Optimisation of Sugar and Blood Feeding Regimen in *Anopheles gambiae* Mass Production System

BY

SEDOFIA BRANSFORD KWASHIE

(10363170)

BSc. Agriculture (University of Cape Coast), 2002

This thesis is submitted to the University of Ghana, Legon, in partial fulfilment of the requirement for the award

of

MPhil Radiation Processing Degree

July, 2013

DECLARATION

This is to certify that this thesis is the result of research work undertaken by BRANSFORD KWASHIE SEDOFIA in the Department of Nuclear Agriculture and Radiation Processing, School of Nuclear and Allied Sciences, University of Ghana, under the supervision of Dr. Delphina A. Adabie-Gomez and Dr. D. D. Wilson, towards the award of Master of Philosophy Degree. This work has not been submitted either in part or full, to this University or elsewhere for the award of any other degree. Literature cited from other people's work has been duly acknowledged.

………………………......... Date………………………...

BRANSFORD K. SEDOFIA

(STUDENT)

………………………........ Date.………………………

DR. DELPHINA A. ADABIE-GOMEZ

(SUPERVISOR)

………………………...... Date………………………..

DR. DAVID. D WILSON

(SUPERVISOR)

DEDICATION

I first and foremost gratefully dedicate this thesis to the Almighty God for bringing me this far. I equally dedicate it to my two daughters Eyram and Makafui, my mother Mrs. Mawunyo Sedofia and my wife Mrs. Josephine Sedofia for their unflinching support and encouragement throughout my study.

ACKNOWLEDGEMENTS

I am most grateful to my supervisors; Dr. Delphina A. Adabie-Gomez, Senior Lecturer and Former Deputy Director of BNARI, GAEC and Dr. D. D. Wilson, Senior Lecturer at the Department of Zoology, University of Ghana for their, patience, suggestions, constructive criticisms and dedicated support in compiling this thesis. I would like to also express my profound gratitude to Mr. Michael Osae, Lecturer, School of Nuclear and Allied Sciences – University of Ghana, Alessi A. Kwawukume, Mr. E. A. Ewusie and Mr. Godfrey K. Damnyag, all of the Radiation Entomology and Pest Management Centre (REPMC) of GAEC for their immense and unflinching support in the laboratory work. My profound appreciation also goes to Mr. Justice Okona Frimpong of Nuclear Agriculture Centre, BNARI-GAEC for his immeasurable contributions to this work. I am very grateful to Dr. Alexander Egyir-Yawson (Centre manager, REPMC) for giving me access to the facilities at the Centre for my work. A note of special thanks is further extended to all REPMC staff, family members and those who endeavoured to assist me in one way or the other, May the good Lord bless you all. Finally, my utmost appreciation goes to the Almighty God for bringing me this far, having shown me such abundant grace, protection and divine guidance to go through this programme of study successfully.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF PLATES

LIST OF FIGURES

Figure **Page**

LIST OF APPENDICES

Appendix **Page**

ABSTRACT

The sterile insect technique (SIT) is being developed for the control of malaria transmitting mosquitoes. Critical to the success of applying the SIT is the establishment of standardized mass production systems for the target species. As part of efforts to develop standardised mass production systems for malaria vectors, this project sought to optimize adult blood and sugar feeding in a mass production system. Different sugar types (glucose, sucrose and honey) were evaluated at 6 % and 10 % concentrations in water to determine the best sugar diet and concentration for feeding adult *An. gambiae*. Different blood feeding methods, restrained Guinea pig, anaesthetised Guinea pig and human arm feeding were evaluated. Adult survival, female insemination and egg production were used as criteria to determine optimum sugar and blood feeding. The effect of anaesthetics on blood feeding response and egg production of female *An. gambiae* was determined by comparing feeding response and egg production of females fed with anaesthetised Guinea pigs as against physically restrained Guinea pigs (Control). The specific effect of different anaesthetic agents on blood feeding response and egg production of female mosquitoes was also determined by comparing the feeding response and egg production of females fed with either Ketamine/Xylazine anaesthetised Guinea pigs or Ketamine/Diazepam anaesthetised Guinea pigs. Effects due to sugar types and concentrations on percentage survival of male and female mosquitoes were observed to be significant at ($p < 0.05$). Honey at 10 % concentration recorded the highest survival of 84.3 % after 1 week and 12.7 % after 6 weeks whilst glucose and sucrose recorded 81.0 % and 78.0 % respectively after 1 week and 1.3 % for both after 6 weeks. Survival up to 35 and 49 days for males and females respectively occurred only in adults fed with honey. The combined effects of blood feeding methods, anaesthetic types and feeding times on the propensity of female *An. gambiae* to feed and produce eggs were not significantly different ($P > 0.05$). However, human arm feeding (HAF) method and Ketamine/Xylazine (KX) anaesthetics fed for 25 minutes recorded higher percentage feeding (76.0 % and 68.0 % respectively) and egg production of 19.0 % and 20.8 % respectively. Anaesthetised Guinea pig feeding (AGF) of adults for 15 minutes followed closely with 60.0 % and 15.1 % blood feeding and egg production respectively whilst restrained Guinea pig feeding (RGF) method and Ketamine/Xylazine (KD) anaesthetic agents recorded the least values. It can be concluded that 10 % honey solution resulted in optimum feeding, survival and female insemination in adult *An. gambiae* compared to other treatments. Although both human arm feeding and Ketamine/Xylaxine anaesthetised Guinea pig feeding resulted in optimum productivity, anaesthetised Guinea pig feeding of adults for 15 minutes is the ideal feeding method for mass production systems. We recommend that 10 % honey solution, anaesthetised Guinea pig feeding method using 0.05 ml Ketamine/Xylazine anaesthetics and 15 minutes feeding time be employed for adult feeding in mass production of this species.

CHAPTER ONE

1.0 INTRODUCTION

1.1 BACKGROUND

Anopheline mosquitoes are known vector species responsible for the transmission of *Plasmodium* parasites to humans in many parts of the world. These vector species vary from region to region but in Africa the parasite is transmitted mainly by members of the *Anopheles gambiae* complex and *Anopheles funestus* group (Kelly-Hope, 2009; Annan *et al.*, 2007; Appawu *et al.*, 2004; Craig *et al.*, 1999). Of these, the *An. gambiae* is found to be the most important and efficient vector species for the parasite in Sub-Saharan Africa (Rodrigues *et al.*, 2012; Obbard *et al.*, 2009; Alavi *et al.*, 2003).

Only the female anophelines are capable of transmitting the malaria parasite from an infected person to a non-infected person or to an already infected person (Cox, 2010; Rhodes, 2008). This is because only females require blood which sometimes do not only contain important protein sources they need to develop their eggs, but *Plasmodium* parasites that equally prefer such blood to mature exceptionally well (Lavazec and Bourgouin, 2008). Even though male Anopheline mosquitoes lack this feature and therefore do not take blood or transmit the parasite, they still play an indirect but very vital role by providing the needed insemination of females with fertile sperm (Gouagna *et al.*, 2010; Jiang, 2008; Nayar and Van Handel, 1971; Clements, 1955). A phenomenon which forms an essential part of the reproductive process that gives rise to progenies that eventually continue to transmit the parasites to millions of people each year. This phenomenon is likely to continue if better and more effective strategies are not put in place to significantly reduce or eliminate the vector.

Currently, though several prevention and control interventions from mechanical, biological through to largely chemical (use of burning marts, smoking coils, indoor residual insecticide sprays, insecticide-treated bed nets and anti-malarial drugs) are in place, malaria infection continues to remain high in many parts of Africa for several reasons (Griffin, 2010). Major among these is the development of multiple insecticide resistance among anopheline mosquitoes, continuous development of *Plasmodium* parasite resistance against anti-malaria drugs, and the short reproductive life cycle of anopheline mosquitoes that enables them to reproduce large numbers at very short periods (Edi *et al.*, 2012; WHO, 1995; Briegel & Hörler, 1993). In addition, the continuous application of insecticides in vector control also tends to cause significant reduction in the population of natural control agents needed to naturally keep vector numbers below destructive thresholds (Marrelli, 2012). The need to incorporate novel control strategies into existing methods to augment their effectiveness or as complementary measures in vector prevention and control becomes imperative (Brelsfoard and Dobson, 2009).

One such promising novel control strategy is the Sterile Insect Technique (SIT); an autocidal approach that provide an area-wide vector control without the accompanying problems of toxicity and the emerging vector/parasite resistance development associated with the widely used chemical approach (Kala, 2012; Parker and Mehta, 2007).

Central to the success of this strategy for area-wide mosquito control is the production and release of large numbers of laboratory-reared, genetically-modified sterile male mosquitoes that can successfully mate with the wild females, cause reproductive failure in the females and in so doing stop them from reproducing (Kala, 2012; Dyck *et al.*, 2005; Knipling, 1955). In this way, the wild vector population will collapse (Morrison *et al.*, 2010) and malaria infection cases reduced considerably.

A sustainable production of these large numbers in the laboratory requires well-maintained brood stock colonies that are readily available for regular and timely supply of sterile adult males for field releases when the need arise. Maintaining the stock colony also requires feeding the colony as well as the adults to be released with appropriate sugar and blood sources, using properly standardised feeding methods and feeding regimens (Clements, 1992).

Sugar is considered the major food resource for all adult mosquitoes (Woodbridge, 1995) as it provides the essential nutrition adults need to generate the energy needed to survive long enough, maintain fitness and reproduce (Gu mail *et al.*, 2011). Adult males for example, require this energy to effectively compete among themselves for females and successfully inseminate females (Gouagna *et al.*, 2010). In the wild, adult male mosquitoes depend on plant juices and exudates (e.g. floral nectar, honeydew, damaged fruits and vegetative tissues etc) for these essential sugars (Woodbridge, 1995). From these different sources, they obtain sucrose, fructose, and glucose among others depending on the source from which the sugar is taken (Benedict, 1997; Clements, 1992; Briegel and Kaiser, 1973, Gillett *et al.*, 1962). Similarly, females also require these sugars for survival throughout their mating, eggs production and oviposition periods and beyond. To this effect, sugar feeding constitutes an indispensable aspect of the life of adult mosquitoes (Woodbridge, 1995).

In a controlled environment such as the laboratory, adult mosquitoes are equally reared on different synthetic sugar sources including glucose, sucrose and honey (artificially extracted) as means of providing food sources similar to what exist in the wild (Benedict, 1997; Clements, 1992; Briegel and Kaiser, 1973; Gillett *et al.*, 1962). Thus, there is the need to evaluate the different synthetic sugar sources and the amounts currently employed in laboratory feeding of mosquitoes in order to determine the specific type and quantity that can result in optimum feeding, survivability and productivity in mass rearing systems.

Though natural sugar sources contain protein needed by females to develop their eggs, the concentrations are not high enough for their reproductive role (Clements, 1992). Blood feeding in female anopheline mosquitoes therefore forms an integral part of their reproductive cycle (Olayemi *et al.*, 2011; Briegel, 1990). In addition, it is a very important component in their mass rearing towards the successful implementation of SIT for area-wide control of the vector. In the wild, female mosquitoes depending on host preference may obtain blood meals from live mammals, reptiles, birds and amphibians (Means, 1968). In controlled environments such as the laboratory, mosquitoes are mostly fed on processed blood meal using artificial delivery systems such as the membrane feeder or by using natural delivery systems such as direct human arm feeding, restrained, and anaesthetised animal feeding methods using human beings, rabbits, Guinea pigs, mice, rats etc (Benedict *et al.*, 2007). The source and the size of blood meal females ingest determines the number of females that develops eggs and the number of eggs developed per female. These variations can be attributed to differences in the type of proteins and their constituent amino acids contained in such blood meals (Prasad, 1987). Thus, blood of superior nutritional quality is necessary for optimum feeding and egg production in mass production systems (Olayemi *et al.*, 2011).

LaFlamme (2011) stated that though female *Anopheles* mosquitoes in most cases prefer human blood to other hosts, this may be practically impossible where very large numbers are to be fed. Similarly, though direct feeding of live mammals (Guinea pigs, rabbits etc) to vector mosquitoes may provide a suitable alternative, its use may require sedating the live mammal with anaesthetic agents in order to immobilise and restrain its movement during feeding. This is because any movement in the live mammal during feeding can disrupt feeding and seriously affect the extent to which the mosquitoes feed. (Gerberg, 1970). Over the years, different anaesthetic agents have been employed for this purpose, however, the presence of an anaesthetic agent in the blood of mammals used as host may affect feeding response and productivity in female mosquitoes fed in a number of ways. For instance, the amount of blood they imbibe, how long they survive to lay eggs and the number of eggs that they lay during the oviposition period may be affected by these anaesthetic agents (Murrieta *et al.*, 2010). It is therefore important to evaluate the effect of these anaesthetic agents on females' blood feeding response and egg production in order to determine the appropriate anaesthetics and the combinations that are optimum for their productivity in mass rearing systems.

1.2 STATEMENT OF THE PROBLEM

Effective vector control and more specifically mosquito control, is a complex and difficult problem, as illustrated by the continuing prevalence (and spread) of mosquito-transmitted diseases (Alphey *et al.*, 2010).

Though the sterile insect technique (SIT) has been reiterated as the much-needed novel approach to stop malaria parasite/disease transmissions, the success of its application for area-wide mosquito control heavily requires inducing reproductive failure in females through unviable matings between laboratory-reared sterile males and wild females. By so doing the reproductive capacity of the wild females will be reduced considerably and the vector will be eliminated over time (Alphey *et al.*, 2010). This outcome can only be achieved if adult males are reared to numbers that can match or exceed the males population in the wild (Feldmann and Hendrichs, 2001) and if the large number of adult males required for field release can be readily obtained at regular time intervals when the need arise. To this effect, it has become necessary to maintain adult stock colonies in mass production systems on the right sugar and blood sources using appropriate feeding methods and feeding time regimens that give optimum feeding and in effect increase the fitness, longevity and productivity of the laboratory stock in mass rearing systems.

Several sugar types, feeding concentrations as well as blood sources, blood feeding methods and time regimens have been identified and explored by researchers over the years to attain the production of these large numbers in the laboratory (Syoziro, 1964). However, these sugars and blood sources and feeding methods/regimens have not been properly developed and standardized for optimum feeding in mass rearing systems (Fabrizio, 2012). This has over the years led to reduced survival, fitness and low productivity among laboratory-reared stocks. Thus, makes it very difficult to turn out the large numbers needed to successfully use the sterile insect technique to control vector mosquitoes especially in sub-Saharan Africa where the vector remains endemic and malaria prevalence is still very high.

1.3 RELEVANCE AND JUSTIFICATIONS

There is very clear evidence that current conventional methods, largely chemical applications have very serious limitations in controlling vector mosquitoes/malaria parasites in the sub-region. Moreover, the deleterious effect of these chemicals on human life and the environment as a result of over dependence on chemical methods to control mosquitoes and malaria parasites are becoming increasingly visible by the day (Marrelli, 2012). These have led to the impetus to include other novel control strategies such as SIT in the effort to eliminate the vector and the parasite.

The Sterile Insect Technique (SIT) which is one such novel methods has the capacity to successfully synergise with good aspects of existing conventional methods (WHO, 2004, 2008) to make vector/malaria control more effective

(Brelsfoard and Dobson, 2009). It is a species-specific, environment-benign, clean (leaving no residues) and sustainable vector control strategy that can prevent, suppress, contain or eliminate particular insect pest or vector populations.

The technique as part of area-wide integrated pest management (AW-IPM) programmes has celebrated many successes in eliminating and suppressing several economically important insect pest populations as well as preventing vector establishment in new areas without the accompanying limitations associated with existing methods (Knipling, 1959; Knipling, 1968; Dyck et al., 2005). For example, it was successfully mounted to control important insect pests and vectors of agricultural and medical importance including tropical fruit flies, *Bactrocera spp*, some species of tsetse flies, *Glossina spp*., the pink bollworm, *Pectinophora gossypiella* (Saunders), and the codling moth, *Cydia pomonella* (Joint FAO/IAEA, 2012; Dyck *et al.*, 2005; Krafsur, 1998; Knipling, 1998, 1979, 1955). There is therefore renewed interest in using sterile insects for managing endemic, as well as emerging or re-emerging vector-borne diseases (Alphey *et al.*, 2010).

However, the development of SIT for use in mosquito AW-IPM programmes is in its infancy, and many fundamental components of the technique still need to be developed, standardised and optimised. These components include aspects of the mass-rearing of the vector and the quality of the sterile males produced in the laboratoryIt has been established that nutrition is one of the important factors that has huge impact on the survival, fitness and productivity of adult vector Anopheline mosquitoes in mass rearing systems (LaFlamme, 201l; Gu mail *et al.*, 2011; Dame *et al.*, 2009; Woodbridge, 1995; Nayar and Sauerman, 1975).

In exploiting the huge prospects of this technique for area-wide vector mosquito control, it has become imperative to thoroughly investigate sugar and blood feeding (an essential aspect of mass rearing of the vector) which constitute an indispensable part of the adults' life, but have not been fully developed and standardised for optimum and sustainable mass production of the insects in the laboratory. This study therefore seeks to evaluate different sugar types, blood feeding methods and feeding time regimens employed in feeding *Anopheles gambiae* mosquitoes in the laboratory so as to properly develop standardised sugar type, blood feeding method and time regimen that will give optimum feeding, survival and productivity in a mosquito mass rearing system.

1.4. Objectives

1.4.1 *General objective*

The general objective of this study is to investigate the effects of three different sugar types and three blood feeding methods on the biology and productivity of *Anopheles gambiae* mosquitoes in a mass production system.

1.4.2 *Specific objectives*

i. To evaluate the effects of 6 % and 10 % concentrations of three sugar types (glucose, sucrose and honey) on the survival and productivity of adult *Anopheles gambiae* mosquitoes.

ii. To evaluate the effects of three different blood feeding methods on feeding response and productivity of female *Anopheles gambiae* mosquitoes.

iii. To determine the effects of Ketamine/Xylazine and Ketamine/Diazepam anaesthetics used to immobilise live animals to feed female mosquitoes on feeding response and productivity of *Anopheles gambiae* mosquitoes.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Brief biology of *Anopheles* mosquitoes

Anopheline mosquitoes have four stages in their life cycle (McCafferty, 1983). According to Miller (2012) their life cycle starts with females laying 50 to 200 boat-shaped eggs singly on the surface of water. The eggs under optimum conditions of temperature and relative humidity may hatch into larvae within 24 hours or more depending on species. The larvae develop through four instars or larval stages with a final moult that give rise to the pupae (Clements, 1992). Adequate nutrition in the larval stage is very crucial to the insects as nutrient reserves from this stage are needed to sustain the insects during their pupal stage where the insect stop feeding completely and enters into a state of structural modifications with the immature tissues breaking down to form adult structures and therefore rely on these reserves for survival (Harzsch and Hafner, 2006; Clements, 1992).

