TITLE PAGE

EFFICACY OF 10% EMULSIFIABLE CONCENTRATE (EC) OF SYPERTIX® FOR TSETSE FLY CONTROL IN KIBOKO MAKUENI COUNTY, KENYA.

By

TRUPHENA WANJA KITHAKA

156/75406/2014

A THESIS SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF THE DEGREE OF MASTER OF SCIENCE (MEDICAL AND VETERINARY ENTOMOLOGY) OF THE UNIVERSITY OF NAIROBI.

2018

DECLARATION

I declare that this research thesis is my original work and has not been presented for a degree or Any other award in any other University.

Signature_____ Date_____

Truphena Wanja Kithaka

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

Signature_____ Date_____

Prof. Florence Oyieke,

School of Biological Sciences,

University of Nairobi

Signature_____ Date_____

Dr. Eunice Owino

School of Biological Sciences,

University of Nairobi

Signature_____ Date_____

Dr. Japhet Kiragu

School of Pure and Applied Sciences,

Machakos University

DEDICATION

To my parents Mr. and Mrs. Francis Kithaka.

ACKNOWLEDGEMENTS

My greatest appreciation goes to the Almighty God for giving me strength and time to pursue postgraduate studies. I appreciate the University of Nairobi for offering me the opportunity to take my course in the institution. I wish to extend my heartfelt gratitude to my supervisors Prof. Florence Oyieke and Dr. Eunice Owino for their unending support, motivation and scientific guidance throughout my research work.

I thank my field supervisor Dr. Japhet Kiragu for imparting me with the skills and knowledge on efficacy trials. I gratefully thank Dr. Ongamo as the Entomology Thematic Head for his guidance on statistical analysis. In addition, I thank Mr. Mwatika who was the officer in charge for allowing me to use their laboratory facilities. Tremendous thanks also go to the technical staff at Kiboko Veterinary and efficacy trial station.

I am also grateful to Madam Karen and Mr. Ogali for their help with the bioassays. My sincere appreciation also goes to my fellow master degree students in Medical and Veterinary Entomology for their cooperation, love and unity throughout the study, creating a very friendly learning environment.

Finally, I would like to express my sincere acknowledgement to the rest of my family members especially my parents, Mr. Francis and Jeremiah Kithaka, for their caring attitude, love, prayers, moral and financial support, and the academic foundation they laid in my life.

TABLE OF CONTENTS

LIST OF ABBREVIATIONS AND ACRONYMS

PCPB	-	Pest Control Product Board
ANOVA	-	Analysis of Variance
EC	-	Emulsion Concentrate
AAT	-	Animal African Trypanosomiasis
BHC	-	Benzene Hexa Chloride
DDT	-	Dichlorodiphenyltrichloroethane
WP	-	Wettable powder
HAT	-	Human African Trypanosomiasis
R.H	-	Relative Humidity
WHO	-	World Health Organization
WG	-	Wettable granules
PAAT	-	Programme against African Trypanosomiasis
FAO	-	Food and Agriculture Organization
PBO	-	Piperonyl Butoxide
SC	-	Suspension concentrate
R H	-	Relative Humidity

DEFINITION OF TERMS

Dry season - One of the two seasons from September to October in 2015 when the bio efficacy was assessed at Mulili, Kaasuvi and Kiboko study sites.

Efficacy: The ability of an insecticide to achieve the desired effect. The effectiveness of drug.

Feeding success: The nutritional status of tsetse flies at the time of taking a blood meal.

Fed: Fully engorged tsetse fly after taking a blood meal.

Knockdown- The state of intoxication and partial paralysis which usually precedes death. This consist of both live and moribund tsetse flies.

Season: One of the four divisions of the year (Wet and Dry) marked by particular weather patterns when the bio efficacy was assessed.

Unfed: Tsetse flies that had not taken the blood meal and their abdomen were not engorged

Wet season - One of the two seasons from May to June in 2015 when the bio efficacy was assessed in Mulili, Kaasuvi and Kiboko study sites.

LIST OF FIGURES

LIST OF TABLES

LIST OF APPENDICES

ABSTRACT

Controlling tsetse flies remain an integral part of trypanosomiasis management. Pour on insecticides have been used in controlling tsetse flies in cattle. Efficacy studies of various pyrethroid insecticides have shown to control tsetse flies. In this study, the feeding success, knockdown effect and efficacy of 10%EC Sypertix®, a pyrethroid insecticide, was assessed on laboratory reared male *Glossina pallidipes* in dry and wet seasons in three study sites namely: Mulili, Kaasuvi and Kiboko in Makueni County, Kenya. Three groups of cattle each comprising of six cows were selected from each study site. A geigy cage holding 30 tsetse flies was strapped at the flank of each cow in each group for ten minutes during the bioassay. The feeding success was ascribed as fed or unfed after the ten minutes of exposure. The knockdown rates were observed at 0, 5, 10 and 20 minutes, after the ten minutes of exposure. Mortality rates were observed after 24 hours post treatment on the experimental cattle.

The feeding success of tsetse flies increased with significant variations from week zero to the third week in the treated groups (Sypertix® and Domenix®), while there were no significant variations in the fourth week in both the dry and wet seasons (P=0.05). There was a significant difference in feeding success from week 0 to week 3 between the treated groups and the control group. There was a 90% knockdown in the insecticide treated groups which occurred 5 minutes post treatment up to the second week in both dry and wet seasons. A higher number of knockdown in tsetse flies, which declined with increase in time, was observed in treated cattle. There was however, significant variation between knockdown observed in treated groups (Sypertix® and Domenix®) compared to the control (untreated) group. A cut off mortality of 80% within 24th hours occurred two weeks post exposure for the insecticide treated groups (Sypertix® and Domenix®) in both dry and wet seasons. Mortalities obtained were comparable and had no significant variation (P=0.05) between the treated groups (Sypertix® and Domenix®) but varied significantly from the control (untreated) group.

The study confirms that Sypertix® is efficacious in both seasons and its persistence in the wet season during the wash off by rains, is a novel quality that shows it can be used as an alternative Alphacypermethrin for control of tsetse flies. The insecticide should therefore be reapplied on the cattle after two weeks of initial application to maintain its efficacy. The findings of this study show the potential of Sypertix® in decreasing the re-invasion of tsetse flies and prevalence of trypanosomiasis.

CHAPTER ONE: INTRODUCTION

1.1 General introduction

Tsetse flies (Diptera: Glossinidae) are important medical and veterinary vectors, responsible for transmitting African trypanosomiasis. The disease leads to high mortality and morbidity in Sub-Saharan Africa (Aksoy, 2003; Bourtzis *et al.*, 2016). Efforts to discover new drugs for the management of this disease have been frustrated by mechanisms of interaction of trypanosomes with their hosts. In addition, the commercially available drugs are either not environmental friendly, or cost effective. A licensed vaccine to control trypanosomiasis is also not available. This has left vector control as the most effective way of managing this disease (Barrett *et al.*, 2003; Bourtzis *et al.*, 2016).

The control of tsetse flies greatly improve the livelihoods of rural dwellers by alleviating the heavy burden of food deficit arising from African trypanosomiasis that keeps many rural communities from vast agricultural land (Ilemobade, 2009). Insecticide based techniques used in field trials to control tsetse flies have minor and short lived environmental impacts (Grant, 2001).

The use of insecticide treated cattle has proved to offer protection against biting arthropods including tsetse flies, as they are highly zoophilic (Banks *et al.*, 2014). Cattle insecticidal bioassay have caused diversion of trypanosomiasis seeking vectors from the cattle, leading to long lasting effect which reduces survivors of blood fed vectors on the treated animals. Most vectors are killed before any subsequent feeding on the treated animals because of the irritancy effect of the insecticides used (Hewitt and Rowland, 1999).

