts of the plant have reported the presence of active compounds especially ricin.

Ricin is the most toxic bioactive component present in seeds but ricinine which is an effective insecticide is located in all parts of the plant (Amandeep & Jasneet, 2016). *R. communis* extracts also have insecticidal, antifeedent and repellent activities against a wide range of arthropod vectors. However, the information on its effects on sand flies is scanty showing that the effects of *R. communis* extracts on sand flies have not been extensively investigated.

R. communis is a tropical plant, known as castor bean, that is distributed widely across the world. It is a small wooden tree which grows to about 6

meters in height (Manpreet *et al.*, 2012). *R. communis* is very common along stream banks, river beds, bottom lands and just about any hot area where the soil is well drained and with sufficient nutrients and moisture to sustain the rigorous growth (Padma & Rupali, 2014). Currently, *R. communis* has been introduced and cultivated in many tropical and subtropical areas of the world sometimes appearing spontaneously.

Ricinus communis is mainly cultivated for leaf and flower colors and for oil production. There are two varieties of *R. communis* which are known: A perennial bushy plant with large fruits and large red seeds which yields about 40% of oil; and a much smaller annual shrub with small grey (white) seed having brown spots and yielding 37% of oil (Jitendra & Ashish, 2012).

Leaves are green or reddish in colour and about 30-60 cm in diameter, alternate, curved, cylindrical, purplish petioles, sub-peltate, drooping, stipules large, ovate, yellowish, united into a cap enclosing the buds, deciduous, blade 6-8 inches across, palmately cut for three quarters of its depth into 7-11 lanceolate, acute, coarsely serrate segments, smooth blue green, paler beneath, red and shining when young (Manpreet *et al.*, 2012) (Plate 2.1).



Plate 2.1: *Ricinus communis*

The stems are varying in pigmentation. The flowers are monoecious and about 30-60 cm long. The seeds are of considerable differences in size and colour. They are oval, somewhat compressed, 8-18 mm long and 4-12 mm broad (Asish *et al.*, 2014)

2.13.2 Uses of *R. communis*

Roots, leaves and seeds are extensively used in different ways. They have been used in the treatment of rheumatic arthritis, paralysis, epilepsy and distension of the uterus. The bark of castor plant is used in dressing ulcers and sores. Extracts from leaves and seed of *R. communis* L. have been used successfully in the management of agricultural pests by causing death through ingestion and contact and may have insectistatic properties (Cinthia *et al.*, 2012).

R. communis possess wound healing activity due to the active constituent of castor oil which produces antioxidant activity and inhibits lipid oxidation (Jitendra & Ashish, 2012). Some phytochemicals in *R. communis* have a toxic effect on adult insects and larvae by interfering with their growth, development and/or reproduction, or by producing attractive or repellent scents (Rania *et al.*, 2014). However, very few studies have been conducted to investigate the insecticidal activity of *R. communis* (Tounou *et al.*, 2011).

Castor seed is the source of castor oil, which has a wide variety of uses. The seeds contain between 40% and 60% oil that is rich in triglycerides, mainly ricinolein. The seed contains ricin, a toxin, which is also present in lower concentrations throughout the plant (Nidhi *et al.*, 2013). Ricin itself has also been evaluated as a potential treatment for cancer and AIDS (Elizabeth *et al.*, 2015). The castor oil obtained from the seeds is widely used traditionally and herbally as a medicine.

Ricin contained in *R. communis* is mainly used as a purgative and laxative. It is also used as a lubricant, lamp fuel, a component of cosmetics, and in the manufacture of soaps, printer's ink, plastics, fibers, hydraulic fluid, brake fluid, varnishes, paints, embalming fluid, textile dyes, leather finishes, adhesives, waxes, and fungicides (Jitendra and Ashish, 2012). Further, the seed is used as anodyne, antidote, bactericide, cathartic, cyanogenetic, discutient, emetic, emollient, expectorant, insecticide, lactagogue, larvicidal, laxative, poison, purgative, tonic and vermifuge (Padma & Rupali, 2014). Seeds are also used as fertilizers after extracting the oil from the seed and cooked to destroy the toxin and incorporated into animal feeds.

2.13.3 *Ricinus. communis* crude extracts and insecticidal properties

Stems of *R. communis* have Anticancer, antidiabetic, antiprotozoal and antibacterial activity (Manpreet *et al.*, 2012). It is also used for the treatment of hepatitis, skin and breast cancer in initial phase (Vandita *et al.*, 2013). The aerial parts of *R. communis* extract have been shown to possess insecticidal activity against *Callosobruchus*

chinensis, *Cosmopolites sordidus*, *Culex pipiens*, *Aedes caspius*, *Culiseta longiareolata* and *Anopheles maculipennis* (Ramos-Lopez *et al.*, 2010).

Further research shows that *R. communis* seed extract has larvicidal effects with 100 % killing activities for *Culex quinquefasciatus*, *Anopheles stephensi* and *Aedes albopictus* larvae (Manpreet *et al.*, 2012). *R. communis* also has antiasthmatic, anti-fertility, antihistaminic, antimicrobial, antidiabetic and wound healing activities (Jitendra & Ashish, 2012). Further, Tounou *et al.*, (2011) found that *R. communis* products have strong larvicidal effect on *P. xylostella*, with 100% mortality on 3rd instar larvae.

Castor bean plant contains ricin toxin, one of the most toxic and easily produced plant toxins worldwide. However, very few studies have been conducted to investigate the insecticidal activity of *R. communis*. It has been shown that aqueous extracts from *R. communis* leaves have high larvicidal activity against 2nd and 4th instar larvae of four mosquito species, *Culex pipiens* (L.), *Aedes caspius* (Pallas), *Culiseta longiareolata* (Aitken) and *Anopheles maculipennis* (Meigen). This high activity of *R. communis* crude extracts could be due to toxic effect of the plant (Tounou *et al.*, 2011).

The larvicidal activity of *R. communis* crude extracts can be attributed to its chemical compounds especially ricin and other compounds which are known to exhibit a wide range of biological activity on insect vectors. The bioactivity of *R. communis* extracts on ants revealed its efficacy in combating hymenopterans, aphids and different species of lice (Lafayette *et al.*, 2013). Sabina *et al.*, (2014) also reported that *Tithonia diversifolia* and *R. communis* had the most toxic extracts against adult *Anopheles gambiae* after 7 days of feeding, with LC₅₀ of 1.52 and 2.56 mg/mL respectively. *R. communis* extracts also exhibited the highest larvicidal activity against *A. gambiae* larvae after 72 h of exposure (LC₅₀ 0.18 mg/mL).

In studying the efficacy of crude extracts of *R. communis* against *Musca domestica*, considerable larval and pupal mortalities were recorded indicating the toxicity of plant extract against the fly (Amandeep & Jasneet, 2016). This plant has also been shown to affect *P. xylostella* adult emergence with 44–79% of adults having

deformed wings and legs (Tounou *et al.*, 2011). Recently, *R. communis* and other Euphorbiaceae like *Jatropha curcas* have gained interest as non-food oil seed trees for biofuel/biodiesel production (Sylvia *et al.*, 2011). *R. communis* is one of the most studied plant species with insecticidal properties against a wide range of insect pests. However, its activity against phlebotomine sand flies is unclear.

2.13.4 Bioactive compounds in *R. communis*

Castor bean plant has steroids, saponins, alkaloids, flavonoids and glycosides (Jitendra and Ashish, 2012). Seeds of castor bean plant contain castor oil, ricin, protein allergens and ricinine. Further, castor bean plant seed contains ricin toxin, one of the most toxic and easily produced plant toxins worldwide. *R. communis* agglutinin (RCA120) is also a much less toxic dimeric protein with high sequence identity to ricin present in the seed. However, ricin is a monomeric AB toxin of about 60 kDa formed by a covalently linked A- and B-subunits while *R. communis* agglutinin is a ~120 kDa homodimer of two A- and B-subunits (Sylvia *et al.*, 2011).

Earlier studies of aerial parts of this plant have reported the presence of ricinine, a low molecular weight alkaloid, which can be found in all parts of the plant and it is a quite strong insecticide (Sylvia *et al.*, 2011). N-demethylricinine and six flavonoids: glycosides kaempferol-3-*O*- β -D-xylopyranoside, quercetin-3-*O*- β -D-xylopyranoside, quercetin-3-*O*- β -D-lucopyranoside, kaempferol-3-*O*- β -rutinoside, quercetin-3-*O*- β -rutinoside, as well as gallic acid, gentistic acid and rutin (Ramos-Lopez *et al.*, 2010) and indole-3-acetic acid has been extracted from the roots while ricinine is present in the stem.

2.13.5 Toxicity of ricin and ricinine

Ricin is a heterodimeric protein from the seeds of *R. communis*. Ricin has cytotoxic activity because of its ability to enter the cell and fatally disrupt protein synthesis. A single molecule of ricin reaching the cytosol can kill the cell due to this property and it can be used to specifically target and destroy cancer cells (Manpreet *et al.*, 2012). Ricinine is another toxic compound present in *R. communis* plant. Ricinine is less toxic as compared to ricin but it causes hyperactivity, seizure and subsequent death due to respiratory arrest in mouse model experiments (Sylvia *et al.*, 2011).

Ricin, with its lectin subunit (B-chain), binds to oligosaccharide residues on the cell surface and undergoes endocytosis via clathrin-dependent and -independent mechanisms that are somewhat dependent on the cell type and polarization status studied (Sylvia *et al.*, 2011). *R. communis* seed is poisonous to people, animals and insects. However, if the seed is swallowed without chewing and there is no damage to the seed coat, it will pass harmlessly through the digestive tract (Padma & Rupali, 2014). Despite this, the seeds of *R. communis* have a long history as medical remedy in various fields.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study site

Field work of this study was carried out during the dry month of July 2014 in Marigat sub-County. Trapping of sand flies was carried out in Rabai, Marigat division, Baringo County. Identification of the captured sand flies was carried out at the Kenya Medical Research Institute, Centre for Biotechnology Research and Development (CBRD), Nairobi.

Baringo is the only region in Kenya where both VL and CL have been found together. It covers an area of approximately 10,000 km² and is located north of the equator in Kenya's Rift Valley Province. Marigat Township is central to this region and a site where most VL studies have been conducted in the past. Baringo County includes a range of landscapes varying between fertile highlands as high as 2,700 m elevation and semiarid lowland at about 900 m elevation. The Tugen hills and the adjacent highlands in the southwest receive 1,200–1,500 mm average yearly rainfall and have daily average temperatures ranging from 10°C to 32°C. In contrast, lowland areas to the west, east, and north of the Tugen Hills receive annual rainfall from 300 to 700 mm and the temperatures vary between 16°C and 42°C. The rainy season is from March to September, with maximum rainfall in May and August. Three main ethnic groups, all classified as agro-pastoralists, inhabit Baringo District: the Tugen, Pokot, and Njemps. The district is sparsely populated due to harsh physical and climatic conditions (Ryan *et al.*, 2006).

3.2 Study design

A comparative experimental design using several baits was used. Their effectiveness was based on the number of sand flies trapped. Efficacy of the different extracts was assessed based on their ability to inhibit larval metamorphosis and adult mortality. Trapping experiments were conducted using a Latin square design with 10 treatments rotated through 10 locations / sites over 10 nights of trapping. The traps were operated from late afternoon to the early morning of the following day (18:00

to 06:00) along different locations with a distance of approximately 150 m between any two trapping sites. The control experiment was set on the windward side and it involved a CDC light trap set at 100-150m away from the treatment area.

3.3 Experimental animals

Syrian golden hamsters were obtained from KEMRI's animal house facility. They were used for blood feeding the sand flies. They were anaesthetized using sodium pentobarbitone (Sagatal[®]) prior to blood feeding for 45 minutes. They were regularly shaved under the belly for convenient feeding. Blood feeding was done twice a week. Hamsters were fed on rat pellets throughout the experimental period. After the experiment, the hamsters were returned to the animal house.

3.4. Sand Fly Colony

A colony of *Phlebotomus duboscqi* Neveu Lemaire (Diptera: Psychodidae) was used for laboratory based experiments. This colony originated from sand flies collected in animal burrows and from termite hill ventilation shafts in Rabai area near the town of Marigat in Baringo County, Rift Valley province in Kenya. This colony is being reared at KEMRI insectaries for research purposes. The colony was established using periodically field captured sand flies and inbreeding according to the methods of Beach *et al.*, (1986). Female sand flies were fed on blood using Syrian golden hamsters for egg development. Blood feeding of sand flies involved anaesthetizing a hamster with sodium pentobarbitone, shaving its lower belly and introducing it into the cage containing sand flies. An equivalent number of males were included for purposes of copulation. Blood fed females were aspirated into rearing jars to lay eggs which were left to hatch.

Feeding of sand flies on hamsters was done at 2 weeks intervals to obtain the larval instars required for bioassays. Adult sand flies were reared at $25 \pm 1^{\circ}\text{C}$, and an average RH of 78-83%, 12:12 (L: D) photoperiod in Perspex insect rearing cages and were fed using slices of apples as a source of energy. Larvae were gently placed into rearing vials using a camel hair brush wetted in distilled water. They were fed on little amounts of larval food which was sprinkled into the vials. Temperature was

maintained at $25\pm 1^{\circ}\text{C}$ relative humidity of 78-83 % and a 12: 12 (light: dark) photoperiod.

3.5 Preparation of *Ricinus communis*

3.5.1. Collection of the plant materials

Stems and leaves of *R. communis* were collected from Suswa, Narok County, Kisii County and Juja, Kiambu County, Kenya and stored separately. Narok County is located on south of Rift Valley. It borders Nakuru, Bomet, Nyamira, Kisii, Kajiado, and Migori counties. It has an elevation of 1827 meters above sea level. It has a minimum of 8°C and a maximum of 28°C in temperature. The county receives two rainy seasons with an average rainfall ranging from 500 to 1800 mm per year. Soils are sandy loam with high contents of the entire major plant nutrients such as calcium and magnesium.

Kisii County is characterized by a hilly topography with several ridges and valleys. Seventy five percent of the county has red volcanic soils which are deep in organic matter. Kisii County exhibits a highland equatorial climate resulting into a bimodal rainfall pattern with average annual rainfall of 1500mm. The maximum temperatures in the county range between 21°C – 30°C while the minimum temperatures range between 15°C – 20°C . Kiambu County is located in central Kenya; it borders Murang'a, Machakos, Nairobi, Kajiado, Nakuru, and Nyandarua counties. Temperatures range from a minimum of 12.8°C to a maximum of 24.6°C with an average of 18.7°C . The average rainfall is 989mm per annum.

Botanical identification of *R. communis* was carried out with the help of taxonomists from the National Museums of Kenya. All the collected parts of the plants were left to dry completely under a shade for one month and then transported to the laboratory where they were left to dry further under room temperature.

3.5.2 Preparation of aqueous extracts of castor bean leaves and bark

The sample extraction procedure was carried out as described by Harborne (1994). Briefly, cold sequential extraction was carried out on plant material with analar grade organic solvents of increasing polarity. The solvents that were used include n-

hexane, dichloromethane, ethyl acetate, ethanol and water to have five different extracts. Six hundred millilitres of n-hexane were added to 300g of the ground plant materials and flasks placed on a shaker and soaked for 48 hours. The residue was filtered using a Buchner funnel under vacuum until the extract dried. The sample was soaked further with 600 ml of n-hexane for 24 h until the filtrate remained clear. The filtrate was then concentrated under vacuum by rotary evaporation at 30 - 35°C. The concentrate was later transferred to a sample bottle and dried under vacuum using a rotary evaporator; the weight of the dry extract was recorded and stored at 4°C until required for bioassay. The process was repeated for dichloromethane, ethyl acetate, ethanol and water successfully.

3.5.3 *R. communis* toxicity test

Ten *Phlebotomus duboscqi* flies were fed on sugar solution mixed with the crude extracts in the ratio of 1:1 of concentrations ranging from 1mg/ml to 20mg/ml of the test extracts. Mortality was assessed daily by counting dead flies in order to evaluate the minimum inhibition concentration (MIC). The lowest concentration of the samples that killed the sand flies was considered the MIC.

3.6 Effects of *R. communis* extracts on *Phlebotomus duboscqi* pre-emergent stages

3.6.1 Ovicidal effects of *Ricinus communis* crude extracts

Five blood-fed *P. duboscqi* females were aspirated into 7-dram plastic vials partially filled with plaster of Paris and fitted with screen tops. They were fed on drops of Karo dark corn sugar syrup (Best Foods, CPC International, Inc., Englewood Cliffs, NJ) placed on the screen tops of the oviposition vials. The set up was then observed for 7-10 days for egg laying. 125 µg/ml, 250 µg/ml and 500 µg/ml of *R. communis* extract were prepared for bioassays. A total of 130 freshly laid eggs were then moistened with 1 ml of 125µg/ml aqueous extract of *R. communis* on 0 day post-oviposition. Another group (the control) was moistened with 1ml distilled water. These eggs were then incubated at 25±2° C and 70±5% RH for hatching while being moistened with 1ml of 125 µg/ml extract daily. Two eggs from each vial were picked daily using a water-moistened applicator stick. They were then transferred to a

microscope slide where a drop of gum chloral was applied. The eggs were then covered with a cover slip and left to dry for one day. They were then observed using a light microscope for any morphological changes on the chorionic membrane. The numbers of eggs that hatched were recorded. The same experiment was repeated for methanol and ethyl acetate leaf and bark extracts of *R. communis* at 125 µg/ml, 250 µg/ml and 500 µg/ml concentrations. Percentage hatchability was calculated as follows using the formula of Khani *et al.*, (2013):

$$\% \text{ hatchability} = \frac{\text{Number of larvae hatched}}{\text{Total number of eggs}} \times 100$$

3.6.2 Larvicidal effects of *Ricinus communis* crude extracts

Larvicidal effects of *R. communis* were assessed according to the methods of Luitgards-Moura *et al.*, (2000). Briefly, after hatching, 1st instar larvae were put into four groups and treated as follows: first group of the larvae were fed on larval food mixed with the crude extract (1:1) at 125 µg/ml, 250 µg/ml and 500 µg/ml, second group was fed on larval food sprinkled with 5ml of the aqueous extract at 125 µg/ml, 250 µg/ml and 500 µg/ml, third group was fed on powdered extract only while the fourth group (control) consisted of larvae fed on larval food only. In each group, 150 larvae were used for bioassays. Larvae were observed daily for any deaths. Larvae that were motionless were considered dead. Any dead larvae were removed and mounted using gum chloral, left to dry for a day then examined for any morphological changes. These larvae were also examined for any transformation to second, third, fourth instars, pupae and adult emergence. The following parameters were evaluated: the length of the larval and pupal periods; the larval and pupal viability, Mean lethal dosage (LD₅₀) after every 24 hours was determined using the formula of Khani *et al.*, (2013).

$$\% \text{ Observed Mortality} = \frac{\text{Test Mortality} - \text{Control Mortality}}{100 - \text{Control Mortality}} \times 100$$

The experiment was stopped when all the larvae or pupae in the controls had died or emerged as adults.

3.7 Adulticidal effects of *Ricinus communis* extract

Insecticidal effects of the plant extracts were determined according to the methods of Moura *et al.*, (2002) with slight modifications. Thirty-five day old adult *P. duboscqi* were carefully aspirated into 7-dram plastic rearing jars partially filled with plaster of Paris and fitted with screen tops. 10% sucrose solution was used to prepare 125 µg/ml, 250 µg/ml and 500 µg/ml of 0.6 % *R. communis* extract by serial dilution and used in the feeding of the flies. Cotton wool pads were soaked in the preparations and placed on the screen tops. Two triplicate series with 10 flies each of *P. duboscqi* was used for each dilution. The first triplicate contained 30 females and the second triplicate had 30 males in each jar. 60 specimens were assayed for each dilution and gender. Sand flies that fed on 10% sucrose solution soaked in cotton wool pads and placed onto the screen tops were used as controls. Males and females were not nested together. Mean lethal concentration designated LC₅₀, was determined at 12, 24, 36, 48 and 72 h of exposure. The set up was maintained at 27±2°C, relative humidity of 78-83 % and a 12: 12 (light: dark) photoperiod. The experiment was stopped when all the flies in the controls had died.

3.8 Design of the trap

The trap consisted of two main components; a 5-liter plastic bottle and a sticky paper trap mounted inside the plastic bottle. The trap was constructed as previously described by Gunter *et al.*, 2011 with slight modifications. Briefly, the sticky trap was constructed from 10 x10 cm white cardboard sheet (with 0.1 cm wide netting separated by 0.3 cm square holes) covered with commercial castor oil as an adhesive. This trap was then mounted vertically inside a clear 5-liter plastic bottle. Three windows were cut in the sides of this plastic bottle. The windows were covered by the steel net to keep larger non-target insects out of the trap (plate 3.1). Below the windows is a reservoir for holding the bait.



Plate 3.1: Design of the trap

The baits used include defibrinated blood from cattle, goat, sheep and chicken; urine from cattle, sheep and goat. The fruits used include mango (*Mangifera indica* L.), banana (*Musa sapientum*), apple (*Malus domestica*) and grapes. The selection of these baits was based on past research which had shown that goat and chicken blood attract a lot of sand flies in the field (Kasili *et al.*, 2009; Mauricio *et al.*, 2010) when compared to other mammals. Ripe fruits also have a great potential in attracting sand flies (Amy *et al.*, 2011). Blood was obtained from slaughter houses (abattoirs) within Marigat area while fruits were bought from local markets.

Blood was obtained from a slaughter house and immediately defibrinated to prevent clotting. A sponge soaked in 0.5 liter of blood/urine (in case of fruits, 0.5kg of ripe or overripe fruits) was placed inside each trap. The baited trap was then hung on a strong stand fixed on the termite mound with the opening 50 cm above the ground (plate 3.2). To eliminate positional bias, traps were rotated clockwise each day. Insects drawn to the traps were collected promptly at 08:00 to prevent degradation. There were four controls. The first control trap was empty, the second trap had only water soaked sponge, the third trap had sponges soaked with fresh 10% sucrose solution and the fourth control was a CDC light trap.



Plate 3.2: Baiting and setting the traps: A shows experimental set up with traps around the termite mound, B shows defibrinated blood in petri dishes, C shows blood and urine in petri dishes ready for transfer into the traps, D shows a CDC light trap set at the entrance of a termite mound

3.9 Mounting and identification of trapped sand flies

The collected sand flies were transferred from the traps by using a camel hair brush wetted in distilled water. Each trap was individually numbered and notes recorded on collection date, the total number of sand flies collected, phlebotomine species composition, sex ratios, number of blood-fed females, and the number of gravid females. The collected sand flies were washed in 2% detergent solution to remove hairs and other debris. Thereafter, the flies were rinsed in phosphate buffered solution (PBS) then transferred to a microscope slide for dissection and mounting. Slides were made of head and genitalia for species confirmation of the *Phlebotomus* spp. The heads were excised and mounted using gum chloral on slides upside-down so as to expose the cibarium and pharynx. The mounted slides were covered with cover slips and allowed to dry on the bench for 1–2 days. Species identification was performed thereafter by observing the cibarial armatures, spermatheca and the pharynx using identification keys of Abonnenc and Minter (1965).

3.10 Ethical and Biosafety considerations

Approval to conduct this research was granted by KEMRI's ethical review committee, Scientific Steering Committee and Animal Care and Use Committee (Appendices 1 & 2). Syrian golden hamsters were injected using a standard 21 gauge needle while anaesthetizing them using 6% sodium pentobarbitone (Sagatal®). Dead hamsters were sterilized by dipping them in 70% ethanol then putting them into biohazard bags before transferring them to the incinerator. Precautionary measures included putting on protective gear while in the laboratory and field to avoid sand fly bites. Confidentiality of data was maintained by keeping the data in note books under key and lock, using passwords only known to the principle investigator and use of genuine soft wares to prevent data and soft ware crush.

3.11 Statistical analysis

Median survival times (MST) was calculated using SPSS version 17.0. The efficacy of the different treatments was compared using the final cumulative mortalities. Differences in mortality rates, rate of pupation, adult emergence, sex ratio, adult deformities and adult longevity and eggs per fly were analyzed by analysis of

variance (ANOVA) and means separated using Student-Newman-Keuls test (PROC MIXED procedure, SAS institute, 1997) and the probability level set at $P = 0.05$. Percentage data and ratios were arcsine-transformed before analysis.

Average larval mortality data was subjected to probit analysis for calculating LC₅₀. LC50 at 24hrs and 48 hrs post treatment were obtained using SPSS version 17.0.

$$\% \text{ Observed Mortality} = \frac{\text{Test Mortality} - \text{Control Mortality}}{100 - \text{Control Mortality}} \times 100$$

CHAPTER FOUR

RESULTS

4.1 Ovicidal effects of *R. communis* extract

4.1.1 Effect of methanol *R. communis* leaf extract on the egg

With 125 µg/ml of *R. communis* leaf extract from Suswa, Narok County, all the eggs were seen to have thin and smooth chorionic layer with very little sculpturing. This observation was made microscopically at 7 days post oviposition. The eggs were also swollen within the first 48 hours post treatment. At a concentration of 250 µg/ml of *R. communis* leaf extract, the exochorionic membrane of the egg was eroded. There was faint sculpturing of the egg hence loss of most of the exochorion layer. At 500 µg/ml, the egg lost its colour becoming more translucent and swollen compared to the control. Exochorion and mesochorion layers of the egg were eroded by the extract and a transverse line was seen on the egg representing the plastron (plate 4.1). The egg in the control vial was amber in colour, outer chorion was sculptured with a series of ridges cross-linked irregularly and both poles were rounded.