Unavailability or inadequate amounts of these nutrient reserves from the larval stage can adversely affect the insects' body size, fitness, survivability and productivity in the adult stage (Araújo *et al.*, 2012; Wheeler, 1996).

According to Helinski *et al.* (2006b) the pupal stage is also an important stage of the insect as it lends itself as a key stage for SIT development and application in the control of Anopheline vector mosquitoes. This is because, apart from the adult stage, sexual sterility in male mosquitoes which is needed to cause reproductive failure in the wild female population can also be induced in the pupal stage of mosquitoes prior to their emergence into adults.

The adult male Anopheline mosquito recognised by its feathery antenna and long palpi usually lives up to one week in nature and has the sole responsibility of inseminating adult females. The females which have a lifespan of 2 to 3 weeks in nature but can survive up to a month or more in the laboratory mate only once in their lifetime. Upon insemination, female Anopheline mosquitoes seek their host in search of blood meals to develop eggs that are laid singly on the surface of water (Cross, 2004).

Soon after oviposition, the female Anopheline mosquito resumes the search for a new blood meal from same or different hosts to lay new batch of eggs after three to four days if conditions are favourable. This provides the link between the human host and the parasite, and accounts for their short reproductive cycle as well as the usually high vector prevalence and malaria infection rates. However, their short reproductive cycle can also be exploited for mass production of male mosquitoes at short time intervals that will enable regular and sustainable supply of sterile males for vector mosquito AW-IPM programmes.

According to the Centres for Disease Control and Prevention (CDC) (2010), the first three stages (egg, larva and pupa) which form the immature stages of the insect are aquatic and last from 5 to 14 days depending on species and ambient temperature. Unlike the adult (female) stage that transmits the malaria parasites, the other stages of the insect do not play a direct role in the transmission of the parasite to humans (WHO, 2009).

2.2 Role of Anopheline mosquitoes as vectors in malaria transmission

Mosquitoes play very important role as vectors in the transmission of disease-causing parasites that account for several diseases and millions of death each year (Rhodes, 2008, Molavi, 2003). According to Molavi (2003), mosquito-borne diseases infect about 700 million people in Africa, South America, Central America and much of Asia yearly and kill over 3 million people globally every year. From this number, over one million deaths are reported to be caused by the infective bites of Anopheline mosquitoes, the main vector responsible for transmitting *Plasmodium* parasites that cause malaria infections especially in Sub-Saharan regions of Africa (Fortin *et al.,* 2002).

In Ghana, over 3.5 million clinical cases of malaria and 3,000 deaths are reported each year, with 8,200 clinical cases occurring daily (Sodzi-Tettey, 2011). According to The Global Poverty Information Bank 2012 report on mosquito/malaria prevention and control in Ghana, the country spends about 6 % of its GDP on malaria prevention and control annually. Aside these huge government spending, residents also spend substantial part of their livelihoods on repellents, insecticides, screens and other anti-mosquito/malaria products in attempts to prevent and or control mosquitoes and malaria infections (Woodbridge, 1995). Despite the huge financial expenditure and vigorous application of existing interventions to manage the vector problem, mosquito/malaria infection cases however continue to show high prevalence rates (Marrelli, 2012).

Evidence provided by the work of Dash *et al.* (2008), revealed that though malaria was nearly eradicated from some parts of the world over the years, the disease in recent times has re-emerged with new features which were not witnessed during the pre-eradication days due to vector resistance to insecticide(s) and the resistance of *Plasmodium parasites* to chloroquine and other anti-malarial drugs. These new features in both the vector and the parasite rendered current control strategies less effective in dealing with the problem.

2.3 Need for novel control strategies

With increasing international attention and effort to bring the situation under control, vector control strategies including the use of insecticide-treated nets (ITNs) and indoor residual spraying (IRS) (as part of available chemical control methods in place) were introduced with the aim to suppress the transmission intensity and the disease burden (WHO, 2007, 2008). However, scale-up applications of these interventions inevitably do not suffice to sustain long-term control efforts (Read *et al.*, 2009; Chambers *et al.*, 2008).

Controlling the disease has therefore become a complex enterprise, and its management will require incorporating other novel control strategies into current control strategies in order to effectively manage the vector problem (Marrelli, 2012; Alphey *et al.*, 2010; Baker *et al.*, 1986). One such important novel control strategy gaining much approval is the Sterile Insect Technique (SIT) which is more of a biological (birth control) method used in several areas to control other key insect pests and vectors of agricultural and medical importance (Klassen, 2005).

According to WHO (2008, 2004), Dyck *et al*. (2005), Krafsur (1998) and Knipling (1998, 1979, 1955), the SIT which exhibited great tendency to effectively synergize with the useful aspects of current prevention/control methods is species-specific, environmental-friendly, clean (leaving no residues) and sustainable vector control tool. It employs mating between released laboratory-reared sexually sterilised males and the native females to reduce or eliminate the reproductive potential of the wild females through production of infertile eggs. This is done by releasing the laboratory-reared sterile males at overflooding ratios over a sufficient period of time.

Over the years, the Sterile Insect Technique has proven to be a safe and effective method to prevent, suppress, contain or eliminate insect pest and vector populations and has been mounted successfully against many vectors by using large numbers of the laboratory-reared sterile males (Helinski *et al*., 2006a). Thus any effort at using SIT for area-wide management of vector mosquitoes must include the mass production of males.

According to Dyck *et al.* (2005) the chances of obtaining such large numbers from the laboratory depend on the fitness, survival (longevity) and productivity of the laboratory-reared population. However, some research findings in this area have shown that mosquitoes reared in the insectary tend to have reduced fitness, survivability and productivity thus making their mass production in the laboratory almost impossible. For example, the work of Kija *et al.* (2005) attributed the general failure of mosquito control programmes launched in the 1970s to poor fitness, survivability and productivity in the laboratory-reared population. Clements

(1955) also attributed this failure largely to poor nutrition among the laboratory-reared stock. These findings were further buttressed by the work of Gouagna *et al.* (2010), Nayar and Sauerman (1975) and House (1961), who indicated that the fitness, survival and reproduction of insects depend significantly on nutrition. Thus, developing optimal adult diet as well as appropriate blood feeding method/feeding time regimen in adult females is very crucial to optimising feeding among the laboratory reared stocks in order to compensate for these survival, fitness and productivity deficits.

2.4 Sugar feeding in adult *Anopheles* mosquitoes

Takken and Verhulst (2012), Gouli *et al.* (2004) and Harrington *et al.* (2001) reported that sugar (carbohydrates) and mammalian blood are critical sources of nutrition among adult mosquitoes. Of these two, sugar is regarded as their basic food since it is commonly and frequently ingested by both sexes to derive the energy needed to maintain fitness, survive long enough and reproduce (Woodbridge, 1995; Nayar and Sauerman, 1975).

Foster and Takken (2004) in their findings also stated the importance of the mosquitoes' need for sugar by reporting that both sexes emerge into adults with little available energy and hence are strongly attracted to nectar-related volatiles which they often prefer to host-related volatiles when they newly emerged under laboratory conditions. This preference for sugar in their early stages indicates that sugar feeding is not only fundamental for maintaining vital activities of mosquitoes in laboratory but an early priority due to the risk of starvation (Magnarelli, 1986). This dependency on sugar is further illustrated by their behavioural, structural and physiological specialisations for finding, feeding and processing these sugars (Woodbridge, 1995).

Foster (1995) and Nayar and Sauerman (1975) further stated that though hematophagous females generally utilize protein from blood meals to develop eggs, they still utilize sugar to help meet their metabolic needs and increase survivorship. In addition, sugar also provides females with a ready source of flight energy (Nayar and Van Handel, 1971; Clements, 1955) and can, in some cases, improve fecundity, both by helping to develop follicles to the resting stage in small females (Magnarelli, 1978, Nayar and Sauerman, 1975) and by increasing the number of follicles undergoing vitellogenesis (Mostowy and Foster, 2004). According to Gary (2005), experiments focusing on the first gonotrophic cycle also suggest that sugar feeding increases fecundity more than blood feeding alone. Gary and Foster (2001) and Straif and Beier (1996) again reported that it is common laboratory practice to maintain female mosquitoes on sugar between blood meals and that sugar in combination with daily blood meals did increase laboratory life span over that of daily blood meals alone, suggesting that though exclusive blood feeding is observed in some mosquitoes it is only a function of sugar scarcity. In buttressing this finding, Morrison *et al.* (1999); Costero *et al.* (1998); Scott *et al.* (1997), Gibson (1996); Foster (1995); Van Handel *et al.* (1994) and Macfie, (1915) reported that though some mosquitoes species seem to have a fitness advantage feeding on blood alone, this is only due to their anthropophilic and endophilic nature that enable them to adapt and live in domestic environments where blood is readily available and sugar is scarce.

Briegel *et al.* (2002), Gary and Foster (2001), Nayar and Pierce (1980), Nayar and Sauerman (1971, 1975) and Briegel and Kaiser (1973), also presented further evidence that many anautogenous species survive longer and/or produce more eggs with sugar as well as vertebrate blood in their diet. Other field studies also presented evidence that sugar-feeding forms part of their normal feeding behaviour and that the continued existence of sugar feeding both in the laboratory and in the field, indicates that sugar meals strongly provide a fitness and productivity advantage to this species (Laarman, 1968).

2.5 Sugars as nutritive food resource for adult mosquitoes

In nature, mosquitoes obtain sugar meals primarily from plant sources such as floral and extra-floral nectaries, damaged fruit, and honeydew (Foster, 1995). Sugars from these sources consist mostly of fructose, glucose, and sucrose (Van Handel, 1972). Other sugars and amino acids may also be present, but usually in trace amounts (Baker and Baker, 1983; Van Handel, 1972, Auclair, 1963).

In the insectary, Anopheline mosquitoes can be fed on different synthetic sugar sources such as glucose, sucrose (granulated table sugar) and honey (Benedict *et al.*, 2007). However, these different sources contain specific carbohydrates with varying amounts of energy yield per calories (Tan, 2013). Glucose as energy source provides approximately 3.75 kilocalories per gram for cellular activities and serves as precursors for the production of more complex biological molecules but lack essential supplemental elements such as proteins, minerals and vitamins (Wikipedia, 2012).

Fructose, though contains 3.89 kilocalories per gram and releases energy more readily, it also lacks essential vitamins, minerals and proteins like the glucose units, (Sefcik, 2010).

On the other hand, sucrose (complex sugar units as found in refined sugars such as Table sugar) contains relatively higher caloric value (3.94 kilocalories per gram) than the simple sugars, but yield their energy more gradually due to chemical bonds between their constituent glucose and fructose units from which they are formed (Lustig, 2013; Wikipedia, 2011). Therefore, in utilizing sucrose, adult mosquitoes tend to expend more energy in hydrolysing these bonds, hence are unable to keep sufficient amount of the energy generated for future use, and in severe cases may deplete their glycogen reserves quickly to satisfy their immediate energy needs. This creates energy deficits that reduce their fitness and longevity. In addition, supplemental elements such as vitamins and minerals are destroyed during their refining processing rendering them deficient of minerals, protein and vitamins (Tan, 2013).

Honey is a mixture of sugars (mostly fructose 38.5 %, glucose 31.0 %, smaller quantities of maltose, sucrose, and other complex carbohydrates) and other compounds such as vitamins (B6, thiamin, niacin, riboflavin and pantothenic acid) (Bogdanov *et al.*, 2008), proteins (trace amounts of all essential amino acid except methionine and histidine, and non-essential amino acids e.g. proline) (Mckenzie, 2011), and minerals (calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium and zinc). It also contains tiny amounts of several compounds thought to function as antioxidants including chrysin, pinobanksin, vitamin C, catalase and pinocembrin, and possess antimicrobial, antifungal and antiviral properties (Molan, 1997 and White, 1975). Unlike sucrose, it digests easily and is absorbed rapidly into the bloodstream providing quick supply of energy (Bogdanov *et al.*, 2008). One gram of honey contains twice the amount of calories (8.2 kilocalories) found in sucrose.

According to Clements (1992), selecting the appropriate sugar type is therefore very critical if feeding is to be optimised to increase fitness, longevity and productivity of the laboratory reared stock.

2.6 Blood feeding in female *Anopheles* mosquitoes

According to Richards *et al.* (2013) and Clements (1992) blood feeding is exclusive to adult female mosquitoes since unlike the males, females have a gonotrophic cycle that begins with a blood meal and ends with oviposition. Thus, blood feeding is not only an essential activity for colonisation and maintenance of female mosquitoes for research work on vector-borne diseases but also for possible production of large numbers of adult mosquitoes needed for AW-IPM programmes (Deng *et al.*, 2011).

According to LaFlamme (2011), adult females apart from the sugars they require to maintain fitness and survive long enough, also take blood meals for supplemental substances such as protein (more importantly threonine) to mature their eggs and enhance reproduction. Takken *et al.* (1998) in their work also confirmed that sugar feeding was not sufficient to bring follicles of *An. gambiae* mosquitoes to resting stage and blood meal was required to help build protein reserves, develops follicles to the resting stage, and initiate vitellogenesis and yolk uptake for oocytes to develop completely. Takken *et al.* (1998) findings was further buttressed by the work of Clements (1992), who stated that primary ovarian follicles of undersized female mosquitoes are arrested in the previtellogenic phase if a meal of blood is not taken. This is because blood has some connection to juvenile hormone suppression, a phenomenon required to stimulate development of follicles to the previtellogenic stage (Clements, 1992).

In satisfying this incessant need for blood, female mosquitoes also ingest *Plasmodium* parasites (agents of malaria infections) from parasite-infected hosts and eventually transmit them to other hosts (either uninfected or already infected) during subsequent blood feeding (Olayemi *et al.*, 2011).

2.7 Effect of blood types and feeding methods on reproduction in female *Anopheles* mosquitoes

Reproduction in adult mosquitoes forms an essential part of their life and influenced by a number of factors (Clements, 1992; Briegel, 1990). According to Billingsley and Hecker (1991) one such factor which is of immense importance is how much blood females ingest during feeding. This according to Billingsley and Hecker (1991) this is very important because it determines how much proteins/amino acids are made available to synthesise egg yolk proteins during reproduction. This was further reiterated by Taylor and Hurd (2003), Lounibos and Conn (1991), Foster and Eischen (1987), who stated that the blood source used and the feeding method and feeding time regimen employed in feeding the mosquitoes is a strong determinant of how females respond to feeding and therefore the amount of blood they eventually ingest to concentrate these essential proteins/amino acids for reproduction.

According to Zimmerman *et al.* (2006) and Burkot (1988), the choice of blood meal in Anopheline mosquitoes is also influenced by several factors including host availability and preference , nutritional requirements and vector density. Laflamme (2011), Lacey and Lacey (1990) and Chandler and Highton (1975) reported that though Anopheline mosquitoes exhibit a wide range of host preferences including preference for humans, livestock, birds, and reptiles, the *Anopheles gambiae* mosquito tend to show greater preference not only for human hosts but for direct ingestion of blood from the skin of the host rather than from other alternative blood sources and feeding methods. This according to Meijerink, *et al.* (2000), Roberts and Janovy, Jr. (2000), Konate, *et al.* (1999), Cork and Park (1996), Knols and De Jong (1996), Kline *et al.* (1991) and Takken and Kline (1989) is because the female *Anopheles gambiae* (which rely on two inherent odorant-binding proteins (OBP) to aid host search) is more attracted to volatiles and microflora present on human skin compared to other hosts.

However, the use of humans as host in mosquito production systems is not practicable where rearing involves large scale production of the insects. Feeding the insects on such scale from time to time will therefore require the use of other alternative blood hosts and or delivery systems other than the human host. Gerberg (1970) stated that Guinea pigs, rats, mice, rabbits etc are alternative hosts that can be used to mass feed vector mosquitoes in the insectary. Coluzzi (1964) also reported that although the use of human arm is the ideal method to feed adult mosquitoes when maximum adult vitality is required, *Anopheles gambiae* mosquitoes can also be induced to feed on Guinea pigs in the laboratory.

2.8 Use of anaesthetics in live animal feeding of adult female *Anopheles* mosquitoes

Various methods including mechanical restraining, and immobilising mammals (sedating with anaesthetic agents) to feed adult mosquitoes are being used in several insectaries as substitute for the human arm feeding method. However, immobilising live mammals with anaesthetic agents prior to blood feeding the mosquitoes can greatly affect their feeding response and ultimately their productivity (Gerberg, 1970). For example, the use of anaesthetic agents in live mammals was found to cause profound physiological changes such as altered peripheral circulation and decreased body surface temperatures that can adversely alter experimental results (Murrieta *et al.*, 2010). Thus, it is important that live mammals are anaesthetised with the right anaesthetics in order to minimise as much as possible the negative impact of these anaesthetics on the productivity of the insects in the laboratory.

Among the several anaesthetics employed to immobilise live mammals to feed adult female mosquitoes are Xylaxine, Ketamine and Diazepam (Green *et al.* 1981). According to Mohammed *et al.* (2012), Murrieta *et al.* (2010), Gonza´ lez Gil *et al.* (2003), the quantity of anaesthetics administered to induce anaesthesia in live animals determines how much anaesthetic is eventually introduced into their bloodstream hence the extent to which normal physiological processes such as blood circulation and body temperature of the live host are altered. Kingsolver (1987) also stated that the body temperature of host animals can affect blood feeding response in female Anopheline mosquitoes. According to Khan and Maibach (1971) female mosquitoes tend to show increased response to blood feed when body temperature of the host is within a range of 34 °C to 36 °C than at lower temperatures. Findings from the work of VanDyk (2007) also revealed that mosquitoes were attracted three times more to hosts with skin temperature of 36.7°C than at a lower skin temperature of 18.3°C or below. Therefore the effect of anaesthetics on blood circulation and body temperature of the host organism can adversely affect feeding response and productivity in Anopheline mosquitoes.

The need to evaluate the effect of these anaesthetic agents or their combinations on the feeding response and productivity of vector mosquitoes is very crucial if productivity is to be optimised among laboratory reared mosquito stocks.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study site

The study was carried out at the Mosquito Insectary of the Radiation Entomology and Pest Management Centre (REPMC) of the Biotechnology and Nuclear Agriculture Research Institute (BNARI), Ghana Atomic Energy Commission, Ghana. The insectary is a four compartments facility consisting of a cleaning room, an experiment room, larval and adult rearing rooms. It is located 20 km north of Accra at an altitude of 76 m above sea level (Figs. 1 and 2).