Synthetic pyrethroids have been widely used in agricultural settings because of low toxicity to mammals, lack of phytotoxicity and reduced application rates (Kolaczinski and Curtis, 2004). They are generally the safest class of insecticides available (Kolaczinski and Curtis, 2004). Use of pyrethroids insecticide on cattle to prevent tsetse flies is an important tool to counter losses caused by tsetse-borne diseases (Eisler *et al.*, 2003). As most farmers do not spray their cattle to prevent African Animal trypanosomiasis, increased prevalence of the disease has negatively affected the economic security and health of the rural populations (Miller, 2017).

Alphacypermethrin is one of the highly active broad spectrum pyrethroid insecticide, effective against tsetse flies via direct contact. It is highly stable to light and elevated temperatures (Hartnik *et al.*, 2008). Sypertix® which is an Alphacypermethrin , an acaricide registered only for control of ticks in Kenya (www.pcpb.or.ke/animalhealthviewform.php/5th Jan/2018) has been ranked as one of the most effective pyrethroids, and is increasingly replacing organophosphates and carbamates (Hartnik *et al.*, 2008). Sypertix® has demonstrated excellent efficacy in control of ticks and it is wash resistance (Norval *et al.*, 1996; Yu *et al.*, 2016).The acaricide is highly effective and shows prolonged residual activity. It has been shown to have an additional advantage over other insecticides of being effective against biting flies and therefore can be used in areas where trypanosomiasis is prevalent (De Meneghi *et al.*, 2016).
In this study, a field trial was performed to determine the efficacy of Alphacypermethrin (10%EC Sypertix®) on laboratory reared male *Glossina pallidipes.* The bio-efficacy was carried out in three trial sites namely Kiboko, Mulili and Kaasuvi in Makueni County, Kenya.

1.1Problem statement

Synthetic pyrethroids continue to be one of the options for tsetse flies and trypanosomiasis integrated control. Prolonged use of insecticides in large quantities and at high concentrations has resulted in insecticide resistance and undesirable environmental effects. The commonly used insecticides against tsetse flies include Deltamethrin, Permethrin and recently new Alphacypermethrin compounds. Deltamethrin the commonly available product is prohibitively expensive for livestock keepers and has led to poor uptake of tsetse control operations (Torr *et al.,* 2000).There is need to re-examine new synthetic pyrethroids like Alphacypermethrin as alternative options . Domenix® an Alphacypermethrin which is registered for the control of ticks and tsetse is expensive compared to Sypertix®. It is also very toxic to aquatic life with long term adverse effect to the aquatic environment, and has deterrent effect on foraging bees (Alliance *et al.*, 2015).

Sypertix®, an Alphacypermethrin acaricide is currently registered in Kenya for the control of ticks. It has been reported to have noble qualities such as being environmental friendly, affordability, low toxicity, lack of residual effects, photo stability and could therefore provide a long lasting solution to tsetse control (Ngaruiya, 2003).

1.2 Justification

Trypanosomiasis is a human infectious and parasitic disease in Africa (Aksoy and Rio, 2005). The disease is a serious constraint to livestock, health and crop production resulting in annual losses estimated at US$ 4.5 billion (Torr and Vale, 2005). Livestock keepers highly rely on animals for food consumption, transport, draught power and income from the sale of animals and their products.

Economically the disease affects African families by creating financial burden. Not only affected families become unproductive but also time and money is spent on their care and treatment

draining resources. Removal of the constraints imposed by tsetse flies and trypanosomiasis on vast agriculture land would open opportunities that reduce food deficit and rural to urban drift thereby improving the livelihood of rural dwellers (Ilemobade, 2009).

Elimination of a vector is the major form of disease control (Aksoy *et al.*, 1995). The control of the vector not only decreases the prevalence of trypanosomiasis and the impact of economic losses but also limits the spreading of the disease in the adjacent areas. If the current methods of controlling tsetse flies are to be successful, additional knowledge is needed before *Glossina* species can be effectively suppressed or eradicated (Gooding and Krafsur, 2005). Use of synthetic pyrethroids such as Sypertix® which do not have any residual activity are highly recommended (Van Nieuwenhove *et al.*, 2001). Sypertix® is an Emulsifiable concentrate acaricide containing Alphacypermethrin 10 % w/v., registered primarily to control ticks. Its efficacy in the control of *Glossina pallidipes*, a major trypanosome vector in Makueni County has not been established, hence the objective of this study.

1.3 Significance of the study

Efficacy of Sypertix®, as a new insecticide of choice for controlling tsetse flies would not only benefit Makueni County livestock keepers but would benefit other livestock keepers elsewhere in Kenya. Scientific data on the novel qualities of Sypertix®, is useful not only to Kenyan livestock keepers but to the scientific community at large. A choice formulation such as Sypertix® that is environmentally friendly and inexpensive will go a long way in improving food security in rural communities across Kenya where local livestock keeping is a major source of food.

1.4 Study objectives

1.4.1 General objective

The main objective of this study was to determine the knockdown rate and efficacy of 10%EC Sypertix® on tsetse flies based on field trials in Kiboko Makueni County, Kenya.

1.4.2 Specific objectives.

(i) To determine the feeding success of tsetse flies on Sypertix® treated cattle.

(ii) To determine the knockdown effect of Sypertix® for control of tsetse flies.

(iii) To determine the efficacy of Sypertix® for tse tse flies control.

1.5 Statistical Hypothesis

1.5.1Null hypotheses

i. There is no successful feeding of tsetse flies on Sypertix® treated cattle.

ii. There is no knockdown effect of Sypertix® on tsetse flies.

iii. Sypertix® is not efficacious for tsetse control.

1.5.2Alternative hypotheses

i. There is a successful feeding of tsetse flies on Sypertix® treated cattle.

ii. There is knockdown effect by Sypertix® on tsetse flies.

iii. Sypertix® is efficacious for tsetse control

CHAPTER TW0: LITERATURE REVIEW

2.1 Classification of Tsetse flies

The current classification of *Glossina* species recognizes 31 species and subspecies. Their grouping in the three subgenera depends on the morphological features of the adult flies but also reflects differences in distributions, habitat and behavior (Kuzoe and Schofield, 2004). There are three subgenera namely *Austenina*, *Nemorhina* and *Glossina* that correspond to *fusca, palpalis* and *mortisan*s species groups, respectively. Tsetse species are confined to Sub-Saharan Africa though *Glossina mortisans submortisans* and *Glossina fuscipes fuscipes* have been identified in the South Western corner of Arabian Peninsula (Cecchi *et al.*, 2008).

The *mortisans* group is found in drier savannah and woodland inhabitants while *Glossina pallidipes* can be found in forests. The *palpalis* group is associated with riverine vegetation with extension in savanna regions between river systems, whereas the fusca group occurs in low rain forest with exception to *Glossina longipennis* and *Glossina brevipalpis* which are found in drier regions of Eastern Africa (Krafsur, 2009). All species and subspecies of *Glossina* are potential vectors of trypanosomes. The *palpalis* group is significant in the transmission of *T.b gambiense* while *T.b rodesiense* transmission is attributed to species of the *mortisans* group. The *fusca* groups are considered to be insignificant vectors of Human African Trypanosomiasis but important vectors of Animal trypanosomiasis. *Glossina pallidipes* is one of the most predominant vector of trypanosomes (Cecchi *et al.*, 2008).

2.2 Biology of *Glossina* species

Both sexes of tsetse flies are obligatory blood feeders and they feed on a variety of vertebrates. They have been shown to thrive best at temperatures around 25°C but some like *Glossina fuscipes fuscipes* can tolerate a variety of temperatures. Sufficient moisture content is necessary for the deposition of the newly formed larva and for it to complete the pupal development (Kuzoe and Schofield, 2004).

Tsetse flies have low reproductive rate, dispersal capacity and genetic variability. This makes them more susceptible to modern pyrethroid insecticides and vulnerable to targets for well executed control measures (Kuzoe and Schofield, 2004). The life expectancy is around 120 days under laboratory conditions and a field mean of 33 days. For the development of pupa the pregnant tsetse feeds for three days. The amount of blood meal taken determines the viability and the size of the offspring (Rock, 2014). All tsetse flies have a similar life cycle and live for three to five months with females living slightly longer than males (Pendleton, 2003).