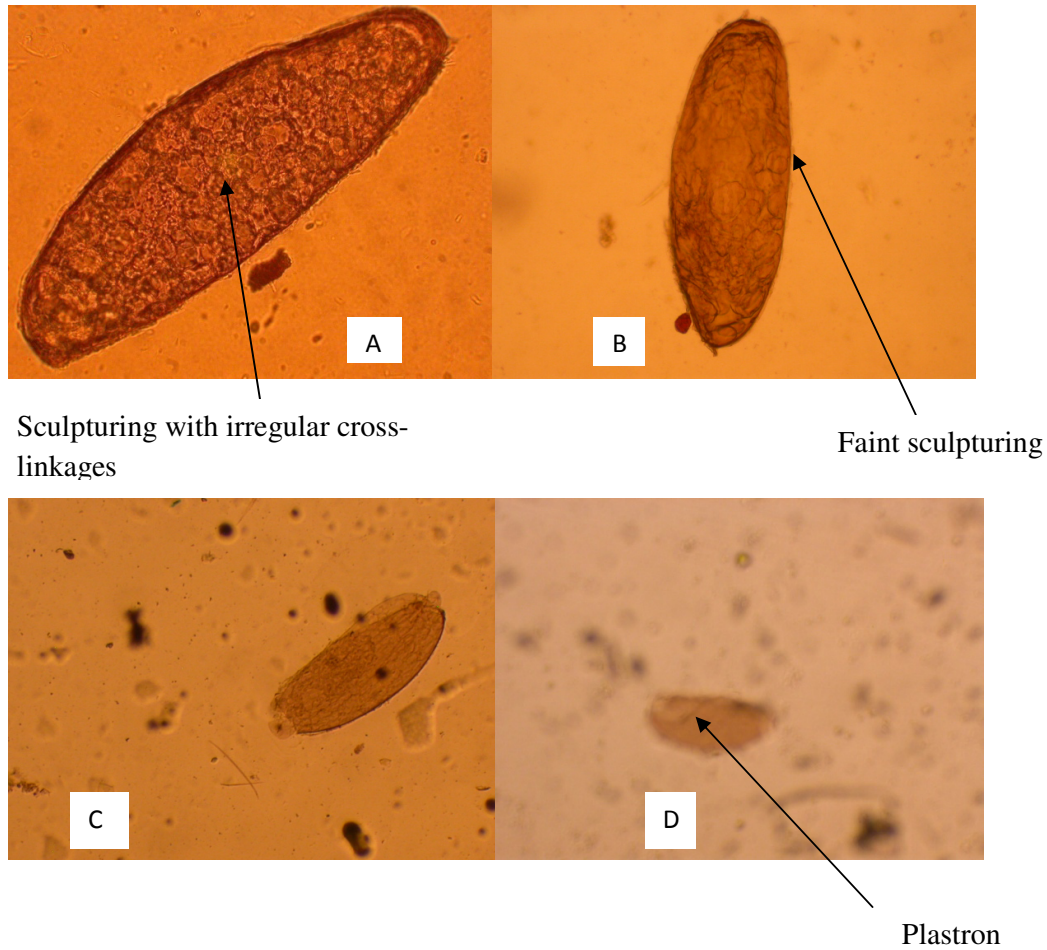


Plate 4.1: (a) *P. duboscqi* egg treated with distilled water (control), (b) egg treated with 125 µg/ml methanol *R. communis* leaf extract, (c) *P. duboscqi* egg at 250 µg/ml methanol *R. communis* leaf extract (d) *P. duboscqi* egg at 500 µg/ml methanol *R. communis* leaf extract. Magnification X200

Treating eggs with aqueous *R. communis* bark extract from Suswa, Narok County showed little loss of exochorion under 125 µg/ml extract concentration and severe loss of exochorion and mesochorion at 500 µg/ml (plate 4.2). At 250 µg/ml and 500 µg/ml these eggs never hatched even after 21 days of incubation.

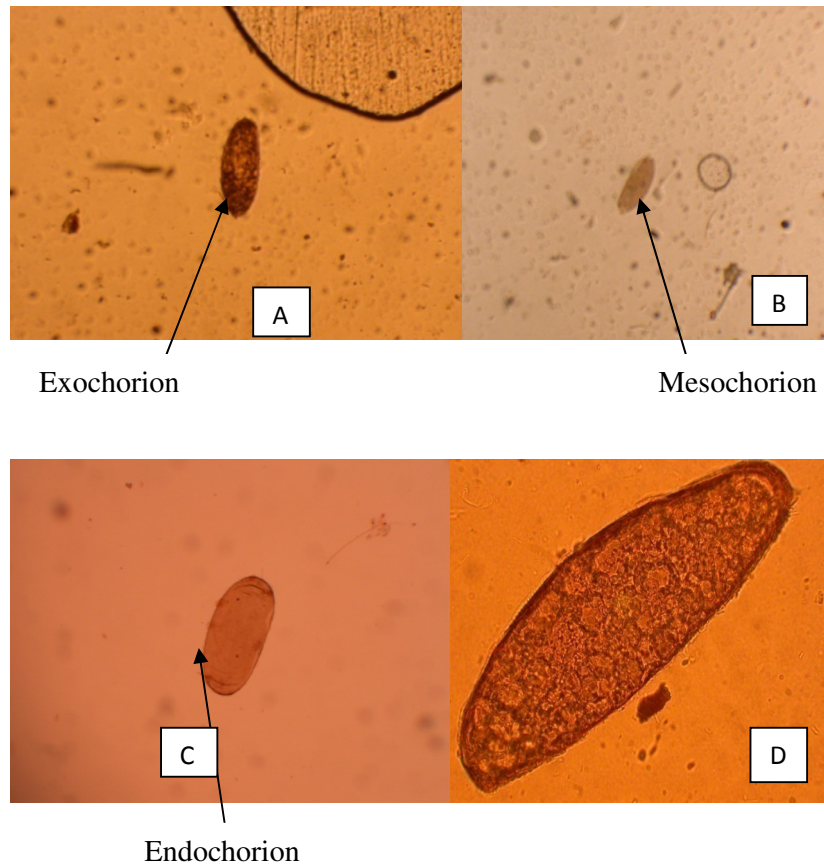


Plate 4.2: (a) *P. duboscqi* egg treated with 125 µg/ml aqueous *R. communis* bark extract (b) *P. duboscqi* egg treated with 250 µg/ml aqueous *R. communis* bark extract (c) *P. duboscqi* egg treated with 500 µg/ml aqueous *R. communis* bark extract (d) Untreated egg (control-treated with distilled water). Magnification, X200

At 14 days post treatment, the egg lost all the exochorion, mesochorion and endochorion as shown below (plate 4.3). These eggs never hatched. In the control, the egg had hatched at day 8 of incubation.



Plate 4.3: Egg at 500 µg/ml aqueous *R. communis* bark extract (14 days post treatment) from Narok, Magnification X200

At 14 days post-oviposition, the eggs treated with 250 µg/ml *R. communis* bark extracts obtained from Juja and Kisii Counties had their sculpturing in place (plate 4.4). No corrosion of the membranes was seen and they were amber in colour just like the egg under distilled water treatment (control).

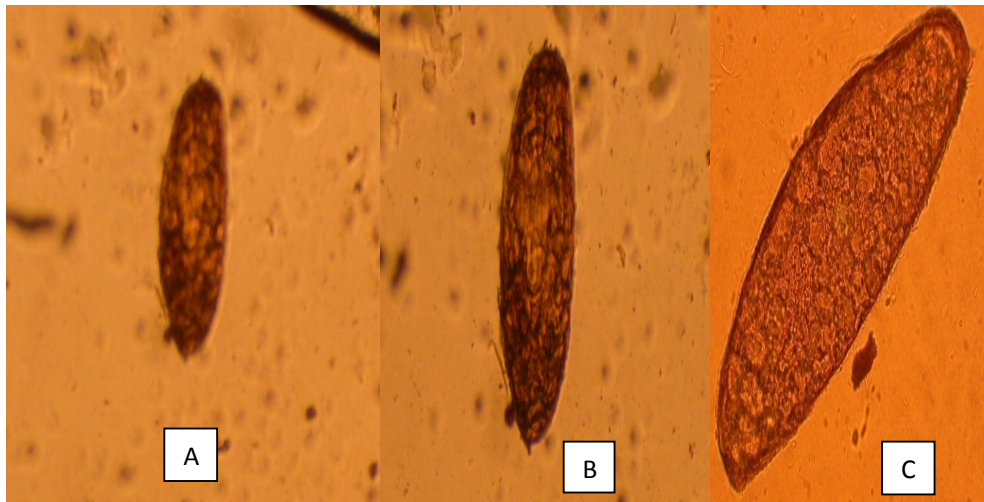


Plate 4.4: (A) Egg treated with methanol *R. communis* leaf extract from Kisii (B) Egg treated with *R. communis* bark from Juja (C) Untreated egg (control-treated with distilled water)

4.1.2 Effect of extract on egg hatchability

Eggs were inhibited from hatching with increase in extract concentration. However, there was no significant difference in effects when bark and leaf extracts were used ($F=1.00$, $P=0.31$). Egg hatching in the treatment group was first observed at 11 days post-oviposition while in the untreated group (control), hatching occurred at 8 days post-oviposition. On average, the incubation period of eggs treated with 125 $\mu\text{g/ml}$ *R. communis* aqueous extract was 15.43 ± 1.37 (mean \pm SE) days while in the control group, hatching occurred at 10.05 ± 2.15 days post-oviposition. At 500 $\mu\text{g/ml}$ concentration, 100% inhibition on egg hatchability in both leaf and bark aqueous extracts was observed.

In the leaf aqueous extract of *R. communis* from Narok County, per cent hatchability was 38% (43.33 ± 1.66) at 125 $\mu\text{g/ml}$, 29% (35.33 ± 1.45) at 250 $\mu\text{g/ml}$, 7% (15.00 ± 2.88) at 500 $\mu\text{g/ml}$ while in the control experiment hatchability was 73% (92.67 ± 3.92). Significant effects were observed when comparing hatchability in the control group with hatchability in all the three concentrations ($P<0.001$). The leaf aqueous extracts obtained from Kisii County gave hatchability of 62% (80.65 ± 2.35), 51% (66.30 ± 1.37) and 38% (49.40 ± 1.35) at 125 $\mu\text{g/ml}$, 250 $\mu\text{g/ml}$ and 500 $\mu\text{g/ml}$ respectively. While the same extract obtained from Juja, Kiambu County had moderate effects on egg hatchability; at 125 $\mu\text{g/ml}$, 51% (66.35 ± 3.00) eggs hatched while 39% (50.73 ± 1.27) and 19% (24.70 ± 1.45) eggs hatched at 250 $\mu\text{g/ml}$ and 500 $\mu\text{g/ml}$ respectively (fig 4.1).

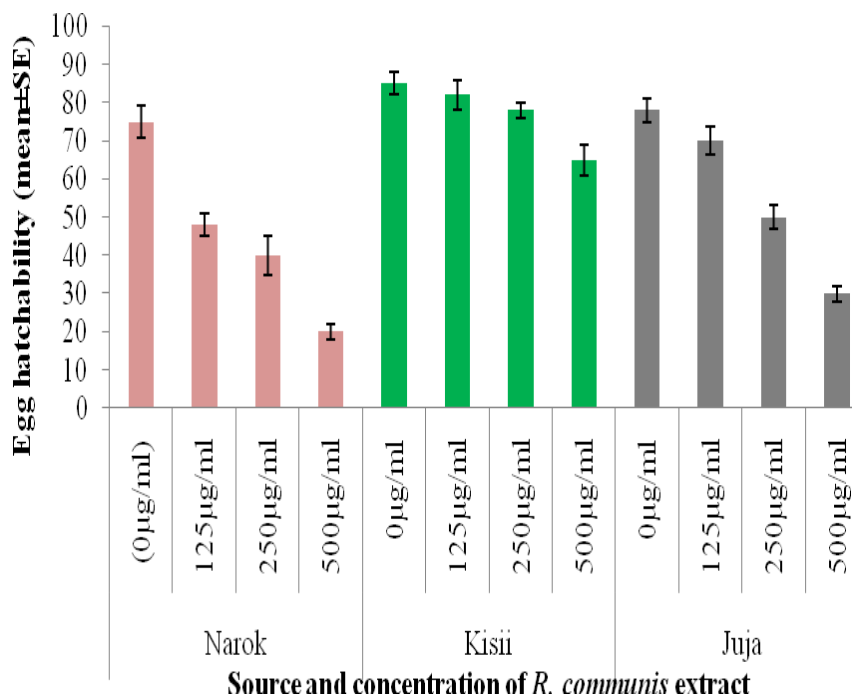


Figure 4.1: Per cent hatchability of eggs after treatment with *R. communis* leaf aqueous extracts from 3 sites: Narok, Juja and Kisii

In the methanolic leaf extracts of *R. communis* from Narok County, % hatchability was 34% (39.03 ± 2.33) at a concentration of 125 µg/ml, 21% (29.67 ± 2.40) at 250 µg/ml, 9% (13.33 ± 1.67) at 500 µg/ml while in the untreated group; hatchability was 84% (96.67 ± 7.26). Severe effects were observed at 500 µg/ml with a significant effect ($P < 0.001$). When methanol leaf extracts from Kisii county were used hatchability was 55% (71.55 ± 1.45), 38% (49.40 ± 2.00) and 27% (35.10 ± 2.45) at 125 µg/ml, 250 µg/ml and 500 µg/ml respectively. In the order of efficacy, the extracts from Narok County were more efficacious followed by extracts from Juja, Kiambu County and the least was extracts from Kisii County. 48% (52.40 ± 2.67) of eggs hatched when the extract from Juja, Kiambu County was administered at a concentration of 125 µg/ml. Hatching was further inhibited at 250 µg/ml and 500 µg/ml concentration where 35% (45.50 ± 1.44) and 25% (32.45 ± 2.33) of eggs had hatched respectively (fig 4.2).

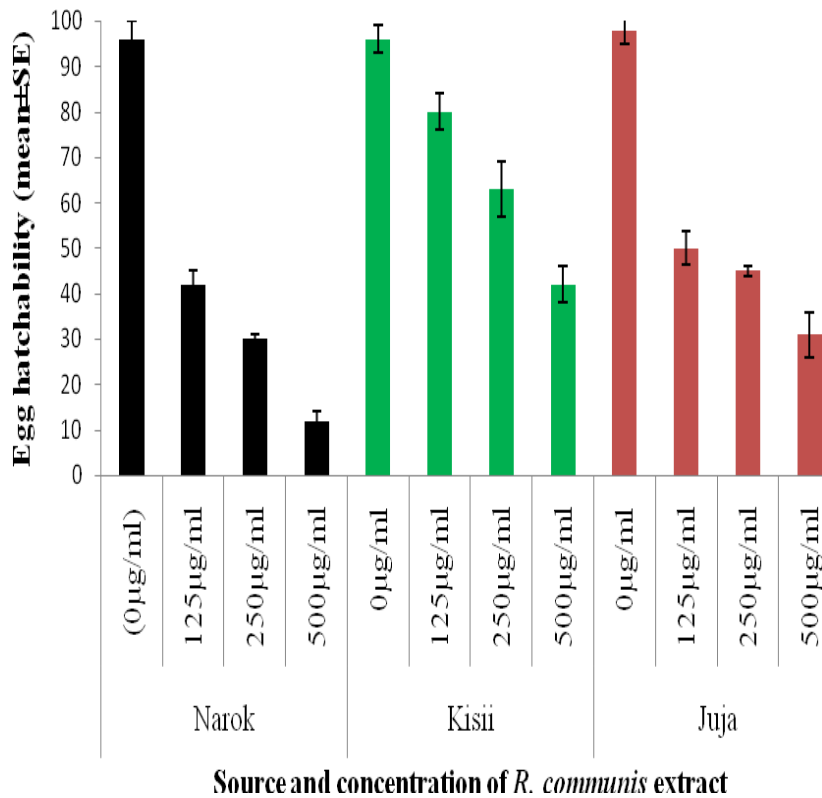


Figure 4.2: Per cent egg hatchability after treatment with *R. communis* leaf methanol extracts from 3 sites; Narok, Juja and Kisii

In the ethyl acetate leaf extract from Narok County, 37% (47.00 ± 1.53) of the eggs hatched at a concentration of 125 $\mu\text{g/ml}$, 36% (41.00 ± 2.08) at 250 $\mu\text{g/ml}$, and 26% (29.67 ± 7.68) at 500 $\mu\text{g/ml}$ while 73% (108.67 ± 1.85) hatched in the control group. No significant difference when comparing hatchability of eggs in eggs treated at a concentration of 125 $\mu\text{g/ml}$ and 250 $\mu\text{g/ml}$ ($P=0.073$). However, a significant difference was noted when comparing hatchability in the treatment group with those in the control group ($P=0.002$). Further inhibition occurred when ethyl acetate leaf extracts from Juja were used with 49% (63.70 ± 1.33), 40% (52.15 ± 0.25) and 34% (44.20 ± 2.35) eggs hatching at 125 $\mu\text{g/ml}$, 250 $\mu\text{g/ml}$ and 500 $\mu\text{g/ml}$ respectively. Using ethyl acetate leaf extract from Kisii county produced 69% (82.37 ± 1.33), 56% (65.25 ± 0.35) and 39% (41.22 ± 2.38) eggs hatchability at 125 $\mu\text{g/ml}$, 250 $\mu\text{g/ml}$ and 500 $\mu\text{g/ml}$ respectively (fig 4.3).

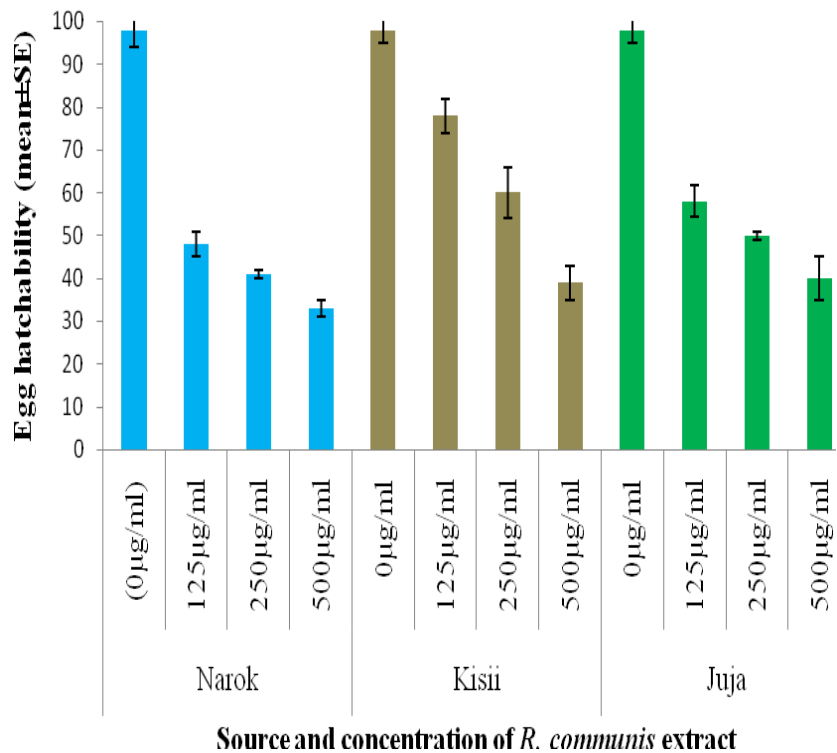


Figure 4.3: Per cent Hatchability of *P. duboscqi* eggs treated with *R. communis* ethyl acetate leaf extracts from 3 sites: Narok, Juja and Kisii

Cumulatively, bark extracts of *R. communis* showed more severe effects on egg hatchability when compared to the leaf extracts from Narok and the observed difference was significant ($P < 0.001$). Severe effects were exhibited by the methanolic extracts of *R. communis* bark at a concentration of 500 µg/ml where 5% (10.00 ± 1.33) of the eggs hatched. At a concentration of 250 µg/ml, 15% (22.33 ± 0.67) of eggs hatched while 29% (38.13 ± 1.67) of eggs hatched at 125 µg/ml. In the control group, 75% (98.67 ± 2.33) of eggs hatched. Significant effects were observed at 500 µg/ml ($F=19.87$, $P=0.011$). Moistening eggs with extracts from Kisii led to 51% (68.85 ± 2.45), 38% (51.33 ± 0.67) and 25% (33.75 ± 1.35) of eggs hatching at 125 µg/ml, 250 µg/ml and 500 µg/ml respectively. However, 43% (58.05 ± 1.65), 29% (39.15 ± 1.45) and 16% (21.63 ± 2.47) of eggs hatched at 125 µg/ml, 250 µg/ml and 500 µg/ml respectively when using bark extracts from Juja (fig 4.4).

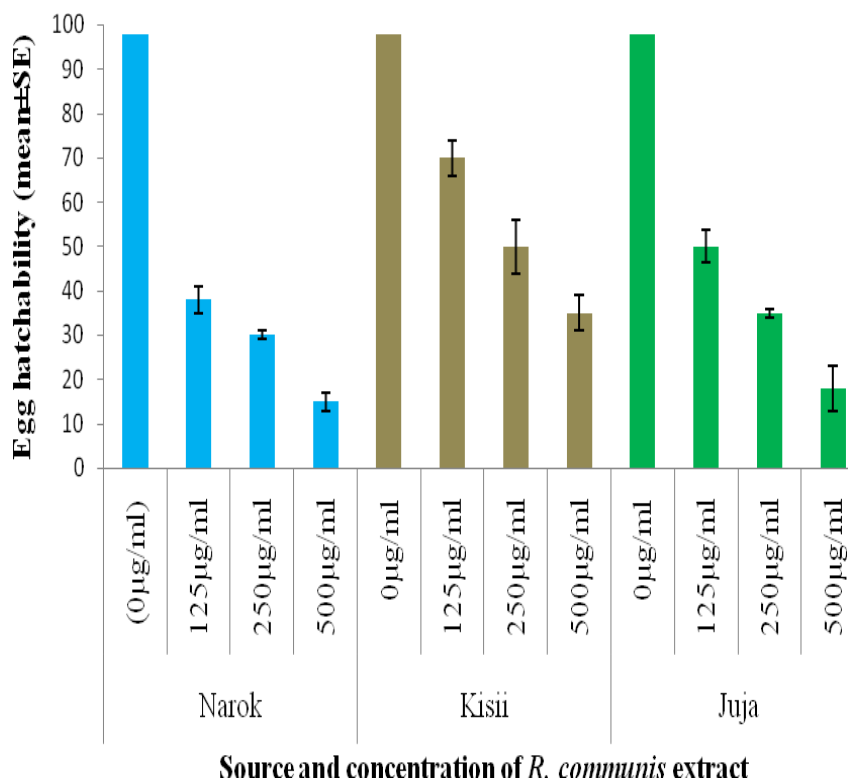


Figure 4.4: Per cent hatchability of eggs after treatment with *R. communis* bark (methanol) extracts from Narok, Juja and Kisii

In the ethyl acetate extracts of *R. communis* (bark) from Narok County, percentage hatchability was 35% (60.00 ± 1.66) at 125 $\mu\text{g/ml}$, 30% (35.13 ± 3.60) at 250 $\mu\text{g/ml}$, while at 500 $\mu\text{g/ml}$, 19% (20.66 ± 1.86) of eggs hatched compared to 88% (105.00 ± 3.33) in the control group ($P=0.000$). There was no significant difference between extracts from Kisii and Juja, Kiambu County ($F=33.7$, $P=0.063$). Despite this, hatchability was inhibited with only 47% (63.45 ± 1.35), 35% (47.25 ± 0.45) and 24% (32.40 ± 2.37) of eggs hatching at 125 $\mu\text{g/ml}$, 250 $\mu\text{g/ml}$ and 500 $\mu\text{g/ml}$ respectively when extracts from Kisii were used. While ethyl acetate of *R. communis* bark extracts from Juja, Kiambu County produced hatchability of 39% (52.65 ± 1.35), 32% (43.25 ± 2.00) and 21% (28.35 ± 1.05) at 125 $\mu\text{g/ml}$, 250 $\mu\text{g/ml}$ and 500 $\mu\text{g/ml}$ respectively (fig 4.5).

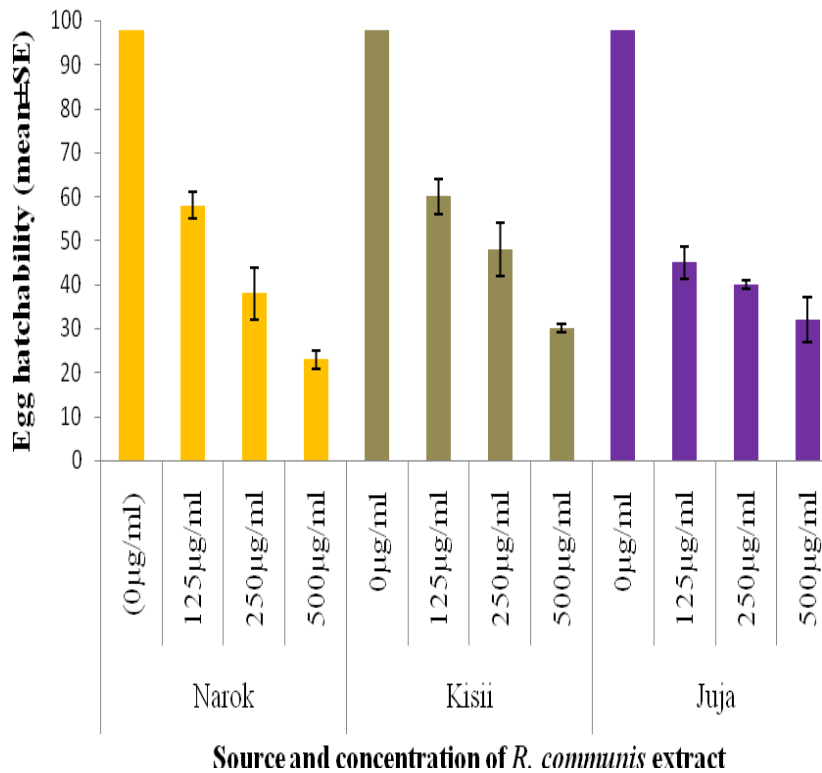


Figure 4.5: Per cent hatchability of eggs after treatment with *R. communis* bark (ethyl acetate) extracts from Narok, Juja and Kisii

R. communis bark aqueous extracts were more severe in inhibiting egg hatchability with only 40% (45.15 ± 0.65), 25% (30.33 ± 0.87) and 8% (17.00 ± 1.67) of eggs hatching at 125 µg/ml, 250 µg/ml and 500 µg/ml respectively. This was in contrast to 86.3% (98.58 ± 2.42) of eggs from the control group that had hatched ($P < 0.001$). At 500 µg/ml this extract showed significant reduction in the hatchability rate compared to the other concentrations and the control group ($F = 44.61$, $P = 0.002$).

Conversely, when the bark aqueous extracts from Kisii County were used, 49% (66.15 ± 3.45), 33% (44.55 ± 0.35) and 21% (28.35 ± 2.65) of eggs hatched at 125 µg/ml, 250 µg/ml and 500 µg/ml respectively. Similar effects were observed when bark aqueous extracts from Juja were used with 47% (63.45 ± 1.15), 29% (39.15 ± 0.25) and 14% (18.93 ± 2.47) eggs hatching at 125 µg/ml, 250 µg/ml and 500 µg/ml respectively (fig 4.6). The observed differences in effects of inhibition between extracts from Kisii and Juja was not significant ($F = 39.83$, $P = 0.071$).