Fig. 1. Map of Ghana

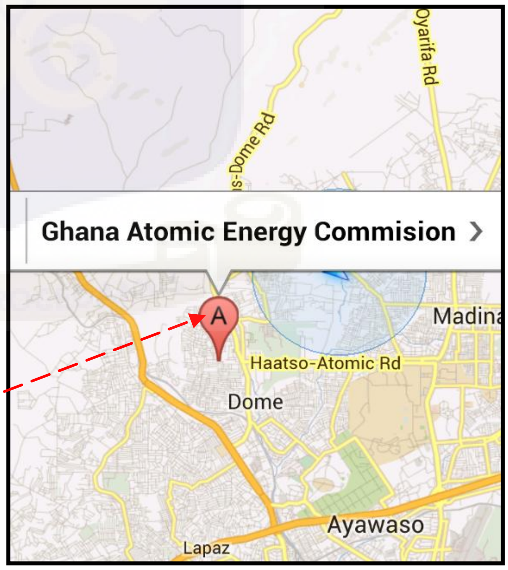

Fig. 2. Map showing location of GAEC in Accra, Ghana.

3.2 Mosquito colony and rearing conditions

The Redco strain of *An. gambiae* established from larval collections around REDCO Flats in Madina near Accra, Ghana were used throughout this study. Adults were maintained under standard rearing conditions of 27 ± 1 °C temperature, and 65 – 80 % relative humidity in a 12 hr day and 12 hr night photoperiod in the insectary. With characteristics similar to the species used in the work of Clements (1955), all adult mosquitoes were sugar feeders and the females which were largely anautogenous (i.e. requiring a blood meal for ovary development), stenogamous (i.e. able to copulate in a small space), human-biting and non-diapausing could easily be cultured in the laboratory.

Eggs obtained from the 66th generation of the laboratory stock colony were used to raise the initial colony for this study. The eggs were set-up in plastic bowls filled with 1.5 L of distilled water to hatch (Plate 1) and the first instar larvae from all egg bowls were mixed and transferred into larval trays at a rearing density of 1000 larvae per tray (40 x 30 x 7 cm^3) containing 1000 ml of water (Plate 2). Larvae in each larval tray were given 0.25 g Baker's yeast on the 1st and 3rd days and 0.25 g Dog biscuit from day five (5) until pupation. Trays were covered with fine polyester mesh after feeding to prevent larvae that pupate and subsequently emerge into adults in the tray prior to collection from escaping into the insectary (Plate 3). Pupae were collected into plastic cups partly filled with water (Plate 4) and placed in 6.6 litre plastic bucket (Instawares, Kennesaw, GA) cages to emerge into adults. Un-emerged pupae were removed from the cage after 24 hours in order to generate same age individuals for the experiments. Mosquitoes were maintained under standard rearing conditions throughout the experiment.

Plate 1: *An. gambiae* eggs set up in plastic bowl

Plate 2: Larval rearing in trays filled with 1000 larvae in 1000 ml water

Plate 3: Mesh-covered trays

Plate 4: *An. gambiae* pupae in a plastic cup

3.3 Sources and handling of adult mosquito sugar diets

Artificially synthesised glucose, sucrose (Table sugar) and honey obtained from the food section of the Accra Shopping Mall, Ghana, made up the adult sugar diet. Each sugar type was prepared to 6 % and 10 % concentrations (current standard concentrations in use in mosquito insectaries) by dispensing 6 and 10 grams respectively of the sugar types into glass reagent bottles (Plate 5). One hundred millilitres of water was added in all cases to obtain the required concentrations.

Plate 5: 6 % and 10 % conc. solutions of sugar types in glass and plastic bottles

3.4 Sources and handling of adult female mosquito blood diets

The blood diets for the blood feeding experiments comprised of fresh human blood fed from human forearm (human arm feeding method) or fresh Guinea pig blood fed from either an anaesthetised guinea pig (anaesthetised Guinea pig feeding method) or restrained live guinea pig (restrained Guinea pig feeding method). Same hosts and time of day were used throughout the study to eliminate errors that might come from variations in blood composition when different hosts and time of day are used. Guinea pigs of same age (2 years), body weight (250 g) and sex (males) were also used in the experiments to ensure uniformity and minimise experimental errors. Animals were clinically examined at the Ghana Atomic Energy Commission Veterinary Clinic to certify their health status prior to their use in the experiments.

For the human arm feeding, each generation of female mosquitoes were blood fed only once in all cases to protect the human host from contracting *Plasmodium* parasites during feeding. In addition, Cages used to blood feed females were thoroughly washed with detergents (e.g. bleach) and sun dried to eliminate any possible chance of the human host contracting other infections.

3.5 Evaluation of the effect of different sugar types on adult mosquito survival

One hundred (100) adult mosquitoes of 50 males and 50 females each were aspirated with hand-held aspirator into 3.3 litre plastic bucket cages with fine mesh screen tops. Sugar feeding was done *ad libitum* in all cases with adults per cage fed using either 6 % or 10 % concentration of one particular sugar type in cotton balls (replaced daily) placed in sugar tubes that were placed inside the cages. In no particular order, each adult cage was fed with 6 % concentration of a particular sugar type in solution. That is, 6 % glucose solution, sucrose solution or honey dilution. Adults in the last cage were given only water to serve as control (Plate 6). The same protocol was followed to feed adults with same sugar types at 10 % concentration. Each experiment was replicated three (3) times using different generations of adults.

Data collection was done similar to the procedure described by (Manda *et al.*, 2007). To determine the effect of the different sugar types and different concentrations fed on adult survival (longevity), dead adult mosquitoes per cage per day were removed into holding cups and the number of adults that survive per cage per day (based on sugar types, concentrations and sex) were counted and recorded. This was continued daily until the last death was recorded in all cases. The sugar type and percentage concentration that optimised adult survival were determined based on the type and concentration that recorded the highest number of days that adults survived.

Plate 6: Adult male and female mosquitoes in holding cages for data collection

3.6 Evaluation of the effect of different sugar types on adult male mosquito mating potential

Hundred adults consisting of 50 male and 50 female mosquitoes each were collected with hand-held aspirator into 3.3 plastic bucket cages with mesh screen tops. Adults per cage were sugar fed *ad libitum* on 10 % concentrations of honey, glucose or sucrose solution in cotton balls and kept for a period of one week in all cases to ensure mating had taken place. Using a procedure similar to the protocol described by Vrzal *et al.* (2010) Thirty (30) females per sugar type were collected into adult holding cups and immobilised in a chiller at 4 ^{o}C for 2 minutes and dissected under dissecting microscope (Radical®, Germany) to remove the spermathecae. The spermatheca from each female was observed under stereoscope (Novel, Holland) to determine the presence or absence of spermatozoa (Plate 7). Females with spermatozoa present in spermatheca were considered inseminated females and those with transparent spermatheca were termed as uninseminated females. The females inseminated per sugar type were counted and recorded. Each experiment was replicated three (3) times.

The sugar type that optimised male mating potential was determined based on the type that gave the highest number of females with sperms present in spermatheca after the one week mating period.

Plate 7: Dissection of females for spermathecae and examination of spermathecae for presence of spermatozoa

3.7 Response of female Anopheles gambiae to different blood feeding methods

One thousand (1000) adult mosquitoes of 500 males and 500 females were collected with hand-held aspirator into a 6.6 litre plastic bucket cage with a mesh screen top. The mosquitoes were fed *ad libitum* on glucose solution prepared at 10 % concentration, soaked in cotton ball and placed in sugar tubes that were placed inside the cage for a one week mating period. Fifty (50) females each from this stock were collected into 3.3 litre plastic bucket cages and allowed to blood feed for 15, 20 and 25 minutes using Human arm feeding (HAF) method (human arm was placed on top of the fine mesh covering of the cage for mosquitoes to feed) or anaesthetised Guinea pig feeding (AGF) method (Guinea pig anaesthetised with Ketamine/Xylazine and placed on top of the fine mesh covering of the cage for the mosquitoes to feed) or by restrained Guinea pig feeding (RGF) method (restrained Guinea pig placed on top of the mesh covering of the cage for mosquitoes to feed). The RGF method served as control and was used as such to determine whether or not the use of anaesthetics in live animal feeding of mosquitoes affect blood feeding response and egg production capacity in *An. gambiae* (Plates 8, 9 and 10). Each experiment was replicated three (3) times.

Following the protocol described by Solarte *et al.* (2007), females with blood present in their abdomen for each of the different blood feeding methods (HAF, AGF and RGF) after the 15, 20 and 25 feeding times were counted as females that blood fed. The number of females that blood fed per feeding method and feeding time were numerically counted and recorded. The feeding method and feeding time that recorded the highest number of female blood feeding was considered as the feeding method and time regimen that optimised blood feeding response in *An. gambiae*.

Plate 8: Human arm feeding (HAF) of adult female *An. gambiae* mosquitoes

Plate 9: Restrained Guinea pig feeding (RGF) of female *An.gambiae* mosquitoes

Plate 10: Anaesthetised Guinea pig feeding (AGF) of female *An. gambiae* mosquitoes

3.8 Evaluation of the effect of different blood feeding methods on egg production of female mosquitoes

For the egg production experiment, twenty females each were randomly collected from females that blood fed per human arm, restrained Guinea pig and Ketamine/Xylazine anaesthetised Guinea pig feeding methods used in the blood feeding response experiments. Each female was Iso-set up in separate oviposition cups to lay eggs for a period of fourteen (14) days. Females in each cup were provided daily with 10 % concentration of glucose solution in cotton pads placed on the mesh screen top of the cups in order to ensure that females survive during the oviposition period (Plates 11, 12 and 13). The Iso-female cups were observed daily for presence of eggs and eggs laid per cup per day were counted and recorded. Each experiment was replicated three (3) times. The feeding method which had females producing the highest number of eggs during the fourteen days period was scored as the method that optimised egg production.

Plate 11: Iso-set up for females that blood fed on human arm

Plate 12: Iso-set up for females that blood fed on restrained Guinea pig

Plate 13: Iso-set up for females blood fed on anaesthetised Guinea pig

3.9 Evaluation of the effect of different anaesthetic agents on blood feeding response of female mosquitoes

One thousand (1000) adult mosquitoes of 500 males and 500 females each were collected with hand-held aspirator into a 6.6 litre plastic bucket cage with a mesh screen top. 10 % Glucose solution soaked in cotton ball was fed *ad libitum* to the mosquitoes for a period of one week. Fifty females each (a week old) from this stock were collected into 3.3 litre plastic bucket cages and fed for 15, 20 and 25 minutes using Guinea pig anaesthetised with 0.05 ml Ketamine/Xylazine (KX) anaesthetic agents (Prepared in the ratio 2:1) and placed on top of the mesh screen cover of the cage in one case and Guinea pig anaesthetised with 2 ml Ketamine/Diazepam (KD) anaesthetic agents in another case (Plates 14 and 15). Variations in the volumes of the different anaesthetics administered was due to the fact that anaesthesia was induced in Ketamine/Xylazine anaesthetised Guinea pigs at relatively lower dose of 0.05 ml whilst in the case of Ketamine/Diazepam, anaesthesia was induced in Guinea pigs at relatively higher doses of 2 ml. This variation in the Guinea pig anaesthesia induction time was similarly reported by Ozkan *et al.* (2010) for same combinations of anaesthetics and attributed to differences in anaesthesia induction properties of the two different anaesthetics. A third cage of female mosquitoes was fed using restrained Guinea pig (RGF) as control. Fed females were observed for presence or absence of blood in their abdomen to determine the blood feeding method and feeding time that optimised blood feeding response. Females with blood present in the abdomen after feeding on either Ketamine/Xylazine, Ketamine/Diazepam or restrained Guinea pigs per 15, 20 and 25 minutes feeding times were considered as females that blood fed. The number of females that blood fed per feeding method and feeding time were counted and recorded. The feeding method and feeding time that recorded the highest number of females blood feeding was considered as the feeding method and time regimen that optimised blood feeding response in *An. gambiae*.

Plate 14: Ketamine/Xylaxine anaesthetised Guinea pig feeding of adult female mosquitoes.

Plate 15: Ketamine/Diazepam anaesthetised Guinea pig feeding of adult female mosquitoes

3.10 Evaluation of the effect of different anaesthetic agents on egg production in female mosquitoes

Twenty (20) females each from the females that blood fed in the Ketamine/Xylazine (KX) and Ketamine/Diazepam (KD) anaesthetised Guinea pig feeding experiments were randomly collected for the experiment. The females collected were Iso-set up in separate oviposition cups to lay eggs and each cup provided with 10 % concentration of glucose solution in cotton pad (replaced daily) placed on top of the mesh screen cover of the oviposition cups to ensure females' survive during the oviposition period (Plates 16 and 17). The Iso-female cups were observed daily for presence of eggs and the eggs laid per cup per day were counted and recorded. Each experiment was replicated three (3) times. The anaesthetic agent used to immobilise guinea pigs to feed the mosquitoes in which female mosquitoes produced the highest number of eggs during the fourteen days period was counted as the method that optimised egg production.

Plate 16: Iso-set up of females fed on Ketamine/Xylazine anaesthetised Guinea pig

Plate 17: Iso-set up of females fed on Ketamine/Diazepam anaesthetised Guinea pig

3.11 Experimental design and data analysis

The various factors studied in these experiments were arranged in a factorial fashion using the complete randomised block design as a base. Data collected for the various parameters determined in the experiment were analysed by following the two way ANOVA procedure using GENSTATS (12th edition). Data collected on adult survival, female blood feeding response and productivity (egg production) were arc sine transformed prior to analysis. Means for the various parameters studied were separated by the LSD method at 5 % probability level when F-test proves significant at 5 %.

Regression analysis was performed to establish a relationship between the % survival values obtained by male and female mosquitoes for the different sugar types fed at different concentration levels. This was done to give an account of variations within the two data sets.

CHAPTER FOUR

4.0 RESULTS AND OBSERVATIONS

4.1 Percentage survival of male *An. gambiae* on 6 % concentration of sugars

From the graph (Fig. 3) it was observed that the different sugars evaluated showed varying effects on percentage survival of adult male *An. gambiae* mosquito for the period monitored. For 60 % survival used as reference point for males, 60 % of the honey fed males survived up to 18 days to mate with females, whiles males fed on glucose recorded same percentage survival at day 11. 60 % of sucrose fed males survived to day 10 with males fed on water (control) recording same percentage survival as early as day 3. For the entire period monitored, honey fed adult males recorded the longest percentage survival period of 28 days followed by glucose and sucrose with 21 and 18 days respectively whiles the control recorded an overall survival period of 4 days. Generally, all sugars recorded a decreasing percentage survival of adult males as the number of days increases with the control showing a relatively sharper decline in percentage survival over the entire period monitored (Fig.1).

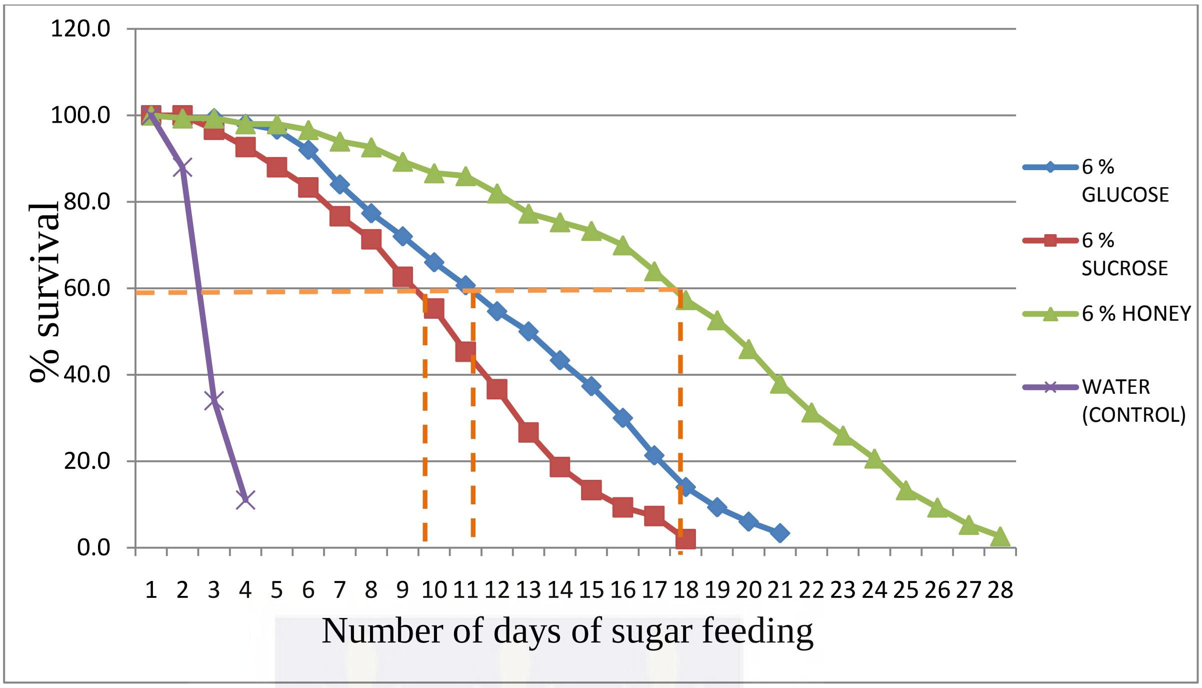

FIG. 3: Survival of adult male *An. gambiae* on 6 % glucose, sucrose and honey solutions

4.2 Percentage survival of male *An. gambiae* on 10 % concentration of sugars

10 % concentration of all three sugars and the control were also observed to produce different effects on the percentage survival of the adult male *An. gambiae*. 60 % of males fed on 10 % honey solution survived to day 18 to mate with females with an overall survival period of 30 days whiles glucose fed males recorded the same 60 % survival at day 14 and survived to 26 days throughout the entire period. 60 % of sucrose fed males survived to 12 day with an entire survival period of 21 days to mate and inseminate females. Adult males fed with water had 60 % of males survived to day 3 with zero % survival recorded as early as day 4 (Fig. 2). A general decreasing trend was observed in the percentage survival of adult males for all the sugar types as the number of days increases. A sharp decline in survival was observed for the control (Fig. 2).