Tsetse flies have unusual viviparous reproductive biology of bearing a live young. Their reproductive system has undergone physiological and morphological modifications. They have two ovarioles per ovary, highly tracheated and muscular uterus and their accessory glands develop into milk gland for supplying nutrients to the developing larvae (Pais *et al.*, 2008). The primary follicles develop and are pushed into vitellanium through division and growth of germarial cells. Ovulation is regulated by hormones and regulatory compounds secreted by neurosecrectory cells, including stimulation from the female reproductive organs, male chemical and physical contact. Unmated virgin tsetse flies do not ovulate (Gooding and Krafsur, 2005).

2.3 Habitat and distribution of tsetse flies

Tsetse flies are endemic in tropical Africa occurring between 15°N and 29°S from Southern edge of Sahara desert to Zimbabwe, Angola and Mozambique (Vreysen, 2001). Studies by Pendleton, (2003) showed that *Glossina mortisans* and *Glossina fuscipes* once inhabited South Western Saudi Arabia. *Glossina* species do not inhabit altitudes exceeding 6000ft but keep moving randomly in their habitats. This random movement has caused an even distribution of flies and homogenized gene frequencies (Pendleton, 2003).

Tse tse fly survival is linked to the presence of water which increases local humidity allowing growth of vegetation that protects them from direct sunlight and wind. Other factors such as sunshine, rainfall and vegetation cover influences their distribution (Pendleton, 2003).

The *mortisans* groups are medium sized flies found in savannahs of West, Central and East Africa. Wild animals and water sources affects their distribution. They primarily feed on wildlife, cattle and only occasionally on human beings (Moore and Messina, 2010). In woodlands they are mostly dispersed in wetter areas. The *mortisans* group is limited by cold winter conditions in south of Zimbabwe and Botswana and hot dry conditions in the North and Central Africa (Moore and Messina, 2010).

The *palpalis* group is mainly limited to the very humid areas of Africa, mangrove swamps, the rain forest, the lake shores and gallery forests along rivers. They are mostly found in lowland rain forests. They habit the drainage systems leading to Mediterranean ocean and from wet mangroves and rainforests along the coastal regions of West Africa to drier Savannah areas (Rogers and Randolph, 1986).

The *fusca* group is made up of forest species which have no economic and medical role in epidemiology. However, *Glossina fusca* and *Glossina medicorum* which are found in West and East African forests are efficient vectors of *Trypanosoma vivax* causing economic burden to the livestock (Glasgow, 1963). Their distribution depends on forest vegetation and climatic factors such as temperature and rainfall (Glasgow, 1963).

In Kenya eight species of tsetse flies are presented in distribution maps. Good climate and land cover has enhanced their spread on the ecologically suitable habitat (McCord *et al.*, 2012). The *mortisans* group has the most expansive distribution in Kenya. The *fusca* distribution overlaps with that of *morsitans* group and primarily distributed in isolated patches of forests along Kenya–Tanzania border while the *palpalis* group is restricted to the shores of lake Victoria and along Kenya-Uganda border (Devisser *et al.*, 2010). The map showing distribution of tsetse flies in Kenya is shown in figure 1.Their distribution limits is based on vegetation type, meteorological records and altitude (Ford, 1971).Generally *Glossina pallidipes* is the major vector of Animal African trypanosomiasis in Kenya which occur in Savannah, shrub lands and grassy woodlands habitat. The suitable conditions of humidity, rainfall and temperature has enabled the *Glossina pallidipes* to keep re-emerging in Coastal ,Western Kenya and lake Victoria belt region (Okeyo *et al*., 2017).*Glossina pallidipes* is also present in other Eastern African countries such as Somalia, Ethiopia and Uganda (Nthiwa 2015).

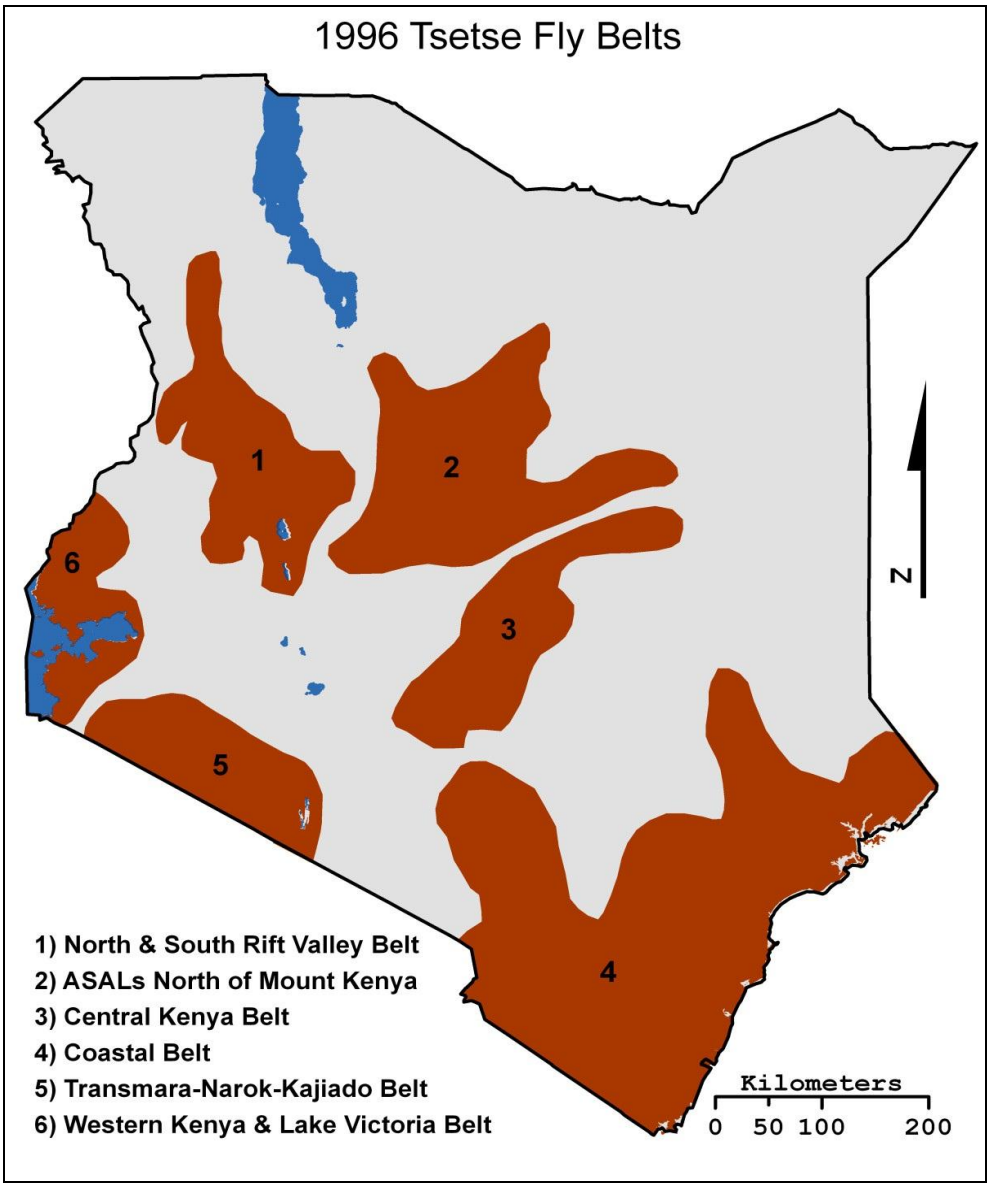


Figure 1: The tsetse flies belt distribution in Kenya (Source: Grady *et al*., 2011).