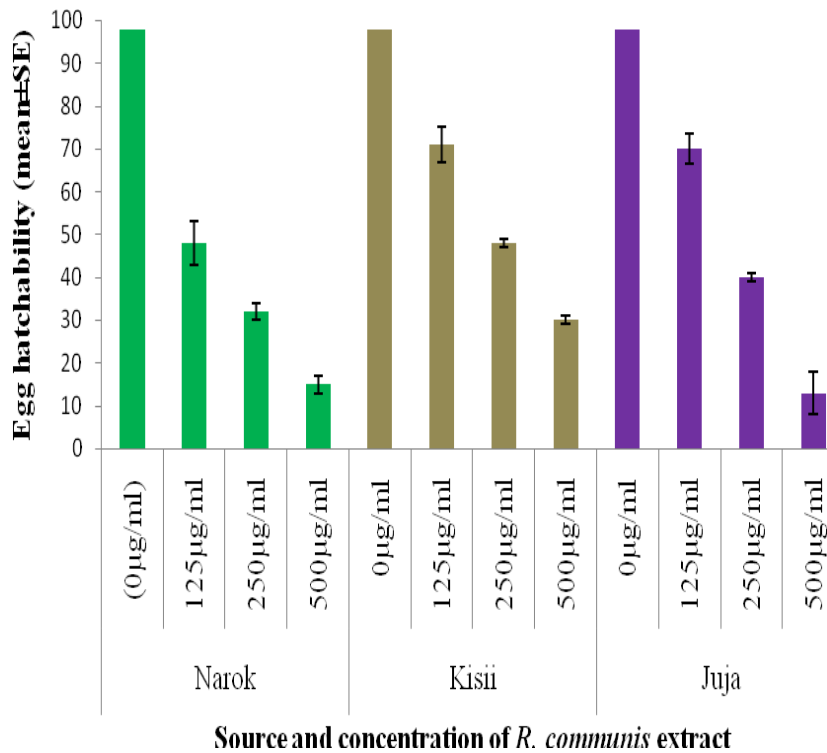


Figure 2: Per cent hatchability of *P. duboscqi* eggs treated with *R. communis* bark extracts (aqueous) from 3 sites: Narok, Juja and Kisii

4.2 Larvicidal effects of *R. communis* bark and leaf extracts

Methanolic extract of *R. communis* bark and leaf were the most active extracts against *P. duboscqi* larvae followed by aqueous extract then ethyl acetate extract. The LC₅₀ values for *R. communis* bark extracts at 24 hours post treatment were 241.45 µg/ml, 219.14 µg/ml and 310.10 µg/ml for aqueous, methanol and ethyl acetate extracts respectively. At 48 hour post treatment, LC₅₀ values observed were 142.20 µg/ml, 127.32 µg/ml and 162.40 µg/ml for aqueous, methanol and ethyl acetate extracts respectively. When *R. communis* leaf extracts were used against *P. duboscqi* larvae, the values for LC₅₀ at 48 hours post treatment were 149.55 µg/ml, 128.51 µg/ml and 222.99 µg/ml for aqueous, methanol and ethyl acetate extracts respectively.

4.3. Larvicidal effects of *R. communis* extract

4.3.1 Larval morphology and mortality after extract ingestion/contact

After emergence, all the first instar larvae treated with 125 µg/ml of aqueous, methanol and ethyl acetate extracts from all the 3 sites showed normal developmental features without any deformity. They all survived and moulted to the second instar larvae despite extract administration. No larval death was recorded due to the extract treatment. However, the extract was suspected to deter larvae from feeding as evidenced by the small size of the larvae and almost empty gut (plate 4.5).

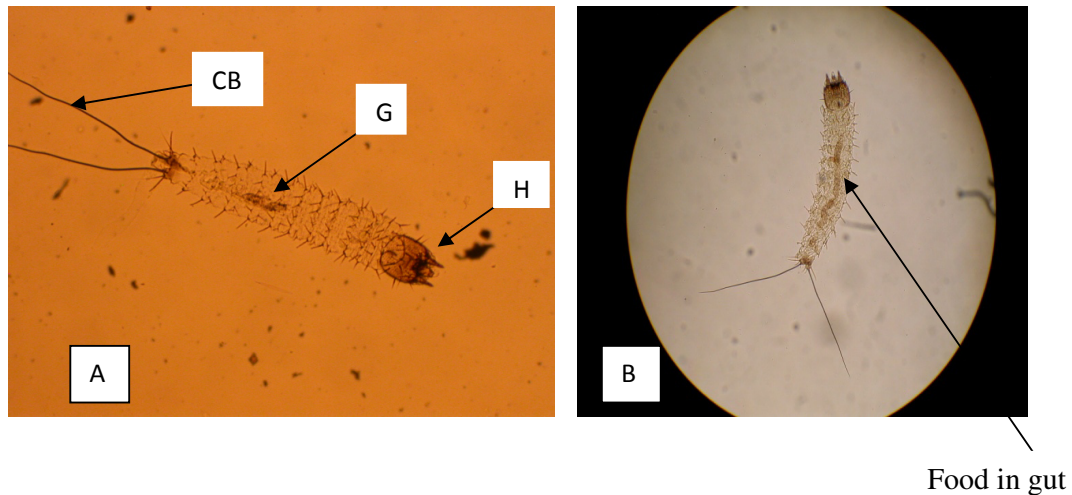


Plate 4.5: Effect of aqueous *R. communis* bark extract on larvae: (A) first larval instar under 125 µg/ml extract concentration with almost empty gut: CB-caudal bristle, G-gut, H-head (B) First larval instar (control) which is fed

At the second larval instar, further inhibition to feeding was observed. With aqueous bark extracts, per cent mortality increased with the dead larvae having a head detached from the gut. Later, the gut was seen to be split followed by disintegration then rotting followed and finally the larvae darkened (Plate 4.6). Similarly, under methanol leaf and bark extracts from Narok, per cent mortality was significantly higher compared to the extracts from Kisii and Juja.

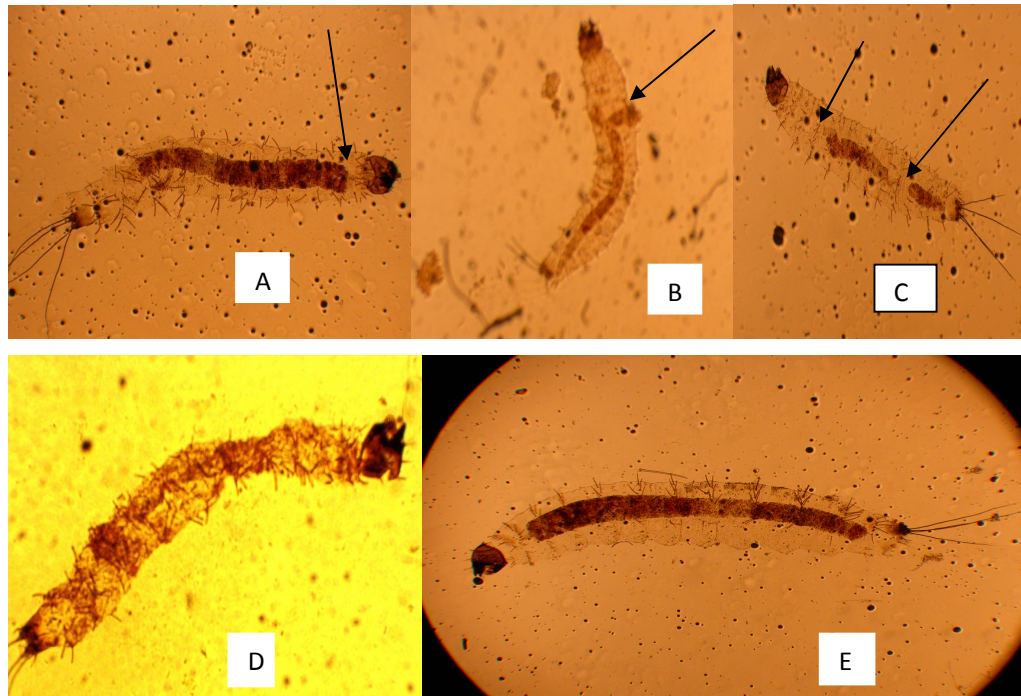


Plate 4.6: Second larval instar treated with 250 µg/ml of methanol *R. communis* leaf extract. Arrows indicate; A-gut detaching from head, B-gut fully detached from the head, C-split gut, D- rotting larvae; E-untreated larvae (control)

Significant larval mortality occurred at the 3rd and 4th instar stages as the extract concentration was increased. Observed mortality when larvae were treated with 250 µg/ml aqueous leaf extract was 25.70±0.58 (n=120), $P<0.001$), 89.33±0.67 (n=120, $P=0.01$) and 98.60±1.69 (n=125, $P=0.017$) for extracts from Kisii, Juja and Narok respectively. In the untreated group, mortality was 4.15±1.65 (n=135). At a concentration of 500 µg/ml, 100% mortality was recorded for the extracts from Narok and Juja while 97% (116.45 ± 2.85) mortality was recorded after administering *R. communis* extract from Kisii.

Severe effects on larvae were observed on the group treated with methanol *R. communis* leaf extract. Mortality was high and most larvae had their guts detached from the head then disintegration and rotting of the larvae started almost immediately (plate 4.7). Mortality reached 100% at the 3rd larval instar hence the life cycle was never completed.

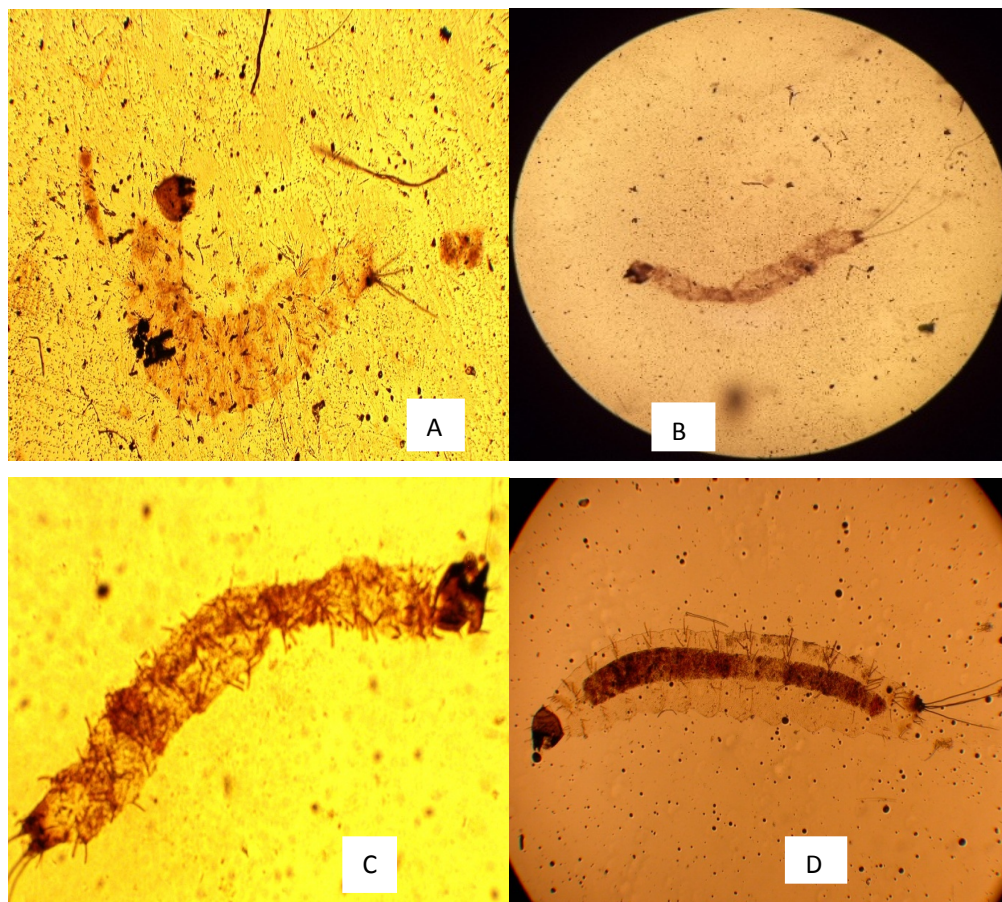


Plate 4.7: Third instar larvae treated with methanol leaf extract of *R. communis*; (A) Dead larvae (B) Rotting larvae (C) rotting larvae (D) Viable larvae from the control group

At a concentration of 250 $\mu\text{g/ml}$ and 500 $\mu\text{g/ml}$, severe larval mortality was observed when *R. communis* methanol leaf extracts from Narok and Juja were used. This extract caused a mortality of 30.33 ± 2.67 ($n=130$, $P=0.019$), 83 ± 0.58 ($n=110$, $P=0.002$) and 114.67 ± 1.33 ($n=125$, $P<0.001$) at a concentration of 250 $\mu\text{g/ml}$ for extracts from Kisii, Juja and Narok respectively for 3rd instar larvae. Larvae that were kept on larval food only showed normal development and only 7.68 ± 1.22 ($n=130$) larvae were dead. At 500 $\mu\text{g/ml}$, 100% mortality was recorded when extracts from Narok and Juja were used. However, treating the larvae with extracts from Kisii caused a mortality of 93% (120.95 ± 1.65).

In the group treated with ethyl acetate *R. communis* leaf extract, larval development was not impaired with at 125 µg/ml compared to methanol and aqueous extract treated groups. At 250 µg/ml, high mortality was observed (plate 4.8).

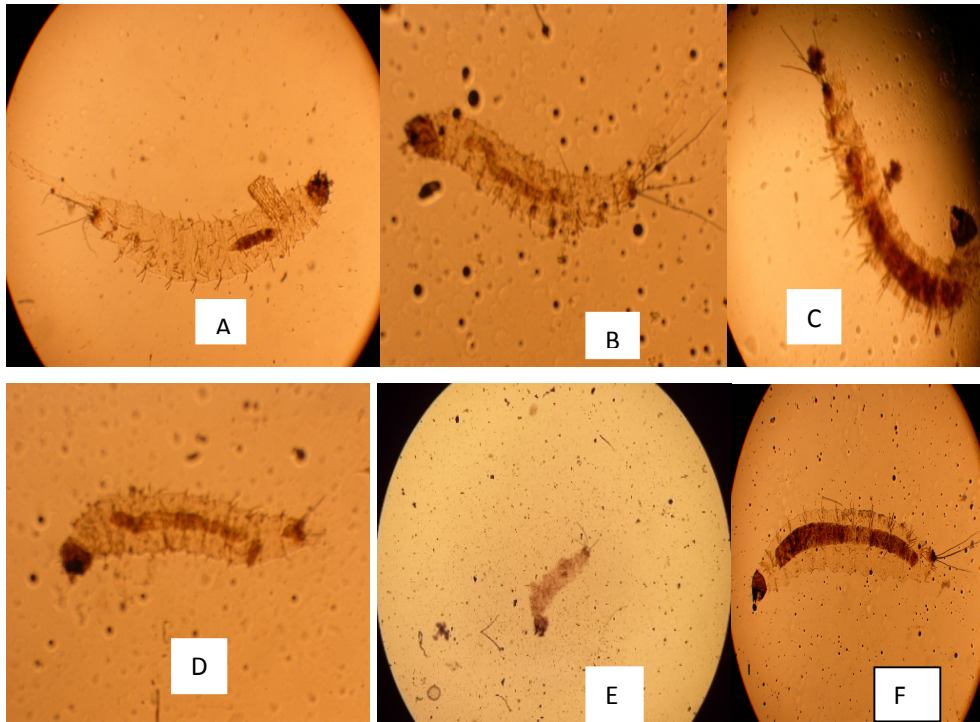


Plate 4.8: Third instar larvae treated with ethyl acetate leaf extract of *R. communis*; A-larvae with deterred feeding, B-Gut starting to detach from head, C- gut splitting D-gut splitting, E-dead larvae, F-Untreated larvae (control)

In order of efficacy, extracts from Narok were more efficacious followed by extracts from Juja and the least was extracts from Kisii (fig 4.7).

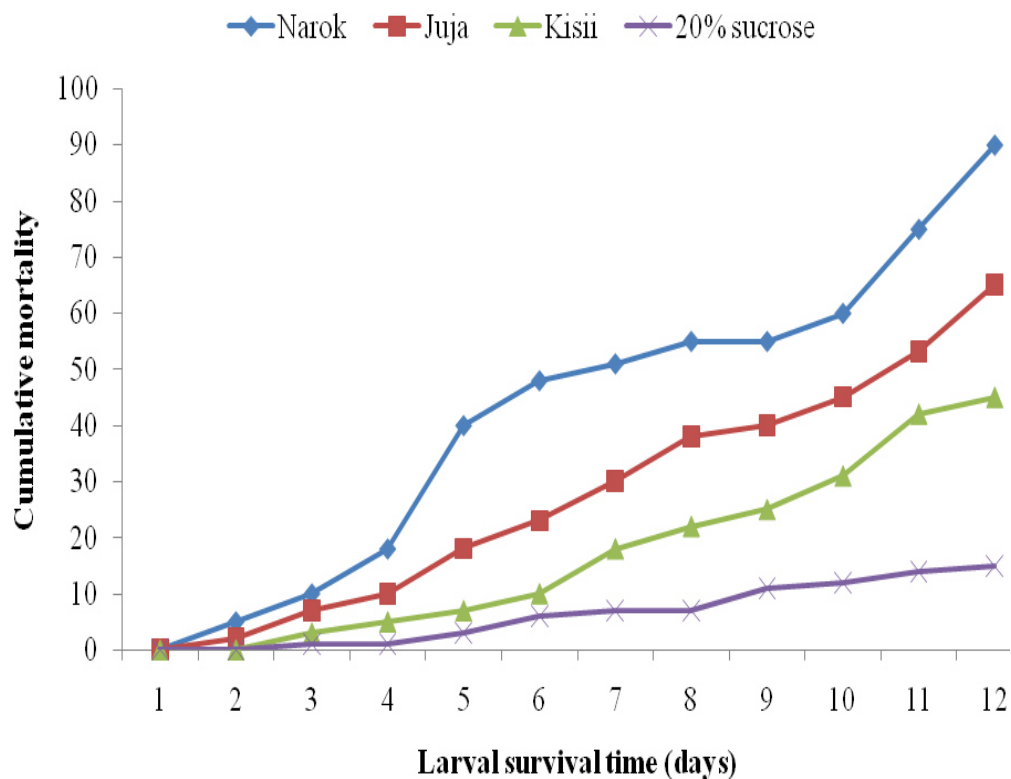


Figure 4.7: Comparing larval mortality after contact with methanol leaf extracts of *R. communis* from Juja, Kisii and Narok Counties

4.3.2 *Phlebotomus duboscqi* larval duration after treatment with *R. communis* bark extracts

For larvae that were treated with aqueous extracts from Kisii, cumulative larval duration was 63.01 ± 1.37 days, 60.33 ± 1.45 days and 80.55 ± 0.35 days at 125 $\mu\text{g/ml}$, 250 $\mu\text{g/ml}$ and 500 $\mu\text{g/ml}$ respectively. Conversely, the cumulative larval duration was 27.37 ± 0.17 days in the control group. However, treating larvae with aqueous extracts from Juja led to a significantly longer period as compared to the group treated with aqueous extracts from Kisii ($P < 0.05$). The larval duration was 65.44 ± 3.15 , 71.83 ± 0.67 and 75.83 ± 0.67 days at 125 $\mu\text{g/ml}$, 250 $\mu\text{g/ml}$ and 500 $\mu\text{g/ml}$ extract concentration respectively. Severe effects were observed when extracts from Narok were used. Larval development was suppressed hence the larval duration was prolonged to 60.33 ± 3.97 , 78.11 ± 0.81 and 91.66 ± 2.50 at 125 $\mu\text{g/ml}$, 250 $\mu\text{g/ml}$

and 500 µg/ml extract concentration respectively (table 4.1). Larvae in the control group had moulted to pupae in 27.37±0.17 days ($P=0.014$).

Table 4.1: Mean (±SE) *P. duboscqi* larval duration after treatment with aqueous *R. communis* bark extracts obtained from Kisii, Juja and Narok regions

Source of <i>R. communis</i> :	Concentration (µg/ml)	Larval duration (days±SE)	<i>P</i>
Kisii	125 µg/ml	63.01±1.37	0.002
Juja	125 µg/ml	65.44±3.15	0.001
Narok	125 µg/ml	60.33±3.97	0.001
Control	0 µg/ml	27.37±0.17	0.014
Kisii	250 µg/ml	60.33±1.45	0.011
Juja	250 µg/ml	71.83±0.67	0.001
Narok	250 µg/ml	78.11±0.81	0.000
Control	0 µg/ml	24.23±0.67	0.001
Kisii	500 µg/ml	80.55±0.35	0.019
Juja	500 µg/ml	75.83±0.67	0.001
Narok	500 µg/ml	91.66±2.50	0.018
Control	0 µg/ml	25.20±1.00	0.011

Under methanol extract treatment, larval duration was 68.11 ± 0.21, 63.33 ± 0.67 and 87.10 ± 1.67 days under 125 µg/ml, 250 µg/ml and 500 µg/ml extract concentration respectively when using extracts from Kisii. When treating larvae with extracts from Juja, the larval duration lasted for 73.00±1.20, 56.45±2.35 and 0 days at 125 µg/ml, 250 µg/ml and 500 µg/ml respectively. All the larvae died when treated with a concentration of 500 µg/ml *R. communis* bark methanol extract compared to 25.95±0.34 days in the control group. The larval duration was inhibited further when 125 µg/ml of *R. communis* extract from Narok was used with larval duration lasting for 87.95±0.15 days. At 250 µg/ml and 500 µg/ml extract concentration, all the

larvae died, terminating the life cycle of *P. duboscqi* compared to a larval duration of 25.95 ± 0.34 days in the control group (table 4.2).

Table 4.2: Mean (\pm SE) *P. duboscqi* larval duration after treatment with methanol *R. communis* bark extracts

Source of <i>R. communis</i> :	Concentration (μ g/ml)	Larval duration (days \pm SE)	<i>P</i>
Kisii	125 μ g/ml	68.11 \pm 0.21	0.012
Juja	125 μ g/ml	73.00 \pm 1.20	0.001
Narok	125 μ g/ml	87.95 \pm 0.15	0.002
Control	0 μ g/ml	25.95 \pm 0.34	0.014
Kisii	250 μ g/ml	63.33 \pm 0.67	0.011
Juja	250 μ g/ml	56.45 \pm 2.35	0.001
Narok	250 μ g/ml	0.0 \pm 0.0	0.000
Control	0 μ g/ml	25.95 \pm 0.34	0.001
Kisii	500 μ g/ml	87.10 \pm 1.67	0.013
Juja	500 μ g/ml	0.0 \pm 0.0	0.000
Narok	500 μ g/ml	0.0 \pm 0.0	0.000
Control	0 μ g/ml	25.95 \pm 0.34	0.021

Ethyl acetate extract was a sharp contrast of methanol extract with the larval duration being significantly low. The larval duration was 37.10 ± 1.37 days, 43.45 ± 0.65 days and 48.33 ± 1.67 days at 125 μ g/ml, 250 μ g/ml and 500 μ g/ml respectively when *R. communis* extract from Kisii was used against larvae. This was in contrast to 28.00 ± 1.65 days in the larvae that formed the control ($P=0.019$). Extracts from Juja showed

a moderate effect on larvae with the larval duration lasting for 43.10 ± 0.44 , 38.00 ± 0.22 and 0 days at 125 $\mu\text{g/ml}$, 250 $\mu\text{g/ml}$ and 500 $\mu\text{g/ml}$ respectively. Larvae that were treated with extracts from Narok region lived for 54.24 ± 1.36 , 0 and 0 days at 125 $\mu\text{g/ml}$, 250 $\mu\text{g/ml}$ and 500 $\mu\text{g/ml}$ respectively. The life cycle was terminated at 250 $\mu\text{g/ml}$ extract concentration with a mortality rate of 100% being recorded compared to a larval duration of 18.05 ± 1.65 days for larvae in the control group (table 4.3).

Table 4.3: Mean ($\pm\text{SE}$) *P. duboscqi* larval duration after treatment with ethyl acetate *R. communis* extracts

Source of <i>R. communis</i> :	Concentration ($\mu\text{g/ml}$)	Larval duration (days $\pm\text{SE}$)	<i>P</i>
Kisii	125 $\mu\text{g/ml}$	37.10 ± 1.37	0.012
Juja	125 $\mu\text{g/ml}$	43.10 ± 0.44	0.001
Narok	125 $\mu\text{g/ml}$	54.24 ± 1.36	0.019
Control	0 $\mu\text{g/ml}$	28.00 ± 1.65	0.004
Kisii	250 $\mu\text{g/ml}$	43.45 ± 0.65	0.013
Juja	250 $\mu\text{g/ml}$	38.00 ± 0.22	0.021
Narok	250 $\mu\text{g/ml}$	0.0 ± 0.0	0.000
Control	0 $\mu\text{g/ml}$	18.05 ± 1.65	0.001
Kisii	500 $\mu\text{g/ml}$	48.33 ± 1.67	0.019
Juja	500 $\mu\text{g/ml}$	0.0 ± 0.0	0.000
Narok	500 $\mu\text{g/ml}$	0.0 ± 0.0	0.019
Control	0 $\mu\text{g/ml}$	18.05 ± 1.65	0.001

4.3.3 *Phlebotomus duboscqi* pupal duration after treatment with *R. communis* bark extracts

9.33 ± 2.58, 11.45 ± 1.35 and 4.11 ± 0.99 (n=130) pupae were viable at 125 µg/ml, 250 µg/ml and 500 µg/ml respectively when larvae were treated with aqueous *R. communis* extract from Kisii. In the control group, 101.55 ± 2.95 (n=130) pupae were viable. The observed difference was highly significant ($P=0.014$). At the same time, the pupal duration was 13.23 ± 0.27 days, 16.44 ± 2.54 days and 21.00 ± 1.55 days at 125 µg/ml, 250 µg/ml and 500 µg/ml respectively against 9.47 ± 0.33 days for the control group. For the larvae treated with *R. communis* extract from Juja, the pupal period lasted for 18.67±0.33, 24.35±2.25 and 17.23±2.27 days at 125 µg/ml, 250 µg/ml and 500 µg/ml respectively (table 4.4). This is in contrast to the pupal period of 10.11±0.21days ($P=0.011$) in the larvae under the control group.

Table 4.4: *Phlebotomus duboscqi* pupal duration after treatment with *R. communis* bark aqueous extracts

Source of <i>R. communis</i> :	Concentration (µg/ml)	Pupal duration (days±SE)	<i>P</i>
Kisii	125 µg/ml	13.23±0.27	0.001
Juja	125 µg/ml	18.67±0.33	0.015
Narok	125 µg/ml	25.05±1.25	0.001
Control	0 µg/ml	9.47±0.33	0.014
Kisii	250 µg/ml	16.44±2.54	0.001
Juja	250 µg/ml	24.35±2.25	0.021
Narok	250 µg/ml	27.20±0.21	0.001
Control	0 µg/ml	15.81±1.90	0.022
Kisii	500 µg/ml	21.00±1.55	0.019
Juja	500 µg/ml	17.23±2.27	0.016
Narok	500 µg/ml	15.95±3.65	0.018
Control	0 µg/ml	10.11±0.21	0.001

Severe *R. communis* effects were seen when larvae were treated with methanol *R. communis* extracts where 100% mortality was recorded when 250 µg/ml and 500 µg/ml extract concentration from Narok were used. Therefore, at 250 µg/ml and 500 µg/ml *R. communis* concentration, there were no viable pupae being recorded. However, the pupal duration was 19.25 ± 0.15 days at 125 µg/ml concentration for *R. communis* bark methanol extracts from Kisii (table 4.5).