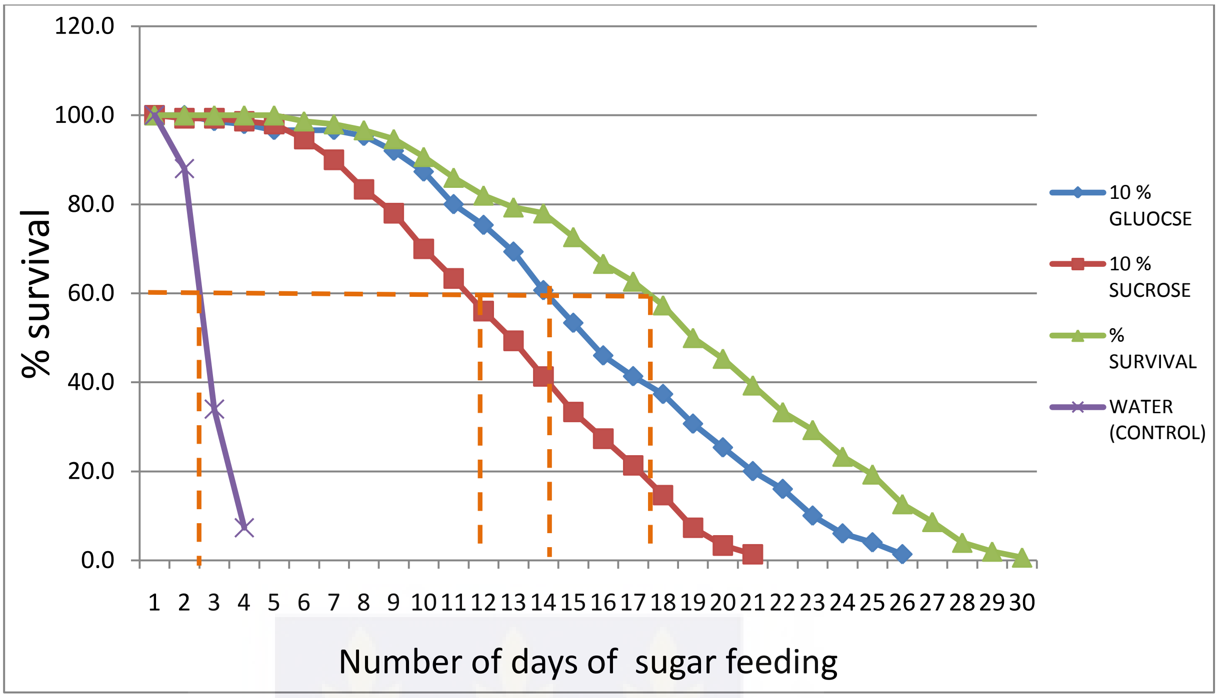

FIG.4: Survival of adult male *An. gambiae* on 10 % glucose, sucrose and honey solutions

4.3 Percentage survival of female *An. gambiae* on 6 % concentration of sugars

The percentage survival of female *An. gambiae* mosquitoes on 10 % concentration of the sugars was observed to be different for all sugar types and the control. For 60 % adults survival, 60 % of sucrose and glucose fed females survived to day 12 and 18 respectively to mate and lay eggs whiles honey recorded a relatively longer survival period of day 21with the control recording the same 60 % survival at day 2. Throughout the entire period monitored, honey fed females recorded the longest percentage survival period of 36 days with glucose and sucrose fed females recording lower values of 29 and 22 days respectively. Survival occurred up to only 4 days in the control. A decreasing trend in percentage survival of females for all sugars as the number of days increased together with a sharp decline in percentage survival of the control were observed over the period (Fig.3).

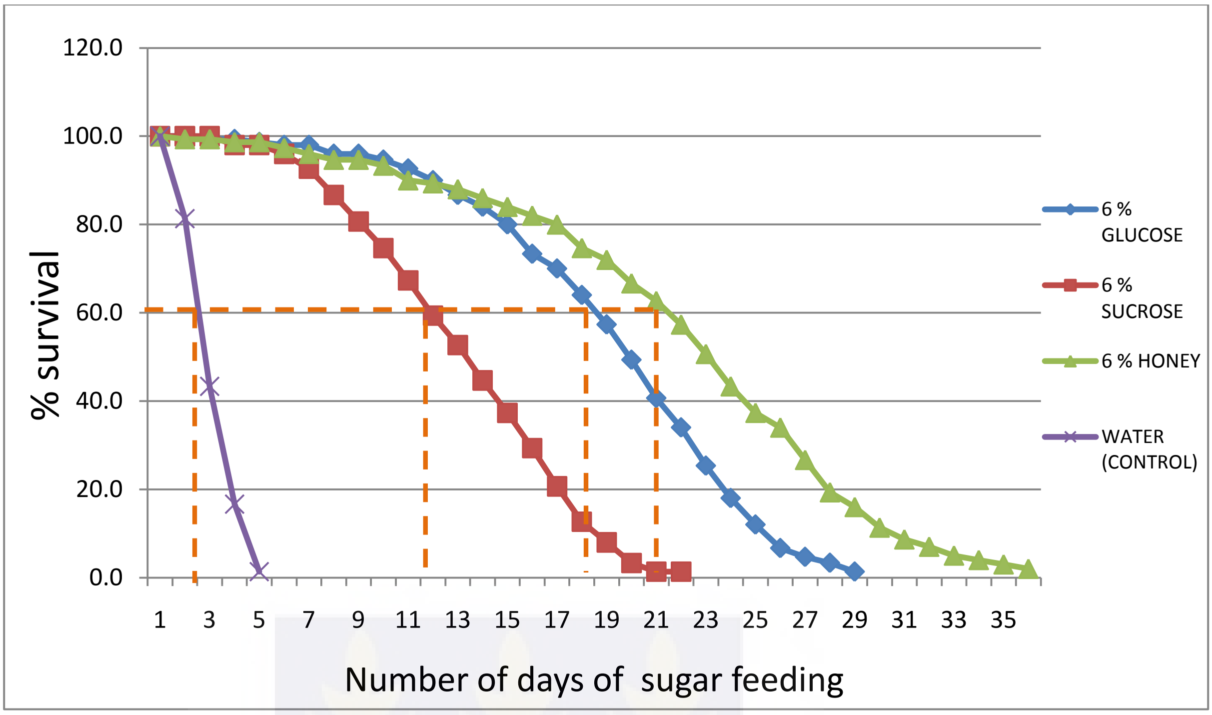

FIG. 5: Survival of adult female *An. gambiae* on 6 % glucose, sucrose and honey solutions

4.4 Percentage survival of female *An. gambiae* on 10 % concentration of sugars

The effects of the 10 % concentration of the sugars on the percentage survival of adult female mosquitoes were also varied. For the same 60 % survival mark, adult females fed on honey survived relatively longer to 24 days to mate with males and lay eggs. 60 % of glucose fed females only survived to 16 days whiles the same percentage of sucrose fed females survived to day 13 with the control recording same 60 % survival as early as day 3. For the entire duration monitored, honey fed females recorded the longest percentage survival period of 45 days whilst glucose and sucrose fed females recorded 34 and 23 days respectively. Survival occurred up to only 4 days in the control. A decreasing trend in percentage survival of females for all sugars as the number of days increased was observed. A sharp decline in percentage survival of the control also occurred over the period (Fig.4).

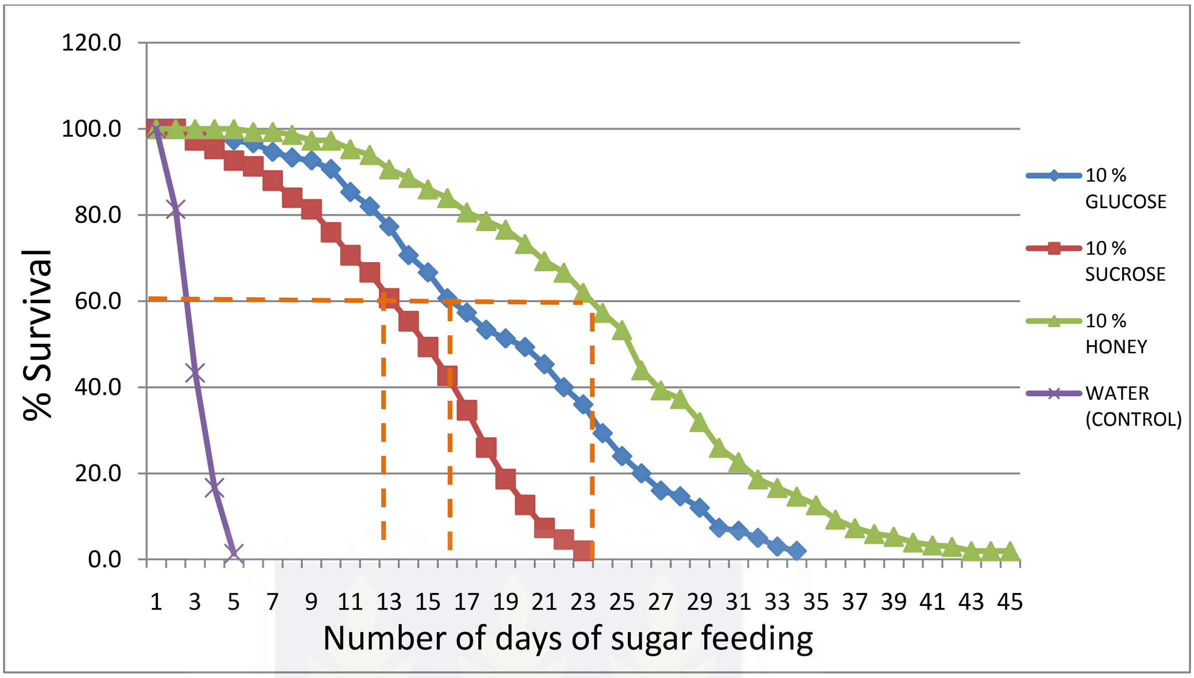

FIG. 6: Survival of adult female *An. gambiae* on 10 % glucose, sucrose and honey solutions

4.5 Relationship between percentage survival of male and female *An. gambiae* mosquitoes

The regression coefficients established between the percentage survival of male and female mosquitoes were found to be extremely significant ($p \leq 0.001$) with the exception of glucose fed at 6 % with p-value of 0.001 (Table 7).

The regression coefficients obtained from the correlation between percentage survival of male and female mosquitoes were in the range of 80 to 100 % (Table 7). Among the regression coefficients (R^2), the correlation between percentage survival of male and female for glucose fed at 6 % produced the least values compared to sucrose and, honey with R^2 values in the range of 90 to 100 %. Similar range of R^2 values were obtained by the control treatment (water).

Table 1: Relationship between concentration and feeding material on % survival of male and female *An. gambiae* mosquitoes

S/C	C	RM	RC	p-value (s)
Water	6 %	$^{a}FM = 0.74\ MM + 0.67$	0.9993	≤ 0.001
	10 %	$^{a}FM = 0.78\ MM + 0.63$	0.9993	≤ 0.001
Sucrose	6 %	$^{a}FM = 1.07\ MM + 2.86$	0.9894	≤ 0.001
	10 %	$^{a}FM = 0.97\ MM + 3.96$	0.9829	≤ 0.001
Honey	6 %	$^{a}FM = 0.98\ MM + 7.08$	0.9740	≤ 0.001
	10 %	$^{a}FM = 0.90\ MM + 13.82$	0.9384	≤ 0.001
Glucose	6 %	$^{a}FM = 1.08\ MM + 9.11$	0.8957	0.001
	10 %	$^{a}FM = 0.91\ MM + 9.28$	0.9472	≤ 0.001

Means of % survival should be compared in columns with respect to the days and concentration levels. Means followed by the same letters in columns were not significantly different. ********* *= extremely significant* ***ns****= not significant.* ***S/C*** *= sugar types/control* ***C*** *= Concentrations* ***RM*** *= Regression models* ***RC*** *= Regression coefficients* ^{a}***FM*** *= Female mosquitoes* ***MM*** *= Male mosquitoes.*

4.6 Potential of male mosquitoes to inseminate females

The spermathecae of inseminated female mosquitoes were found to contain long thread-like spermatozoa which appear as fine concentric threads (Figure 3). Uninseminated females were observed to have a fairly transparent spermathecae (Figure 4)

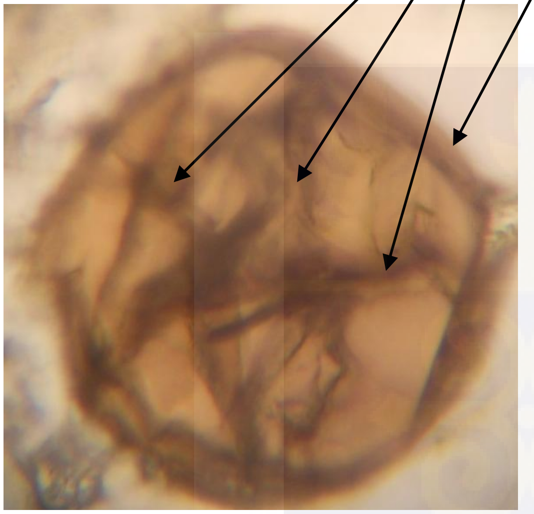

Fig. 7: Inseminated spermatheca with bundles of sperm

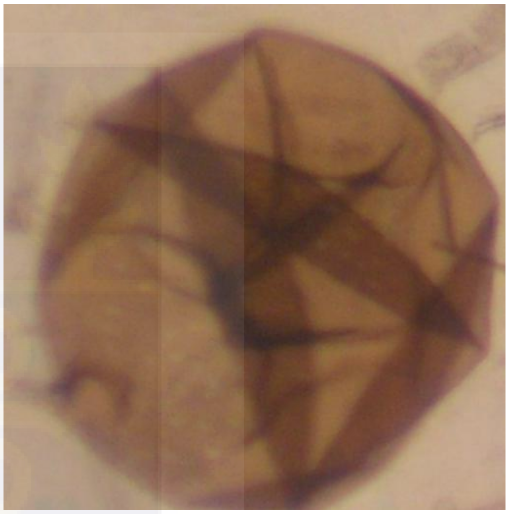

Fig. 8: Spermatheca of uninseminated female

No statistical significance was observed among the three sugar types regarding the potential of male mosquitoes to inseminate females, however, adult males fed with honey showed higher potential to inseminate females (Figure 5).

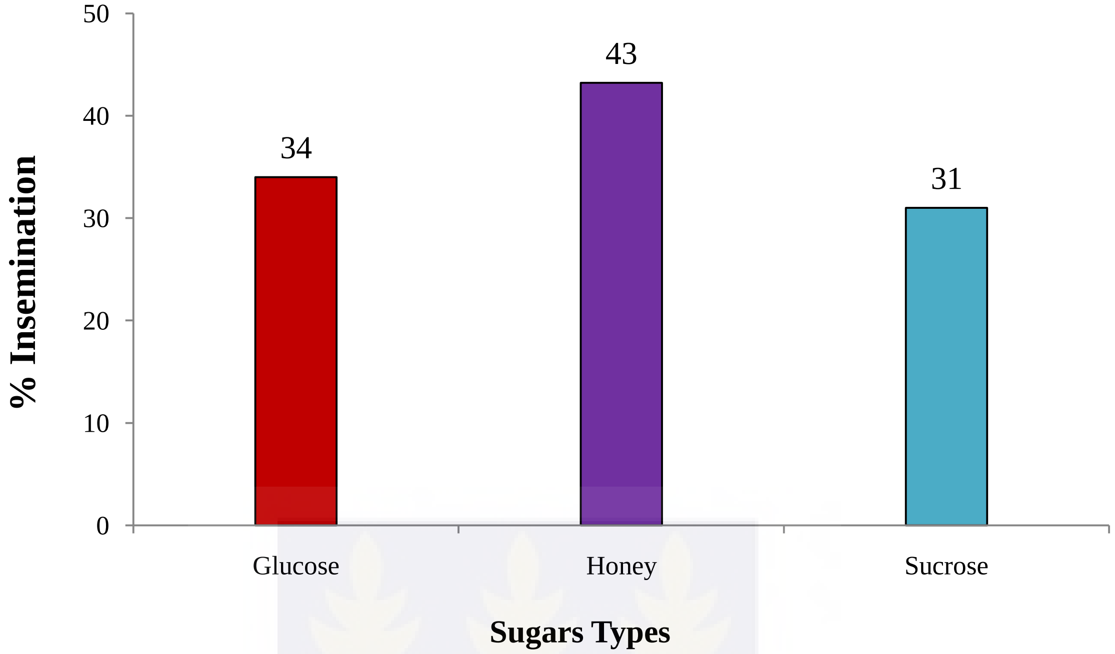

Figure 9: Effect of 10 % concentration of sugar types on the potential of male mosquitoes to inseminate female mosquitoes

4.7 Percentage blood feeding of female *An. gambiae* fed using different blood feeding methods and time regimens

The effects of blood feeding methods on blood feeding response in female *An. gambiae* mosquitoes were observed to be significant (p = 0.1). The Human arm feeding (HAF) method recorded the highest percentage feeding response of 70.6 %, followed by 50.1 % for anaesthetised guinea pig feeding (AGF) and 33.1 % for restrained guinea pig feeding (RGF) method. The control (RGF) recorded the least percentage feeding response of 33.1 % (Fig. 1). For the effect of feeding time on percentage blood feeding response in females , 57.6 %, 48.3 % and 49.0 % blood feeding response were recorded at 15, 20 and 25 minutes feeding times respectively. The highest percentage blood feeding of 57.6 %

was recorded when adults were fed for 15 minutes whiles the least percentage of 48.3 % was obtained at 20 minutes feeding time(Fig. 2).