2.4 Seasonality, abundance and distribution of tsetse flies

Tsetse flies occurrence in the environment is influenced by both abiotic and biotic factors. The abiotic factors include temperature, rainfall and humidity while biotic factors include vegetation, host preference and human activity (Majekodunmi, 2012). Low ambient temperatures provide lower rate of breeding. The low abundance of tsetse flies in wet seasons can be due to presence of vegetation cover which causes many areas to be water logged making them to attract predators such as ants and dragon flies. This causes the flies to move from riverine vegetation into open woodland. Female flies are more abundant than males in both wet and dry season due to natural longevity. Human activities such as fishing and other activities around water bodies also influence the distribution of tsetse flies in an area (Lukaw *et al.*, 2014).

2.4.1 Effects of temperature, humidity and rainfall on tsetse longevity and abundance

Tsetse flies thrive in areas with temperature between 19°C and 30°C.Temperatures below 19°C lowers their activity and general physiology whereas temperatures below 15°C increases fly mortality. High temperatures affect their survival and at temperatures more than 36°C tsetse flies have a survival capacity of close to zero (Albert *et al.*, 2015). Temperature influences the biting rate, mortality and parasite development rate in tsetse flies. The mortality rates are high at extreme low and high temperatures (Terblanche *et al.*, 2008).

Tsetse flies are more active in the dry weather than in the wet causing the flies to be hungrier in the dry season than in the wet season. This may be caused by high metabolic rates associated with high temperatures and flight activities (Nnko *et al.*, 2017). Previous Studies by Geiger *et al.*, (2015) showed that high temperatures induces high blood digestion in tsetse flies causing them to feed more rapidly. High temperatures and low humidity are probably unfavorable to the flies because the life of flies is much longer at 65% r.h than at 88% r.h. Therefore, intermediate humidity enhances survival and activity of flies compared to high humidity which is unfavorable.

Humidity and rainfall have effect on mortality rate on both wet and dry season. A humid environment causes the tsetse flies to escape from sheltered habitat provided that the temperatures are not too high. The humidity of the soil also affects pupal development. The population of tsetse flies is much smaller in the dry season than in the wet season. Low temperatures, lower humidity and shade abundant causes the flies to congregate in gallery forests (Majekodunmi, 2012).

The relationship between temperature and tsetse mortality rate depends on species and sex of the flies. Studies by Munang'andu *et al.*, (2012), postulated that age structure of tsetse flies population is dependent on temperature and relative humidity. Tsetse birth rate is low during cold seasons when the pupal and interlarval periods are at their maximum. Studies by Syed and Guerin, (2004), revealed that tsetse flies will seek cover from extreme temperatures to conserve energy and for shelter.

2.4.2 Vegetation and tse-tse distribution

Different species of tsetse flies requires particular vegetation type that would provide favorable environments for survival and growth (Dumesa and Demessie, 2015). All forms of woodland from savannah to rainforest provide conducive habitat for tsetse flies. The abundance and distribution of *Glossina pallidipes* will depend on availability of adequate environmental conditions and mammalian hosts (Ouma, 2005). *Glossina pallidipes* prefers dense evergreen vegetation, heavier shade and humid habitats which are close to riverine thickets (Nthiwa 2005). Resting sites are divided into night resting sites where flies spend time being inactive at night and the day resting sites where flies rest on hot weather (Davis *et al.*, 2011).

2.4.3 Human Activity and tsetse distribution

Human settlements, crop farming and clearing of the land through felling of trees reduce the distribution of tsetse flies. This removes the tree cover and the host game animals. Activities by

humans leading to forest fires tend to clear trees and bushes. The changes in the environment lead to tsetse migration to shadier and moister areas. If the cultivated land is abandoned for some time the tsetse flies may move back as the trees and bushes grow. Introduction of herds of cattle in an area and prevention of hunting of wild animals increases reinvasion of tsetse species in a habitat (Pollock, 1982).

As the human population increases there is a decline in the populations of savannah and riverine species due to humans interfering with their habitat. Certain riverine species have however, been found to survive in densely populated villages, living on groves of trees planted near human dwelling on the manmade housing structures (Reid *et al.*, 2000).

Human activities like crop farming and livestock farming may create new habitats favoring the persistence of peri-domestic fly populations which are not easily detected. These act as major foci for animal and human trypanosomiasis (Hendrick *et al.*, 2004).

2.4.4 Tse tse host preference and location

The growth of tsetse population is affected by availability of food (Hargrove, 2003). All tsetse flies feed on blood mainly from mammals and also on reptiles and birds. Blood analyses show that tsetse species have different host preferences. Cattle and donkeys are good domestic hosts of tsetse flies compared to goats and sheep (Franco *et al.*, 2015). Savannah flies are attracted to buffalo urine which contains phenolic compounds whereas the body odor from the waterbuck repels tsetse flies. On the other hand riverine species are attracted to reptile's odors (Torr and Mangwiro, 2000). *Glossina mortisans* and *Glossina fusca* feed mainly on *Bovidae* and *Suidae* while species of *Glossin*a *palpalis* have no defined host preferences and can feed on the most available host species (Bekele, 2004).The distribution and abundance of *Glossina mortisans* and *Glossina pallidipes* is associated with the number and habitat of wild animals, and correlates to the densities of wild animals and low human population (Regasa *et al.*, 2015).

2.5 Control of tse tse flies

2.5.1 Ecological control of tsetse flies

Ecological control involves modification of the biotope by making it less suitable for tsetse flies. Bush clearing is most applicable for riverine species. It is customary to clear almost all shrubs and trees in selected bands in the country borders to check tsetse flies encroachment or dispersal and provide effective barriers (Hocking *et al.*, 1963).

Clearing of vegetation about 100 yards on each side of river crossings and at water places reduces the contact between the man and the fly. Mechanical bush clearing encourages growth of grass instead of woodland vegetation. Elimination of game animals has proven to be effective in eliminating tsetse flies in South Africa. The number of game species can be lowered to a point where they cannot generally support tsetse flies (Hocking *et al.*, 1963).Tsetse fly density is closely related to that of wild animals and destruction of all host vertebrates would inevitably lead to reduction of fly numbers through starvation (Leak, 1999).

Wild animals are not only the source of food for tsetse flies but also trypanosome carriers. Elimination of wild animals is difficult to accomplish especially when eliminating small animals like warthog, bush pig and small antelope which are preferred by tsetse flies (Affected, 2009).

2.5.2 Biological control

Ecological hazards and development of pesticide resistance due to use of chemicals has led to the use of biological control as an integral part of pest management (Ebrahim, 2015).

This method works by lowering the density of a pest population to an equilibrium position below economic threshold (Kennedy, 2004).

Microorganisms like *Bacillus spp*, microsporidian protozoans, fungi and virus can be useful in controlling tsetse flies. Ants, asilids and spider's causes decrease on tsetse populations with mutilid wasps being recorded in East Africa tsetse biotopes. The hymenopteran (*Synthomosphyrum indicum*) has shown positive results in East Africa. (Van der Vloedt, 1992).

Glossina species are also susceptible to entopathogenic fungi. The contaminated flies with the fungus, contaminate other organisms of the same species in the field during mating and body contact (Maniania and Takasu, 2006).Predators such birds, mongoose, rodents, snakes, ants and bats predate on tsetse flies whereas parasitoids deposit their eggs in the tsetse flies pupae laid on the ground. Pathogens have however, not yet been used in biological control (Reichard, 2002) of tse tse.

2.5.3 Genetic control

Tsetse flies are thought to be susceptible to genetic methods because of their low reproductive rate. Genetic method includes introduction of sterile males into a population, cytoplasmic incompatibility and use of semi sterility (Vreysen and Marc, 2006).

The sterile Insect Technique involves the large scale production of insects in laboratory colonies in mass rearing facilities. The males are sexually sterilized by exposing them to precise and specific dose of ionizing radiation. Afterwards the sterile males are sequentially released into the target insect population in large numbers to out compete the wild male files for wild virgin females, thus no viable progeny. Sustaining of Sterile Insect Technique ensures that the size of the target population declines and eventually can become extinct (Vreysen and Marc, 2006).