Table 4.5: *Phlebotomus duboscqi* pupal duration after treatment with *R. communis* extracts

Source of <i>R. communis</i> :	Concentration (µg/ml)	Larval duration (days±SE)	<i>P</i>
Kisii	125 µg/ml	19.25±0.15	0.002
Juja	125 µg/ml	25.05±3.15	0.007
Narok	125 µg/ml	28.75±1.15	0.011
Control	0 µg/ml	9.55±0.15	0.014
Kisii	250 µg/ml	63.33±0.67	0.001
Juja	250 µg/ml	28.43±1.67	0.001
Narok	250 µg/ml	0.0 ± 0.0	0.000
Control	0 µg/ml	9.55±0.15	0.011
Kisii	500 µg/ml	0.0 ± 0.0	0.000
Juja	500 µg/ml	0.0 ± 0.0	0.000
Narok	500 µg/ml	0.0 ± 0.0	0.000
Control	0 µg/ml	9.55±0.15	0.001

For experiments with ethyl acetate extracts, the pupal duration was long compared to larvae treated with aqueous and methanol extracts. The duration was 37.10 ± 0.27 days, 38.54 ± 1.46 days and 40.00 ± 1.67 days at $125 \mu\text{g/ml}$, $250 \mu\text{g/ml}$ and $500 \mu\text{g/ml}$ when extracts from Kisii was used against larvae respectively (table 4.6).

Table 4.6: *Phlebotomus duboscqi* pupal duration after treatment with *R. communis* bark methanol extracts from three different regions

<i>Source of R. communis:</i>	Concentration ($\mu\text{g/ml}$)	Larval duration (days\pmSE)	P
Kisii	125 $\mu\text{g/ml}$	37.10 \pm 0.27	0.042
Juja	125 $\mu\text{g/ml}$	15.33 \pm 1.67	0.020
Narok	125 $\mu\text{g/ml}$	26.00 \pm 0.11	0.001
Control	0 $\mu\text{g/ml}$	8.05 \pm 1.65	0.004
Kisii	250 $\mu\text{g/ml}$	38.54 \pm 1.46	0.001
Juja	250 $\mu\text{g/ml}$	18.65 \pm 2.05	0.001
Narok	250 $\mu\text{g/ml}$	0.0 \pm 0.0	0.000
Control	0 $\mu\text{g/ml}$	8.05 \pm 1.65	0.021
Kisii	500 $\mu\text{g/ml}$	40.00 \pm 1.67	0.001
Juja	500 $\mu\text{g/ml}$	0.0 \pm 0.0	0.000
Narok	500 $\mu\text{g/ml}$	0.0 \pm 0.0	0.000
Control	0 $\mu\text{g/ml}$	9.55 \pm 0.15	0.001

4.3.4 *Phlebotomus duboscqi* larval duration after treatment with *R. communis* leaf extracts

The methanol leaf extracts produced 25% (8.37 ± 2.33) larval viability at a concentration of $250 \mu\text{g/ml}$ and 0% at $500 \mu\text{g/ml}$ for larvae treated with *R. communis* extracts obtained from Narok and Juja. However, at a concentration of $125 \mu\text{g/ml}$, larval viability was 75% (28.05 ± 1.65) for *R. communis* from Kisii, Narok and Juja

leaf extracts. The larval duration was 42.82 ± 1.25 , 41.06 ± 0.14 and 53.11 ± 0.15 days for larvae treated with *R. communis* aqueous extracts from Kisii at 125 $\mu\text{g/ml}$, 250 $\mu\text{g/ml}$ and 500 $\mu\text{g/ml}$ respectively. At the same time, larvae from the control group which were kept on larval food only had a larval duration of 24.23 ± 0.67 days, the observed difference was significant ($P=0.018$). A similar trend was observed when extracts from Narok and Juja were used (table 4.7).

Table 4.7: *Phlebotomus duboscqi* larval duration after treatment with *R. communis* aqueous leaf extracts

Source of <i>R. communis</i> :	Concentration ($\mu\text{g/ml}$)	Larval duration (days \pm SE)	<i>P</i>
Kisii	125 $\mu\text{g/ml}$	42.82 ± 1.25	0.001
Juja	125 $\mu\text{g/ml}$	45.14 ± 1.15	0.001
Narok	125 $\mu\text{g/ml}$	51.37 ± 1.67	0.018
Control	0 $\mu\text{g/ml}$	27.37 ± 0.17	0.004
Kisii	250 $\mu\text{g/ml}$	41.06 ± 0.14	0.001
Juja	250 $\mu\text{g/ml}$	52.85 ± 0.55	0.031
Narok	250 $\mu\text{g/ml}$	59.15 ± 1.55	0.001
Control	0 $\mu\text{g/ml}$	24.23 ± 0.67	0.001
Kisii	500 $\mu\text{g/ml}$	53.11 ± 0.15	0.000
Juja	500 $\mu\text{g/ml}$	57.10 ± 1.33	0.000
Narok	500 $\mu\text{g/ml}$	62.05 ± 2.51	0.000
Control	0 $\mu\text{g/ml}$	24.23 ± 0.67	0.001

Methanol leaf extracts proved to be more efficacious against larvae especially the methanol extracts of *R. communis* from Narok and Juja. Treating larvae with this extracts caused 100% larval mortality at 500 $\mu\text{g/ml}$ extract concentration. For these extracts, larval viability was insignificant at high extract concentrations compared to

the untreated larvae. For the larvae that were treated with *R. communis* from Kisii, the larval duration was 49.81±0.23, 51.36±1.86 and 71.55±0.15 days compared to 25.95±0.34 days for larvae that were untreated (table 4.8).

Table 4.8: *Phlebotomus duboscqi* larval duration after treatment with methanol *R. communis* leaf extracts

Source of <i>R. communis</i>	Concentration (µg/ml)	Larval duration (days±SE)	<i>P</i>
Kisii	125 µg/ml	49.81±0.23	0.012
Juja	125 µg/ml	57.07±1.33	0.001
Narok	125 µg/ml	62.35±0.15	0.011
Control	0 µg/ml	25.95±0.34	0.018
Kisii	250 µg/ml	51.36±1.86	0.001
Juja	250 µg/ml	59.35±2.44	0.001
Narok	250 µg/ml	0.0 ± 0.0	0.000
Control	0 µg/ml	25.95±0.34	0.001
Kisii	500 µg/ml	71.55±0.15	0.000
Juja	500 µg/ml	0.0 ± 0.0	0.000
Narok	500 µg/ml	0.0 ± 0.0	0.000
Control	0 µg/ml	25.95±0.34	0.011

With ethyl acetate extracts, larval viability was high, 76.9%, (47.65 ± 0.15) 47% when *P. duboscqi* larvae were treated with *R. communis* extracts from Kisii. The larval duration was 41.95±0.23, 45.33±1.67 and 52.50±0.15 days compared to 17.95±0.34 days for the control untreated group (*P*=0.001). However, treating larvae

with *R. communis* extracts from Juja at 125 µg/ml and 250 µg/ml led to a larval duration of 39.17±0.13 and 41.40±1.25 days respectively. Using extracts from Narok, 100% larval mortality was recorded at 250 µg/ml and 500 µg/m (table 4.9).

Table 4.9: *Phlebotomus duboscqi* larval duration after treatment with *R. communis* ethyl acetate leaf extract from three different regions

Extract from:	Concentration (µg/ml)	Larval duration (days±SE)	<i>P</i>
Kisii	125 µg/ml	41.95±0.23	0.001
Juja	125 µg/ml	39.17±0.13	0.002
Narok	125 µg/ml	51.64±2.26	0.001
Control	0 µg/ml	18.00±1.65	0.017
Kisii	250 µg/ml	45.33±1.67	0.001
Juja	250 µg/ml	41.40±1.25	0.021
Narok	250 µg/ml	0.0 ± 0.0	0.000
Control	0 µg/ml	18.00±1.65	0.001
Kisii	500 µg/ml	52.50±0.15	0.004
Juja	500 µg/ml	0.0 ± 0.0	0.000
Narok	500 µg/ml	0.0 ± 0.0	0.000
Control	0 µg/ml	17.95±0.34	0.010

Pupal viability decreased further reaching 15%, 8% 0% at 125 µg/ml, 250 µg/ml and 500 µg/ml respectively for extracts from Narok and Juja. Consequently, the pupal duration was 12.67±2.33, 12.20±0.85 and 0.0 ± 0.0 days at 125 µg/ml, 250 µg/ml and 500 µg/ml respectively when aqueous extracts from Kisii were administered.

With *R. communis* aqueous extracts from juja, 13.00±0.33, 17.15±0.05 and 0 days at 125 µg/ml, 250 µg/ml and 500 µg/ml respectively while 15.95±1.15, 17.37±0.63 and 0.0 ± 0.0 days were recorded after using *R. communis* extract from Narok. A pupal duration of 9.47±0.33 days was recorded from the untreated larval group (table 4.10).

Table 4.10: *Phlebotomus duboscqi* pupal duration after treatment with *R. communis* aqueous leaf extract from three different regions

Source of <i>R. communis</i> :	Concentration (µg/ml)	Larval duration (days±SE)	P
Kisii	125 µg/ml	12.67±2.33	0.011
Juja	125 µg/ml	13.00±0.33	0.021
Narok	125 µg/ml	15.95±1.15	0.001
Control	0 µg/ml	10.11±0.21	0.018
Kisii	250 µg/ml	12.20±0.85	0.001
Juja	250 µg/ml	17.15±0.05	0.001
Narok	250 µg/ml	17.37±0.63	0.000
Control	0 µg/ml	11.00±1.65	0.001
Kisii	500 µg/ml	0.0 ± 0.0	0.000
Juja	500 µg/ml	0.0 ± 0.0	0.000
Narok	500 µg/ml	0.0 ± 0.0	0.000
Control	0 µg/ml	9.47±0.33	0.001

R. communis methanol extracts were more efficacious against *P. duboscqi* larvae compared to the aqueous and ethyl acetate extracts. In all the extracts, 100% larval mortality was recorded at 500 µg/ml extract concentration. The extracts from Narok caused 100% larval mortality at 250 µg/ml and 500 µg/ml extract concentrations. Pupal duration was 11.25±2.05, 15.20±0.87 and 0.0 ± 0.0 days at 125 µg/ml, 250

$\mu\text{g/ml}$ and $500 \mu\text{g/ml}$ respectively for larvae that were treated with *R. communis* extracts from Kisii. The untreated larvae had a pupal duration of 10.47 ± 0.33 , while pupal duration was 18.65 ± 2.05 and 0.0 ± 0.0 at $125 \mu\text{g/ml}$ and $250 \mu\text{g/ml}$ respectively for larvae treated with *R. communis* extracts obtained from Narok (table 4.11).

Table 4.11: *Phlebotomus duboscqi* pupal duration after treatment with methanol *R. communis* leaf extracts from three different regions

Source of <i>R. communis</i> :	Concentration ($\mu\text{g/ml}$)	Larval duration (days \pm SE)	<i>P</i>
Kisii	$125 \mu\text{g/ml}$	11.25 ± 2.05	0.008
Juja	$125 \mu\text{g/ml}$	15.15 ± 1.15	0.015
Narok	$125 \mu\text{g/ml}$	18.65 ± 2.05	0.001
Control	$0 \mu\text{g/ml}$	10.11 ± 0.21	0.014
Kisii	$250 \mu\text{g/ml}$	15.20 ± 0.87	0.001
Juja	$250 \mu\text{g/ml}$	21.97 ± 1.63	0.001
Narok	$250 \mu\text{g/ml}$	0.0 ± 0.0	0.000
Control	$0 \mu\text{g/ml}$	10.00 ± 1.65	0.001
Kisii	$500 \mu\text{g/ml}$	0.0 ± 0.0	0.000
Juja	$500 \mu\text{g/ml}$	0.0 ± 0.0	0.000
Narok	$500 \mu\text{g/ml}$	0.0 ± 0.0	0.000
Control	$0 \mu\text{g/ml}$	10.47 ± 0.33	0.011

For larvae treated with ethyl acetate *R. communis* extracts from Kisii, the pupal viability 67%, 54% and 0%, at $125 \mu\text{g/ml}$, $250 \mu\text{g/ml}$ and $500 \mu\text{g/ml}$ respectively; whereas the pupal viability rates at $125 \mu\text{g/ml}$, $250 \mu\text{g/ml}$ and $500 \mu\text{g/ml}$ were 10%, 0%, and 0%, respectively for larvae treated with extracts from Narok. The larval duration for larvae that were treated with *R. communis* extract from Kisii was

10.67±1.33, 13.23±0.67 and 0.0 ± 0.0 days. For larvae that formed the control group, the larval duration was 8.05±1.65 days. There was no significant difference when compared to larval duration at 125 µg/ml ($P=0.081$). However, the pupal duration was 16.81±0.27 and 0.0 ± 0.0 days when larvae were treated with extracts from Narok at 125 µg/ml and 250 µg/ml respectively (table 4.12).

Table 4.12: *Phlebotomus duboscqi* pupal duration after treatment with *R. communis* ethyl acetate leaf extracts from three different regions

Source of <i>R. communis</i> :	Concentration (µg/ml)	Larval duration (days±SE)	<i>P</i>
Kisii	125 µg/ml	10.67±1.33	0.082
Juja	125 µg/ml	12.35±1.65	0.011
Narok	125 µg/ml	16.81±0.27	0.001
Control	0 µg/ml	8.05±1.65	0.018
Kisii	250 µg/ml	13.23±0.67	0.031
Juja	250 µg/ml	13.33±2.37	0.001
Narok	250 µg/ml	0.0 ± 0.0	0.000
Control	0 µg/ml	8.05±1.65	0.001
Kisii	500 µg/ml	0.0 ± 0.0	0.000
Juja	500 µg/ml	0.0 ± 0.0	0.000
Narok	500 µg/ml	0.0 ± 0.0	0.000
Control	0 µg/ml	8.05±1.65	0.011

4.4 *Phlebotomus duboscqi* adult emergence after *R. communis* larval treatment

From the group that was fed on larval food mixed with aqueous *R. communis* leaf extract from Kisii, emerged adults were 4.00 ± 1.37, 3.33 ± 1.37 and 1.00 ± 1.55 for 125 for 125 µg/ml, 250 µg/ml and 500 µg/ml respectively. Cumulatively, 8.25 ± 2.65 adult flies emerged (3 males and 5 females) compared to 98.55 ± 1.45 adults (43

males and 55 females) that emerged from the control group ($P=0.018$). The emerged adults had normal developmental features although a high percentage died within the first 24 hours of emergence. The life cycle of *P. duboscqi* was significantly long lasting for 91.00 ± 2.35 , 91.25 ± 1.45 and 116.05 ± 1.37 days at 125 $\mu\text{g/ml}$, 250 $\mu\text{g/ml}$ and 500 $\mu\text{g/ml}$ respectively. In the control group, the life cycle was completed in 46.77 ± 1.33 days ($P=0.021$).

Under all methanol extract treatments, emerged adults were 3.67 ± 2.33 (mean \pm SE) and they had no deformity; all were females. The entire life cycle lasted for 101.01 ± 2.67 days at 125 $\mu\text{g/ml}$. However, at a concentration of 250 $\mu\text{g/ml}$ and 500 $\mu\text{g/ml}$, the life cycle was never completed since mortality was 100% at the larval stage.

For larvae that were treated with bark ethyl acetate extract, the entire life cycle lasted for 89.23 ± 1.67 , 96.21 ± 2.33 and 103.24 ± 1.54 days in 125 $\mu\text{g/ml}$, 250 $\mu\text{g/ml}$ and 500 $\mu\text{g/ml}$ respectively. In the control group, the life cycle was completed in 46.77 ± 1.33 days.

For *R. communis* leaf extracts from Narok, 11.15 ± 0.75 adults emerged when 125 $\mu\text{g/ml}$ concentration was used. However, at 250 $\mu\text{g/ml}$ and 500 $\mu\text{g/ml}$ mortality was 100% hence no adult emerged. The life cycle lasted for 121.05 ± 2.55 days while in the control group the life cycle was completed in 46.77 ± 1.33 days ($P=0.017$). For, extracts from Juja, the life cycle lasted for 115.13 ± 1.95 days and only 5.67 ± 1.33 adults emerged.

4.5 Comparing larvicidal effects of *R. communis* bark and leaf extracts from Kisii, Narok and Juja

Feeding the larvae on powdered bark and leaf extracts of *R. communis* only had no effect on the larvae compared to the untreated larvae. After feeding on leaf and bark powders from Kisii, Narok and Juja, larvae were able to digest the extract without any detrimental effect hence they grew very fast with insignificant mortality (plate 4.9). There was no larval mortality observed.

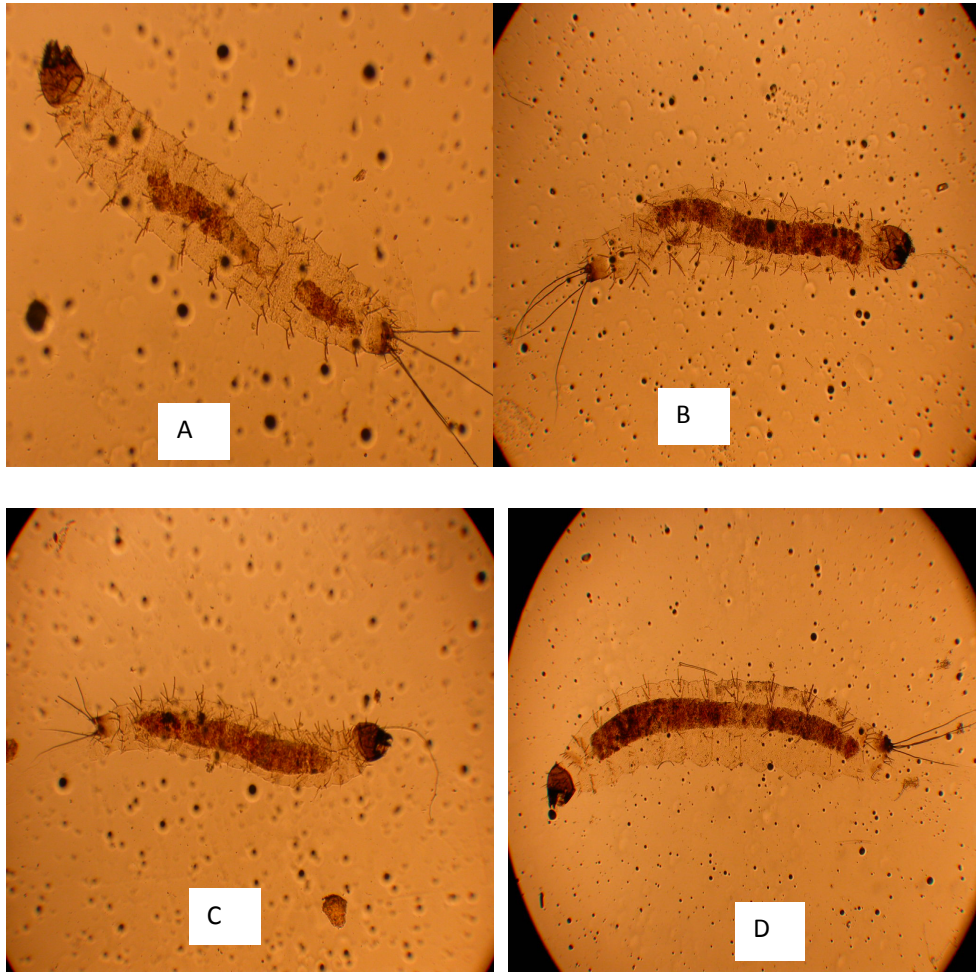


Plate 4.9: Comparing larvae that had fed on aqueous *R. communis* powder obtained from Kisii, Narok and Juja. A: Larvae that had fed on *R. communis* leaf powder from Narok, B: Larvae that had fed on *R. communis* bark powder from Kisii, C: Larvae that had fed on *R. communis* bark powder from Juja, D: Larvae fed on larval food only (control)

4.6 Adulticidal effects of *R. communis* bark and leaf extracts on *P. duboscqi*

From early studies, it was noted that there was no significant difference in the effects of *R. communis* extracts obtained from Kisii and Juja regions of the country. As a result, only *R. communis* extracts from Narok County were considered in this section.

4.6.1 Mortality of sand flies by ingestion of methanol leaf extracts of *R. communis* from Narok County

After feeding on methanol *R. communis* leaf extract, mortality for both male and female sand flies was observed. However, mortality values between male and female sand flies were not significantly different ($F = 1.51$, $P = 0.218$). However, individual sexes showed significant mortality when different extract concentrations were used. With methanol bark extracts, low or no mortality was recorded at 1, 4, 8, 12, 24 hours post treatment and hence cumulative mortality was reported at 96 hours post feeding (table 4.13). Cumulative mortality was 24.05 ± 2.35 (n=30) and 23.75 ± 2.95 (n=30), for male and female respectively when *P. duboscqi* flies were fed on methanol *R. communis* leaf extracts. In contrast, methanol *R. communis* bark extract caused a cumulative mortality of 19.33 ± 1.67 and 22.90 ± 2.15 for males and females respectively. LC_{50} and LC_{90} at 48 hours was $121.15 \mu\text{g/ml}$ and $173.78 \mu\text{g/ml}$ respectively for methanol *R. communis* bark extract.

Table 4.13: Cumulative mortality of female *P. duboscqi* after feeding on *R. communis* leaf methanol extracts from Narok County

Type of extract	8hrs	12hrs	24hrs	48hrs	72hrs	96hrs	Total dead flies
MeOHL	2	1	4	4	8	5	24
MeOHB	3	3	6	2	5	8	27
AqL	1	0	2	5	8	3	19
AqB	4	4	3	6	4	2	23
EACL	0	2	8	4	3	4	21
EACB	5	2	3	1	6	5	23
Total dead flies	15	12	26	22	34	27	137

KEY: MeOHL-methanol leaf, MeOHB-methanol bark, AqL-aqueous leaf, AqB-aqueous bark, EACL-ethyl acetate leaf, EACB-ethyl acetate bark

Significant female mortality was recorded in methanol extract of both leaf and bark *R. communis* extracts at 48 hours. When female sand flies were fed on leaf extracts, recorded mortality was 15.57 ± 0.63 (n=30), 19.67 ± 1.45 (n=30) and 21.00 ± 0.58 (n=30) at 125 $\mu\text{g/ml}$, 250 $\mu\text{g/ml}$ and 500 $\mu\text{g/ml}$ respectively. A significant difference was noted ($F=13.38$, $DF_{6, 14}$, $P=0.001$). The LC_{50} and LC_{90} for females was 126.21 $\mu\text{g/ml}$ ($\chi^2 = 23.4$) and 292.86 $\mu\text{g/ml}$ ($\chi^2 = 31.3$) respectively. Equally, significant male mortality was recorded ($P<0.001$) with LC_{50} and LC_{90} being 146.78 $\mu\text{g/ml}$ ($\chi^2 =29.01$) and 323.59 $\mu\text{g/ml}$ ($\chi^2 =39.20$) respectively.

Likewise, mortality increased with increase in concentration when sand flies fed on *R. communis* bark methanol extracts. At 48 hours post feeding, recorded mortality was 17.23 ± 1.15 (n=30), 20.67 ± 0.33 (n=30) and 25.01 ± 0.58 (n=30) at 125 $\mu\text{g/ml}$, 250 $\mu\text{g/ml}$ and 500 $\mu\text{g/ml}$ respectively and the observed difference was significant ($F=79.75$, $DF_{6, 14}$, $P=0.001$). LC_{50} and LC_{90} for females was 125.05 $\mu\text{g/ml}$ ($\chi^2 = 18.9$) and 288.40 $\mu\text{g/ml}$ ($\chi^2 = 26.2$) respectively. Mortality for males was 13.63 ± 2.55 (n=30), 15.64 ± 1.37 (n=30) and 22.25 ± 0.55 (n=30) at 125 $\mu\text{g/ml}$, 250 $\mu\text{g/ml}$ and 500 $\mu\text{g/ml}$ respectively. A significant difference ($P=0.001$) occurred when comparing female and male mortality at a concentration of 250 $\mu\text{g/ml}$. Mortality increased steadily in both sexes at a concentration of 500 $\mu\text{g/ml}$ and as time of exposure increased, mortality rate approached 100%. Sand fly mortality was significantly higher in *R. communis* extract treatments than in the control treatments ($P < 0.001$) (Fig. 4.8).

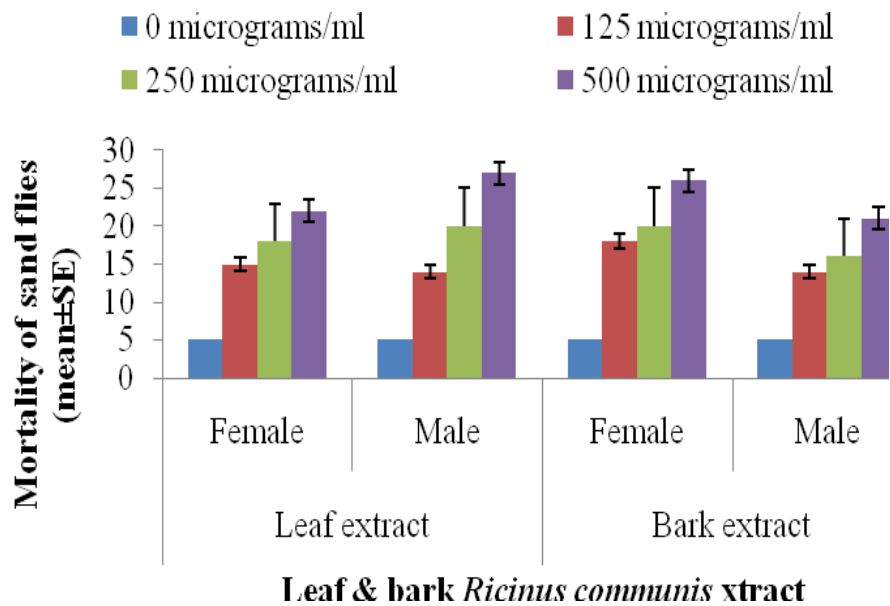


Figure 4.8: Comparing male and female mortality rates of *P. duboscqi* after feeding on *R. communis* leaf and bark methanol extracts from Narok

For aqueous extract treatment, similar mortality rates were recorded which were significantly higher than those of the controls. Significant mortality rates were noted when comparing males and females ($F=63.58$, $P<0.001$). At 96 hours post feeding on *R. communis* leaf extract, male mortality was 14.00 ± 1.47 , 16.67 ± 0.33 and 20.48 ± 2.32 ($n=30$) at 125 $\mu\text{g/ml}$, 250 $\mu\text{g/ml}$ and 500 $\mu\text{g/ml}$ respectively. Female mortality rates were 6.40 ± 2.31 , 11.00 ± 0.67 and 15.85 ± 0.55 ($n=30$) at 125 $\mu\text{g/ml}$, 250 $\mu\text{g/ml}$ and 500 $\mu\text{g/ml}$ respectively. LC_{50} and LC_{90} values were 121.15 $\mu\text{g/ml}$ ($\chi^2 = 16.31$) and 301.39 $\mu\text{g/ml}$ ($\chi^2 = 23.42$) respectively. Cumulatively, more males were dying as compared to the females, although the difference was not significant ($P=0.131$) (fig. 4.9).