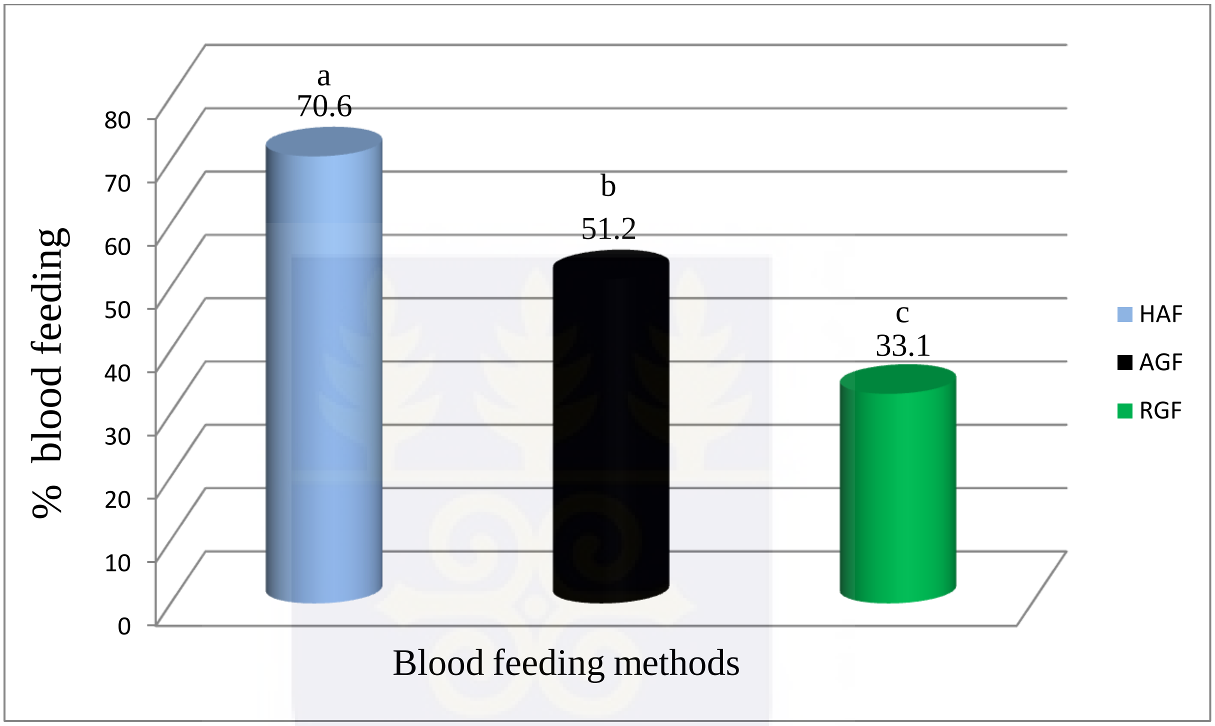

Fig 10: Effect of blood feeding methods on percentage blood feeding response of *An. gambiae*

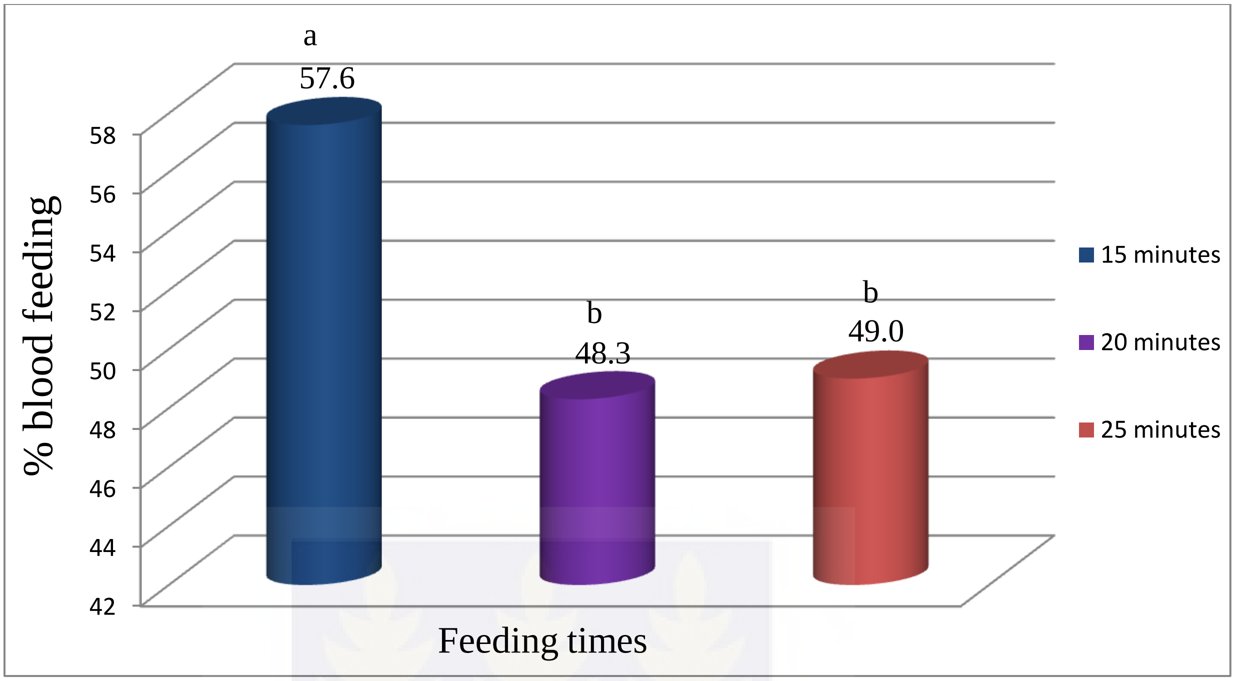

Fig 11: Effect of feeding times on percentage blood feeding response of *An. gambiae*

4.8 Percentage egg production in female *An. gambiae* fed using different blood feeding methods

Significant differences ($p \leq 0.001$) were observed for the effect of blood feeding methods on % egg production of female *An. gambiae* over the period monitored. HAF recorded percentage egg production of 28.3 % whiles AGF and RGF (control) recorded 23.8 % and 14.6 % respectively. The highest percentage egg production of 28.3 % was obtained using the HAF method whilst the least percentage egg production of 14.6 % was recorded for the control (RGF) (Fig. 3).

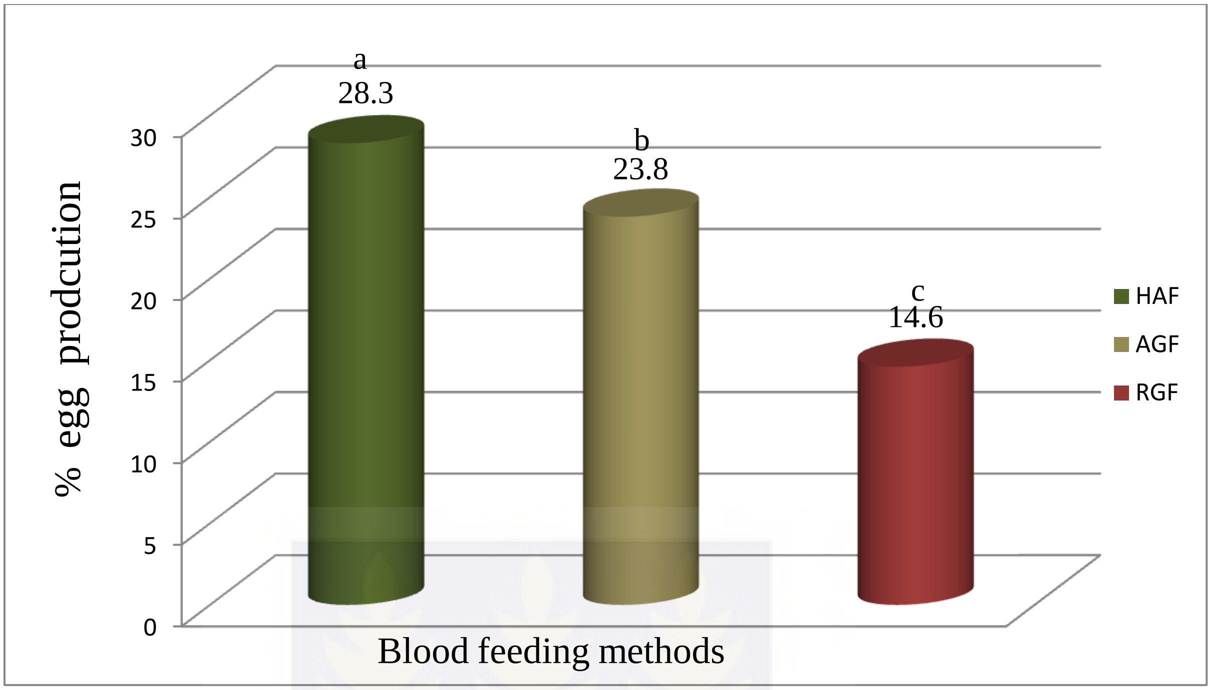

Fig. 12: effect of blood feeding methods on percentage egg production of female *An. gambiae*

4.9 Percentage blood feeding response of female *An. gambiae* fed using Guinea pigs anaesthetised with different anaesthetics and time regimens

The effects of anaesthetics on the percentage blood feeding of female *An. gambiae* mosquitoes were observed to be significant ($p \leq 0.001$). Ketamine/Xylazine (KX) anaesthetics recorded the highest percentage feeding of 66.3 % followed by 50.1 % for Ketamine/Diazepam (KD) anaesthetics and 31.6 % for the control (RGF) (Fig: 4). For the effect of feeding time on percentage blood feeding response of females, 15 minutes feeding time recorded 55.6 % whiles 20 and 25 minutes obtaining 41.5 % and 44.2 % respectively. The highest percentage blood feeding of 55.6 % was therefore obtained at 15 minutes whiles the least percentage of 41.5 % was recorded when 20 minutes feeding time was allowed (Fig. 5).

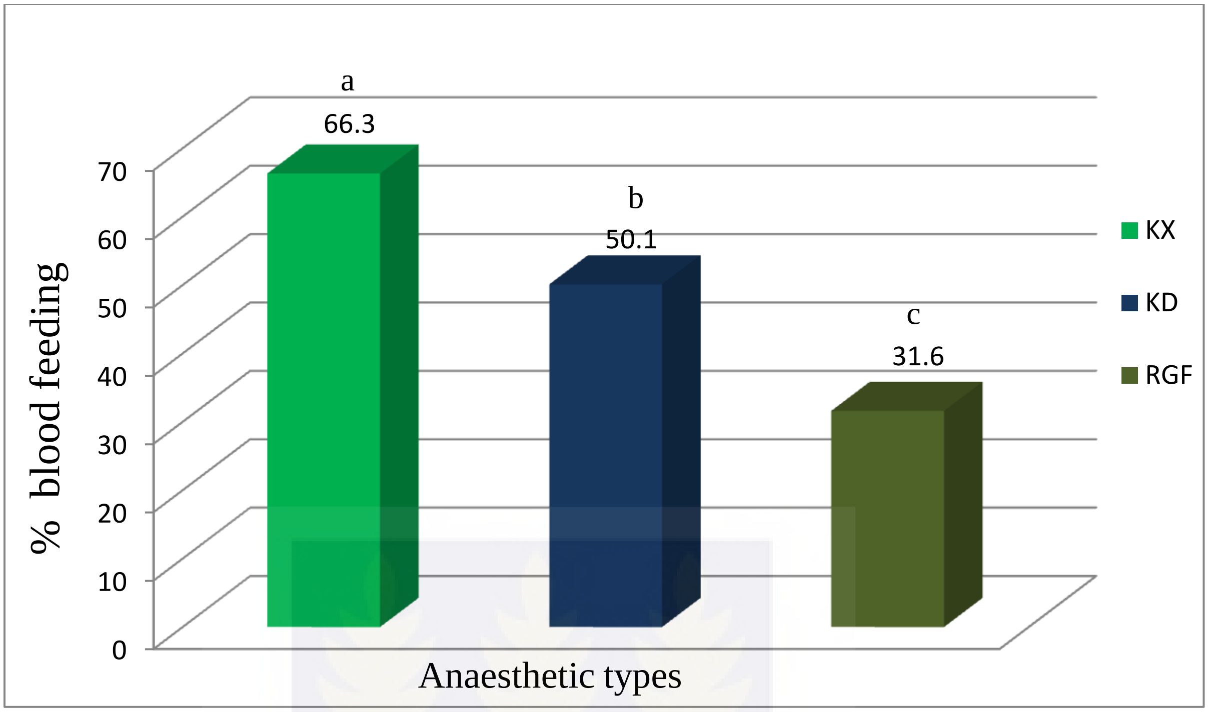

Fig. 13: effect of anaesthetics on percentage blood feeding of *An. gambiae*

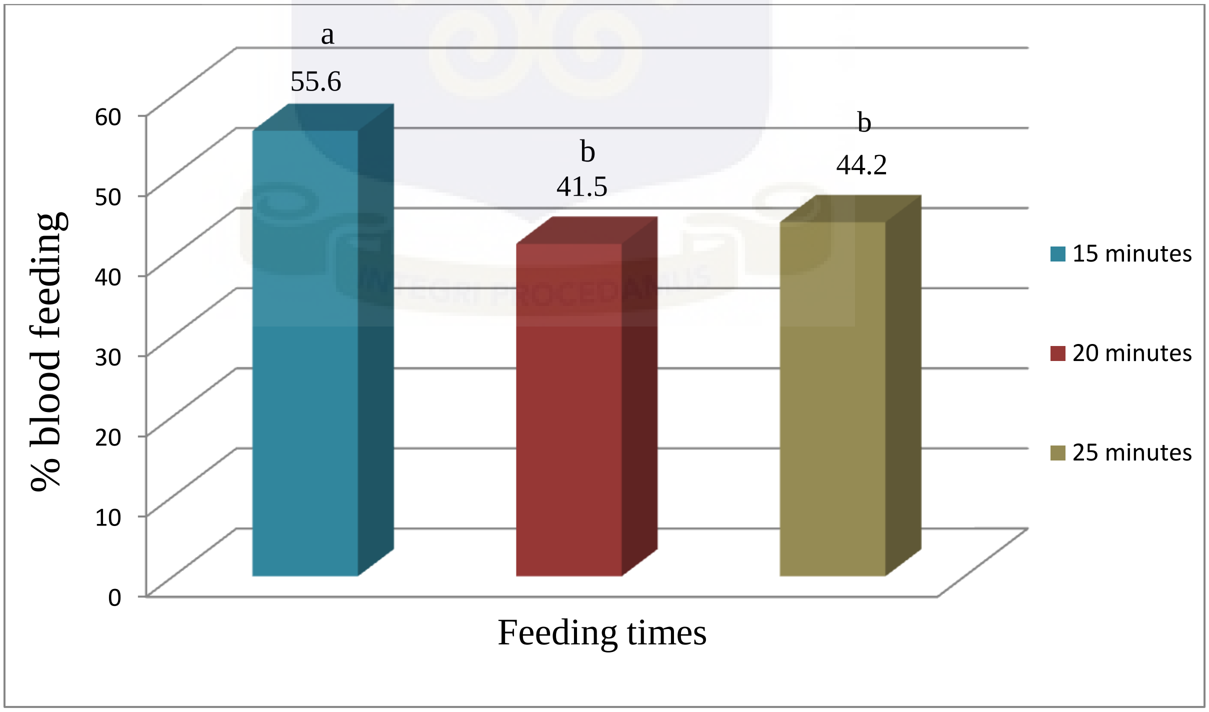

Fig. 14: effect of feeding times on percentage blood feeding response of *An. gambiae*

4.10 Percentage egg production in female *An. gambiae* fed using Guinea pigs anaesthetised with different anaesthetics

The effects of the different anaesthetic types on percentage egg production were observed to be significantly different ($p \leq 0.001$). Percentage egg production of 21.2 %, 29.4 % and 16.9 % were recorded for Ketamine/Diazepam, Ketamine/Xylazine and Restrained guinea pig (RGF) (control) respectively (Fig. 6). The highest percentage egg production of 29.4 % obtained over the period was recorded when females were fed Guinea pigs anaesthetised with the KX anaesthetics whiles the least percentage egg production of 16.9 % was obtained with the use of the RGF (control).

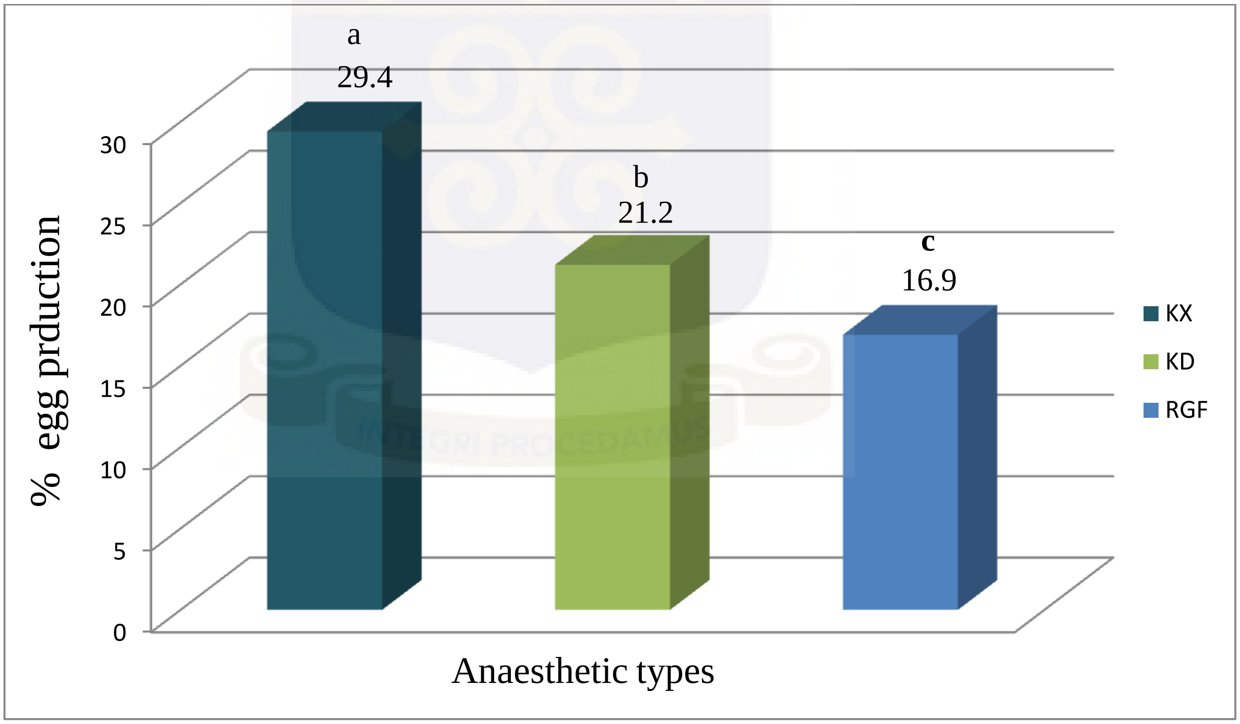

Fig. 15: effect of anaesthetics on percentage egg production of *An. gambiae*

CHAPTER FIVE

5.0 DISCUSSION

The type of sugar as well as amount obtained by adult Anophelines determines how long they survive (Coluzzi, 1964). Findings from this study similarly revealed that under optimum laboratory conditions, the survival of the adult *An. gambiae* is dependent on sugar type as well as the concentration used to feed adult Anopheles mosquitoes. For the different sugars used in the study, honey-fed males and females survived longer than those fed on glucose and sucrose solutions.

These findings agree with the work of Coluzzi, (1964) who reported that honey has superior natural dietary substances such as amino acids, vitamins and trace mineral elements absent in glucose, sucrose and water (control) and therefore might be responsible for the increased survival and productivity of honey fed adults in the laboratory as compared to glucose and sucrose. Bogdanov *et al.* (2008) and Molan (1997) further reported that honey apart from possessing antimicrobial, antifungal and antiviral properties that prevent its rapid deterioration in quality, it also contains relatively higher caloric content (about twice the amount found in sucrose and glucose) and other compounds including antioxidants, enzymes and metabolites that enhance their digestion and utilisation.

Even though Coluzzi (1964) stated that 10 % sucrose solution can be successfully used as a suitable substitute for honey to sustain survival of adult mosquitoes in the laboratory, findings from this work have shown that sucrose at both 6 and 10 % concentrations proved least suitable in sustaining the survival of both sexes of the species for a longer period as compared to honey and glucose. According to Ashley (2011) this might be due to the fact that although artificially synthesised sucrose like glucose contains high calorific content vital in the survival of adult mosquitoes, it lacks all other essential compounds such as proteins, vitamins and minerals equally needed to enhance survival.

For the two concentrations (6 % and 10 %) evaluated, the 10 % concentration of the sugars in solution sustained the survival of both male and female *An. gambiae* for relatively longer period as compared to the 6% concentration. This outcome might be largely attributed to differences in the quantity of sugar each concentration contains as different grams of the sugars were used to obtain the two different concentrations. Rudloff (2013) and Robergs and Kravitz (2000) also reported that the quantity of sugar present in solution determines its calorific content hence its energy yield. It can therefore be deduced from this premise that the 10 % sugar solutions tend to contain higher sugar content (grams), calorific content and energy yield that sustained adults for relatively longer period as compared to the 6 % concentration.