Glossina austeni has been successfully eradicated from Zanzibar using this method (Vreysen *et al*., 2000). Current research on genetic control of tsetse flies involves the development of transgenic symbionts of the tsetse fly (Aksoy, 2005). The reproductive potential of vector is altered through Sterile Insect Technique via irradiation or chemical sterilization (Vreysen and

Marc, 2006). Male sterile flies should live sufficiently long to transfer their sterile sperm to many virgin females as possible. During mating with the sterile male the spermathecae of the female is filled up with unfertile sperms. Subsequent mating with fertile males does not influence the female sterility due to mechanical barrier by the filled spermathecae.

Further studies by Kariithi *et al.*, (2013) revealed that control of the target populations would be eradicated when release of sterile males is applied on an area wide on entire insect pest populations. However, Sterile Insect technology can only be successful mainly in isolated foci.

2.5.4 Chemical control

Studies by Committee, (2011) showed that the persistence of an insecticide will depend upon the physical chemistry of the insecticide and its formulations. Synergists can be implemented in the insecticides in reduction of metabolic resistance of the flies and increase the killing effect and efficacy of the insecticides. Hossain, (2015) further showed that when insecticides are released in the environment they undergo many processes where they vaporize and enter the atmosphere and breakdown through microbial and chemical pathways into less toxic compounds. When synthetic pyrethroids are exposed to sunlight, water and oxygen their decomposition is accelerated. Absorbed insecticides may be broken down on the animal skin or be released into the environment hence loss of toxicity (Hossain, 2015).

Studies have shown that the insect's enzyme in the body detoxifies the insecticides enhancing the longevity of the vectors and prevents the insecticide reaching the intended site of action (World health Organization, 2012). This leads to control failure and reduction of efficacy, calling for new strategies for managing tsetse population (World health Organization, 2012) Some insecticides with different mode of action have a shorter residual efficacy requiring more than one round of spraying (World health Organization, 2012) making them expensive.

Studies by Barnard, (2000) figured out that pyrethrum insecticides contains several active compounds which are toxic to insect and tend to be effective and persistent thus causing biting flies which are landing to die or quickly become incapacitated. Pyrethrum insecticides acts on insects by causing immediate paralysis and evidenced by the immediate knockdown of household aerosol sprays. Fabric treated with pyrethroid insecticide such as Permethrin repels insects at a distance and even kills them before feeding.

Some factors such as rate of absorption, penetration of the insecticide on the skin and chemical modification on the skin causes variation in the knockdown effect and efficacy of the insecticides. Evaporation, abrasion, perspiration and washing of the treated surfaces also causes physical loss of the repellents (Barnard, 2000).The repellent effects of insecticide has a positive impact on the knockdown rates because flies that cannot feed are more sensitive to the exposure of the administered dose and mostly likely to die (Gimonneau *et al.,* 2016) .

Findings by Hertz, (2007) showed that certain factors will enhance attraction of flies to their feeding host. Surfaces which are more attractive to the flies cause faster mortalities because of increased exposure to the insecticides. Dark, rough surfaces are more favorable to light, smooth surfaces. Flies landing on the cords receive large dose of insecticide through the thorax and abdomen in comparison to those landing on the flat surfaces of the lattice where the insecticide is absorbed through their tarsi or imbibed (Hertz, 2007).

Dichlorodiphenyltrichloroethane (DDT) and Benzene Hexa Chloride (BHC) were the available synthetic compounds applied as ground spraying by 1945. DDT was earlier used as a selective application to tsetse habitats by ground spraying. It has long persistence and its single application can remain for 2-3 months in the environment (Food and Agricultural Organization 1999). DDT and BHC have also been used extensively to control fly strike although they have been replaced by Aldrin and Dieldrin which are more effective. Organophosphates and organic pyrethroids have since replaced DDT in controlling sheep cab. DDT and BHC have been abandoned due to

their effect on non-target organisms and residual effects on the environment (Kagbadouno *et al.*,2011).

Since synthetic pyrethroids are hardly broken down by sunlight, they tend to stick on animal body surfaces in the dry season for weeks enabling the prolonged effect. For instance, Deltamethrin residual effect has been reported to last for 5 and 6 weeks post treatment hence it is a good acaricide for the farmer's to use (Ouma, 2010). Deltamethrin impregnated on traps and targets also has a direct killing effect on biting flies coming into contact with the treated surfaces or indirectly through knockdown effect (Bett and Gatuma 2008). In livestock breeding areas, tsetse flies will tend to feed on belly or legs of the insecticide treated host. Therefore dipping of cattle regularly in a solution of 0, 0038% to 0,005% of Deltamethrin is an approved form of vector control (Kotlyar, 2010; Torr *et al.,* 2007) .Deltamethrin is however highly toxic to aquatic life, particularly fish, is and must be used with caution around water. Although generally considered safe to use around humans, it is still a neurotoxic to humans. Deltamethrin is able to pass from a woman's skin through her blood and into her breast milk (Buowman *et al,* 2006). In term of cost Deltamethrin is more expensive than Sypertix®.

Other synthetic pyrethroids like Cypermethrin have been used to control a wide range of ectoparasites; however exposure to sunlight, water and oxygen will accelerate its decomposition. Cypermethrin is also highly toxic to fish, bees and aquatic insects, according to the National Pesticides Telecommunications Network (NPTN, 1997) (http://ace.orst.edu/info/extonet/ 15 Jan /2018).

2.6 Efficacy of Insecticides used in tsetse control

Insecticide treated cattle (ICT) by pyrethroid pour-on solutions are used in reduction of trypanosomiasis transmission (Maia, 2009). Application of pyrethroid based insecticides causes' reduction of ectoparasites and transmission of tick borne diseases. The efficacy of the insecticide is enhanced by applying it on the predilection sites such as legs and belly of the animals. Studies

by Quarcoo *et al.*, (2010), showed that some chemicals are effective at lower rate. The isomeric ratios in the pyrethroids vary in the toxicities of the insecticides with their efficacy and the selectivity defending on the chemical and physical properties.

Studies in Chad revealed that treating of cattle with 0.005% Deltamethrin and Alphacypermethrin using foot baths is cost effective and preserve the demonstrated efficacy of the insecticides by significantly reducing the apparent densities of *Glossina pallidipes gambiensis and Glossina tachnoides.*

Deltamethrin has also successfully controlled bovine trypanosomiasis in some parts of Africa. Its persistence in the field trials reduces the prevalence of trypanaosome and tsetse apparent density. Deltamethrin pour on insecticide has a repellency effect which decreases the number of flies landing and eventually paralyzes flies before they can take a blood meal. Cases of *Glossina mortisans submortisans* transmission rate reduction have been noticed during the use of Deltamethrin pour on formulations (Rowlands *et al.*, 2001).

Effective repellent effect and safety in application have been observed with Permethrin, however the breakdown of Permethrin by sunlight, deterioration and its loss in the environment decreases its knockdown ability and the kill effect. Weathering of the material by environmental factors such as temperature and humidity also reduces its effectiveness (Banks *et al.*, 2014). It is known to be highly toxic to fish, bees and other animals (http://npic.orst.edu/factsheets/PermGen.html#wildlife,9th Feb/ 2018).

Studies by Mangwiro *et al.*, (1999) showed that 0.1% Lambdacyhalothrin applied on cloth resulted into tsetse mortalities of above 70% in two consecutive weeks of exposure. Increasing its concentration on material decreases its decomposition rate hence increasing the insecticide efficacy. The insecticide poor performance is affected by its rapid loss in the atmosphere and it is easily washed by heavy rains which causes rapid loss of active ingredients during the wet season.

Insecticide like Fipronil can also be used as an alternative insecticide in controlling tsetse flies. It however does not prevent tsetse flies from feeding unlike to other pyrethroids like Deltamethrin, although high mortalities of the feeding flies have been recorded (Bauer and Baumann, 2015).