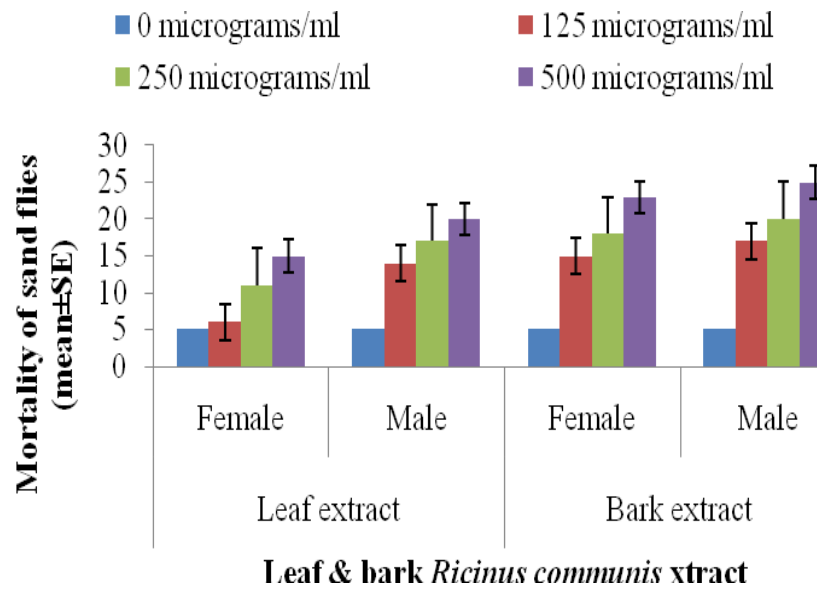


Figure 4.9: Mortality rate of *P. duboscqi* sand flies after feeding on aqueous *R. communis* extracts from Narok County

In *R. communis* ethyl acetate extract treatment in both leaf and bark, males were more affected than the females with mortality of over 60% in the lowest concentration of 125 $\mu\text{g/ml}$ at 48 hours of exposure and beyond. There was also significant difference in mortality between the three different concentrations used for the study ($F=37.12$, $P=0.01$). The LC_{50} for females at 48 hours was 169.25 $\mu\text{g/ml}$ ($\chi^2=31.7$) while the LC_{50} for males at 48 hours was 134.02 $\mu\text{g/ml}$ ($\chi^2=26.5$). Female mortality was 11.33 ± 2.33 , 13.63 ± 1.45 and 17.23 ± 1.85 ($n=30$) at 125 $\mu\text{g/ml}$, 250 $\mu\text{g/ml}$ and 500 $\mu\text{g/ml}$ respectively. For males, mortality was 12.67 ± 1.63 , 16.00 ± 2.83 and 21.42 ± 1.30 ($n=30$) at 125 $\mu\text{g/ml}$, 250 $\mu\text{g/ml}$ and 500 $\mu\text{g/ml}$ respectively (fig. 4.10).

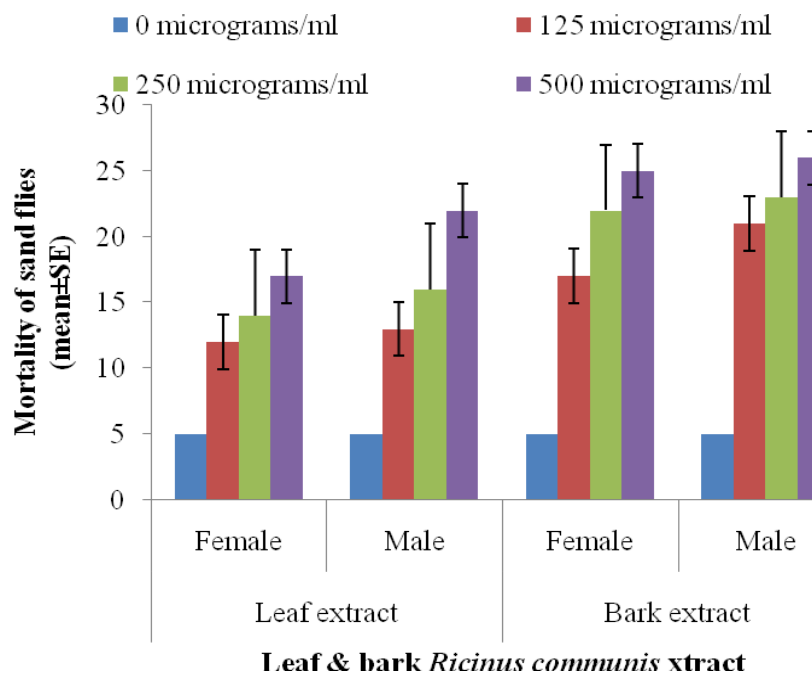


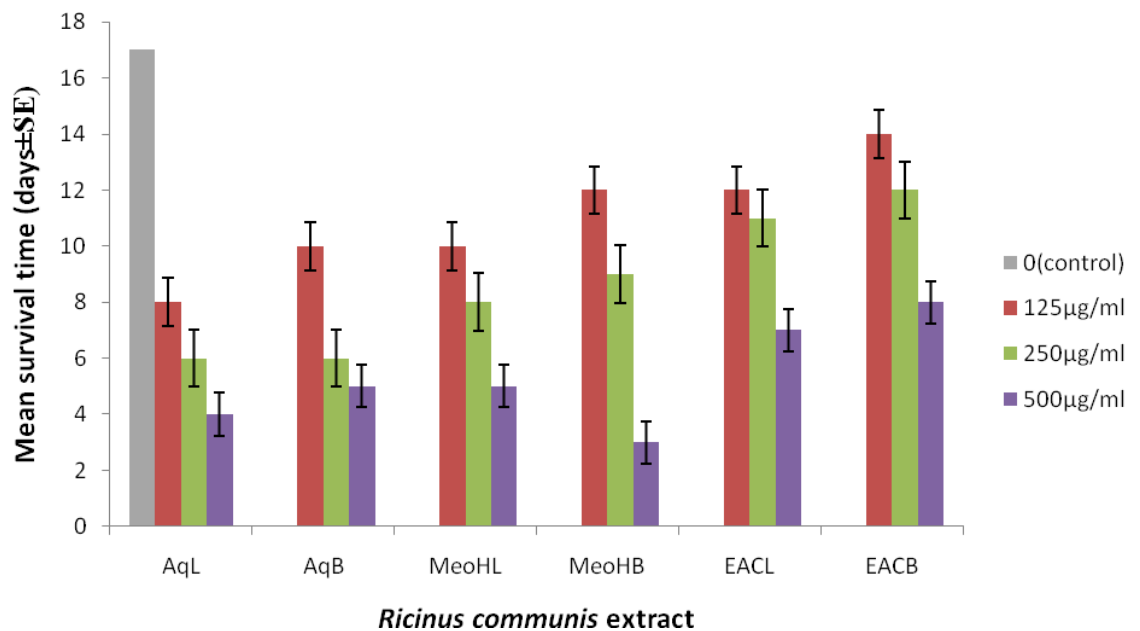
Figure 4.10: Comparison of male and female *P. duboscqi* mortality rates after feeding on *R. communis* ethyl acetate extract from Narok County

4.6.2 Mean survival time after feeding on *R. communis* leaf and bark extracts

Mean survival time decreased with increase in concentration of the extract used. At a concentration of 125 $\mu\text{g/ml}$ there was no significant difference in survival time ($P=0.31$) for sand flies that had fed on methanol, aqueous and ethyl acetate extracts. However, there was a significant difference when compared to the control group ($P<0.001$). Longevity in days was highest in the sand flies that had fed on ethyl acetate *R. communis* leaf extract, 12.67 ± 1.33 days ($n=30$, $P=0.011$) followed by the sand flies that had fed on methanol extract, 10.44 ± 1.46 days ($n=30$, $P=0.011$) while the sand flies that had fed on aqueous extract had the lowest longevity of 8.67 ± 0.33 days ($P=0.021$). Sand flies in the control group had lived for 17.37 ± 1.63 days.

Significant decline in longevity was noted when high extract concentrations were used. At a concentration of 250 $\mu\text{g/ml}$, mean survival time was 6.00 ± 0.58 days and 8.37 ± 2.01 days for sand flies that had fed on aqueous and methanol extracts respectively. However, sand flies in the control group lived for 15.00 ± 1.30 under

similar conditions. The sand flies that had fed on ethyl acetate extract had the highest longevity of 11.60 ± 2.54 days ($P=0.021$). At $500 \mu\text{g/ml}$, longevity was further suppressed across all the three extracts used. In the sand flies that had fed on aqueous extract, mean survival time was 4.41 ± 1.58 days, significantly different compared to the sand flies in the control group ($P<0.001$). Feeding the sand flies on methanol and ethyl acetate extracts resulted in 5.95 ± 0.55 and 7.81 ± 1.18 days of survival respectively (fig. 4.11).



Key: AqL-aqueous leaf, AqB-aqueous bark, MeOHL-methanol leaf, MeOHB-methanol bark, EACL-ethyl acetate leaf, EACB-ethyl acetate bark

Figure 4.11: Mean survival time of *P. duboscqi* sand flies after feeding on different *R. communis* leaf and bark extracts from Narok County

4.7 Potential of a modified trap for sand fly surveillance

A total of 1302 sand flies were caught over 3 nights of trapping. Out of these, 576 were males while 726 were females. These traps attracted significantly more females than males ($t=-2.334$, $P=0.02$) with a male/female ratio of less than 0.80. A significant difference was found among the different attractants used ($P<0.001$).

Traps baited with goat blood were the most effective followed by banana fruit baited traps while cattle urine baited traps were the least.

4.7.1 Cattle, sheep, goat and chicken blood baited traps

The trap baited with goat blood yielded more sand flies, 202.33 ± 2.85 (mean \pm S. E) as compared to the rest (fig. 4.12). This was followed by sheep blood-baited trap which caught 142.67 ± 4.92 , cattle blood-baited trap 121.00 ± 4.00 while chicken blood baited trap was the least 65.00 ± 1.53 . CDC light trap was significantly different from the experimental traps, 346.33 ± 10.84 ($P < 0.001$). There was no significant difference between the negative controls (water and sugar baited traps) which had 9.67 ± 1.20 and 13.00 ± 1.00 ($P = 0.99$) respectively. When averaged over the 3 nights, a significant difference was found among the four blood baited traps ($f = 579$, DF 6, 14, $P = 0.001$). However, the difference between sheep blood baited trap and cattle blood baited trap was not significant ($P = 0.08$). 76% of the sand flies trapped by blood baited traps were females while 24% were males.

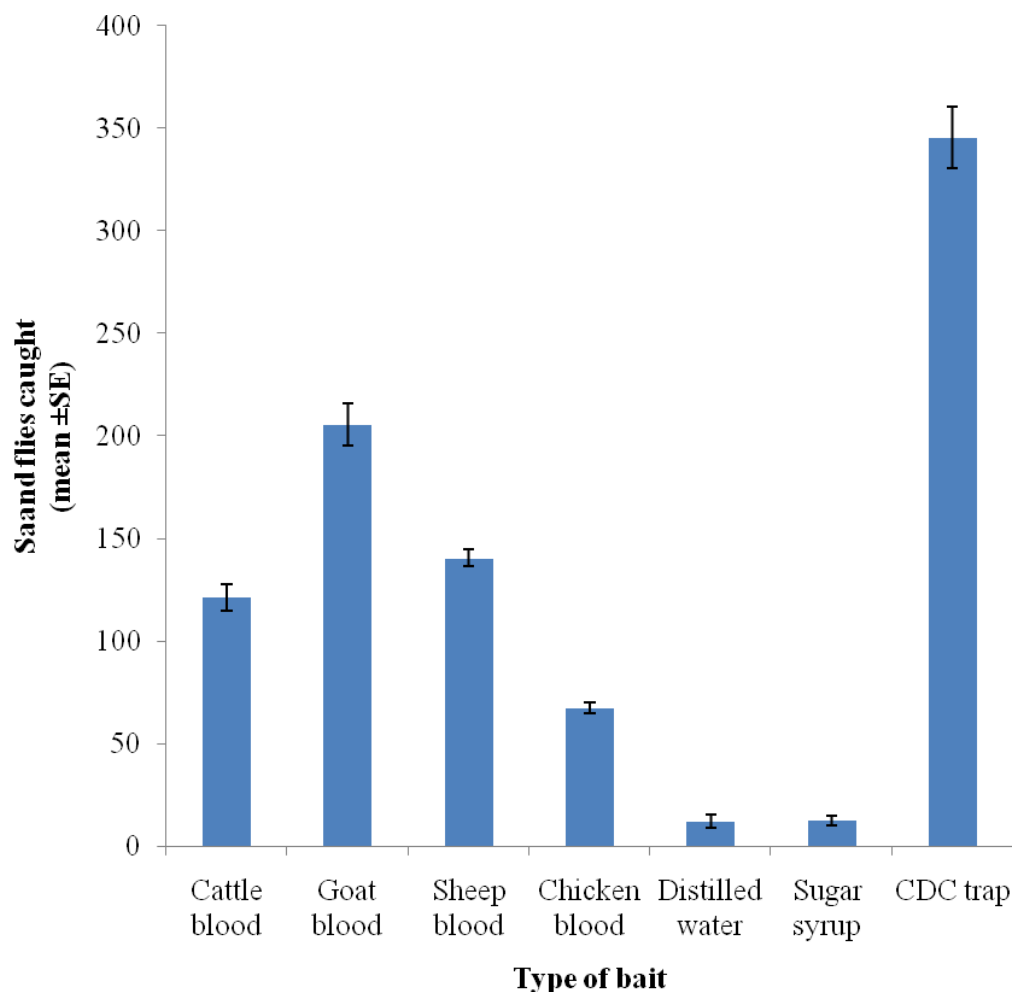


Figure 4.12: Mean number of sand flies caught by cattle, goat, sheep and chicken blood baited traps compared to positive and negative controls

4.7.2 Potential of cattle, sheep and goat urine Baited traps

More males than females (56% males, 44% females) were attracted by these traps. There was no significant difference among the three baited traps ($P=0.21$). Sheep urine baited trap captured the highest number of sand flies, 37.67 ± 2.96 , followed by goat blood baited trap 33.00 ± 1.53 and the least was cattle urine baited trap, 26.67 ± 1.45 . Post hoc analysis revealed that there was no significant difference among these three traps ($P=0.87$). However, these catches were significantly different as compared to the positive control CDC light trap which captured 379.67 ± 10.11

($P=0.01$). The negative control captured 11.67 ± 0.88 (mean \pm SE) sand flies (fig.4.13).

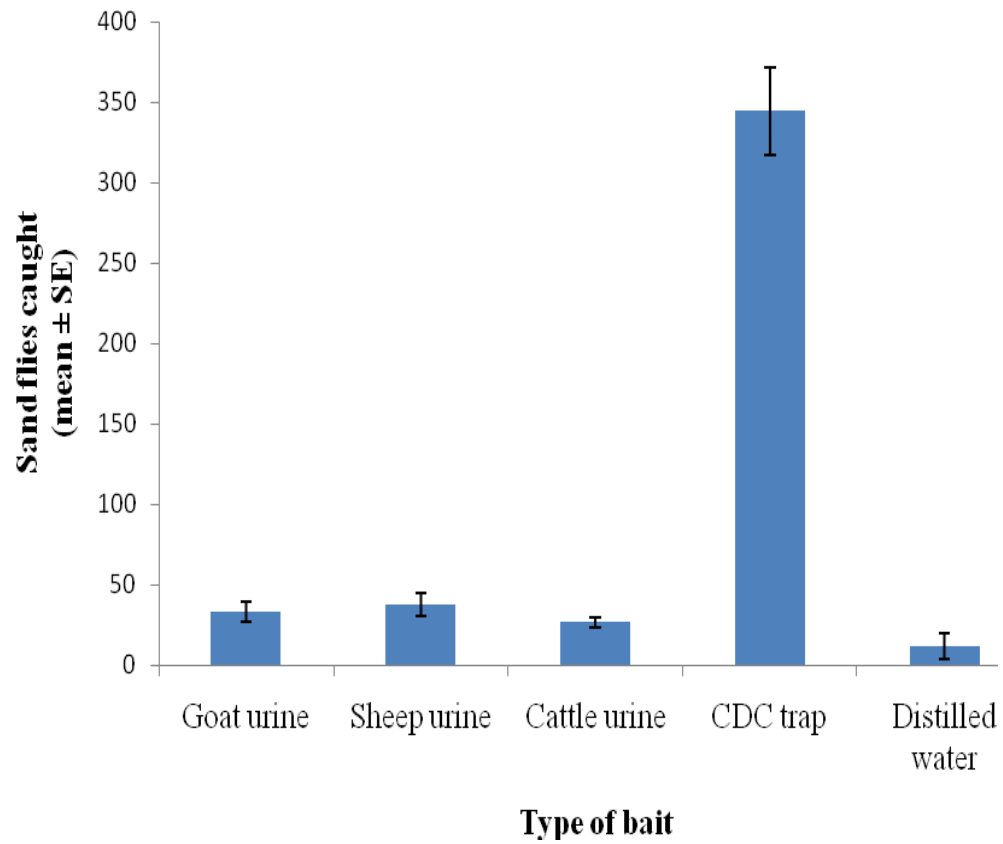


Figure 4.13: Mean number of sand flies caught by cattle, sheep and goat urine baited traps

Comparing blood baited traps with urine baited traps revealed a significant difference in their catches ($P<0.001$) (fig. 4.14).

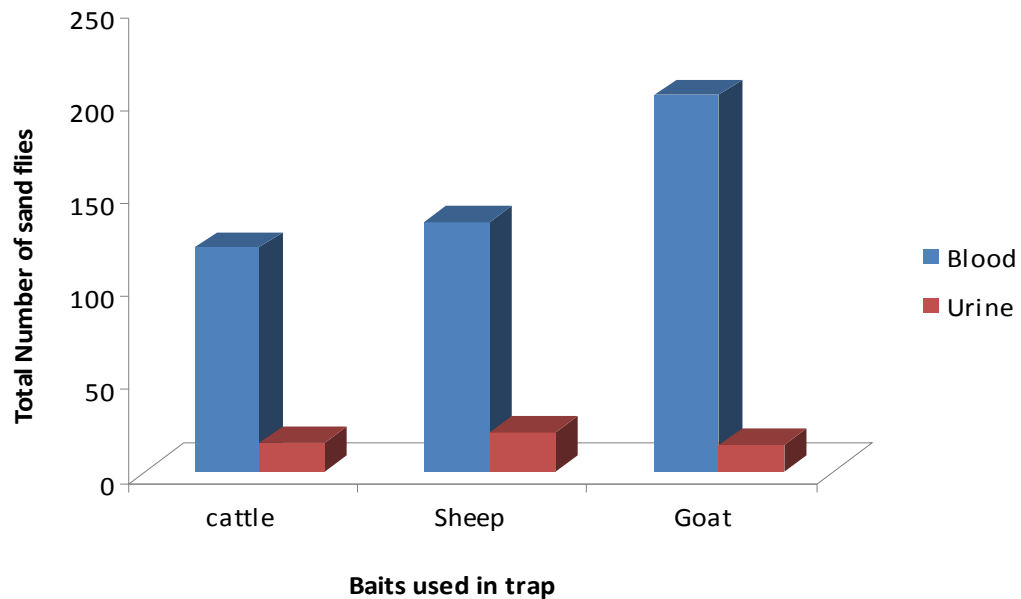


Figure 4.14: Comparison of number of sand flies caught by blood and urine baited traps

4.8 Ripe banana, apple, grapes and mango fruit baited traps

Fruits used as baits included bananas, apples, grapes and mangoes while CDC light trap and water were the controls. The average catch of the baited traps as compared to the controls was significantly different ($f=259.364$, $DF 6, 14$, $P<0.001$). Banana baited trap had caught the highest number of sand flies, 94.33 ± 4.63 followed by mango baited trap which caught 89.67 ± 2.33 (mean \pm S. E). Apple and grapes baited traps caught 78.00 ± 4.62 and 57.00 ± 5.29 (mean \pm S. E) sand flies respectively (fig. 4.15). The difference between banana baited trap and apple baited trap was not significant ($P=0.68$). The CDC light trap caught a significantly high number of sand flies ($P<0.001$) while the negative controls; water and sugar syrup baited traps caught 18.67 ± 6.17 and 17.00 ± 2.08 (mean \pm S. E) respectively. Fruit baited traps attracted more males (66%) than females (34%).

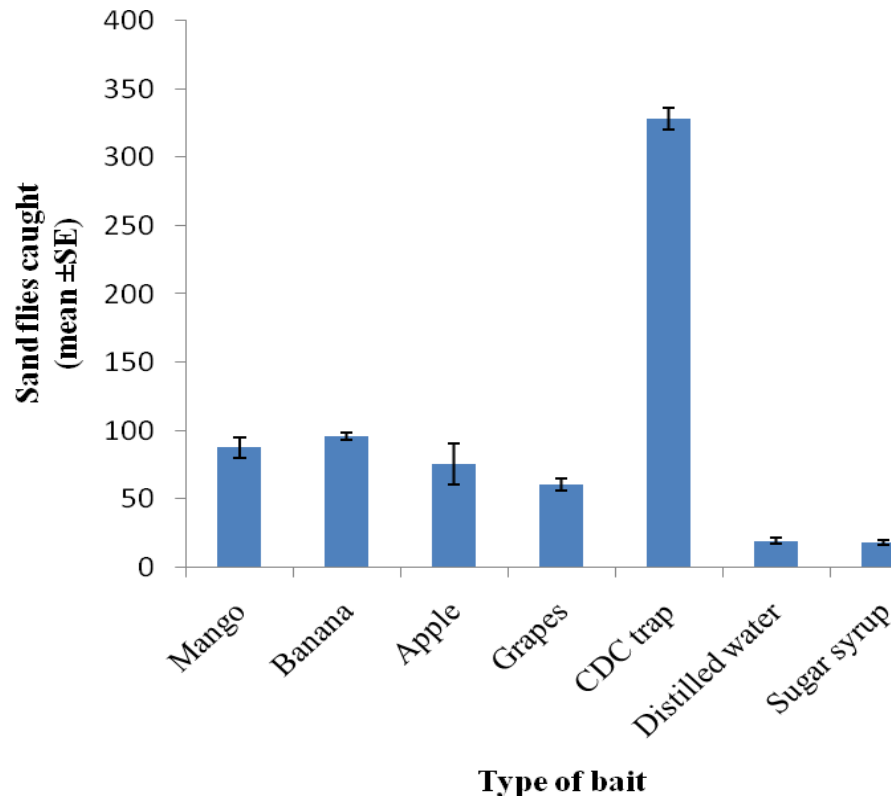


Figure 4.15: Comparison of number of sand flies caught by fruit baited traps (ripe banana, mango, grapes and apple)

4.9 Identification of collected sand flies

A total of 1302 sand flies representing two genera and 7 species were collected using traps baited with three different types of baits: defibrinated blood, urine and fruits. 64.3% of the collected sand flies belonged to the genus *Sergentomyia* and 35.7% to *Phlebotomus*. In all the traps *Phlebotomus martini* dominated with a relative abundance of 35.7%. This was followed by *Sergentomyia schwetzi* (34.1%), *S. antennata* (15.7%), *S. clydei* (7.9%), *S. africana* 3.5%, *S. squamipleuris* (2.2%) while *S. durenii* (0.9%) was the least in relative abundance. Sand flies collected by blood baited traps were twice as numerous as those from fruit baited traps.

Blood baited traps collected the highest number of sand flies with a relative abundance of 64.6% followed by fruit baited traps (27.2%) and urine baited traps

(8.2%). Blood baited traps collected sand flies representing 7 species. *P. martini* dominated with a relative abundance of 23% then *S. schwetzi* (19.5%), *S. antennata* (9.9%), *S. clydei* (5.8%), *S. africana* 2.9%, *S. squamipleuris* (2.1%) while *S. durenti* (0.9%) was the least. In the fruit baited traps *S. schwetzi* was the dominant species (11%), followed by *P. martini* (9.3%), *S. antennatus* (4.6%) while *S. clydei* was the least with 2.1%. *S. durenti*, *S. squamipleuris* and *S. afrikanus* were not collected in the fruit baited traps. Urine baited traps collected the least in terms of abundance and diversity. The collected sand flies represented four species: *S. schwetzi* (3.5%), *P. martini* (2.9%), *S. antennata* (1.2%) and *S. africanus* (0.6%). *S. clydei*, *S. durenti* and *S. squamipleuris* were not represented.

There was a significant difference when *P. martini* ($\chi^2=17.964$, $P=0.001$) was compared with *S. schwetzi* ($\chi^2=6.366$, $P=0.01$). The abundance of these two species was significantly different ($P=0.001$) in the goat blood baited trap but in sheep blood they were not significantly different ($P=0.061$). Among the blood, urine and fruit baited traps, the sand flies collected by blood baited trap was significantly different from the rest ($\chi^2=11.464$, $P=0.003$). Comparing the abundance of the two genera (*Sergentomyia* and *Phlebotomus*) showed a significant difference among the species ($\chi^2=9.458$, $P=0.009$). In terms of physiology, only 3 sand flies were gravid while 6 were blood fed.

CHAPTER FIVE

DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 Effect of *R. communis* on egg shell membranes

Synthetic chemical pesticides have been used widely as the main method for controlling insect pests. However, there are many problems associated with the extensive use of these compounds, such as build-up of pesticide resistance, negative impact on natural enemies, in addition to negative environmental and health impacts (Ramos-López *et al.*, 2010). It has been proven that plant extracts and plant compounds are eco-friendly, target-specific, less expensive, and highly efficacious pesticides for the control of vectors (Appadurai *et al.*, 2015). Therefore, natural products are proving to be potential sources of new and selective agents for the treatment of important tropical diseases and the control of vectors.

In this study, when *P. duboscqi* eggs were treated with *R. communis* extract at various concentrations, there was loss of chorionic layers irrespective of the type of extract and part of the plant (leaf or bark) used. Active compounds in *R. communis* were capable of eroding the exochorion, mesochorion and endochorion layers of the egg leaving a thin chorionic layer on the egg. This concurs with past research which showed that moistening incubated *P. duboscqi* eggs with 1ml of *Mundulea sericea* (Anjili *et al.*, 2014) led to massive loss of chorionic layers. At a scanning electron microscopy level, the egg shell of all phlebotomine sand flies is characterised by the outer chorion forming a series of ridges cross-linked in various places to form a specific pattern (Fausto *et al.*, 1992). All these cross-linkages are lost to an extent of revealing the hidden plastron once incubated eggs are moistened with plant extracts (Aritho *et al.*, 2017). These corrosive effects of *R. communis* extracts are due to the presence of certain phytoconstituents in the plant especially ricinine, a water soluble glycoprotein concentrated in various parts of the plant (Manpreet *et al.*, 2012).