Variations seen in the longevity of both sexes of mosquitoes fed with the same 10 % honey solution can be attributed to sex differences among same species of organisms rather than the concentrations as male mosquitoes by nature survive for relatively fewer days as compared to females (Styer *et al.*, 2007; Carey *et al.*, 1995).

Similar adult survival rate seen in the first 3 to 5 days for all sugars and concentrations used, and the steady decline in survival as the monitoring period increases was similarly reported in the work of Manda *et al.* (2007). It is reported that nutrient reserves carried from the larval stage to the adult stage might have provided sufficient nourishment for adults in the first few days, thus, the distinct effects of the sugars and concentrations used might have been masked during that period (Telang *et al.*, 2005). However, the effects of the sugars and concentrations on the adult survival of adult *Anopheles* mosquitoes became apparent as the larval reserves depleted with time.

Although, results obtained on the potential of male *An. gambiae* to inseminate females showed no significant differences for the sugars, honey fed males showed greater potential to inseminate females compared to glucose and sucrose fed males. According to Rehan *et al.* (1975) this might be due to the fact that presence of proteins and other compounds in honey might have produced some positive effects on sperm production in honey fed males hence the relatively greater female insemination recorded for females that were mated with honey-fed males.

For the blood feeding methods evaluated in this work, the study revealed that the effect of the different blood feeding methods and feeding times on female *An. gambiae* blood feeding response and egg production varied significantly ($p \leq 0.001$). As evident in the result obtained in this study, female anophelines showed relatively high inclination to blood feed when feeding was done using the human arm (HAF) method as compared to the anaesthetised Guinea pig feeding (AGF). Feeding was however higher in females fed with the AGF method when the AGF was used against the RGF (restrained Guinea pig feeding) method. These findings were similarly reported by Coluzzi (1964) who stated that adult *An. gambiae* mosquitoes fed with the HAF method recorded higher percentage blood feeding as compared to those fed using other live hosts. This might be due partly to the fact that odorant proteins (proteins that aid host search) found in female *An. gambiae* mosquitoes tend to show stronger affinity for certain volatiles and microflora present on the human skin, making humans the natural host for the vector and partly due to its greater preference for large size hosts (Takken and Verhulst, 2012; Kweka *et al.*, 2010; Cork and Park, 1996; Coluzzi, 1964)..

Again, Murrieta *et al.* (2010) reported that anaesthetics tend to alter peripheral blood circulation, decrease body surface temperatures and cause physiological changes that could adversely alter experimental results. These possible changes in the body temperature and general physiology of anaesthetised hosts might have also accounted for the relatively low percentage blood feeding and egg production recorded by AGF as against the HAF method.

The relatively low percentage blood feeding and egg production obtained in the control (RGF) might be attributed to feeding disruptions that might have occurred from body movements (wriggling) of live hosts during feeding. Thus, females were unable to ingest sufficient blood to mature enough eggs as compared to HAF and AGF.

For the effect of feeding times on percentage blood feeding, feeding response was optimum when females were exposed to their host for15 minutes as compared to 20 and 25 minutes feeding times. This might be due to fact that as feeding time increases, the anaesthetic agents severely affected the hosts' physiology and body temperature, affecting feeding response negatively in the case of the AGF method. For the HAF method, this might be attributed to the fact that feeding response was not significantly different among all three feeding times used and therefore there is no need to exceed the 15 minutes feeding time. This was similarly reported in the work of Turley *et al.* (2009) and might confirmed why the 15 minutes feeding time is used as the standard feeding time by most mosquito insectaries to feed adult female mosquitoes.

For percentage egg production in females, the human arm feeding method (HAF) again recorded higher egg production of 28.3 % as against 23.8 % and 14.6 % scored for AGF and RGF respectively emphasising a positive relationship between feeding method and egg production.

This means that the method used to blood feed female *An. gambiae* mosquitoes can significantly affect the number of females that will blood feed, the amounts of blood ingested, the number of females that lay eggs and the number of eggs eventually laid by females (Taylor and Hurd, 2001; Billingsley and Hecker, 1991; Lounibos and Conn, 1991; Foster and Eischen, 1987; Roitberg and Prasad, 1987).

Although findings from this work and the work of other researchers including Coluzzi (1964) established that the HAF method which uses human beings as live hosts is the ideal method to blood feed female mosquitoes in the insectary, this cannot be the case for a mass production system where mass feeding is required and therefore becomes practically impossible to use human as host where large scale rearing is required. On this premise, AGF with an added advantage of being used when same generation of *An. gambiae* are to be fed more than once (without any risk of malaria infections as possible with human hosts) becomes a suitable method to blood feed adult *Anopheles gambiae* mosquitoes as similarly reported by Coluzzi (1964).

For the experiments carried out on the effect of anaesthetic agents on blood feeding response and productivity of *An. gambiae* fed with live animals immobilised with anaesthetics, significant differences were observed due to the effect of the anaesthetics. A positive correlation was again established between the type of anaesthetic used to immobilise the live animals used to feed the mosquitoes and the amount of blood they ingest as well as the number of eggs produced. This is because the relatively high percentage blood feeding response recorded for females that were blood fed with Guinea pigs anaesthetised with Ketamine/Xylazine (KX) anaesthetics as against the Ketamine/Diazepam (KD) anaesthetics was again recorded in the egg production of the females fed with KX anaesthetised Guinea pigs as compared to KD and the control. These results therefore established that aside the fact that anaesthetic agents produce effects on blood feeding and egg production in female *An. gambiae* mosquitoes, these effects are varied depending on the anaesthetic used. The relatively lower feeding response and egg production recorded for KD anaesthetics might be attributed to a greater effect of the KD anaesthetics on the physiology of the host as compared to the KX anaesthetics which might have equally affected blood feeding and egg production in the vector as reported by Murrieta *et al*. (2010).

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

The number of *An. gambiae* mosquitoes that survive and how long they survive in a mass production system is primarily dependent on sugar type and concentration. Although 10 % glucose and sucrose solutions performed relatively well, their suitability as sugar sources to feed adult mosquitoes in the laboratory as compared to honey may be greatly influenced by other factors such as availability and cost other than merely nutritional composition which is very critical and indispensable to the utmost survival of the *An. gambiae* mosquito in the insectary. Honey solution which contains several nutritional elements and properties absent in glucose, sucrose and water (control) increased the survival and longevity of both sexes of *An. gambiae* mosquitoes in the laboratory against all the sugar types as well as the control (water) evaluated in this study.

Furthermore, the potential of male *An. gambiae* mosquitoes to inseminate females was relatively greater when male mosquitoes fed on 10 % honey solution as compared to 10 % glucose and sucrose solutions.

It was concluded that 10 % honey solution resulted in optimum feeding, survival and productivity (male potential to inseminate females) of adult *An. gambiae* mosquitoes in the insectary.

For blood feeding, it was established that the amount of blood meal female *An. gambiae* mosquitoes obtain to mature eggs and the number of eggs they produce in a mass production system is also strongly dependent on the type of feeding method, time regimen and anaesthetic agents used to immobilise live animals. For the three methods evaluated in this study, the HAF method is well preferred by female *An. gambiae* mosquitoes as evident in the relatively higher percentage blood feeding response and egg production recorded for females that were blood fed using the HAF method as compared to the AGF and RGF methods. The RGF which recorded the least number of females that blood fed also recorded the least number of females that laid eggs and the number of eggs laid for all three methods. Although the HAF method was determined as the ideal method to blood feed adult female *An. gambiae* mosquitoes in the insectary, its suitability in a production system is restricted to small scale production units because of the dependence of the method on human beings as live hosts to feed adult female mosquitoes. On the other hand, The AGF method which uses anaesthetised Guinea pigs instead of humans as live host in feeding female mosquitoes has the capacity to support large scale and sustainable feeding of female *An. gambiae* mosquitoes in mass production systems. This method also obtained relatively higher percentage blood feeding and egg production in female *An. gambiae* mosquitoes as compared to the RGF. We therefore conclude that the

AGF method is a more suitable blood feeding method for adult female *An. gambiae* mosquitoes in a mass production system as compared to the HAF and the RGF.

For the two different combinations of anaesthetic agents and blood feeding regimen evaluated in this study, KX anaesthetics administered to immobilise live animals (Guinea pigs) to feed female *An. gambiae* mosquitoes at 15 minutes feeding time recorded the highest number of females that blood fed and produced eggs as against the other treatments and feeding time regimens used. We also conclude that KX anaesthetics and 15 minutes feeding time resulted in optimum blood feeding and productivity of adult female *An. gambiae* mosquitoes in a mass production system.

6.2 Recommendations

We recommend that 10 % honey solution provided ad-libitum,, anaesthetised Guinea pig feeding (AGF) method using Ketamine/Xylazine (KX) anaesthetics and 15 minutes feeding time be used in mass rearing of this species.

Further studies should be carried out to evaluate the potential of other sugar sources (propolis and juice from riped fruits such as oranges) to optimise feeding, survival, and the potential of male *An. gambiae* to inseminate females.

Further work should be done to evaluate the effects of other blood feeding methods and anaesthetic agents used in live animal feeding on *An. gambiae* productivity in a mass production system.

The outcome of this study and other future works could be useful in the mass production of the *An. gambiae* and consequently facilitate the speedy implementation of the sterile insect technique programme for area-wide control of the mosquito vectors in the near future.

REFERENCES

Alavi, Y., Arai, M., Mendoza, J., Tufet-Bayona, M. and Sinha, R. (2003). The dynamics of interactions between *Plasmodium* and the mosquito: A study of the infectivity of *Plasmodium berghei* and *Plasmodium gallinaceum* and their transmission by *Anopheles stephensi*, *Anopheles gambiae* and *Aedes aegypti*. *Int. J. Parasitol*, **33**: 933 - 943.

Alphey. L., Benedict, M., Bellini, R., Clark, G. G., Dame, D. A., Service, M. W. and Dobson S. L. (2010). Sterile insect methods for control of mosquito-borne diseases: an analysis. *Vector Borne Zoonotic Dis.*, 10(**3**): 295 - 311.

Annan, Z., Durand, P., Ayala, F. J., Arnathau, C., Awono-Ambene, P., Simard, F., Razakandrainibe, F. G., Koella, J. C., Fontenille, D. and Renaud F. **(**2007). Population genetic structure of *Plasmodium falciparum* in the two main African vectors, *Anopheles gambiae* and *Anopheles funestus*. *Proc. Natl. Acad. Sci. U. S. A.* **104***:* 7987 - 7992.

Appawu, M., Owusu-Agyei, S., Dadzie, S., Asoala, V. and Anto, F. (2004). Malaria transmission dynamics at a site in Northern Ghana proposed for testing malaria vaccines. *Trop. Med. Int. Health*, **9**: 164 - 170.

Araújo**,** M. S., Gil, L. H. S. and e-Silva, A. A. (2012). Larval food quantity affects development time, survival and adult biological traits that influence the vectorial capacity of *Anopheles darlingi* under laboratory conditions. *Malaria Journal,* **11**: 261.

Ashley J. (2011). What is the difference between sucrose, glucose & fructose http://www.livestrong.com/article/499857-what-is-the-difference-between-sucrose-glucose-fructose. Retrieved 06/03/2013.

Auclair, J. L. (1963). Aphid feeding and nutrition. *A. Rev. Ent*., **8**: 439 - 490.

Baker, H. G., and Baker, I. (1983). Floral nectar sugar constituents in relation to pollinator type. In: Jones, C. E. and Little, R. J. (Eds.), *Handbook of experimental pollination biology*. New York: Van Nostrand Reinhold. pp. 117 - 141.

Baker, J. R., Apperson, C. S. and Arends, J. J. (1986). Insect and related pests of man and animals: Some important, common, and potential pests in the southeastern United States. *North Carolina Agricultural Extension Service* Publication AG-369: (i) - iv, 1 - 92. (treats many North Carolina species) http://dx.doi.org/10.1046/j.1365-3156.2003.01162.x.

Bart, G. J. K. and De Jong, R. (1996). Limburger cheese as an attractant for the malaria mosquito *Anopheles gambiae s.s. Parasitol. Today*. **12**: 159 - 161.

Benedict, M. Q. (1997). Care and maintenance of anopheline mosquito colonies. In: Crampton, J. M., Beard C. B., and Louis, C. (eds) *The molecular biology of insect disease vectors*. Chapman & Hall, New York, pp 2 - 12.

Benedict, M. Q., Howell, P. and Wikins, L. (2007). Methods in malaria research. *Anopheles* adult diet. "Methods in *Anopheles* research manual, MR4 protocol." MR4 Protocol: 1 - 2.

http://www.mr4.org/Portals/3/Pdfs/Anopheles/2.4.7%20Anopheles%20Adult%20Diet%20v%201.pdf. Accessed 23 / 02 / 2013.

Billingsley, P. F. and Hecker, H. (1991). Blood digestion in the mosquito, *Anopheles stephensi* Liston (Diptera: Culicidae): Activity and distribution of trypsin, aminopeptidase, and alpha-glucosidase in the midgut. *J. Med. Entomol.* **28** (6): 865 - 871.

Bogdanov, S., Jurendic, T., Sieber, R. and Gallmann, P. (2008). Honey for nutrition and health**:** A Review. *J. Am. Coll. Nutr.*, 27 (**6**) 6677 - 6689.

Brelsfoard, C. L. and Dobson, S. L. (2009). *Wolbachia*-based strategies to control insect pests and disease vectors. *AsPac J. Mol. Biol. Biotechnol.*, 17 (**3**): 55 - 63.

Briegel, H., Hefti, M. and DiMarco, E. (2002). Lipid metabolism during sequential gonotrophic cycles in large and small female *Aedes aegypti*. *J. Insect Physiol.*, **48**: 547 - 554.

Briegel, H. (1990). Fecundity, metabolism and body size in *Anopheles* (Diptera: Culicidae), vectors of malaria. *J. Med. Entomol.*, **27**: 839 - 850.

Briegel, H. and Horler. E. (1993). Multiple blood meals as a reproductive strategy in *Anopheles* (Diptera: Culicidae). *J. Med. Entomol.*, **30**: 975 - 985.

Briegel, H. and Kaiser, C. (1973). Life-span of mosquitoes (Culicidae, Diptera) under laboratory conditions. *Gerontologia*, **19**: 240 - 249.

Burkot, T. R. (1988). Non-random host selection by *Anopheles* mosquitoes. *Parasitol Today*, **4**:158 - 162.

Carey, J. R., Liedo, P., Orozco, D., Tatar, M. and Vaupel, J. W. (1995). A male-female longevity paradox in medfly cohorts. *Journal of animal ecology*, **64**,107 - 116.

Centers for disease control and prevention (2010). *Anopheles* mosquitoes. http://www.cdc.gov/malaria/about/biology/mosquitoes/. Accessed 27/09/2012.

Chambers, R. G., Gupta, R. K. and Ghebreyesus, T. A. (2008). Responding to the challenge to end malaria deaths in Africa. *Lancet*, **371**: 1399 - 1401.

Chandler, J. A. and Highton, R. B. (1975). The succession of mosquito species (Diptera, Culicidae) in rice fields in the Kisumu area of Kenya and their possible control. *Bull Ent. Res*., **65**: 295 - 302.

Clements, A. N. (1955). The sources of energy for flight in mosquitoes. *J. Exp. Biol.,* ***32****:* 547 - 554.

Clements, A. N. (1992). The biology of mosquitoes - Development, nutrition and reproduction. *J. Exp. Biol., Vol.***1**. 509 pp.10.

Coluzzi, M. (1964). Maintenance of laboratory colonies of *Anopheles* mosquitoes. *Bull. Wild Hlth. Org*., 31, 441 - 443.

Cork, A. and Park, K. C. (1996). Identification of electro physiologically-active compounds for the malaria mosquito, *Anopheles gambiae*, in human sweat extracts. *Med. Vet. Entomol*., **10**: 269 - 276.

Costero, A., Edman, J. D., Clark, G. G. and Scott T. W. (1998). Life table study of *Aedes aegypti* (Diptera: Culicidae) in Puerto Rico fed only human blood versus blood plus sugar. *J. Med. Entomol*., **35**: 809 - 813.

Cox, F. E. G. (2010). History of the discovery of the malaria parasites and their vectors. *Parasites and vectors*. London, UK. pp. 144 - 170.

Craig, M. H., Snow, R. H. and Le-Sueur, D. (1999). A climate-based distribution model of malaria transmission in sub-Saharan Africa. *Parasitol. Today*, **15**: 105 - 111. http://www.ncbi.nlm.nih.gov/pubmed/10322323.

Cross, C. (2004). The life cycle of *Anopheles* mosquitoes. http://malaria.wellcome.ac.uk/doc_WTD023872.html. Accessed 27/09/2012.

Dame, D. A., Curtis, C. F., Benedict, M. Q., Robinson, A. S. and Bart, K. J. G. (2009). Historical applications of induced sterilisation in field populations of mosquitoes. *Malar J.,* **8**: S2.

Dash, A. P., Valecha, N., Anvikar, A. R. and Kumar, A. J. (2008). Malaria in India: Chalenges and opportunities. *Biosci*., 33(**4**) 583 - 592.

Deng, L., Koou, S. Y., Png, A. B., Ng, L. C. and Lam-Phua, S. G. (2011). A novel mosquito feeding system for routine blood-feeding of *Aedes aegypti* and *Aedes albopictus*. *Tropical Biomedicine*, 29(**1**): 169 - 174.

Dyck, V. A., Hendrichs, J, and Robinson, A. S. (editors) (2005). Sterile Insect Technique: Principles and Practice in Area-Wide Integrated Pest Management. Dordrecht: Springer. Netherlands. 787 pp.

Edi, C. V. A., Koudou, B. G., Jones, C. M., Weetman, D. and Ranson, H. (2012). Multiple-insecticide resistance in *Anopheles gambiae* mosquitoes, southern Côte d'Ivoire - Emerging Infectious Diseases. *Eid journal*, 18(**9**): 1508 - 1511.

Fabrizio, B. (2012). New rearing system may aid sterile insect technique against mosquitoes. http://www.sciencedaily.com/releases/2012/05/120507102328.htm. Retrieved 30/03/2013.

Feldmann, U. and Hendrichs, J. (2001). Integrating the Sterile Insect Technique as a key component of area-wide tsetse and trypanosomosis intervention. PAAT Technical and Scientific Series, No. 3. p. 12.

Flecknell, P. (2003). Laboratory animal anesthesia, 2nd Ed. *Elsevier*, 2003.

Fortin, A., Stevenson, M. M. and Gros, P. (2002). Susceptibility to malaria as a complex trait: big pressure from a tiny creature. *Human Molecular Genetics*, **11**: 2469 - 2478.