2.7 Knockdown effect of insecticides on tsetse flies

Knockdown is a highly significant mode affecting the mortality directly because 85% of the flies' knocked down will later die through predation. Studies by Barnard, (2000) figured out that pyrethrum insecticides contains several active compounds which are toxic to insects and tend to be effective and persistent in causing biting flies to die or quickly become incapacitated.. The repellent effects of insecticide has a positive impact on the knockdown rate as it causes immediate paralysis on landing insects, The flies that cannot feed are more sensitive to the exposure of the adminis tered dose and are mostly likely to die (Arora, 2000).

Studies by Rehman *et al.*, (2014), showed that synthetic insecticides such as Deltamethrin have a disabling effect on the feeding insects. Tsetse flies feeding on the insecticide treated cattle will be killed by picking up a lethal deposit of the dose used on the ventral tarsal spines and pre-tarsi when feeding. This increases the mortality of tsetse flies when exposed to insecticide treated traps (Bett and Gathuma, 2008).

Knockdown of fed *Glossina pallidipes* on Deltamethrin treated cattle at the 12h of exposure exceeds 90% for 15 days and 7% knockdown of the unfed tsetse flies on the sprayed oxen. The effect of Deltamethrin (Decatix®) is long-lasting and more effective than that of Alphacypermethrin or Flumethrin (Leak 1999). About 2.5% of the female tsetse flies in population are killed per day. The knockdown effects contribute to the controlling of tsetse flies with 95% of the population being eradicated within 12 months. Further laboratory experiments have shown Deltamethrin Spot on ® to cause 90% mortality for up to 20 days' post exposure ,and a significant knockdown of up to 75 days. Low concentration of Deltamethrin results to

prolonged knockdown and mortality of tsetse flies which has been indicated in control of *Glossina tachnoides* in pigs (Leak 1999). Deltamethrin is however costly and has some undesirable effect already discussed above.

Sypertix® is an acaricide currently registered for control of ticks on cattle by dipping and spraying. Use of Sypertix® has been reported to be a possible cost effective control method that can be adopted by farmers in controlling both ticks and tsetse flies (Bardosh *et al.*, 2013). It has high levels of toxicity for most insects including tsetse flies. It's very stable and only mildly toxic to mammals. Sypertix® is mostly safe to handle with no undesirable effects on other non-target animals (https://www.rti.org/sites/default/files/kaves_persuap.pdf 20th/Feb/2018).

CHAPTER THREE: MATERIALS AND METHODS

3.1 Description of the study site.

The study was conducted in the wet and dry seasons in Mulili, Kaasuvi and Kiboko study sites located in Kiboko Makueni County (Figure 2). Kiboko is an area located between latitudes of 1.71° and 2.63° south and longitudes of 36.71° and 37.94° East in Makueni in the semi-arid land of Kenya. The study sites comprises of arid and semi-arid land that receives bimodal rains characterized by dry and wet seasons. It receives annual rainfall of 600mm.The long rainy season occurring from March to May is characterized by high rainfall density, high humidity and low temperatures (Obiero *et al.*, 2014). The total amount of rainfall recorded in May and June was 70.1 mm and 1 mm respectively. The minimum and maximum temperatures of the area ranges from 13°^{C} to 42°^{C} but during the field trials the mean minimum and maximum temperatures ranged from 15°^{C} to 28°^{C} whereas the humidity was >80%. The dry season of bioassays was done between September and October. This season had no rains and it was characterized by high temperatures of >30°C. *Glossina pallidipes* which is a major vector for animal trypanosomiasis is widely distributed in the area. Livestock keeping with cases of Animal trypanosomiasis has been reported with distribution of *Glossina pallidipes, Glossina longipennis* and *Glossina brevipalpis* species widely spread in the area. Historically, the area has low agricultural potential due to high tsetse infestation. This has also reduced the population density of the people (Nthiwa *et al.*, 2015)

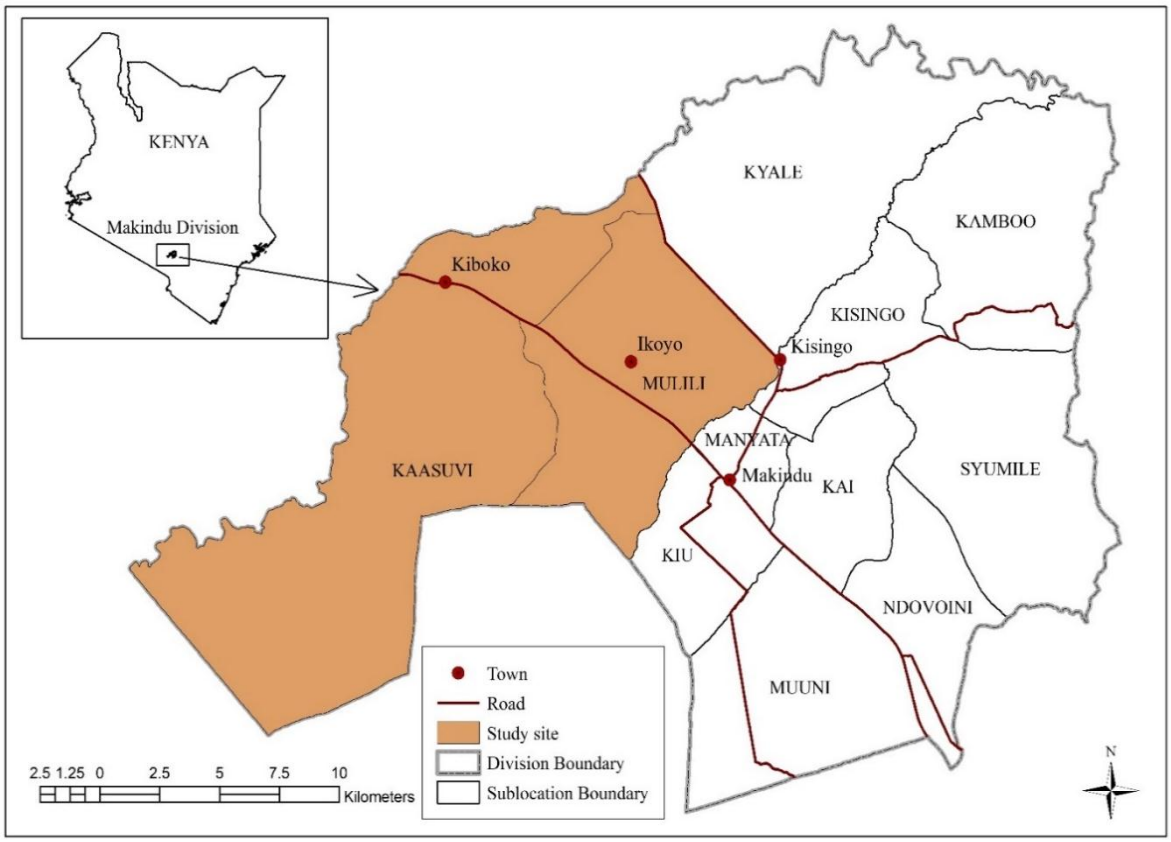


Figure 2: Map of the three trial sites at Kiboko, Mulili and Kasuvi, Makueni County, Kenya, 2015

3.2 Choosing of the study sites

The three sampled study sites were Mulili, Kaasuvi and Kiboko in Makueni Kenya. The three sampled study sites were selected from within Kiboko in Makueni County. The three study sites that is; Mulili, Kaasuvi and Kiboko were located at a distance of approximately 1K.M apart to have no significant differences in climate change and general weather conditions in both wet and dry seasons. The study sites were conveniently selected because of the extensive grazing systems by Kamba people. The study sites were accessible and had large herds of cattle with a history of trypanosomiasis and invasion of tsetse flies (Adrian, 2012). The group I cattle which was located at Mulili study site, received the trial product (Sypertix®).The group II- Domenix® (Positive reference) treated group was located at Kaasuvi study site. The group III cattle –the Control (untreated) group was located at Kiboko study site and received no treatment. The three study sites were used in both dry and wet seasons.

3.3 Selection of experimental cattle

The study animals were short honed zebu cattle. The owner's willingness to participate in the study determined the criteria of selection of the study animals. The herd of the farmers selected in the study had a capacity of 20 -35 number of cattle. Six female cattle were chosen in each study site. The animals selected were based on the following criteria (i) The animals had no history of the application of the insecticides for the last one month (ii)The age was between 3-5yrs (iii) The cattle had no trypanososomiasis.