The insect egg shell has several roles that it performs so as to ensure viability of the embryo. The egg shell serves as a boundary between the external environment and the developing embryo. Other vital functions of the egg shell include protection

against desiccation, enemies and enhancing gaseous exchange (Fausto *et al.*, 1992). Therefore, any change in the structure of the egg shell will affect the developing embryo. The loss of chorionic structures may account for the loss of embryo viability that was observed in this study and the subsequent low number of emerging larvae. The embryo might have died due to desiccation or poor regulation of gaseous exchange due to the loss of chorionic membranes.

5.2 Effect of *R. communis* extract on hatchability of eggs

In this study, hatchability of eggs was found to be inversely proportional to the extract concentration. Aqueous and methanolic extracts showed significant reduction in egg hatchability revealing the harmful effects of these extracts. However, at 125 µg/ml concentration, a significant number of eggs hatched showing the inability of the extracts to reach the embryo. This observation is in agreement with past research on mosquitoes which revealed that hatchability rate is inversely proportional to the concentration of *Artemisia annua* extract (Shao-Xiong *et al.*, 2013).

At the lower concentrations, the extracts eroded the chorionic egg layers but did not inhibit the eggs from hatching. This might be due to the inability of the extract to penetrate the egg membranes to reach the embryo. However, at 500µg/ml concentration, 95% inhibition of egg hatching was observed meaning that the high concentration of the toxic compounds reached the embryo and inhibited or killed it.

These findings concur with past research which showed that sand flies feeding on *R. communis* and Bougainvillea leaves produced fewer larvae compared to the control and post hoc analysis revealed that fewer eggs had hatched (Kaldas *et al.*, 2014). Hexane extracts of *Andrographis lineat*, *A. paniculata* and *Tagetes erecta* showed 100% ovicidal activity against *An. Subpictus* (Mann & Kaufman, 2012). Elsewhere, hatchability of eggs was reduced by petroleum ether extracts of *Piper nigrum* and *Jatropha curcas* by 59% and 58% respectively at the lowest concentration of 2µL/ml (Khani *et al.*, 2013). This reduction in egg hatchability shows that plant extracts possess compounds which are toxic to the developing embryo.

Besides inhibiting hatching, *R. communis* extracts also had pronounced effect on the incubation period of eggs and developmental period of larvae. Under normal insectary conditions, phlebotomine eggs take averagely 10 days to hatch into larvae but when these eggs were moistened with the extract, the incubation period took 15 days. This may suggest that bioactive compounds from plant extracts can have pronounced effects on the developmental period of various insect stages. This may reveal that, exposure of insect vectors to active botanical derivatives can result in an extension of the duration of development (Mann & Kaufman, 2012) hence, exposing susceptible stages to the effects of these botanicals.

Comparing the effects of leaf and bark extracts shows that *R. communis* bark extracts have severe effects on hatchability of *P. duboscqi* eggs. Acute application of extracts in all of the concentrations did not significantly inhibit hatchability of eggs. At 125µg/ml concentration of bark methanol extract, 29% of eggs hatched while at 500µg/ml, only 5% of eggs hatched. In the methanol leaf extract, 34% and 9% of eggs hatched at 125µg/ml and 500µg/ml respectively. This implies that the active compounds in *R. communis* especially ricinine is differentially concentrated in leaves and bark. Ethyl acetate and aqueous extracts showed moderate effects.

5.3 Effect of *R. communis* extract on larvae

At 125µg/ml extract concentration, no mortality or deformity was observed. This observation is in agreement with Anjili *et al* (2014) who observed that feeding *P. duboscqi* larvae with 1ml *Mundulea sericea* powdered leaves resulted in the survival of all first instar larvae. An explanation for this may be due to the low content of active compounds in the low concentrated or powdered extract. Therefore, the extract was unable to penetrate the larval cuticle hence no toxic effect was experienced by the larvae.

Increasing the concentration to 250µg/ml and 500µg/ml saw significant larval mortality with deformity especially when using *R. communis* bark extracts. High larvicidal activity was seen against 2nd, 3rd and 4th larval instars. Methanol extract was more effective in mortality as shown by the LC₅₀ values. Similar results were obtained by Ramos-Lopez *et al.*, (2010) who obtained 0% larval viability rate with

methanol leaf extracts of *R. communis* at 24000µg/ml. Tounou *et al.*, (2011) also showed that in topical and ingestion toxicity tests, aqueous extracts and oil emulsion of castor bean plant caused significantly higher *Plutella xylostella* larval mortalities than the control.

Extracts from *R. communis* have been shown to possess larvicidal and insecticidal activities against a wide range of insects of medical, veterinary and agricultural importance (Ramos-Lopez *et al.*, 2010). Phytochemical studies of *R. communis* extract reveal the presence of steroids, saponins, alkaloids, flavonoids, and glycosides which might be responsible for the observed mortalities (Jitendra & Ashish, 2012). The killing effects of these extracts may have been due to the presence of ricinine compound in the bark extract (Jitendra & Ashish, 2012) which probably killed the larvae through ingestion and contact.

A relatively high mortality was noted when *R. communis* bark extracts were used as opposed to leaf extracts. This might be due to the difference in the levels of ricinine in these extracts since insecticidal effects of plant extracts vary according to plant species, insect species, geographical varieties and plant parts used (Anupam *et al.*, 2012). It has also been shown that roots and bark have a high partitioning for the photosynthates or exudates which act as toxins (Okello *et al.*, 2010). Past investigation suggests that ricinine is the compound responsible for this activity but it occurs in minor concentrations in the leaf (Ramos-Lopez *et al.*, 2010). Several compounds in the extract might be responsible for the killing action of *R. communis* extracts. Ricin is known to cause larval mortality by causing acute cell death by inactivating ribosomal RNA hence inhibiting protein synthesis. Also the burning effect of the extract might have caused larval mortality by contact (Tounou *et al.*, 2011).

The effectiveness of an insecticide depends on several factors including chemical nature of the molecule, amount of active ingredients used, frequency of applications, time interval between applications, size and age of insect, area and time of contact with the insect (Cinthia *et al.*, 2012). In the current study, larvae of *P. duboscqi* were sprayed with 5ml of the extracts on a two day interval throughout the experimental

period. Hence, larvae were completely in contact with the extract and this may explain why mortality was very high. It has also been shown that plants produce numerous chemicals, many of which produce chemical factors and metabolites that have larval, adulticidal or repellent activities against different species of insects (Anupam *et al.*, 2012). Where larvae survived, the explanation could be because the extract did not break the cuticle to penetrate into the vital internal organs hence toxicity was very low. This was observed in all the low concentration throughout the experimental period.

These extracts have a wide range of biological activity against vectors and the toxic effects of these extracts may be comparable to extracts from other medicinal plants. From this study, it was observed that the killing of larvae by these extracts was stepwise through lysis of the gut. First there is disintegration of the gut which detaches first from the head. Later, splitting of the gut was observed at specific points. After this, rotting occurred which caused darkening of the larvae and the final step in the mechanism of larvae killing was the rotting of the whole larvae. This concurs with past research on *Leishmania infantum* which showed that saponins in *R. communis* extract increased membrane permeability of the parasite leading to their lysis (Rondon *et al.*, 2012) and eventual death.

R. communis extracts were also found to prolong the larval, pupal and the entire life cycle duration for *P. duboscqi* flies (for more than 80 days). These developmental stages were either delayed or inhibited completely (like the pupal stage) because most plants have been found to contain compounds like repellents, feeding deterrents, toxins, and growth regulators (Marta & Sarah, 2011) which have varying effects to the insects. The long larval duration of up to 87 days in methanolic extract shows that this plant extract can act as a growth regulator. This way, this extracts could be effective against all the developmental stages of sand flies. Some plant phytochemicals have a toxic effect on insect adults and larvae by interfering in their growth, development or reproduction (Rania *et al.*, 2014). Also Neem oil has been shown to act as larvicidal, oviposition inhibitor and growth regulator against *Culex quinquefasciatus*, *Anopheles culicifacus*, *Anopheles stephensi* and *Aedes aegypti* (Man & Kaufman, 2012).

5.4 Adulticidal effects of *Ricinus communis* extract

Extensive use of synthetic compounds in the control of vectors of medical, agricultural and veterinary importance has led to the build-up of pesticide resistance, negative impacts on the environment and they are not target specific. Due to this, botanical insecticides have been found to be suitable alternatives for new and selective agents for the treatment of important tropical diseases and the control of vectors (Mong'are *et al.*, 2016). In the present study, results obtained from *R. communis* extracts concur with early research showing that botanical insecticides are potential in controlling vectors of medical importance.

This study has shown that *R. communis* compounds extracted from either leaves or the bark have the potential of killing sand flies through ingestion or contact. The killing effect of an insecticide depends not only on the active compounds contained in an insecticide but also the part of the insect in contact with the insecticide (Degri *et al.*, 2013). This may explain why ingestion of *R. communis* extract caused high mortality in *P. duboscqi* sand flies. Similar to this study is past research which had shown that ingestion or contact with *R. communis* extracts caused high mortality of up to 100% in various insect vectors (Collavino *et al.* 2006; Ghosh, 2012).

The observation that low concentrations produced low mortality rates while high concentrations gave mortalities of up to 100% shows that *R. communis* has insecticidal properties. This concurs with past research which showed that high concentrations of *Acalypha fruticosa* and *Tagetes minuta* extracts were insecticidal to *Phlebotomus duboscqi* sand flies (Ileri *et al.*, 2010). This means that most plants contain compounds including repellents, feeding deterrents, toxins, and growth regulators which are insecticidal to haematophagous arthropods and because of this, plants have been used for centuries in the form of crude fumigants where plants were burnt to drive away nuisance biting insects (Marta & Sarah, 2011).

The efficacy of most plant extracts varies with insect species or the developmental stage, and also the formulation type or combination and concentration tested (Degri *et al.*, 2013). Larval stages are highly susceptible compared to adult insects. In this

study, the recorded mortality was concentration dependent and despite the low concentrations used mortality was recorded with very low LC₅₀ values indicating the high insecticidal properties present in *R. communis*. Biologically active flavonoids like quercetin and rutin have been identified in extracts of *R. communis* and may be responsible for these high insecticidal properties (Cinthia *et al.*, 2012). Furthermore, quercetin is a flavonoid which has been found to interfere with the iron metabolism (Rania *et al.*, 2014).

The current results are in agreement with past research which has shown that most medicinal plants have excellent results in controlling vectors including sand flies. Plants like *Azadirachta indica*, *Ricinus communis*, *Solanum jasminoides*, *Bougainvillea glabra* and *Capparis spinosa* have been shown to act as future alternatives for the control of sand flies (Diwakar *et al.*, 2014). Extracts from these plants contain a wide range of active compounds which can act concertedly on physiological processes of the vectors to either kill or deform them. This might be the reason why the mean survival time of sand flies fed with *R. communis* extracts was significantly low compared to the untreated sand flies.

In this study, sugar solution was mixed with the crude extracts to act as bait for the flies. Female sand flies need blood for egg production, but sugar is their main source of energy and the only food taken by males (Gunter *et al.*, 2011). It has been shown that sand flies can feed on aqueous sucrose solutions mixed with noxious plant juices and have their lifespan greatly reduced (Schlein *et al.*, 2001). Therefore, the sugar that was mixed with the crude extracts might have attracted more sand flies which in turn fed more on the crude extracts leading to their death. This property of sugar feeding behaviour in sand flies has been shown to influence longevity, fecundity, dispersal, host seeking behavior and ultimately blood feeding and disease transmission (Gunter *et al.*, 2011).

Among the extracts used, methanolic extract was highly efficacious followed by ethyl acetate extract and the least was aqueous extracts. Phytochemical analysis of methanolic extractions shows the presence of tannins, saponins, flavonoids, alkaloids

and terpenoids (Rania *et al.*, 2014) which acts in a variety of ways to cause death. Besides, killing arthropods, *R. communis* extracts have been found to be active against a wide range of protozoa, bacteria and fungi (Nidhi *et al.*, 2013; Vandita *et al.*, 2013)

5.5 Potential of blood baited traps in attracting Phlebotomine sand flies

Sticky traps, CDC light trap and CO₂ baited traps are mainly used for monitoring sand flies in order to understand their vector ecology so that effective traps for surveillance can be designed (Signorini *et al.*, 2013). It is generally known that CDC light trap and CO₂ baited traps capture the highest number of sand flies in the field hence they are highly preferred for sand fly surveillance. However, these traps have shortcomings for example; they are expensive, need regular maintenance, are heavy and can stay in the field for only a limited amount of time (Gunter *et al.*, 2011). Therefore, there is a need to come up with new, cheap and readily available traps for sand flies and other haematophagous insects. Further, there is a need for new devices which are dependent on host odours in trapping these vectors.

The findings that blood baited traps captured more sand flies than the rest of the baits shows that compounds released from animal hosts dominate over plant host. Significantly high numbers of female sand flies were attracted to the blood baited traps showing the significance of blood to the sand flies. Host blood is a combination of volatiles emitted by breath, hair, skin, urine and faeces making it a strong olfactory cue in host seeking (Logan & Birkett, 2007).

It has been shown that vertebrate blood is significant for reproduction and growth of haematophagous insects (Aurelie *et al.*, 2009). Female biting midges use volatile animal odours to recognize and discriminate between potential hosts on which to blood feed on (Logan *et al.*, 2009). Therefore, identifying and maximizing the use of these chemicals to attract or repel sand flies will be an important step in developing new strategies effective against phlebotomine sand flies.

The high number of sand flies captured by goat blood baited trap reveals that the goat is the preferred reservoir host for sand flies. These findings are consistent with

previous studies which revealed that goat blood is the preferred blood meal for *P. martini* although other species prefer mixed feeding (Philip *et al.*, 1992). Therefore, it is likely that a goat has a strong cue which attracts sand flies for blood feeding (Kasili *et al.*, 2009).

It was also observed that chicken blood was the least in attracting sand flies compared to goat, sheep and cattle blood. This was contrary to other investigations that have shown that chickens can be suitable hosts for *Lutzomyia longipalpis* population (Mauricio *et al.*, 2010; Mutuku *et al.*, 1986). This may be because sand flies prefer habitats that are close to chicken coops; hence it is easier to locate chicken for blood feeding compared to human, dog or rabbit.

Further research has shown that female sand flies which feed on chicken blood have delayed egg development compared to those that feed on rabbits (Katerina *et al.*, 2013). Therefore, chicken blood may not be favoured as a source of blood meal in the presence of goat blood.

Cattle and sheep blood baited traps had a stronger attraction index for sand flies than chicken blood. This shows that cattle and sheep can be alternative hosts for sand flies in case the favoured hosts are not available. Sand flies prefer blood feeding on mammalian hosts but some prefer reptilian hosts. Therefore, the attraction of sand flies to avians and bovids shows the role of these animals in the transmission of *Leishmania* parasites. Attraction of haematophagous insects to hosts may depend on the natural host preference or the availability and accessibility of the hosts within the local environment (Irma *et al.*, 2002). This explains why goat's blood was the preferred attractant in this study bearing in mind that almost every homestead in Rabai, Marigat division keeps goats more than any other domestic animal.

5.6 Potential of urine baited traps in attracting phlebotomine sand flies

Contrary to blood, urine baited traps attracted very few sand flies showing that there are no attractants for sand flies in goat, sheep and cattle urine. This may also be attributed to the fact that urine has no role in the physiology of sand flies. In the field, haematophagous insects are attracted to host odour components and male

pheromones which stimulate mating (Mara *et al.*, 2012). Little is known on the role of urine as a potent cue for biting insects. Very few sand flies were attracted to urine baited traps and this concurs with other findings which showed that feeding stimulants reported to attract mosquitoes and sand flies including host urine did not stimulate *L. shannoni* feeding (Rajinder & Phillip, 2010).

However, urine from host animals may attract or influence physiological conditions of other haematophagous insects including repelling or attracting them. Horse urine has been depicted as the best attractant during sampling of *Glossina morsitans submorsitans* (Enrih *et al.*, 2007). Apart from *morsitans* flies, mosquitoes are also attracted to host urine which contains 4-methylphenol and 3-n-propylphenol as active substances (Enrih *et al.*, 2007).

Other studies have shown that addition of cow urine to habitats attracted oviposition by anopheline and culicine species (Eliningaya *et al.*, 2011). The same might be expected in the case of sand flies since mosquitoes and sand flies have a closely related physiology. This finding shows that the presense of these animals in endemic areas might increase the risk of leishmaniasis or increase the breeding of sand flies in those areas. Therefore, incorporating sheep urine in traps might increase the efficiency of such traps hence reduction of sand flies from the locality. Consequently, volatiles from goat, sheep, cattle or chicken blood, sweat, urine and faeces may be used to synthesize pheromones which could attract blood-feeding females away from feeding on people. Instead, they could be attracted towards insecticide-treated areas where they can be killed and this will reduce their effects on man.

5.7 Potential of fruit baited traps in attracting phlebotomine sand flies

Sand flies generally feed on plant tissues like leaves, stems and flowers. They also feed on various sweet substances like honey dew and ripe fruits which contain a lot of sucrose, glucose, fructose and raffinose (Nataly *et al.*, 1995). Once adults emerge, their first activity is sugar feeding which is the only source of energy for sand flies. Little research has been done on the attractiveness of sand flies to ripe fruits. However, the observation that banana and mango baited traps attracted more sand

flies may be due to the speculation that bananas and mangoes contain high concentrations of sucrose and fructose for energy which the vectors use for host seeking, survival and reproduction.

It is probable that as these fruits ripen and rot, they release volatile chemicals which attract sand flies and other insects. This can be supported by the observation that mosquitoes have been collected from peaches, apples, grapes and watermelons, particularly those damaged by rain and rot (Amy *et al.*, 2011). All the fruits used were attractive to the sand flies but the order of preference was banana, mango, apple and the least was grapes. The actual attractants are not known but sugar and CO₂ emitted as part of metabolism can be some of the attractants since fermenting sugar yeast mixtures have been used as a source of carbon dioxide to attract and trap sand flies (Oscar *et al.*, 2013).

More males were attracted to the fruit baited traps because sugars are their main sources of food and energy. In the field, the main source of the sugar meal is from honey dew excreted by aphids and coccids and by feeding directly on plant tissues in the field (Umakant & Sarman, 2008). Sugar/yeast mixture when allowed to ferment, it produces carbon dioxide which attracts sand flies in the same way as CO₂ baited traps (Aviad *et al.*, 2013). There are also speculations that the attractants for sand flies in fruits are the volatile compounds affecting olfactory receptors or an insect's ability to detect CO₂ emissions (Amy *et al.*, 2011).

Water and sugar syrup baited traps which were the negative controls caught the lowest number of sand flies. This shows that there were no attractants in the negative controls except moisture which is also present in the ripe fruits. Apples and grapes attracted significantly more sand flies as compared to the negative controls, an observation in agreement with previous studies where these fruits attracted more sand flies than the controls (Amy *et al.*, 2011). This shows that ripe and rotting fruits release volatile compounds with emission rates peaking at ripening stage. The released volatile compounds include esters, alcohols, aldehydes, ketones, lactones and terpenoids which insects are sensitive to (Ana *et al.*, 2013). Arthropods

especially insects use them for social communication and some use them as sex pheromones or sex pheromone precursors (Ana *et al.*, 2013). Hence, volatiles emitted by fruits can be used by flies to locate their vertebrate hosts or plants for sugar feeding.

Fruit location is a key issue for feeding, mating and reproduction of specialist insects, and involves the perception of a sequence of olfactory and visual cues (Ana 2013). This shows that some of these volatile compounds may mimic insect hormones used during mating. It is likely that as overripe fruits are fermenting, they emit chemicals which are similar to host seeking cues that stimulate either blood feeding or mating behavior in haematophagous insects and biting insects are believed to be attracted to ripe fruits based on attractive volatile compounds released by plants (Amy *et al.*, 2011). As a consequence, emanations from fermenting sugars can be used as a source of CO₂ to attract and trap sand flies (Oscar *et al.*, 2013). Carbon dioxide can enhance trap collections (Ulrich *et al.*, 2008); hence if traps can be baited with odours from host animals together with carbon dioxide, the attractive efficiency of these traps will be greatly improved. Such traps will be easy and less costly to use as compared to CDC light traps.

5.8 Identification of sand flies

Human landing collections, the CDC light trap and the sticky paper traps are the standard surveillance techniques used to determine sand fly densities in a given locality (Hoel *et al.*, 2010). Phlebotomine sand flies have a wide distribution and diversity in Marigat Division. Females and males depend on sugar as a source of energy but females also need blood meals for egg development (Daniel *et al.*, 2011). Effective vector sampling is necessary for predicting disease outbreaks in a given area.

In this study, lures from different vertebrates and fruits were used in place of light and CO₂ which attracted sand flies from two genera. These traps collected sand flies from seven species within Rabai, Marigat division with *P. martini*, *S. schwetzi* and *S. antennata* being the dominant species. This study concurs with studies using CDC

light traps in Marigat which trapped 11 species of sand flies with *P. martini* and *S. schwetzi* being the dominant species (Kasili *et al.*, 2010).

The high number and dominance of *P. martini*, *S. schwetzi* and *S. antennata* in all the baited traps suggest that these three species of sand flies have a wide range of blood meal and source of sugars hence they may show mixed feeding in nature. This shows that host blood and ripe fruits can mimic human hosts in the field hence these traps can yield a true representative picture of sand fly species in a given locality. *Phlebotomus martini* is the main vector for visceral leishmaniasis (Tonui, 2006) in Kenya although others may be there. The high number of trapped *P. martini* concurs with the high prevalence of visceral leishmaniasis in Marigat division. There is little literature showing the relationship between host urine and sand flies although cow, goat and sheep attracted 4 species of sand flies (*S. schwetzi*, *P. martini*, *S. antennata* and *S. africana*).

The result showed that very few blood fed females were collected despite a very high population density of sand flies that were caught by the traps. This shows that majority of the sand flies in Marigat division blood feed on wild animals or they find their way to human habitation for blood feeding. This observation is in agreement with previous studies which suggested that the majority of sand flies either migrate to villages for feeding on their preferred hosts or those feeding outdoors on wild animals are probably as widely dispersed as their wild hosts are, thus, becoming rare in the trap collections (Teshome *et al* 2010).

This study has shown that volatiles from plant and animal hosts can strongly attract sand flies when incorporated into traps. Hence, they may be used instead of light or carbon dioxide to improve the efficiency of traps for haematophagous insects. This study has led to the innovation of a trap from solid wastes like plastic bottles, ripe fruits and wastes from animal slaughter houses which are becoming a nuisance both to urban and rural dwellers. Synthetic compounds can be processed from ripe fruits and goat blood to be used in place of dry ice, light or live animal (in case of Disney traps) in attracting sand flies to the trap. This trap is easy to manufacture and it is

very cheap compared to a CDC miniature light trap; one piece can cost USD 2 compared to USD 106 for a piece of CDC miniature light trap.

5.9 Conclusions

1. *Ricinus communis* extracts have ovicidal and larvicidal activities against sand flies pre-emergent stages
2. *Ricinus communis* extracts have insecticidal properties against adult sand flies.
3. Ripe fruits, goat, cattle, sheep and chicken blood baited traps attracts sand flies to the modified trap.

5.10 Recommendations

1. The extracts of *R. communis* can be processed into insecticides that can be used against all sand fly stages in line with WHO guidelines.
2. To reduce *Leishmania* infections and other effects of sand flies to humans, *R. communis* plants can be planted as barriers for sand flies in leishmaniasis endemic areas.
3. Ripe fruits and blood from chicken, cattle, sheep and goat can be processed into a suitable formulation and incorporated into traps used for sand fly surveillance instead of using other attractants like carbon dioxide and light.

REFERENCES

- Abonnenc, E. & Minter, D. M. (1965). Keys for the identification of the sand flies of the Ethiopian region.
- Adebayo, O. L., Suleman, D. & Samson, A. A. (2013). Natural products in anti-leishmanial drug discovery: A review. *Journal of Asian Scientific Research*, 3(2), 157-173.
- Ahluwalia, I. B., Bern, C., Costa, C., Akter, T., Chowdhury, R., Ali, M... & Maguire, J. H. (2003). Visceral leishmaniasis: Consequences of a neglected disease in a Bangladeshi community. *American Journal of Tropical Medicine and Hygiene* 69(6), 624–628.
- Akendengue, B., Roblot, F., Loiseau, P. M., Bories, C., Ngou-Milama, E., Laurens, A. & Hocquemiller, R. (2002). Klaivanolide, an antiprotozoal lactone from *Uvaria klaineana*. *Phytochemistry* 59, 885–888.
- Akilov, O. E., Khachemoune, A. and Hassan, T. (2007). Clinical manifestations and classification of Old World Cutaneous Leishmaniasis. *International Journal of Dermatology* 46, 132-142.
- Alexander, B. (2000). Sampling methods for phlebotomine sand flies. *Medical and Veterinary Entomology*, 14, 109-122.
- Alten, B., Ozbel, Y., Ergunay, K., Kasap, O. E., Cull, B., Antoniou, M... & Medlock, J. M. (2015). Sampling strategies for phlebotomine sand flies (Diptera: Psychodidae) in Europe. *Bulletin of Entomological Research* 105, 664–678.
- Amandeep, S. & Jasneet, K. (2016). Toxicity of leaf extracts of *Ricinus communis* L. (Euphorbiaceae) against the third instar larvae of *Musca domestica* L. (Diptera: Muscidae). *American Journal of BioScience*, 4(3-1), 5-10.
- Amy, J., Gunter, C. M. & Yosef, S. (2011). Attraction of *Phlebotomus papatasi* to common fruit in the field. *Journal of Vector Ecology* 36(1), 206-211.

- Ana, R., Berta, A. & Leandro, P. (2013). Fruit aromas in mature fleshy fruits as signals of readiness for predation and seed dispersal. *New Phytologist*, 197, 36–48.
- Anjili, C. O., Mugambi, R., Siele, D. K., Langat, B., Kamanyi, K., Nyasenda, S., & Ngumbi, P. (2014). The effects of *Mundulea sericea* (Fabales: Fabaceae) water extracts on *Phlebotomus duboscqi* (Diptera: Psychodidae) eggs and larvae. *African Journal of Pharmacology and Therapeutics* 3(2), 47-50.
- Anupam, G., Nandita, C., & Goutam, C. (2012). Plant extracts as potential mosquito larvicides. *Indian Journal of Medical Research*; 135(5), 581–598.
- Appadurai, D. R., Munusamy, R. G., Micheal, G. P., & Savarimuthu, I. 2015. Ovicidal and oviposition deterrent activities of medicinal plant extracts against *Aedes aegypti* L. and *Culex quinquefasciatus* Say Mosquitoes (Diptera: Culicidae), Osong. *Public Health Research Perspective*. 6(1), 64–69.
- Arfan, U. B, & Simeen, B. R. (2008). Cutaneous leishmaniasis: an overview of parasitology and host-parasite-vector inter relationship. *Journal of Pakistan Association of Dermatologists*; 18, 42-48.
- Aritho, N. M, Ndungu, P. K., Inyangala, J., Mwangi M., Michael, M. G, Joseph, J. N. N. & Christopher, O. A. (2017). Efficacy of allopatric *Tephrosia vogelii* (Fabales: Fabaceae) against pre-emerginal stages of *Phlebotomus (Phlebotomus) duboscqi* (Diptera: Psychodidae). *Open Journal of Plant science* 2(1), 007-010
- Armed Forces Pest Management Board (2015). Technical Guide No. 49. Sand Flies (Diptera: Psychodidae: Phlebotominae): Significance, Surveillance, and Control in Contingency Operations. Published and Distributed by the Armed Forces Pest Management Board, Information Services Division, Fort Detrick Forest, Glen Annex Bldg. 172, Forney Road Silver Spring, MD 20910-1230.