Foster, W. A. (1995). Mosquito sugar feeding and reproductive energetics. *Annu. Rev.Entomol*., **40**: 443 - 474.

Foster, W. A. and Eischen, F. A. (1987). Frequency of blood-feeding in relation to sugar availability in *Aedes aegypti* and *Anopheles quadrimaculatus* (Diptera: Culicidae). *Ann. Entomol. Soc. Am.*, **80**: 103 - 108.

Foster, W. A. and Takken, W. (2004). Nectar-related vs. human-related volatiles: Behavioural response and choice by female and male *Anopheles gambiae* (Diptera: Culicidae) between emergence and first feeding. *Bull. Entomol. Res.*, **94**: 145 - 157.

Gandomani, M. J., Ghashghaii, A., Tamadon, A., Attaran, H. R., Behzadi, M. A. and Javdani, Z. (2011). Comparison of anaesthetic effects of Ketamine-Xylazine and Ketamine-Diazepam combination in Budgerigar. 6 (**1**): 81.

Gary, R. (2005). Biology of the malaria vector *Anopheles gambiae*: Behavioral and reproductive components of sugar feeding. http://rave.ohiolink.edu/etdc/view. Retrieved 2 /03/2013.

Gary, R. E. Jr. and Foster, W. A. (2001). Effects of available sugar on the reproductive fitness and vectorial capacity of the malaria vector, *Anopheles gambiae* (Diptera:Culicidae). *J. Med. Entomol.*, **38**: 22 - 28.

Gerberg, E. J. (1970). Manual for mosquito rearing and experimental techniques. *AMCA Bulletin No. 5*, p. 12.

Gibson, G. (1996). Genetics, ecology and behavior of anophelines. In Bock, G. R. and Cardew, G. (Editors). *Olfaction in mosquito-host interactions*. Wiley, New York. pp. 22 - 47.

Gillett, J. D., Haddow, A. J., Corbet, P. S. (1962). The sugar-feeding cycle in a cage-population of mosquitoes. *Entomologia Exp. Appl.*, **5**: 223 - 232.

Gonza´ lez Gil, A., Illera, J. C. Silva´n, G. and Illera, M. (2003). Effects of the anaesthetic=tranquillizer treatments on selected plasma biochemical parameters in NZW rabbits. *Laboratory Animals*, **37**, 155 - 161.

Gouagna, L-C., Poueme, R. S., Dabire, K. R., Ouedrago, F. and Simard, F. (2010). Patterns of sugar feeding and host plant preferences in adult males of *An. gambiae* (Diptera: Culicidae). *Journal of Vector Ecology*, 35 (**2**): 267 - 276.

Green, J., Knight, J., Precious, S. and Simpkin, S. (1981). Ketamine alone and combined with diazepam or xylazine in laboratory animals: A10 year experience. *Lab Anim.* **15**: 163-170.

Griffin, J. T., Hollingsworth, T. D., Okell, L. C., Churcher, T. S., White, M., Hinsley, W., Bousema, T., Drakeley, C. J., Ferguson, N. M., Basáñez, M. and Ghani A. C. (2010). Reducing *Plasmodium falciparum* malaria transmission in Africa: A Model-based evaluation of intervention strategies *PLoS Med.*, 7(**8**): e1000324.

Gu mail, W., mailto:wgu@uab.edu Müller, G., Schlein, Y., Novak, R. J. and Beier, J. C. (2011). Natural plant sugar sources of *Anopheles* mosquitoes strongly impact malaria transmission potential. *PLoS One*, 6(**1):** e15996. www.plosone.org.

Guoli, Z., Pennington, J. E. and Wells, M. A. (2004). Utilization of pre-existing energy stores of female *Aedes aegypti* mosquitoes during the first gonotrophic cycle. *Insect Biochem. Mol. Biol.*, 34(**9**): 919 - 925.

Harrington, L. C., Edman, J. D. and Scott, T.W. (2001). Why do female *Aedes aegypti* (Diptera: Culicidae) feed preferentially and frequently on human blood? *J. Med. Entomol.*, **38**: 411 - 426.

Harzsch, S. and Hafner, G. (2006*).* "Evolution of eye development in arthropods: Phylogenetic aspects". *Arthropod Structure and Development*, 35 (**4**): 319 - 340.

Helinski, M. E. H., El-Sayed, B. and Bart. G. J. K. (2006a). The Sterile Technique: Can established technology beat malaria? *Entomol berichten*, 66(**1**): 13 - 20.

Helinski, M. E. H.**,** Parker, A. G. and Bart, G. J. K**. (**2006b). Radiation-induced sterility for pupal and adult stages of the malaria mosquito *Anopheles arabiensis. Malaria Journal*, **5**: 41.

House, H. L. (1961). Insect nutrition. *Ann. Rev. Entomol.,* **6**:13 - 26.

Hurd, H. (2003). Manipulation of medically important insect vectors by their parasites. *Ann. Rev. Vector Ecol.*, **48**: 141 - 161.

Jiang , F. (2008). No love for deadly mosquitoes. science, sex, superflies, *IAEA Bulletin*, Vol. **48/2**. p.10.

JOINT FAO/IAEA Programme. (2012). Sterile Insect Technique, Insect pest control. http://www-naweb.iaea.org/nafa/ipc/sterile-insect-technique.html. Retrieved 23/03/ 2013.

Kala, C. (2012). Sterile insect technique for eradicating harmful insects. http://www.biotecharticles.com/. Retrieved 23/03/2013.

Kelly-Hope, L.A., Hemingway, J. and McKenzie, F. E. (2009). Environmental factors associated with the malaria vectors *Anopheles gambiae* and *Anopheles funestus* in Kenya. *Malar. J.,* **8**: 268 - 268. http://dx.doi.org/10.1186/1475-2875-8-268.

Khan, A.A. and Maibach, H.I. (1971). A Study of the Probing Response of Aedes aegypti: Effect of dry and moist heat on probing. *J. Econ.Entomol.,* **64**: 442 - 443.

Kija, R. N., Bernadette, J., Gamba, N., Bart, G. J. K., Gerry, F. K. and Heather, M. F. (2005). Effect of larval crowding on mating competitiveness of *Anopheles gambiae* mosquitoes. *Malar J.*, **4**: 49.

Kingsolver, J. G. (1987). Mosquito host choice and the epidemiology of malaria. *The American Naturalist*, 130 (6): 811 - 827.

Klassen, W. (2005). Area-wide integrated pest management and the sterile insect technique. In: Dyck, V. A., Hendrichs J. and Robinson, A. S. (editors), *Sterile Insect Technique. Principles and Practice in Area-Wide Integrated Pest Management*. Dordrecht, The Netherlands: Springer; pp. 39 - 68.

Kline, D. L., Wood, J. R. and Cornell, J. A. (1991). Interactive effects of 1-octen-3-ol and carbon dioxide on mosquito (Diptera: Culicidae) surveillance and control. *J. Med. Entomol.*, **28**: 254 - 258.

Knipling, E. F. (1955). Possibilities of insect control or eradication through the use of sexually sterile males. *J. Econ. Entomol.*, **48**: 459 - 462.

Knipling, E. F. (1959). Sterile-male method of population control. *Science*, **130**: 902 - 904.

Knipling, E. F. (1979). The basic principles of insect population suppression and management. U.S. Dept. Agric. Handbook No. 512. p. 41.

Knipling, E. F. (1998). Role of parasitoid augmentation and sterile insect techniques for area-wide management of agricultural insect pests. *J. Agric. Entomol.*, **15**: 273 - 30l.

Knipling, E. F., Laven, H., Craig, G. B., Pal, R., Smith, C. N. and Brown, A. W. A. (1968). Genetic control of insects of public health importance. *Bull World Health Organ.*, **38**: 421 - 438.

Knols, B. G. J. and De Jong, R. (1996). Limburger cheese as an attractant for the malaria mosquito *Anopheles gambiae s.s. Parasitol. Today, 12(**4**): 159 - 161.*

Konate, L., Faye, O., Gaye, O., Diouf, M. and Diop, A. (1999). Observations on the feeding patterns and the alternative hosts selection of the malaria vectors in Senegal. *Parasite*, 6(**3**): 257 - 267.

Krafsur, E. (1998). Sterile insect technique for suppressing and eradicating insect populations: 55 years and counting. *J. Agric. Entomol.*, **15**: 303 - 317.

Kweka, E. J., Mwang'onde, B. J., Lyaruu, L., Tenu, F. and Mahande, M. A. (2010) Effect of different hosts on feeding patterns and mortality of mosquitoes (Diptera: Culicidae) and their implications on parasite transmission. *J. Glob. Infect. Dis.*, 2 (**2**): 121 -123.

Laarman, J. J. (1968). The intake of sugars by females of wild *Anopheles gambiae* and *Anopheles funestus*. *Acta Leiden.* **36**: 137 - 144.

Lacey, L. A. and Lacey, C. M. (1990). The medical importance of Riceland mosquitoes and their control using alternatives to chemical insecticides. *J. Am. Mosq. Control Assoc.*, **6**: 1 - 93.

LaFlamme, B. (2011). Human blood contains the secret ingredient for mosquito eggs. http://www.healthmedicinecentral.com/mucus-in-lungs/. Retrieved 17/02 /2013.

Lavazec, C. and Bourgouin, C. (2008). Mosquito-based transmission blocking vaccines for interrupting *Plasmodium* development. *Microbes Infect.*, 10(**8**): 845 - 849.

Lounibos, L. P. and Conn. J. (1991). Fecundity, parity and adult feeding relationships among *Nyssorhynchus* malaria vectors from Venezuela. *Mem. Inst. Ostvaldo Cruz*, **86**, 57 - 66.

Lustig, R. H. (2013). Sugar, the bitter truth. www.amazon.com/Real-Truth-About-Sugar-Lustigs/dp/1468161776. Retrieved 28/02/2013.

Macfie, J. W. S. (1915). Observations on the bionomics of *Stegomyia fasciata*. *Bull. Entomol. Res.*, **6**: 205 - 229.

Magnarelli, L. A. (1978). Nectar-feeding by female mosquitoes and its relation to follicular development and parity. *J. Med. Entomol.*, **14**: 527 - 530.

Magnarelli, L. A. (1986). Energy reserves in natural populations of *Aedes triseriatus* (Diptera: Culicidae). *Ann. Entomol. Soc. Am.*, **79:** 829 - 832.

Manda, H., Gouagna, L. C., Woodbridge, A. F., Jackson, R. R., Beier, J. C., Githure, J. I. and Hassanali, A. (2007). Effect of discriminative plant-sugar feeding on the survival and fecundity of *Anopheles gambiae*. *Malaria Journal*, **6**:113

Marrelli, M. T. (2012). Genetic control of mosquitoes: Population suppression strategies. *Rev. Inst. Med. trop. S. Paulo*, 54(5): 287 - 292.

McCafferty, W. P. (1983). Illustrations by Arwin V. Provonsha; Jones and Bartlett Publishers, Boston, Massachusetts, USA.

Mckenzie, D. (2011). The essential amino acids in honey. http://www.livestrong.com. Accessed 12/01/2013.

Means, R. G. (1968). Host preference of mosquitoes (Diptera: Culicidae) in Suffolk country, New Yolk. *Ann. Entomol. Soc. Am.*, **61**: 116 - 120.

Meijerink, J., Braks, M., Brack, A. Adam, W. and Dekker, T. (2000). Identification of olfactory stimulants for *Anopheles gambiae* from human sweat samples. *Journal of Chemical Ecology*, 26(**6**): 1367 - 1382.

Miller, K. (2012)**.** Mosquitoes. http://intellectualventureslab.com/?p=5448. Accessed 20/04/2013.

Mohammed, E-N. A., Abdelnabi1, M. A. and Modlinski, J. A. (2012). Evaluation of anesthesia and reproductive performance upon diazepam and xylazine injection in rats. *Animal Science Papers and Reports,* 30 (**3**): 285 - 292.

Molan, P. C. (1997). Honey as an antimicrobial agent. In Mizrahi, A. and Lensky, Y. (Editors): "Bee products: Properties, applications and apitherapy." New York: Plenum Press, pp. 27 - 37.

Molavi, A. (2003). "Africa's malaria death toll still "outrageously high". National Geographic. http: // news.nationalgeographic.com/news/2003/06 / 0612_030612_malaria.html. Accessed 13/12/2012.

Morrison, A. C., Costero, A., Edman, J. D., Clark, G. C. and T. W. Scott. (1999). Increased fecundity of *Aedes aegypti* fed human blood before release in a mark-recapture study in Puerto Rico. *J. Am. Mosq. Control Assoc*., **15**: 98 - 104.

Morrison, N. I., Franz, G., Koukidou, M., Miller, T. A., Saccone, G., Alphey, L. S., Beech, C., Javaregowda, J. N., Simmons, G. S. and Polito, L. C. (2010). Genetic improvements to the Sterile Insect Technique for agricultural pests. *AsPac J. Mol. Biol. Biotechnol.,* 18 (**2**): 275 - 295.

Mostowy, W. M. and Foster, W. A. (2004). Antagonistic effects of energy status on meal size and egg-batch size of *Aedes aegypti* (Diptera: Culicidae). *J. Vector. Ecol.,* **29**:84 – 93.

Murrieta, C. M., Bennet, K. E., Stuart, M. A., West, M. and Miller, M. M. (2010). A comparison of Hamster anaesthetics and their effects on mosquito (Diptera: Culicidae) blood feeding. *J. Entomol. Sci.,* 45 (**4**): 388 - 391.

Nayar, J. K. and Pierce, P. A. (1980). The effect of diet on survival, insemination and oviposition of *Culex nigripalpus Theobald. Mosq. News,* **40**: 210 - 217.

Nayar, J. K. and Sauerman, D. M. (1971). The effects of diet on life-span, fecundity, and flight potential of *Aedes taeniorhynchus* adults. *J. Med. Entomol.*, **8**: 506 - 513.

Nayar, J. K. and Sauerman, D. M., (1975). The effects of nutrition on survival and fecundity in Florida mosquitoes Part 3. Utilization of blood and sugar for fecundity *J. Med. Entomol.*, **12**: 220 - 225.

Nayar, J. K. and Van Handel, E. (1971). The fuel for sustained mosquito flight. *J. Insect Physiol.*, **17**: 471 - 481.

Obbard, D. J., Welch, J. J. and Little, T. J. **(**2009). Inferring selection in the *Anopheles gambiae* species complex: An example from immune-related serine protease inhibitors. *Malaria Journal*, **8**:117.

Olayemi, I. K., Ande, A. T., Danlami, G. and Abdullahi, U. (2011). Influence of blood meal type on reproductive performance of the malaria vector, *Anopheles gambiae* s.s. (Diptera: Culicidae). *Journal of Entomology*, **8:** 459 - 467.

Ozkan, F., Çakır-Ozkan, N., Eyibilen, A., Yener, T., Erkorkmaz, U. (2010). Comparison of Ketamine - diazepam with Ketamine - xylazine anaesthetic combinations in sheep spontaneously breathing and undergoing maxillofacial surgery. *Bosn J. Basic Med. Sci.,* 10(**4**): 297 - 302.

Parker, A. and Mehta, K. (2007). Sterile insect technique: A model for dose optimization for improved sterile insect quality. *Florida Entomological Society*, 90**(1):** 88 - 95.

Prasad, R. S. (1987). Nutrition and reproduction in haematophagous arthropods. *Proc. Indian Acad. Sci. (Anim. Sci.),* **96**: 253 - 273.

Read, A. F, Lynch, P. A. and Thomas, M. B. (2009). How to make evolution-proof insecticides for malaria control. *PLoS Biol.,* **7(4)**: e1000058.

Rehan, N., Sobrero, A. J. and Fertig, J. W. (1975). The density of human semen and the validation of weight as an indicator of volume: A multicentre study. *Fertil Steril*., 26 (**6**): 492 - 502.

Rhodes, J. (2008). The reason why only female mosquitoes suck blood. www.helium.com/knowledge/8503-the-reason-why-only-female-mosquitoes-suck-blood. Accessed 28/02/2013.

Richards, S. L., Anderson, S. L. and Smartt, C. T. (2013). The females mosquito's quest for blood: Implications for disease cycles. http://edis.ifas.ufl.edu. Retrieved 28/ 02/2013.

Robergs, R. A. and Kravitz, L. (2000). Making sense of calorie-burning claims. http://www.unm.edu/~lkravitz/Article%20folder/caloricexp.html#Anchor-44591. Retrieved 15/02/2013.

Roberts, L. and Janovy, J. Jr. (2000). *Foundations of Parasitology Sixth edition*. United States: McGraw-Hill Companies. www.lsic.ucla.edu/../eebiol181_syl11w.pdf. Accessed 24/01/2013.

Rodrigues, J., Oliveira, G. A., Kotsyfakis, M., Dixit, R., Molina-Cruz, A., Jochim, R. and Barillas-Mury, C. (2012). An epithelial serine protease, AgESP, is required for *Plasmodium* invasion in the mosquito *Anopheles gambiae*. *PLoS ONE* 7(**4**): e35210.

Roitberg, B. D. and Gordon, I. (2005). Does the *Anopheles* blood meal-fecundity curve, curve? *J. Vector Ecol.*, 30(**1**): 83 - 86.

Rudloff, W. (2013). How to calculate calories in food http://www.answers.com/guides/how-to-calculate-calories-in-food#ixzz2Se3Edq9Q. Retrieved 30/01/2013.

Scott, T. W., Naksathit, A., Day, J. F., Kittayapong, P. and Edman, J. D. (1997). A fitness advantage for *Aedes aegypti* and the viruses it transmits when females feed only on human blood. *Am. J. Trop. Med. Hyg*., **57**: 235 - 239.

Sefcik, L. (2010). Nutrition facts in fructose, a natural sweetener. http://www.livestrong.com. Accessed 24/02/2013.

Shahid, A. A, Parveen, T. and Reisen, W. K. (1980). Changes in weight, calories and triglyceride content of sucrose fed *Culex tritaeniorhynchus* Giles and *Anopheles stephensi* Liston. *Pakistan J. Zool.*, **12**: 163 - 169.

Sodzi-Tettey, S. (2011). 'Malaria control in Ghana: Challenges and opportunities'. Article 208399, p. 1**.** Accessed 24 / 02 / 2013.

Solarte, Y., Manzano, M. R., Rocha, L., Castillo, Z., James, M. A., Herrera, S. and Arévalo-Herrera, M. (2007). Effects of anticoagulants on *Plasmodium vivax* oocyst development in *Anopheles albimanus* mosquitoes. *Am. J. Trop. Med. Hyg.*, 77**(2)**: 242 - 245.

Straif, S. C. and Beier, J. C. (1996). Effects of sugar availability on the blood feeding behavior of *Anopheles gambiae* (Diptera: Culicidae). *J. Med. Entomol.*, **33**: 608 - 612.