The selected eighteen experimental cows in all the three groups were ear tagged for case of identification (Adrian, 2012).

3.4Chemical treatments and formulations

Cattle were treated separately with one of the following commercial formulations of Alphacypermethrin: Sypertix® 10g/ L Emulsion Concentrate of Alphacypermethrin diluted with

water to concentration of 0.01g/L. (b) Domenix® 100g/L Emulsion Concentrate of Alphacypermethrin diluted with water to concentration of 0.1 g/L . Insecticides were applied according to the instructions of the manufactures. Both insecticides were applied with a knapsack sprayer to the entire body of the cattle. Treatments used in each group were applied only once in week zero of the dry and wet seasons. The efficacy of the Sypertix® spray was assessed using the experimental design below.

Group I cattle

The group I cattle –Treated with sypertix®, was selected from Mulili study site in Kiboko. This comprised of six female cows which were all ear tagged for identification and ready for use in both dry and wet seasons. The cattle were sprayed as recommended for tick control since the product is also expected to control them, thus 10 ml of Sypertix® to 20 l of water. A thorough whole body spray of cattle was carried out only once at the beginning of the bioassay in both wet and dry seasons.

Group II cattle

The group II cattle –Treated with Domenix® was located at Kaasuvi study site in Kiboko. The six selected cows in this study site were all ear tagged for identification. They were treated with positive reference (Domenix®) an Alphacypermethrin stock spray, already registered with Pest Control Product Board for tsetse control. The same selected cattle were used in both wet and dry seasons during the assessment of the bioassay.

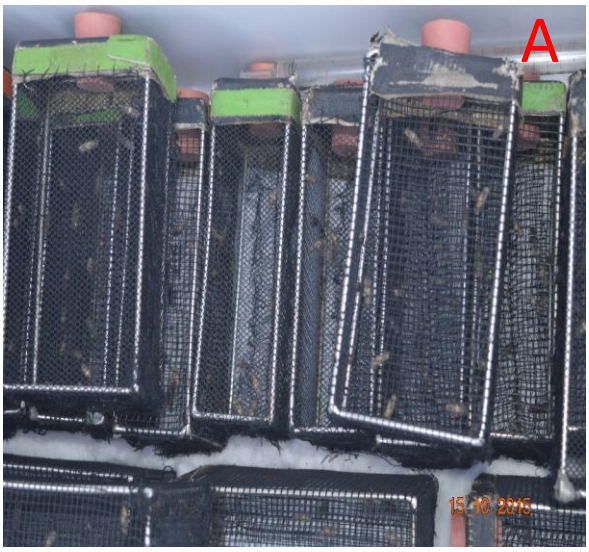
Group III cattle

The group III cattle–The untreated group was located at Kiboko study site. All the six cows used in this group were ear tagged for identification and acted as a control to group I and II.The cattle in this group received no insecticide treatment and were used in both dry and wet seasons.

3.5 Assessment of bioassays

Experimental *Glossina pallidipes* were obtained from KARI-TRC insectary and the exposure of tsetse flies to the experimental cattle was carried out weekly for four consecutively weeks in both dry and wet seasons. The exposures were carried out the next day following insecticide application to allow the insecticide to dry on body of the cattle. All the three groups in each study site were held in separate pens during the bioassay assessment. Male tsetse flies from the laboratory colony were exposed to clipped netting over each treatment for exposure time of ten minutes. Percentage feeding success and knockdown effect of the flies was recorded after exposure to netting exposed in the field site.A Geigy cage containing 30 male *Glossina pallidipes* was exposed to each of the six cattle per treatment for all the 18 experimental cattle. New batches of live tsetse flies were used each time of the bioassay (Figure 3A). The exposure involved strapping the cage of flies onto the flank of the cattle for 10 minutes after which the cages were removed from the cattle flanks. The cages were covered with a black cloth during the feeding of the tsetse flies (Figure 3 B). Feeding success was determined as the number of flies that were fully engorged from each cow. Failure to feed was noted as blood feeding inhibition. Feeding success was observed immediately after 10 minutes of exposure. The knockdown effect was observed at an interval of 0,5,10 and 20 minutes whereas mortality was recorded at the 24^{th} hour post treatment (Alemu 2013; Bauer and Baumann, 2015).

Batches of unfed tsetse flies



Tsetse feeding on the cow

Figure 3: Batches of live laboratory reared male *Glossina pallidipes (*A) and a cage strapped on the cow flank (B)

3.6 Duration effect of the studied parameters

This is the significant interaction between the treatment effect and the treatment type-The time action of the treatment after the ten minutes of the exposure, where the following parameters of feeding success, knockdown effect and mortality were assessed through bioassays at weekly intervals. This led to the determination of efficacy-which is the Function of the number of flies that contact the animal for the sufficient time to pick the lethal dose of insecticide (Torr *et al.*, 2009) or the assessment of the innate level of activity for the active substance being tested which is most effective.

(i) Determination of feeding success

Feeding success is the nutritional status of the tsetse flies at the time of taking the blood meal. It was observed immediately after the ten minutes of exposure. The level of engorgement on the tsetse flies abdomen was observed after feeding and the number of flies that fed was determined at two categories of fed and unfed .Fed tsetse flies successfully took a blood meal from the experimental cattle and had an engorged abdomen whereas unfed tsetse flies were not successful in taking the blood meal from the cattle and therefore their abdomen was not engorged (Figure 4A). Failure of the flies not to feed was ascribed as feeding blood inhibition.

(ii)Determination of knockdown effect

This is when an insect is unable to carry coordinated movement but has not been killed (Figure 4B). In this study influence of time after treatment on knockdown and recovery from Alphacypermethrin (10%EC Sypertix®) and Alphacypermethrin (100%EC Domenix®) was carried out on *Glossina pallidipes* during the wet and dry seasons. The knockdown effect of the insecticides was observed after exposing the caged tsetse flies on the flanks of the experimental cattle for ten minutes ,after which the cages were removed and knockdown rates observed at intervals of 0, 5, 10 and 20 minutes (post exposure) for 4 consecutive weeks in both dry and wet seasons

(iii)Evaluation of mortality

The mortalities in each treated groups (Sypertix® and Domenix®) and the Control (untreated group) was recorded after 24th hour (Post exposure) of continuous duration of effect in both dry and wet seasons. This constituted of only dead tsetse flies after exposure to the experimental cattle. The corrected mortalities were determined using Abbott formulae. In cases where the exposed tsetse flies to the experimental cattle were not knocked down in 20 minutes, their fate was recorded after 1, 2, 4,8,20 and 24 hours post exposure to help in evaluating the mortality rate

at the 24th hour. The mortality after the 24th hour was used to determine the efficacy of the insecticide. The efficacy of the insecticide was determined when at least 80% of flies were knocked down after 24 hour (Post exposure) after which 80% knockdown was achieved within 24 hours was ascribed to be the effective duration of insecticidal action, after which re-treatment will be required. 80% was considered to be most efficacious. This was termed as effective duration of insecticidal action (Alemu, 2013).