- Armed Forces Pest Management Board Technical Guide No. 49. Sand Flies (Diptera:Psychodidae: Phlebotominae): Significance, Surveillance, and Control in Contingency Operations .
- Ashford, R. W. (2000). The leishmaniasis as emerging and re-emerging zoonoses. *International Journal of Parasitology* 30, 1269-1281.
- Asish, B., Swapna, M., Sucharitha, M., Sravya, S., Lavanya, M. & Siraj, A. M. D. (2014). Phytochemical and antimicrobial screening of various extracts of castor Fruit-seeds (*Ricinus communis* L). *International Journal of Information Research and Review*, 1(10), 124-127.
- Assimina, Z., Charilaos, K., & Fotoula, B. (2008). Leishmaniasis: an overlooked public health concern. *Health Science Journal*. 2(4), 196-205.
- Aurelie, B., Clement, V. & Claudio, R. L. (2009). Behavioural and physiological state dependency of host seeking in the blood sucking insect *Rhodnius prolixus*. *The Journal of Experimental Biology* 212, 2386-2393.
- Aviad, M., Araya, G., Oscar, K., Roy, F., Teshome, G., Asrat, H. & Alon, W. (2013). Attraction of phlebotomine sand flies to baited and non-baited horizontal surfaces. *Acta Tropica* 126, 205-210.
- Bates, P. A., Depaquit, J., Galati, E. A. B., Kamhawi, S., Maroli, M., McDowell, M. A... & Warburg, A. (2015). Recent advances in phlebotomine sand fly research related to leishmaniasis control. *Parasites & Vectors* 8, 131-139.
- Berman, J. D. (1997). Human Leishmaniasis: clinical, diagnostic, and chemotherapeutic developments in the last 10 years. *Clinical Infectious Diseases* 24, 684–703.
- Berman, J. D. (2006). Visceral Leishmaniasis in the New World and Africa. *Indian Journal of Medical Research* 123, 289-294.
- Boakye, D. A., Wilson, M. D. & Kweku, M. (2005). A review of Leishmaniasis in West Africa. *Ghana Medical Journal* 39, 94-97.
- Bray, D. P., Carter, V., Alves, G. B., Brazil, R. P., Bandi, K. K. & Hamilton, J. G. C. (2014). Synthetic Sex Pheromone in a Long-Lasting Lure Attracts the Visceral Leishmaniasis Vector, *Lutzomyia longipalpis*,

- for up to 12 Weeks in Brazil. *PLoS Neglected Tropical Diseases* 8(3): e2723. doi:10.1371/journal.pntd.0002723
- Camargo, L. B. & Langoni, H. (2006). Impact of leishmaniasis on public health. *Journal of Venomous Animals and Toxins including Tropical Diseases* 12(4), 527-548
- Chappuis, F., Sundar, S., Hailu, A., Ghalib, H., Rijal, S., Peeling, R. W., Alvar, J. & Boelaert, M. (2007). Visceral leishmaniasis: what are the needs for diagnosis, treatment and control? *Nature Reviews, Microbiology* 5, 873-882.
- Cinthia, P. S., Patricia, V. A., Roberto, M. B. & Alfredo, J. P. (2012). Effects of *Ricinus communis* extracts on weight and mortality of *Scyphophorus acupunctatus* (Coleoptera: Carculionidae). *International Journal of Applied Science and Technology*; 2(1), 83-94.
- Claborn, D. M. (2010). The Biology and Control of Leishmaniasis Vectors. *Journal of Global Infectious Diseases* 2(2), 127–134.
- Claborn, D. M. (2010). The Biology and Control of Leishmaniasis Vectors. *Journal of Global Infectious Disease*, 2(2), 127–134.
- Collavino, M., Pelicano, A., & Giménez, R. A. (2006). Actividad insecticida de *Ricinus communis* L. sobre *Plodia interpunctella* HBN. (Lepidoptera: Phycitinae). *Revista Facultad de Ciencias Agropecuarias, Universidad Nacional de Cuyo*, 38, 13-18.
- Conjivaram, V. & Ruchir, A. (2007). Leishmaniasis. *eMedicine journal*.
- Croft, S. L., Seifert, K. & Yardley, V. (2006). Current scenario of drug development for leishmaniasis. *Indian Journal of Medical Research* 123, 399-410.
- Daily nation (May 19, 2016). Kala-azar: Silent killer disease ruins livelihoods in rural Kenya. www.nation.co.ke/counties/nakuru/Sand-fly-bites-agony/-/1183314/2305548/-/n3kwes/-/index.html
- Daily nation (May 6, 2014). Sand fly bites leave family in agony.<http://www.nation.co.ke/counties/nakuru/Sand-fly-bites-agony//1183314/2305548/-/n3kwes/-/index.html>

- Daniel, L. K., Jerome, H. & Gunter, M. (2011). Comparison of various configurations of CDC-type traps for the collection of *Phlebotomus papatasi* Scopoli in Southern Israel. *Publications from USDA-ARS/UNL Faculty*. Paper 1020.
- Dawit, G. & Shishay, K. (2014). Epidemiology, public health impact and control methods of the most neglected parasite diseases in Ethiopia: A Review. *World Journal of Medical Sciences* 10(2), 94-102.
- Degri, M. M., Duna, M. M. & Joshua W. W. (2013). Efficacy of aqueous leaf extracts and synthetic insecticide on pod-sucking bugs infestation of cowpea (*Vigna unguiculata* (L.) Walp) in the Guinea Savanna Region of Nigeria. *Advances in Entomology*, 1(2), 10-14.
- Desjeux, P. (2004). Leishmaniasis. *Nature Reviews Microbiology* 2,692-693.
- Diwakar, S. D., Seema, K., Vibhishan, P., Jainendra, K., Nisha, K., Prahlad, K., ... & Pradeep, D. (2015). Insecticidal effect of plant extracts on *Phlebotomus argentipes* (Diptera: Psychodidae) in Bihar, India. *Indian Journal of Medical Research*, 142, 95-100.
- Diwakar, S. D., Seema, K., Vijay, K. & Pradeep, D. (2014). The potentiality of botanicals and their products as an alternative to chemical insecticides to sand flies (Diptera: Psychodidae): A review. *Journal of Vector Borne Disease* 51, 1-7.
- Dostalova, A. & Volf, P. (2012). *Leishmania* development in sand flies: parasite-vector interactions overview. *Parasites and Vectors* 5, 276-288.
- Dujardin, J. C., Campino, L., Canavate, C., Dedet, J. P., Gradoni, L., Soteriadou, K., ... & Boelaert, M. (2008). Spread of Vector-borne Diseases and Neglect of Leishmaniasis, Europe. *Emerging Infectious Diseases*; 14(7), 1013-1018.
- Dutari, L. C. & Loaiza, J. R. (2014). American cutaneous leishmaniasis in Panama: a historical review of entomological studies on anthropophilic *Lutzomyia* sand fly species. *Parasites and Vectors*, 7, 218-228.

- Edessa, N., Endalamaw, G., Yamuah, L., Engers, H., Jemal H., Hailu, A... & Abraham, A. (2008). Outbreak of Cutaneous Leishmaniasis in Silti Woreda, Ethiopia: Risk Factor Assessment and Causative Agent Identification. *Royal Society of Tropical Medicine and Hygiene*, 1-8. doi:10.1016/j.trstmh.2008.03.021
- Eliningaya, J. K., Eunice, A. O., Beda, J. M., Aneth, M. M., Mramba, N. & Franklin, M. (2011). The role of cow urine in the oviposition site preference of culicine and Anopheles mosquitoes, *Parasites & Vectors* 4, 184-189.
- Elizabeth, I. H., Rudolph, C. J., John, O., & Jerry, T. (2015). Evaluation of Ricinine, a Ricin Biomarker, from a Non-Lethal Castor Bean Ingestion. *Journal of Anal Toxicology* 36(9), 660–662.
- Enrih, M., Stjepan, K., Mirta, S. B. & Zeljka, J. (2007). Response of mosquitoes to different synthetic and natural olfactory attractants (Diptera: Culicidae). *Entomology General* 30(4), 252-261.
- Fausto, A.M., Maroli, M. & Mazzini, M. (1992). Ootaxonomy and eggshell ultrastructure of Phlebotomus Sand flies. *Medical and Veterinary Entomology*, 6, 201-208.
- Garg, R. & Dube, A. (2006). Animal models for vaccine studies for visceral leishmaniasis. *Indian Journal of Medical Research* 123, 439-454.
- Getti, G., Durgadoss, P., Dominguez-Carmona, D., Martin-Quintal, Z., Peraza-Sanchez, S., Pena-Rodriguez, L. M. & Humber, D. (2009). Leishmanicidal Activity of Yucatecan Medicinal Plants on *Leishmania* Species Responsible for Cutaneous Leishmaniasis. *Journal of Parasitology* 95(2), 456-460.
- Ghalib, H. & Modabber, F. (2007). Consultation meeting on the development of therapeutic vaccines for Post Kala azar Dermal Leishmaniasis. *Kinetoplastid Disease* 6, 128-134.
- Ghosh, S., Tiwari, S.S., Srivastava, S., Sharma, A.K., Kumar, S., Ray, D.D. & Rawat, A.K. (2012). Acaricidal properties of *Ricinus communis* leaf extracts against organophosphate and pyrethroids resistant

- Rhipicephalus (Boophilus) microplus*. *Veterinary Parasitology*, 18, 259- 267.
- Gossage, S.M., Rogers, M. E. & Bates, P. A. (2003). Two separate growth phases during the development of *Leishmania* in sand flies: implications for understanding the life cycle, *International Journal of Parasitology* 33, 1027–1034.
- Guerin, P. J., Olliaro, P., Sundar, S., Boelaert, M., Croft, S. L., Desjeux, P... & Bryceson, A. D. (2002). Visceral leishmaniasis: current status of control, diagnosis and treatment, and a proposed research and development agenda. *The lancet Infectious Diseases*, 2, 494-501.
- Gunter, C. M., Vasily, D. K., Leonid, R. & Yosef, S. (2011). Characteristics of resting and breeding habitats of adult sand flies in the Judean desert. *Journal of Vector Ecology*, 36(S1), S195-S205
- Hailu, A., Mudawi, M. A., Royce, C. & Wasunna, M. (2005). Visceral Leishmaniasis: New Health tools are needed. *PLoS Medicine* 2, 7.
- Hamilton, J. G. C. (2008). Sand fly pheromones: their biology and potential for use in control programs. *Parasites*, 15, 252-256
- Handman, E. & Bullen, D. V. R. (2002). Interaction of *Leishmania* with the host macrophage. *Trends in Parasitology* 18, 332–334.
- Harborne, J. B. (1994). Phytochemical methods: A guide to modern Techniques of Plant Analysis. Chapman and Hall Ltd, London, 1994, 279.
- Herwaldt, B. L. (1999). Leishmaniasis. *The Lancet*. 354(9185), 1191–1199.
- Hesam-Mohammadi, M., Rassi, Y., Abai, M. R., Akhavan, A. A., Karimi, F., Rafizadeh, S... & Sharafkhah, M. (2014). Efficacy of different sampling methods of sand flies (Diptera: Psychodidae) in endemic Focus of cutaneous leishmaniasis in Kashan district, Isfahan Province, Iran. *Journal of Arthropod-Borne Diseases*, 8(2), 156–162.
- Hoel, D.F., Kline, D. L., Hogsette, J. A., Bernier, U. R., El-Hossary, S. S., Hanafi, H. A... & Szumlas, D. E. (2010). Efficacy of commercial mosquito traps in capturing Phlebotomine sand flies (Diptera: Psychodidae) in Egypt. *Journal of Medical Entomology* 47(6), 1179-1184.

- Igor, A. R., Ana, M. M., Veronica C., Renan L. A., Ana, C. F. A., Jefferson, R. d. A... & Alane, B. V. (2015). Natural products: Insights into leishmaniasis inflammatory response. *Mediators of inflammation*, 2015, 1-12.
- Ileri, L. N., Kongoro, J., Ngure, P., Mutai, C., Langat, B., Tonui, W... & Mucheru, O. (2010). The potential of the extracts of *Tagetes minuta* Linnaeus (Asteraceae), *Acalypha fruticosa* Forssk (Euphorbiaceae) and *Tarhonanthus camphoratus* L. (Compositae) against *Phlebotomus duboscqi* Neveu Lemaire (Diptera: Psychodidae), the vector for *Leishmania major* Yakimoff and Schokhor, *Journal of Vector Borne Diseases* 47, 168–174.
- Irma, A., Erlinda S., Beverly, G., & Dora, M. F. (2002). Feeding behavior of *Lutzomyia pseudolongipalpis* (Diptera: Psychodidae), a putative vector of visceral leishmaniasis in Venezuela. *Journal of Medical Entomology*, 39(3), 440-445.
- Jitendra, J. & Ashish, K.G. (2012). *Ricinus communis* Linn.: A phytopharmacological review. *International journal of pharmacy and pharmaceutical sciences*; 4(4), 25-29
- Jorge, A., Ivan, D. V., Caryn, B., Merce, H., Philippe, D., Jorge, C... & Margriet, den B. (2012). Leishmaniasis worldwide and global estimates of its incidence. *PLoS One*; 7(5), e35671.
- Kaldas, R. M., El Shafey, A. S., Magdi, G. S., Samy, A. M. & Villinski, J. T. (2014). Experimental effect of feeding on *Ricinus communis* and *Bougainvillea glabra* on the development of the sand fly *Phlebotomus papatasi* (diptera: psychodidae) from Egypt. *Journal of the Egyptian Society of Parasitology*, 44(1), 1-12.
- Kandagal, A. S. & Khetagoudar, M. C. (2011). Biological potency of crude aqueous Extracts of clerodendr on inerme (Verbenaceae) in management of lepidopteron pest *S.litura* (Lepidoptera: Noctuidae). *WebmedCentral Zoology* 2(12), WMC002793.
- Kasap, O. E., Belen, A., Kaynas, S., Simsek, F. M., Biler, L., Ata, N. & Alten, B. (2009). Activity patterns of sand fly (Diptera: Psychodidae) species

and comparative performance of different traps in an endemic cutaneous leishmaniasis focus in cukurova plain, Southern Anatolia, Turkey. *ACTA Veterinary*, 78, 327–335.

- Kasili, S., Kutima, H., Mwandawiro, C., Ngumbi, P. M. & Anjili, C. O. (2009). Comparative attractiveness of CO₂-baited CDC light traps and animal baits to *Phlebotomus duboscqi* sand flies. *Journal of Vector Borne Diseases* 46, 191–196.
- Kasili, S., Ngumbi, P. M., Helen, K., Francis, G. N., Elizabeth, K., & Nicholas, O. (2010). Comparative performance of light traps, lunar influence and sand fly abundance in Baringo district, Kenya. *Journal of vector borne diseases*; 47, 108-112.
- Katerina, P., Jan, V. & Petr, V. (2013). The effect of avian blood on *Leishmania* development in *Phlebotomus duboscqi*, *Parasites & Vectors* 6, 254.
- Khan, I. & Umar, M. N. (2015). Evaluation of crude extract of *Melia azedarach* Linn. against attenuated Amphotericin B resistant *Leishmania tropica* strain. *Journal of Basic & Applied Sciences*, 11, 314-317.
- Khani, M., Awang, R. M., Omar, D. & Rahmani, M. (2013). Toxicity, antifeedant, egg hatchability and adult emergence effect of *Piper nigrum* L. and *Jatropha curcas* L. extracts against rice moth, *Corcyra cephalonica* (Stainton). *Journal of Medicinal Plants Research*, 7(18), 1255-1262.
- Kishore, K., Kumar, V., Kesari, S., Dinesh, D. S., Kumar, A. J., Das, P. & Bhattacharya, S. K. (2006). Vector control in leishmaniasis. *Indian Journal of Medical Research* 123, 467-472.
- Kolaczinski, J. H., Reithinger, R., Worku, D. T., Ocheng, A., Kasimiro, J., Kabatereine, N. & Brooker, S. (2008). Risk factors of visceral leishmaniasis in East Africa: a case-control study in Pokot territory of Kenya and Uganda. *International Journal of Epidemiology*, 37(2), 344-352.
- Kumar, N. P., Srinivasan, R. & Jambulingam, P. (2012). DNA barcoding for identification of sand flies (Diptera:Psychodidae) in India, *Molecular Ecology Resources* 12(3), 414-420.

- Lafayette, P. C., Monica, T. C. & Eduardo, B. B. (2013). Bioactivity of plant extracts on the larval and pupal stages of *Aedes aegypti* (Diptera, Culicidae). *Revista da Sociedade Brasileira de Medicina Tropical* 46(4), 420-425.
- Logan, J. G. & Birkett, M. A. (2007). Semiochemicals for biting fly control: their identification and exploitation. *Pest Management Science*, 63(7), 647-657.
- Malaria consortium (2010). Leishmaniasis control in Eastern Africa: past and present efforts and future needs. Situation and gap analysis.
- Mann, R. S. & Kaufman, P. E. (2012). Natural product pesticides: Their development, delivery and use against insect vectors. *Mini-Review in Organic chemistry*; 9, 185-202.
- Manpreet, R, Hitesh, D, Bharat, P. & Shivani, S. (2012). *Ricinus communis* L.-A Review. *International Journal of Pharmtech Research*; 4(4), 1706-1711.
- Mara, C. P., Daniel, P. B., Alvaro, E. E., Henrique, P. C. & Camila, P. P. (2012). Attraction of the cutaneous leishmaniasis vector *Nyssomyia neivai* (Diptera: Psychodidae) to host odour components in a wind tunnel. *Parasites & vectors* 5, 210.
- Marlet, M. V., Sang, D. K., Ritmeijer, K., Muga, R. O., Onsongo, J. & Davidson, R. N. (2003). Emergence or re-emergence of visceral Leishmaniasis in areas of Somalia, northeastern Kenya, and south-eastern Ethiopia in 2000-01. *Transactions of the Royal Society for Tropical Medicine and Hygiene* 97, 515-518.
- Maroli, M., Feliciangeli, M. D., Bichaud, L., Charrel, R. N. & Gradoni, L. (2012). Phlebotomine sandflies and the spreading of leishmaniasis and other diseases of public health concern. *Medical and Veterinary Entomology*, (2012), doi: 10.1111/j.1365-2915.2012.01034.x
- Marta, F. M. & Sarah, J. M. (2011). Plant-based insect repellents: a review of their efficacy, development and testing. *Malaria Journal*; 10(1), S11.
- Marziel, H. M, Yavar, R., Mohammad, R. A., Amir, A. A., Fatemeh, K., Sina, R... & Maryam, S. (2014). Efficacy of different sampling methods

- of sand flies (Diptera: Psychodidae) in endemic focus of cutaneous leishmaniasis in Kashan district, Isfahan province, Iran. *Journal of Arthropod-borne Diseases*; 8(2), 156-162.
- Mauricio, R. V., Alexandre, N., Bruce, A., Erin, D., Reginaldo, R. C., Hector, M. D... & Rod J. D. (2010). Chicken blood provides a suitable meal for the sand fly *Lutzomyia longipalpis* and does not inhibit *Leishmania* development in the gut. *Parasitology vectors* 3, 3 doi: 10.1186/1756-3305-3-3
- Mishra, B. B., Kale, R. R., Prasad, V., Tiwari, V. K. & Singh, R. K. (2011). Scope of natural products in fighting against leishmaniasis. *Opportunity, Challenge and Scope of Natural Products in Medicinal Chemistry, 2011*, 121-154.
- Mohammad, M. E. & Mahbobeh, Y. (2008). Entomological survey of phlebotomine sand flies (Diptera: Psychodidae) in a focus of visceral leishmaniasis in central Iran. *Journal of Vector Borne Diseases* 45, 38–43.
- Mong'are S., Ng'ang'a Z., Maranga R., Osiemo Z., Ngure P., Ngumbi P... & Tonui W. (2013). Assessing the Potential Effects of Selected Kenyan Medicinal Plants on Longevity of *Phlebotomus duboscqi* (Diptera: Psychodidae). *Asian Journal of Pharmaceutical and Health Sciences* 3(1), 648-653.
- Mong'are, S., Ng'ang'a, Z., Ngumbi, P., Johnstone, I. & Ngure P. (2015). Comparative Analysis of the Effectiveness of Sand Fly Traps with Different Baits. *IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)*, 10(4), 16-24.
- Mong'are, S., Ng'ang'a, Z., Ngumbi, P., Johnstone, I. & Ngure, P. (2016). Ovicidal and Larvicidal effects of *Ricinus Communis* extracts on *Phlebotomus Duboscqi*. *European Journal of Medicinal Plants*, 11(3), 1-14.
- Monzote, L. (2009). Current treatment of leishmaniasis: A Review. *The Open Antimicrobial Agents Journal* 1, 9-19.

- Moura, T. R., Oliveira, F., Novais, F. O., Miranda, J. C., Clarencio, J. (2002). Enhanced *Leishmania braziliensis* Infection Following Pre-Exposure to Sand fly Saliva. *PLoS Neglected Tropical Diseases*; 1(2), 84.
- Mousa, K., Rita, M. A., Dzolkhifli, O. & Mawardi, R. (2013). Toxicity, antifeedant, egg hatchability and adult emergence effects of *Piper nigrum* L. and *Jatropha curcas* L. extracts against rice moth *Corcyra cephalonica* (Stainton). *Journal of Medicinal Plants Research*; 7(18), 1255-1262.
- Mueller, Y. K., Kolaczinski, J. H., Koech, T., Lokwang, P., Riongoita, M., Velilla, E... & Chappuis, F. (2014). Clinical Epidemiology, Diagnosis and Treatment of Visceral Leishmaniasis in the Pokot Endemic Area of Uganda and Kenya. *American Journal of Tropical Medicine and Hygiene* 90(1), 33–39.
- Mukhopadhyay, S. & Mandal, C. (2006). Glycobiology of *Leishmania donovani*. *Indian Journal of Medical Research* 123, 203-220.
- Muller, G. C., Revay, E. E. & Boier, J. C. (2011). Simplified and improved monitoring traps for sampling sand flies. *Journal of Vector Ecology*, 36(2), 454-457.
- Murray, H. W., Berman, J. D., Davies, R. C. & Saravia, N. G. (2005). Advances in Leishmaniasis. *The Lancet Infectious Diseases* 366, 1561–1577.
- Mutuku, J. M., Kyai, F. M., Kamau, C. & Omogo, D. M. (1986). Epidemiology of leishmaniasis in Kenya-II. Host preference studies using various types of animal baits at animal burrows in marigat, Baringo District. *Insect Science Applications*, 7(2), 191-197.
- Nataly, A. S., Claudia, A. A., Andre, F. B., Mauricio, L. V., Elizabeth, F. R. & Maria, P. D. (1995). The influence of sugar and amino acids on the blood feeding behavior, oviposition and longevity of laboratory colony of *Lutzomyia longipalpis* (Lutz and Neiva, 1912) (Diptera: Psychodidae, Phlebotominae). *Mem Inst Oswaldo cruz, Rio de Janeiro* 90(6), 751-757.

- Ngure, P., Kimutai, A., Tonui, W. & Ng'ang'a, Z. (2009). A review of Leishmaniasis in Eastern Africa. *The Internet Journal of Parasitic Diseases*. 4, 1-10.
- Ngure, P., Kimutai, A., Tonui, W. & Ng'ang'a, Z. (2008). A Review of Leishmaniasis in Eastern Africa. *The Internet Journal of Parasitic Diseases*, 4(1), 1-10.
- Niamba, P., Lompo, O. G., Traore, A., Traore, F. B. & Soudre, R. T. (2007). Diffuse Cutaneous Leishmaniasis in an HIV positive patient in Western Africa. *Australasian journal of Dermatology* 48, 32-34.
- Nidhi, R., Sandhya, M., Sudhanshu, & Ekta, M. (2013). Assessment of Phytochemical Screening, Antioxidant and Antibacterial Potential of the Methanolic Extract of *Ricinus communis* L. *Asian Journal of Pharmacy and Technology* 3(1), 20-25.
- Nisha, S., Bhuwan, B. M., Surabhi, B., Rakesh, K. S. & Vinod, K. T. (2014). Natural product based leads to fight against leishmaniasis. *Bioorganic & Medicinal Chemistry*, 22, 18-45.
- Nisha, S., Manish, K. & Rakesh, K. S. (2012). Leishmaniasis: Current status of available drugs and new potential drug targets. *Asian Pacific Journal of Tropical Medicine*, 2012, 485-497.
- Okello, S. V., Nyunja, R. O., Netondo, G. W. & Onyango, J. C. (2010). Ethnobotanical study of medicinal plants used by Sabaots of Mt. Elgon Kenya. *African Journal of Traditional, Complementary and Alternative medicines*; 7(1), 1-10.
- Oscar, D. K., Roy, F., Araya, G., Asrat, H. Teshome, G., & Alon, W. (2013). Attraction of Ethiopian Sand flies (Diptera: Psychodidae) to light and sugar-yeast mixtures (CO₂). *Parasites and vectors* 6, 341-348.
- Padma, L. L. & Rupali B. K. (2014). *Ricinus communis* (Castor): an overview. *International Journal of Research in Pharmacology and Pharmacotherapeutics*, 3(2), 136-144.
- Peta, D. & Pathipati, U. R. (2011). Insect growth regulatory activity of the crude and purified fractions from *Solanum melongena* L., *Lycopersicum*

- esculentum* Mill. and *Capsicum annuum* L. *Journal of Biopesticides*, 4(2), 118-130.
- Philip, M. N., Philip, G. L., Richard, N. J., Gabriel, K. & Charles, A. (1992). Identification of phlebotomine sand fly blood meals from Baringo district, Kenya, by direct enzyme-linked immunosorbent assay (ELISA). *Medical and Veterinary Entomology* 6, 385-388.
- Picado, A., Dash, A. P., Bhattacharya, S. & Boelaert, M. (2012). Vector control interventions for visceral leishmaniasis elimination initiative in South Asia, 2005-2010. *Indian Journal of Medical Research*, 136, 22-31.
- Pigott, D. M., Bhatt, S., Golding, N., Duda, K. A., Battle, K. E., Brady, O. J... & Hay, S. I. (2014). Global distribution maps of the leishmaniasis. *Epidemiology and global health Microbiology and Infectious Disease*, 3, 1-21.
- Piscopo, T. V. & Mallia, A. C. (2006). Leishmaniasis. *Postgraduate Medical Journal* 82, 649-657.
- Rab M. A., Azmi F. A. & Iqbal J. (1986). Cutaneous leishmaniasis in Balochistan. Reservoir hosts and sand fly vector in Uthal, Lasbella. *Journal of the Pakistan Medical Association*; 36, 134-8.
- Rajeshree, S. P., Mohini, S. P., Sandip, S. K., Praful. S. C., Jayendrasing, P. B. & Rajesh, J. O. (2012). Synthetic and natural products against leishmaniasis: A review. *World Journal of Public Health Sciences*, 1(1), 7-22.
- Rajinder, S. M. & Phillip, E. K. (2010). Colonization of *Lutzomyia shannoni* (Diptera: Psychodidae) utilizing an artificial blood feeding technique, *Journal of Vector Ecology* 35 (2), 286–294.
- Ramalho-Ortigao, J. M., Kamhawi, S., Joshi, M. B., Reynoso, D., Lawyer, P. G., Dwyer, D. M... & Valenzuela, J. G. (2005). Characterization of a blood activated chitinolytic system in the midgut of the sand fly vectors *Lutzomyia longipalpis* and *Phlebotomus papatasi*. *Insect Molecular Biology* 14, 703–712.