Styer, L. M., Carey, J. R., Wang, J. L. and Scott, T. W. (2007). Mosquitoes do senesce: Departure from the paradigm of constant mortality. *Am. J. Trop. Med. Hyg*., 76 (**1**): 111 -117.

Syoziro, A. (1964). Food material and feeding procedures for mosquito larvae. *Bull. Wld. Hlth. Org.*, 31(**4**): 465 – 466.

Takken, W., Klowden, M. J. and Chambers, G. M. (1998). The effect of body size on host seeking and blood meal utilization in *Anopheles gambiae s.s.* (Diptera: Culicidae): The disadvantage of being small. *J. Med. Entomol*., **35**: 639 - 646.

Takken, W. and Kline, D. L. (1989). Carbon dioxide and 1-octen-3-ol as mosquito attractants. *J. Am. Mosq. Control Assoc*., **5**: 311 - 316.

Takken, W., van Loon, J. J. A. and Adam, W. (2001). Inhibition of host-seeking response and olfactory responsiveness in *Anopheles gambiae* following blood feeding. *Journal of Insect Physiology*, **47**: 303 - 310.

Takken, W. and Verhulst, N. O. (2012). Host preferences of blood-feeding mosquitoes. *Ann. Rev. of Entomol*., **58**: 433 - 453.

Tan, R. (2013) Honey vs sugar. http://www.honeyvssugar.com. Accessed 01/01/2013.

Taylor, P. J. and Hurd, H. (2003). The influence of host haematocrit on the blood feeding success of *Anopheles stephensi*: Implications for enhanced malaria transmission. *Parasitology*, **122**: 491 - 496.

Telang, A., Li, Y., Noriega, F. G. and Brown, M. R. (2005). Effects of larval nutrition on the endocrinology of mosquito egg development. *J. Exp. Biol.*, **209**, 645 – 655.

The Global poverty information Bank (2012). Malaria and extreme poverty http://www.globalpovertyproject.com/infobank/malaria. Accessed 17/02/2013.

Turley, A. P., Moreira, L. A., O'Neill, S. L. and McGraw, E. A. (2009). *Wolbachia* infection reduces blood-feeding success in the Dengue fever mosquito, *Aedes aegypti* *PLoS Negl. Trop. Dis*., 3 (**9**): e516.

VanDyk, J. (2007). Mosquito host-seeking: A partial review. http://www.ent.iastate.edu/dept/research/vandyk/hostseek.html. Accessed 13/08/2012.

Van Handel, E. (1965). The obese mosquito. *J. Physiol.*, **181**: 478 - 486.

Van Handel, E. (1972). The detection of nectar in mosquitoes. *Mosq. News*, **32**: 458 - 458.

Van Handel, E., Edman, J. D., Day, J. F., Scott, T. W., Clark, G. G., Reiter, P. and Lynn, H. C. (1994). Plant-sugar, glycogen, and lipid assay of *Aedes aegypti* collected in urban Puerto Rico and rural Florida. *J. Am. Mosq. Control Assoc.*, **10**: 149 - 153.

Van Handel, E. and Lum. P. T. M. (1961). Sex as regulator of triglyceride metabolism in the mosquito. *Science*, **134**: 1979 - 1980.

Vrzal, E. M., Allan, S. A. and Hahn, D. A. (2010). Amino acids in nectar enhance longevity of female *Culex quinquefasciatus* mosquitoes. *Journal of Insect Physiology*, **56**: 1659 – 1664.

Wheeler, D. (1996). The role of nourishment in Oogenesis. *Annu. Rev. Entomol.*, 41: 407 - 431.

White, J. W. (1975). Composition of honey. In Crane E (ed): *Honey. A comprehensive survey*. London: Heinemann Edition, pp.157 - 206.

WHO. (1995). Malaria: resistance to drugs. Vol. 30, Supplement 4, p. 68.

WHO. (2004). Global strategic framework for integrated vector management. WHO/CDS/CPE/PVC/2004/ **10**: 1 - 12.

WHO. (2005). World Malaria Report 2005. Geneva: World Health Organisation. http://rbm.who.int/wmr2005. Accessed 20/09/2012.

WHO. (2007). Insecticide-treated mosquito nets: A position statement. Global Malaria Programme. Geneva: World Health Organization. www.who.int/malaria/publications/atoz/itnspospaperfinal.pdf. Accessed 20/09/2012.

WHO. (2008). WHO position statement on integrated vector management. *Wkly Epidemiol Rec.*, **83**:177 - 181.

WHO (2009). Do all mosquitoes transmit malaria? http://www.who.int/features/qa/10/en/. Accessed 15/03/2013.

Wikipedia. (2012). Glucose. http://en.wikipedia.org/wiki/Glucose. Retrieved 28 /07/2012.

Wikipedia. (2011) Sucrose.. http://en.wikipedia.org/wiki/Sucrose. Retrieved 06/12/2013.

The All-Party Parliamentary Malaria Group (APPMG), 2007. Tackling malaria in developing countries. www.appmg-malaria.org.uk. Accessed 10/10/2012.

Woodbridge, A. F. (1995). Mosquito sugar feeding and reproductive energetic. *Malaria Journal*., **40**: 443 - 474.

Zimmerman, R. H., Galardo, A. K. R., Lounibos, L. P., Arruda, M. and Writz, R. (2006). Blood meal hosts of *Anopheles* species (Diptera: Culicidae) in a malaria-endemic areas of the Brazillian Amazon. *J. Med. Entomol.*, **43**: 947 - 956.

APPENDICES

Appendix 1: Analysis of variance (ANOVA) for weekly effect of sugar types and concentrations on adult mosquitoes survival.

Table 1: ANOVA for adult males in week 1 (7 days)

Source of Variation	Degree of Freedom	Sum of Square	Mean square	Variance ratio	F.pr.
R	2	59.37	29.69	4.76	
C	1	20.39	20.39	3.27	0.092
S	3	1351.81	450.60	72.21	≤ 0.001
C x S	3	59.27	19.76	3.17	0.058
E	14	87.36	6.24		
Total	23	1578.19			

CV= 3.3 % R = Replication C = Concentration S = Sugar types E = Error

Table 2: ANOVA for adult males in week 2 (14 days)

Source of Variation	Degree of Freedom	Sum of Square	Mean square	Variance ratio	F.pr.
R	2	303.07	151.53	6.23	
C	1	237.51	237.51	9.77	0.007
S	3	5858.92	5286.31	217.45	<.001
C x S	3	218.92	72.97	3.00	0.066
E	14	340.35	24.31		
Total	23	16958.76			

CV= 11.1 % R = Replication C = Concentration S = Sugar types E = Error

Table 3: ANOVA for adult males in week 3 (21 days)

Source of Variation	Degree of Freedom	Sum of Square	Mean square	Variance ratio	F.pr.
R	2	342.45	171.23	5.25	
C	1	94.64	94.64	2.90	0.111
S	3	7118.17	2372.72	72.73	<.001
C x S	3	109.35	36.45	1.12	0.375
E	14	456.71	32.62		
Total	23	8121.33			

CV= 23.2 % *R = Replication* *C = Concentration* *S = Sugar types* *E = Error*

Table 4: ANOVA for adult males in week 4 (28 days)

Source of Variation	Degree of Freedom	Sum of Square	Mean square	Variance ratio	F.pr.
R	2	18.776	9.388	2.99	
C	1	54.180	54.180	17.27	<.001
S	3	2013.573	671.191	213.90	<.001
C x S	3	126.701	42.234	13.46	<.001
E	14	43.930	3.138		
Total	23	2257.160			

CV= 20.4 % *R = Replication* *C = Concentration* *S = Sugar types* *E = Error*

Table 5: ANOVA for adult males in week 5 (35 days)

Source of Variation	Degree of Freedom	Sum of Square	Mean square	Variance ratio	F.pr.
R	2	9.054E-05	4.527E-05	2.01	
C	1	1.244E+01	1.244E+01	5.537E+05	<.001
S	3	3.751E+01	1.250E+01	5.564E+05	<.001
C x S	3	3.733E+01	1.244E+01	5.537E+05	<.001
E	14	3.146E-04	2.247E-05		
Total	23	8.728E+01			

CV= 0.2 % *R = Replication* *C = Concentration* *S = Sugar types* *E = Error*

Table : 6 ANOVA for adult females in week 1 (7 days)

Source of Variation	Degree of Freedom	Sum of Square	Mean square	Variance ratio	F.pr.
R	2	11.777	5.889	2.00	
C	1	5058.673	1686.224	571.70	<.001
S	3	0.653	0.653	0.22	0.645
C x S	3	19.432	6.477	2.20	0.134
E	14	41.293	2.949		
Total	23	5131.829			

CV= 2.4 % *R = Replication* *C = Concentration* *S = Sugar types* *E = Error*

Table : 7 ANOVA for adult females in week 2 (14 days)

Source of Variation	Degree of Freedom	Sum of Square	Mean square	Variance ratio	F.pr.
R	2	99.556	49.778	5.43	
C	1	20273.050	6757.683	737.53	<.001
S	3	0.001	0.001	0.00	0.993
C x S	3	85.122	28.374	3.10	0.061
E	14	128.277	9.163		
Total	23	20586.005			

CV= 6.0 % *R = Replication* *C = Concentration* *S = Sugar types* *E = Error*

Table : 8 ANOVA for adult females in week 3 (21 days)

Source of Variation	Degree of Freedom	Sum of Square	Mean square	Variance ratio	F.pr.
R	2	170.32	85.16	6.19	
C	1	12410.04	4136.68	300.62	<.001
S	3	8.70	8.70	0.63	0.440
C x S	3	100.71	33.57	2.44	0.108
E	14	192.65	13.76		
Total	23	12882.41			

CV= 10.6 % *R = Replication* *C = Concentration* *S = Sugar types* *E = Error*

Table : 9 ANOVA for adult females in week 4 (28 days)

Source of Variation	Degree of Freedom	Sum of Square	Mean square	Variance ratio	F.pr.
R	2	222.63	111.32	10.58	
C	1	6217.57	2072.52	196.93	<.001
S	3	175.45	175.45	16.67	0.001
C x S	3	70.91	23.64	2.25	0.128
E	14	147.34	10.52		
Total	23	6833.90			

CV= 16.8 % *R = Replication* *C = Concentration* *S = Sugar types* *E = Error*

Table : 10 ANOVA for adult females in week 5 (35 days)

Source of Variation	Degree of Freedom	Sum of Square	Mean square	Variance ratio	F.pr.
R	2	0.18968	0.09484	4.91	
C	1	1455.12255	485.04085	25087.29	<.001
S	3	274.97032	274.97032	14222.02	<.001
C x S	3	275.37219	91.79073	4747.60	<.001
E	14	0.27068	0.01933		
Total	23	2005.92542			

CV= 1.8 % *R = Replication* *C = Concentration* *S = Sugar types* *E = Error*

Table : 11 ANOVA for adult females in week 6 (42 days)

Source of Variation	Degree of Freedom	Sum of Square	Mean square	Variance ratio	F.pr.
R	2	6.659E-06	3.329E-06	1.28	
C	1	4.888E+01	4.888E+01	1.873E+07	<.001
S	3	1.468E+02	4.892E+01	1.875E+07	<.001
C x S	3	1.466E+02	4.888E+01	1.873E+07	<.001
E	14	3.653E-05	2.609E-06		
Total	23				

Table : 12 ANOVA for adult females in week 7 (49 days)

Source of Variation	Degree of Freedom	Sum of Square	Mean square	Variance ratio	F.pr.
R	2	0.002611	0.001305	1.00	
C	1	0.001305	0.001305	1.00	0.334
S	3	0.003916	0.001305	1.00	0.422
C x S	3	0.003916	0.001305	1.00	0.422
E	14	0.018275	0.001305		
Total	23	0.030024			

CV= 2.8 % *R = Replication* *C = Concentration* *S = Sugar types* *E = Error*

Appendix 2: Regression and correlation analysis for sugar types and concentrations

Table : 1 Summary of analysis for 6 % concentration of glucose

Source of Variation	Degree of Freedom	Sum of Square	Mean square	Variance ratio	F.pr.
Regression	1	6624.7	6624.7	42.92	0.001
Residual	5	771.8	154.4		
Total	6	7396.5	1232.7		

Percentage variance accounted for 87.5

Standard error of observations is estimated to be 12.4

Table : 2 Summary of analysis for 10 % concentration of glucose

Source of Variation	Degree of Freedom	Sum of Square	Mean square	Variance ratio	F.pr.
Regression	1	5645.7	5645.74	89.72	<.001
Residual	5	314.6	62.93		
Total	6	5960.4	993.40		

Percentage variance accounted for 93.7

Standard error of observations is estimated to be 7.93

Table : 3 Summary of analysis for 6 % concentration of honey

Source of Variation	Degree of Freedom	Sum of Square	Mean square	Variance ratio	F.pr.
Regression	1	6007.54	6007.54	468.78	<.001
Residual	5	64.08	12.82		
Total	6	6071.61	1011.94		

Percentage variance accounted for 98.7

Standard error of observations is estimated to be 3.58

Table : 4 Summary of analysis for 10 % concentration of honey

Source of Variation	Degree of Freedom	Sum of Square	Mean square	Variance ratio	F.pr.
Regression	1	5785.7	5785.6	76.20	<.001
Residual	5	379.6	75.93		
Total	6	6165.3	1027.55		

Percentage variance accounted for 92.6

Standard error of observations is estimated to be 8.71.

Table : 5 Summary of analysis for 6 % concentration of sucrose

Source of Variation	Degree of Freedom	Sum of Square	Mean square	Variance ratio	F.pr.
Regression	1	6007.54	6007.54	468.78	<.001
Residual	5	64.08	12.82		
Total	6	6071.61	1011.94		

Percentage variance accounted for 98.7

Standard error of observations is estimated to be 3.58

Table : 6 Summary of analysis for 10 % concentration of sucrose

Source of Variation	Degree of Freedom	Sum of Square	Mean square	Variance ratio	F.pr.
Regression	1	5713.15	5713.15	288.14	<.001
Residual	5	99.14	19.83		
Total	6	5812.29	968.71		

Percentage variance accounted for 98.0

Standard error of observations is estimated to be 4.45

Table : 7 Summary of analysis for 6 % concentration (Control)

Source of Variation	Degree of Freedom	Sum of Square	Mean square	Variance ratio	F.pr.
Regression	1	1868.114	1868.1138	7653.02	<.001
Residual	5	1.221	0.2441		
Total	6	1869.334	311.5557		

Percentage variance accounted for 99.9

Standard error of observations is estimated to be 0.494.

Table : 8 Summary of analysis for 10 % concentration (Control)

Source of Variation	Degree of Freedom	Sum of Square	Mean square	Variance ratio	F.pr.
Regression	1	1852.002	1852.0017	6831.79	<.001
Residual	5	1.355	0.2711		
Total	6	1853.357	308.8929		

Percentage variance accounted for 99.9

Standard error of observations is estimated to be 0.521.

Appendix 3: Analysis of variance (ANOVA) for the effect of sugar types on male potential to inseminate females

Table : 1 ANOVA for percentage male insemination of females

Source of Variation	Degree of Freedom	Sum of Square	Mean square	Variance ratio	F.pr.
R	2	28.40	14.20	0.56	
S	2	242.97	121.48	4.78	0.087
E	4	101.65	25.41		
Total	8	373.02			

*CV=*14.0 % *R = Replication* *S = Sugar types* *E = Error*

Appendix 4: Analysis of variance (ANOVA) for the effect of blood feeding methods on adult female mosquitoes productivity.

Table : 1 ANOVA for adult females blood feeding response

Source of Variation	Degree of Freedom	Sum of Square	Mean square	Variance ratio	F.pr.
R	2	212.52	106.26	7.43	
FM	2	6311.63	3155.81	220.67	<.001
TR	2	476.07	238.04	16.64	<.001
FM x TR	4	73.26	18.31	1.28	0.319
E	16	228.81	14.30		
Total	26	302.30			

CV= 2.8 % *R = Replication FM = Feeding method TR =Feeding regimen FM x TR = Feeding method x Time regimen E = Error*

Table : 2 ANOVA for adult females egg production in week 1

Source of Variation	Degree of Freedom	Sum of Square	Mean square	Variance ratio	F.pr.
R	2	4.993	2.497	1.24	
FM	2	484.968	242.484	120.50	<.001
TR	2	3.009	1.505	0.75	0.489
FM x TR	4	10.294	2.573	1.28	0.319
E	16	32.197	2.012		
Total	26	535.460			

CV= 11.2 % R = Replication FM = Feeding method TR =Feeding regimen FM x TR = Feeding method x Time regimen E = Error

Table : 3 ANOVA for adult females egg production in week 2

Source of Variation	Degree of Freedom	Sum of Square	Mean square	Variance ratio	F.pr.
R	2	6.597	3.299	0.72	
FM	2	161.123	80.562	17.63	<.001
TR	2	46.224	23.112	5.06	0.020
FM x TR	4	45.004	11.251	2.46	0.087
E	16	73.123	4.570		
Total	26	332.072			

CV= 22.4 % R = Replication F = Feeding method TR =Feeding regimen FM x TR = Feeding method x Time regimen E = Error

Appendix 5: Analysis of variance (ANOVA) for the effect of anaesthetics on adult female mosquitoes productivity.

Table : 1 ANOVA for adult females blood feeding response

Source of Variation	Degree of Freedom	Sum of Square	Mean square	Variance ratio	F.pr.
R	2	75.47	37.73	1.72	
FM	2	5443.78	2721.89	123.81	<.001
TR	2	26.54	13.27	0.60	0.559
FM x TR	4	220.76	55.19	2.51	0.083
E	16	351.76	21.98		
Total	26	6118.31			

CV= 9.5 *% R = Replication AT= Anaesthetic types TR = Time regimen AT x TR = Anaesthetic types x Time regimen E = Error*

Table : 2 ANOVA for adult females egg production in week 1

Source of Variation	Degree of Freedom	Sum of Square	Mean square	Variance ratio	F.pr.
R	2	2.004	1.002	0.12	
FM	2	434.530	217.265	25.92	<.001
TR	2	18.584	9.292	1.11	0.354
FM x TR	4	32.859	8.215	0.98	0.446
E	16	134.131	8.383		
Total	26	622.107			

CV= 22.8 *% R = Replication AT= Anaesthetic types TR = Time regimen AT x TR = Anaesthetic types x Time regimen E = Error*

Table : 3 ANOVA for adult females egg production in week 2

Source of Variation	Degree of Freedom	Sum of Square	Mean square	Variance ratio	F.pr.
R	2	2.6955	1.3477	1.52	
AT	2	57.1904	28.5952	32.24	<.001
TR	2	23.3420	11.6710	13.16	<.001
AT x TR	4	100.3805	25.0951	28.29	<.001
E	16	14.1931	0.8871		
Total	26	197.8015			

CV= 9.6 %   R = Replication   AT= Anaesthetic types   TR = Time regimen   AT x TR = Anaesthetic types x Time regimen   E = Error