- Ramos-Lopez, M. A., Perez, G. S., Rodriguez, C. H., Guevara, F. P. & Zavala, M. A. (2010). Activity of *Ricinus communis* (Euphorbiaceae) against *Spodoptera frugiperda* (Lepidoptera: Noctuidae). *African Journal of Biotechnology*; 9(9), 1359-1365.
- Rania, M. K., Azza, S., Shafey, E. L., Magdi, G. S., Abdallah, M. S. & Jeffrey, T. V. (2014). Experimental effects of feeding on *Ricinus communis* and *Bougainvillea glabra* on the development of the sand fly *Phlebotomus papatasi* (Diptera: Psychodidae) from Egypt. *Journal of the Egyptian Society of Parasitology*; 44(1), 1-12.
- Ready, P. D. (2010). Leishmaniasis emergence in Europe. *European Surveillance* 15(10), pii=19505.
- Reithinger, R., Dujardin, J. C., Louzir, H., Pirmez, C., Alexander, B. & Brooker, S. (2007). Cutaneous Leishmaniasis. *The Lancet Infectious Diseases* 7, 581–596.
- Roberts, M. T. M. (2006). Current understandings on the immunology of Leishmaniasis and recent developments in prevention and treatment. *British Medical Bulletin* 75 & 76, 115–130
- Rogers, M. E., Chance, M. L. & Bates, P. A. (2002). The role of promastigote secretory gel in the origin and transmission of the infective stage of *Leishmania mexicana* by the sand fly *Lutzomyia longipalpis*. *Parasitology* 124, 495–508.
- Rondon, F. C. M., Bevilaqua, C. M. L., Accioly, M. P., Morais, S. M., Andrade-Júnior, H. F. and Carvalho, C. P. (2012). *In vitro* efficacy of *Coriandrum sativum*, *Lippia sidoides* and *Copaifera reticulata* against *Leishmania chagasi*. *Revista Brasileira de Parasitologia Veterinária*; 21(3), 185-191.
- Rosypal, A. C., Troy, G. C., Zajac, A. M., Duncan, R. B. Waki, K., Chang, K. P. & Lindsay, D. S. (2003). Emergence of zoonotic canine leishmaniasis in the United States: Isolation and immunohistochemical detection of *Leishmania infantum* from foxhounds from Virginia. *Journal of Eukaryotic Microbiology* 50, 691–693.

- Ryan, J. R., Mbui, J., Rashid, J. M., Wasunna, M. K., Kirigi, G., Magiri, C... & Chan, A. S. T. (2006). Spatial clustering and epidemiological aspects of visceral leishmaniasis in two endemic villages, Baringo district, Kenya. *American Journal of Tropical Medicine and Hygiene*, 74 (2), 308–317.
- Sabina, W. W., Sabar, O., Julia, W. J., Martin, W., Hans, T. A., David, R. S... & Baldwin, T. (2014). Toxicity of six plant extracts and two pyridine alkaloids from *Ricinus communis* against the malaria vector *Anopheles gambiae*. *Parasites and Vectors*, 7:312-320.
- Sadlova, J., Dvorak, V., Seblova, V., Warburg, A., Votypka, J. & Volf, P. (2013). *Sergentomyia schwetzi* is not a competent vector for *Leishmania donovani* and other *Leishmania* species pathogenic to humans. *Parasites and Vectors*, 6,186-196.
- Schlein, Y., Jacobson R. L. & Muller G. R. (2001). Sand fly feeding on noxious plants: a potential method for the control of leishmaniasis. *American journal of Tropical medicine and Hygiene* 65(4), 300-303.
- Seaman, J., Mercer, A. J. & Sondorp, E. (1996). The epidemic of visceral Leishmaniasis in Western Upper Nile, southern Sudan: course and impact from 1984 to 1994. *International Journal of Epidemiology*. 25, 862-871.
- Secundino, N. F., Eger-Mangrich, I., Braga, E. M., Santoro, M. M. & Pimenta, P. F. (2005). *Lutzomyia longipalpis* peritrophic matrix: formation, structure and chemical composition, *Journal of Medical Entomology* 42, 928–938.
- Sen, G., Mukhopadhyay, S., Ray, M. & Biswas, T. (2008) Quercetin Interferes with Iron Metabolism in *Leishmania donovani* and Target Ribonucleotide Reductase to Exert Leishmanicidal Activity; *in Vivo* Study. *Journal of Antimicrobial Chemotherapy*, 61, 1066-1075.
- Shao-Xiong, C. Jia-Wei, T., Lai-Keng, C. & Zairi, J. (2013). Larvicidal, oviposition, and ovicidal effects of *Artemisia annua* (Asterales: Asteraceae) against *Aedes aegypti*, *Anopheles sinensis*, and *Culex*

- quinquefasciatus* (Diptera: Culicidae). *Parasitology Research* 112, 3275–3282.
- Sharma, U. & Singh, S. (2008). Insect vectors of Leishmania: distribution, physiology and their control. *Journal of Vector Borne Diseases*, 45, 255–272.
- Sharquie, K. E., Noaim, A. A. & Saleh, B. A (2016). Loranthus europaeus as an Alternative Medicine in Treatment of Acute Cutaneous Lesihmaniasis: Review Article. *Journal of Cosmetics, Dermatological Sciences and Applications*, 6, 24-33.
- Shiddo, S. A., Akuffo, H. O., Mohamed, A. A., Huldt, G., Nilsson, L. A., Ouchterlony, O. & Thorstensson, R., (1995). Visceral leishmaniasis in Somalia: prevalence of leishmanin-positive and seropositive inhabitants in an endemic area. *Transactions of the Royal Society of Tropical Medicine & Hygiene* 89(1), 21-4.
- Shyamapada, M. (2010). Exploration of larvicidal and adult emergence inhibition activities of *Ricinus communis* seed extract against three potential mosquito vectors in Kolkata, India. *Asian Pacific Journal of Tropical Medicine*, 2010, 605-609.
- Signorini, M., Drigo, M., Marcer, F., Frangipane, D. R. A., Gasparini, G., Montarsi, F... & Cassini, R. (2013). Comparative field study to evaluate the performance of three different traps for collecting sand flies in Northeastern Italy. *Journal of Vector Ecology* 38(2), 374-378.
- Sthenia, S. A. A., Claudia, M. L. B., Francisco, M C F., Nilza, D A. & Michelline, V. M. (2009). Control of phlebotomine (Diptera: Psychodidae) leishmaniasis vectors. *Neotropical Entomology* 38(3), 303-310.
- Stoops, C. A., Heintshcel, B., El-Hossary, S., Kaldas, R. M., Obenauer, P. J., Farooq, M. & Villinski, J. T. (2013). Sand fly surveillance and control on Camp Ramadi, Iraq, as part of a leishmaniasis control program. *Journal of Vector Ecology*, 38(2), 411-414.

- Subathra, D. C., Harini S., Renuka E. J., Surya K. & Mohana S. V. (2014). Leishmaniasis: A review on global epidemiological trends. *Research Journal of Pharmacy and Technology*, 7(5), 569-574.
- Sylvia, W., Kernt, K., Diana, P., Marc-Andre, A., Martin, S., Martin, B. D. & Brigitte, G. D. (2011). *Ricinus communis* Intoxications in Human and Veterinary Medicine—A summary of real cases. *Toxins*, 3(10), 1332-1372.
- Teshome, G. M., Meshesha, B., Nega, B., Asrat, H. & Yalemtehay, M. (2010). Further studies on the phlebotomine sand flies of the kala-azar endemic lowlands of Humera-Metema (north-west Ethiopia) with observations on their natural blood meal sources, *Parasites and Vectors* 3(6), 1-7.
- Tonui, W. K. (2006). Situational analysis of Leishmaniasis research in Kenya. *African Journal of Health Sciences* 13, 7-21.
- Tounou, A. K., Mawussi, G., Amadou, S., Agboka, K., Gumedzoe, Y. & Mawuena, D. (2011). Bio-insecticidal effects of plant extracts and oil emulsions of *Ricinus communis* L. (Malpighiales: Euphorbiaceae) on the diamondback, *Plutella xylostella* L. (Lepidoptera: plutella) under laboratory and semi-field conditions. *Journal of Applied Biosciences*; 43, 2899-2914.
- Tounou, A. K., Mawussi, G., Amadou, S., Agboka, K., Gumedzoe, Y., Mawuena, D. & Sanda, K. (2011). Bio-insecticidal effects of plant extracts and oil emulsions of *Ricinus communis* L. (Malpighiales: Euphorbiaceae) on the diamondback, *Plutella xylostella* L. (Lepidoptera: Plutellidae) under laboratory and semi-field conditions. *Journal of Applied Biosciences* 43, 2899 – 2914.
- Ulrich, R. B., David, F. H., Jerome, A. H. J. R., Hanafi, A. H. & Daniel, L. K. (2008). Effect of lures and trap placement on sand fly and mosquito traps. Proceedings of the sixth international conference on urban pests, William H. Robinson and Daniel Bajomi (Editors). *Publication from USDA-ARS/UNL Faculty*. 1029.

- Umakant, S. & Sarman, S. (2008). Insect vectors of *Leishmania*: distribution, physiology and their control. *Journal of Vector Borne Diseases*. 45, 255–272.
- Vandita, P., Nirali, A., Khyati, P. & Monisha, K. (2013). Effect of Phytochemical Constituents of *Ricinus Communis*, *Pterocarpus Santalinus*, *Terminalia Belerica* on Antibacterial, Antifungal and Cytotoxic Activity, *International Journal of Toxicological and Pharmacological Research*, 5(2), 47-54.
- Veronesi, E., Pilani, R., Carrieri, M. & Bellini, R. (2007). Trapping sand flies (Diptera: Psychodidae) in the Emilia-Romagna region of northern Italy. *Journal of Vector Ecology*, 32(2), 313-318.
- Volf, P. & Volfova, V. (2011). Establishment and maintenance of sand fly colonies. *Journal of Vector Ecology*, 36(1), S1-S9.
- Wasunna, M. K., Rashid, J. R., Mbui, J., Kirigi, G., Kinoti, D., Lodenyo, H... & Horton, A. J. (2005). A phase ii Dose-Increasing study of sitamaquin for the treatment of Visceral Leishmaniasis in Kenya. *American Journal of Tropical Medicine and Hygiene* 3(5), 871-876.
- World Health Organization (2006).
http://www.who.int/gb/ebwha/pdf_files/EB118/B118_4-en.pdf.
 Accessed 21st July 2015.
- World Health Organization (2000). WHO Report on Global Surveillance of Epidemic-prone Infectious Diseases, <http://www.who.int/emc>
- World Health Organization (2010). Report of a meeting of the WHO Expert Committee on the Control of Leishmaniasis Geneva, 22–26 March 2010.
- World Health Organization (2012). Diagnosis and management of visceral leishmaniasis (Kala Azar). National guidelines for health workers, Revised January 2012.
- World Health Organization (2014). Strategic framework for leishmaniasis control in the WHO European Region 2014–2020.
- World Health Organization (WHO) (2007). Report on global surveillance of epidemic-prone infectious diseases.

World Health Organization. (WHO) (2005). Leishmaniasis: Burden of disease.

<http://www.who.int/leishmaniasis>

Yusuf O., Chizu S., Bulent A., Masahito A., Jerome D., Yasunobu M... & Yoshitsugu M. (2011). Distribution and ecological aspects of sand fly (Diptera: Psychodidae) species in Sri Lanka. *Journal of Vector Ecology*, 36(1), S77-S86.

APPENDICES

APPENDIX 1: KEMRI SCIENTIFIC STEERING COMMITTEE APPROVAL



KENYA MEDICAL RESEARCH INSTITUTE

P.O. Box 54840-00200, NAIROBI, Kenya
Tel (254) (020) 2722541, 2713349, 0722-205901, 0733-400003; Fax: (254) (020) 2720030
E-mail: director@kemri.org info@kemri.org Website:www.kemri.org

KEMRI/SSC/102556

3rd March, 2014

Mong'are Samuel

Thro'

Director, CBRD
NAIROBI

[Handwritten signature] 5/3/14

REF: SSC No. 2758 (Revised) – Evaluating the potential of blood baited traps with castor oil adhesive and the effects of *ricinus communis* extracts on sandflies

I am pleased to inform you that the above mentioned proposal, in which you are the PI, was discussed by the KEMRI Scientific Steering Committee (SSC), during its 211th meeting held on 4th, February, 2014 and has since been approved for implementation by the SSC.

Kindly submit 4 copies of the revised protocol to SSC within 2 weeks from the date of this letter i.e, 17th March, 2014.

We advise that work on this project can only start when ERC approval is received.

[Handwritten signature]

Sammy Njenga, PhD
SECRETARY, SSC

APPENDIX 2: KEMRI ETHICS REVIEW COMMITTEE APPROVAL



KENYA MEDICAL RESEARCH INSTITUTE

P.O. Box 54840-00200, NAIROBI, Kenya
Tel (254) (020) 2722541, 2713349, 0722-205901, 0733-400003; Fax: (254) (020) 2720030
E-mail: director@kemri.org info@kemri.org Website:www.kemri.org

KEMRI/RES/7/3/1

May 23, 2014

TO: SAMUEL MONG'ARE (PRINCIPAL INVESTIGATOR)

**THROUGH: DR. KIMANI GICHUHI
DIRECTOR, CBRD
NAIROBI**

Handwritten signature and date: 27/5/14

Dear Sir,

RE: SSC PROTOCOL NO. 2758 (RESUBMISSION): EVALUATING THE POTENTIAL OF BLOOD BAITED TRAPS WITH CASTOR OIL ADHESIVE AND THE EFFECTS OF RICINUS COMMUNIS EXTRACTS OF SANDFLIES

Reference is made to your letter dated 12th May 2014. The ERC Secretariat acknowledges receipt of the revised document on 14th May 2014.

This is to inform you that the Ethics Review Committee (ERC) reviewed the document submitted, and is satisfied that the issues raised at the 226th meeting, have been adequately addressed.

This study is granted approval implementation effective this **May 23, 2014**. Please note that authorization to conduct this study will automatically expire on **May 22, 2015**. If you plan to continue with data collection or analysis beyond this date please submit an application for continuing approval to the ERC secretariat by **April 10, 2015**.

You are required to submit any amendments to this protocol and other information pertinent to human participation in this study to the SSC and ERC for review prior to initiation.

You may embark on the study.

Yours faithfully,

Handwritten signature: EAB

**DR. ELIZABETH BUKUSI,
ACTING SECRETARY,
KEMRI/ETHICS REVIEW COMMITTEE**

In Search of Better Health

APPENDIX 3: STANDARD OPERATING PROCEDURES
KENYA MEDICAL RESEARCH INSTITUTE (LEISHMANIASIS LABORATORY)

Research project: Determination of insecticidal effects of *Ricinus communis* crude extracts and blood baited traps with castor oil adhesive on phlebotomine sand flies (Diptera: Psychodidae)

Protocol I: Preparing R. communis stock solutions

Responsible personnel: Samuel Mong'are

Procedure

1. Determine the amount of solute you need and weigh it out with an electronic balance.
2. Weigh the required amount of solute, place it in an appropriate volumetric measuring device and add enough solvent until the total volume of the solution reaches the desired amount
3. Carefully add water to your measuring device until the bottom of the meniscus reaches the level of the final volume of your solution.
4. Seal the top of the measuring device so that no solution can leak out.
5. Gently invert the measuring device several times until all of the solute is dissolved.
6. To make parallel dilutions (125 µg/ml, 250 µg/ml and 500 µg/ml), the following formula was used to calculate the amount of stock solution needed for each dilution:

$$C_1 \times V_1 = C_2 \times V_2$$

-C₁ is the concentration of the starting (stock) solution

-V₁ is the volume of the starting (stock) solution needed to make the dilution (unknown volume)

-C₂ is the desired concentration of your final (dilute) solution

-V₂ is the desired volume of your final (dilute) solution

Protocol II: Sand fly larval food preparation

1. Weigh and mix equal amounts of rabbit chow and dried feces
2. Grind into fine powder and spread the powder in a layer of 3-4 cm deep in a large plastic tray.
3. Thoroughly wet the mixture with tap water and cover it tightly in a plastic paper bag to prevent drying.
4. Keep the mixture at ambient temperature for 4-6 weeks to age, while stirring up 2-3 times per week to aerate and disrupt fungal growth.
5. Several tablespoons of beef liver powder may be added to enrich it.
6. The mixture is allowed to dry, sterilized by autoclaving and kept at room temperature until required for use.

Protocol III: Ovicidal bioassays

1. Aspirate *P. duboscqi* females into each oviposition vial partially filled with plaster of Paris and fitted with screen tops.
2. Feed each sand fly with drops of Karo dark corn sugar syrup (Best Foods, CPC International, Inc., Englewood Cliffs, NJ) placed on the screen tops of the oviposition vials.
3. Keep the set up under insectary condition ($25\pm 2^{\circ}\text{C}$ and $70\pm 5\%$ RH) for 7-10 days for egg laying.
4. Remove the dead insects and leave only the eggs in the vials
5. Moisten the eggs with 1 ml of the aqueous extract of *R. communis* on 0 day post-oviposition. Another group (the control) should be moistened with 1ml distilled water.
6. Incubate the eggs at $25\pm 2^{\circ}\text{C}$ and $70\pm 5\%$ RH for hatching while moistened them with 1ml of extract daily.
7. Examine two eggs from each vial daily for any change on the egg membrane, then transfer them to a microscope slide with a drop of gum chloral.
8. Cover the eggs with a cover slip and leave it to dry for one day.
9. Observe using a light microscope for any morphological changes on the chorionic membrane.
10. Record the number of eggs that are hatching.

11. Repeat this experiment for methanolic and ethyl acetate leaf and bark extracts of *R. communis*.
12. Calculate percentage hatchability as follows using the formula of Khani *et al.*, 2013:

$$\% \text{ hatchability} = \frac{\text{Number of larvae hatched}}{\text{Total number of eggs}} \times 100$$

Protocol IV: Larvicidal bioassay

1. Transfer larvae (groups of 100-150) using a camel hair brush into rearing vials
2. Have four groups and treat them as follows: first group of the larvae give larval food mixed with the powdered crude extract (1:1), second group give larval food sprinkled with 5ml of the aqueous extract, third group give powdered extract only while the fourth group (control) feed the larvae on larval food only.
3. Observe these larvae daily for any defect/death.
4. Any motionless larvae should be considered dead and should be removed and mounted using gum chloral, left to dry for a day then examined for any morphological changes.
5. Also examine the live larvae for any transformation to second, third, fourth instars, pupae and adult emergence.
6. Assess the following parameters: the length of the larval and pupal periods; the larval and pupal viability, Mean lethal dosage (LD₅₀) after every 24 hours. Calculate % mortality using the formula of Khani *et al.*, 2013

$$\% \text{ Observed Mortality} = \frac{\text{Test Mortality} - \text{Control Mortality}}{100 - \text{Control Mortality}} \times 100$$

Reference: Moura, T. R., Oliveira, F., Novais, F. O., Miranda, J. C. & Clarencio, J. (2002). Enhanced Leishmania braziliensis Infection Following Pre-Exposure to Sand fly Saliva. PLoS Neglected Tropical Diseases; 1(2), 84-88.

Protocol V: Adulticidal bioassays

1. Aspirate 35 day old adult *P. duboscqi* into plastic rearing jars partially filled with plaster of Paris and fitted with screen tops.
2. Use 10% sucrose solution to prepare 125 µg/ml, 250 µg/ml and 500 µg/ml of 0.6 % *R. communis* extract by serial dilution and use in feeding the flies.
3. Soak cotton wool pads in the preparations and place on the screen tops.
4. Determine mean lethal concentration (LC₅₀) at 12, 24, 36, 48 and 72 h of exposure.
5. Maintain the set up at 27±2°C, relative humidity of 78-83 % and a 12: 12 (light: dark) photoperiod.
6. Stop the experiment when all the flies in the controls die.

Reference: **Moura, T. R., Oliveira, F., Novais, F. O., Miranda, J. C. & Clarencio, J. (2002).** Enhanced *Leishmania braziliensis* Infection Following Pre-Exposure to Sand fly Saliva. *PLoS Neglected Tropical Diseases; 1(2)*, 84.

Protocol VI: Design of the trap

Materials

- 5 L plastic bottles
- white cardboard sheet
- 0.1 cm wide steel netting separated by 0.3 cm square holes
- Castor oil

Procedure

1. Cut 3 windows on the sides of the 5-liter plastic bottle.
2. Cover the windows with the steel net to keep larger non-target insects out of the trap
3. Cut a 10 x10 cm white cardboard sheet
4. Paint the white cardboard (both sides) with castor oil to make a sticky paper trap.
5. Mount this trap vertically inside a clear 5-liter plastic bottle (plate).



Reference: Gunter, C. M., Vasilij, D. K., Leonid, R. & Yosef, S. (2011).
Characteristics of resting and breeding habitats of adult sand flies in
the Judean desert. *Journal of Vector Ecology*, **36(S1)**, S195-S205

Prepared by: Samuel Mong'are

Signature _____

Checked by: Name: _____

Signature _____

APPENDIX 4: PUBLICATIONS

IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)

e-ISSN: 2278-3008, p-ISSN:2319-7676. Volume 10, Issue 4 Ver. II (Jul - Aug. 2015), PP 16-24 www.iosrjournals.org

Comparative Analysis of the Effectiveness of Sand Fly Traps with Different Baits

Mong'are samuel¹, Ng'ang'a Zipporah¹, Ngumbi Philip², Johnstone Ingonga² and Ngunge Peter³

1. *Jomo Kenyatta University of Agriculture and Technology, P.O Box 62000, Nairobi, Kenya*

2. *Kenya Medical Research Institute, KEMRI, P.O. Box 43640 – 00100 Nairobi, Kenya*

3. *Technical University of Kenya, P. O Box 52428-00200 Nairobi, Kenya*

Mong'are Samuel, Jomo Kenyatta university of Agriculture and Technology, Zoology

Department P.O Box 62000-00200, Nairobi, Kenya

Abstract: Sand flies are small haematophagous insects that transmit *Leishmania* parasites. Infection with *Leishmania* parasites results in diseases known as leishmaniases which can be grouped into three main forms: cutaneous, mucocutaneous and visceral leishmaniases. The CDC light trap is the standard surveillance technique used to determine sand fly densities in a given locality, although this technique has been hampered by several logistic issues. Therefore, this study sought to use host blood, urine and ripe fruits as baits in a modified trap to come up with a cheap device for sand fly surveillance. This study also aimed at identifying the sand fly species attracted to this new device. Defibrinated blood from cattle, goat, sheep and chicken; urine from cattle, sheep and goat and fruits: grapes, mangoes, bananas and apples were placed inside each trap to act as sand fly attractants. A total of 1302 sand flies were collected within 3 trapping nights. Blood baited trap yielded more sand flies (202.33 ± 2.85) while chicken baited trap trapped the least (65.00 ± 1.53). Among the fruit baited traps, bananas attracted the highest number of sand flies (94.33 ± 4.63) followed by mango baited trap which caught (89.67 ± 2.33) although the difference between these two was not significant ($P=0.682$). 64.3% of the collected sand flies belonged to the genus *Sergentomyia* and 35.7% to *Phlebotomus*. The collected sand flies belonged to 7 species with *P. martini* (35.7%), *S. schwetzi* (34.1%) and *S. antennata* (15.7%) being the dominant species. Volatiles from plant and animal hosts may be used instead of light or carbon dioxide to improve the efficiency of traps for haematophagous insects. These volatiles can also be used to synergize each other and be incorporated in traps targeting sand flies.

Key words: Trap, sand flies, attractant, bait, leishmaniasis, fruit, blood, urine.

Introduction

Leishmaniasis is a major vector-borne disease caused by obligate intramacrophage protozoa of the genus *Leishmania* infecting numerous mammalian species, including humans [1]. Leishmaniasis is endemic in areas of the tropics, subtropics, and southern Europe. Specifically, it is endemic in 88 countries and is the only tropical vector-borne disease that has been endemic to southern Europe for decades [2]. Currently, leishmaniasis has a wider geographical distribution pattern than understood before and it is considered to be a growing public health issue.

Leishmaniasis has been endemic in Kenya for a long time. The most prevalent forms are cutaneous leishmaniasis, visceral leishmaniasis and post kala-azar dermal leishmaniasis (PKDL) [3]. Visceral leishmaniasis is found predominantly in the arid, low-lying areas of the Rift Valley, Eastern and North Eastern provinces, whereas cutaneous leishmaniasis occurs over a wide range of environmental conditions, from semi-arid lowlands to high plateaus in the Eastern, Rift Valley, Central and Western provinces [4]. Visceral Leishmaniasis is endemic in Baringo, Koibatek, Turkana, West Pokot, Kitui, Meru, Keiyo, Marakwet, Mwingi, Tana River and Machakos districts [5].

Since anti-leishmanial vaccines are still being developed, the current control strategies for leishmaniasis rely on case management. However, case management is difficult to be conducted since it is restricted by factors like lack of access to affordable, active drugs, incorrect prescribing and poor compliance [1]. The best method of interrupting any vector-borne disease is to reduce man-vector contact. Sand fly control programmes in most visceral leishmaniasis foci have advanced slowly when compared to that of other haematophagous arthropods like mosquitoes, ticks and black flies. Measures employed include spraying houses with insecticides where sand flies are endophilic and using treated and untreated bed nets where sand flies are endophagic [6]. Personal protection using repellents and nets is an important aspect. In endemic areas, spraying with dichlorodiphenyltrichloroethane (DDT) and other residual insecticides is effective in sand fly control [7].

DOI: 10.9790/3008-10421624

www.iosrjournals.org

16 | Page 1