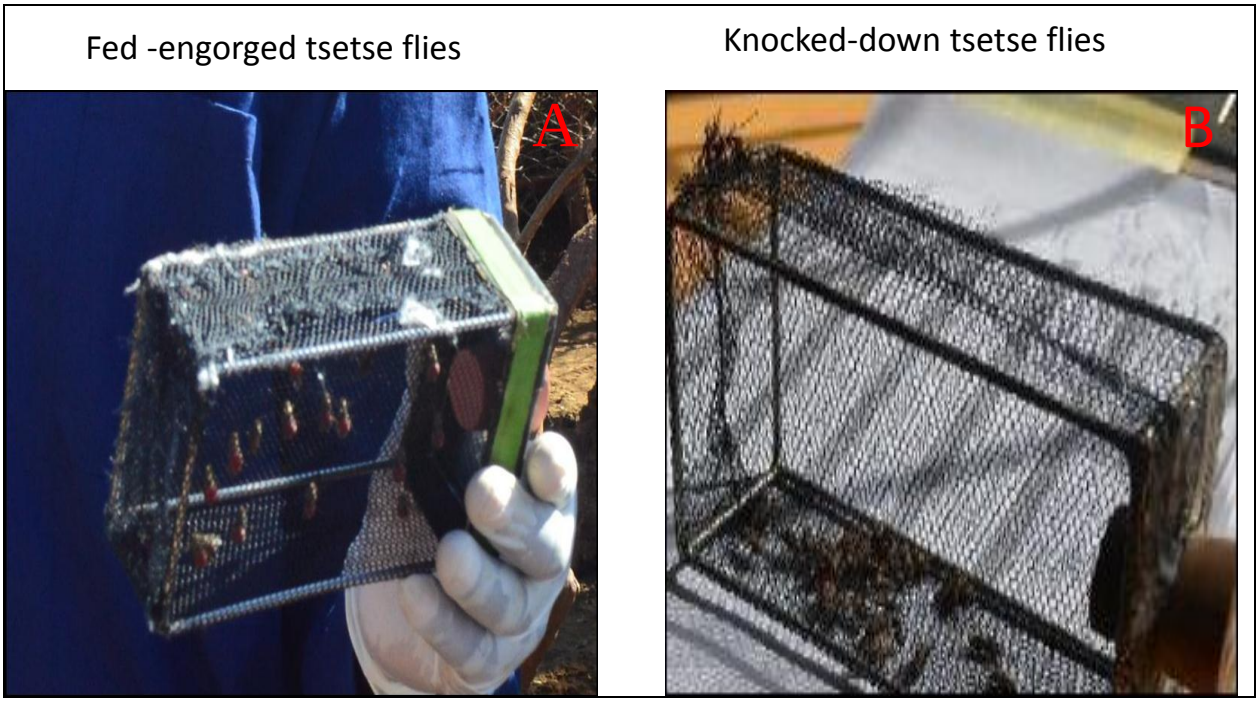


Figure 4: A Batch of fed and engorged (A) and a cage of knocked-down male *Glossina Pallidipes* (B) after the bioassay.

3.7Data analysis

Data obtained from the study was entered in Microsoft Excel (2000) spreadsheet, computed into percentages. All statistical analysis were performed using GENSTAT a statistical package. Treatment means were compared and separated using Analysis of Variance. Separate and different means that were significant in groups were separated using Tukey's HSD test. Significance to all test were determined at $P \leq 0.05$. For analysis purposes, numbers of dead tsetse

flies were expressed as proportion of total tsetse flies exposed (N=30).The arcsine square root transformation was employed for all variables prior to analyses to normalize variances. The feeding success, knockdown effect and efficacy of treated groups (Sypertix® and Domenix®) and Control (untreated group) among the groups was determined and different means considered significant different or not accordingly (Simmonds *et al.*, 2005). As a result of substantial mortality occurring in the control treatment due to natural causes, the observed mortality of the treated samples was corrected by Abbort formula, as follows:

$$\text{Observed mortality} = \frac{\text{Total number of dead tsetse flies}}{\text{Total sample (N)}} \times 100$$

Corrected mortality %=((% Survival control (X) - %Survival treated(Y) / %Survival control) ×100 (World Health Organization, 2009)

CHAPTER FOUR: RESULTS

4.2Feeding success of tsetse flies fed on insecticide treated cattle

The feeding success in the wet and dry seasons is shown in table 1 below. Three groups of cattle (treated with 10% EC Sypertix®, treated with 100% Domenix® and Control (untreated) group each comprising of six cattle were used to determine the feeding success of tsetse flies. The feeding success of tsetse flies increased with significant variations from week zero to the third week in the treated groups (Sypertix® and Domenix®), while there were no significant variations in the fourth week in both the dry and wet seasons (P=0.05). There was a significant difference in feeding success from week 0 to week 3 between the treated groups and the control group. An average of 90% tsetse flies fed successfully on the Control (untreated) group in all the weeks of the bioassay in wet and dry seasons in this study. In week 4 of the wet and dry seasons, no significant variation (P=0.05) was observed between the treated groups (Sypertix® and Domenix®) and the Control (untreated) group. The average feeding success of tsetse flies was 42.7% for Sypertix® and 56.2% for Domenix® during the wet season and, 46% and 38.9% in Sypertix® and Domenix® treated groups in the dry season, respectively.

Table 1: Average feeding success of laboratory reared male *Glossina pallidipes* on treated cattle (10% EC Sypertix® and 100 EC Domenix®) and Control (untreated) group in the dry and wet seasons

Seasons	Treatments	Percentage feeding success				
		Weeks (Post Exposure)				
		0	1	2	3	4
Wet	Sypertix®	11.12 ± 3.4^{g}	20.55 ± 1.8^{fg}	45.55 ± 5.49^{de}	56.68 ± 6.20^{cd}	79.45 ± 5.47^{ab}
	Domenix®	5.02 ± 2.7^{f}	35.55 ± 6.86^{ef}	75.57 ± 7.34^{bc}	88.35 ± 0.74^{ab}	76.67 ± 4.87^{ab}
	Control	96.12 ± 1.60^{a}	94.43 ± 2.54^{ab}	95 ± 2.55^{a}	87.22 ± 2.00^{ab}	83.9 ± 2.0^{ab}
	F=30.66;df=8,89; P=0.05					
Dry	Sypertix®	23.9 ± 1.12^{ef}	31.17±2.13d^{ef}	$42.21^{cde}\pm7.17$	51.1 ± 2.44^{cd}	81.67 ± 2^{ab}
	Domenix®	5.57 ± 1.22^{f}	11.11 ± 3.67^{f}	39.45 ± 14.78^{cde}	57.77 ± 2.78^{bc}	80.55 ± 4.65^{ab}
	Control	98.9 ± 0.74^{a}	92.78 ± 2.95^{a}	87.25 ± 2.54^{a}	92.67 ± 2.78^{a}	82.78 ± 4.03^{ab}
	F=13.56 ; df=8, 89; P =0.05					

Means Means followed by the same letter within columns are not significantly different (Tukey's HSD at P ≤0.05).
HSD-Honest Significant Difference

4.2The knockdown effect of 10%EC Sypertix® on tse tse flies

The results of each week were compared for the persistence of the insecticides (Sypertix® and Domenix®) in both wet and dry seasons. There was a significant higher number of tsetse knockdown in the treated groups (Sypertix® and Domenix®) than in Control (untreated) group (P=0.05). The knockdown rates of *Glossina pallidipes* exposed to control (untreated) group were <2%. As these percentages were so low in the control group, the knockdowns for the various insecticidal treatments (Sypertix® and Domenix®) were not corrected for the control by Abbott formula. During the wet season the percentage knockdown across the three groups: Alphacypermethrin (Sypertix®), Alphacypermethrin (Domenix®) treated cattle and Control group

had no significant variation (P=0.05) from week zero to the 4th week but varied significantly (P =0.05) between the treated groups (Sypertix® and Domenix®) and the control (untreated) group (Table 2). In week zero of the wet season, high insecticide knockdown effects of 99.7% and 86.1% was observed in Domenix® and Sypertix® treated groups, respectively. A knockdown rate of 0.69% was observed in the control (untreated) group.

In week one of this study, Alphacypermethrin (Domenix®) had the highest average knockdown of 87.4%, whereas Alphacypermethrin (Sypertix®) had 78.2% and 1.1% knockdown effect was recorded in the Control (untreated) group. The highest percentage knockdown of 66.2% was observed in Alphacypermethrin (Sypertix®) treated group whereas an average of 42.2% was observed in Alphacypermethrin (Domenix®) treated group and the least was 0.85 % in the Control group in the second week of this study in the wet season (Table 2 and Figure 5).

In week three of this study, Alphacypermethrin (Sypertix®) recorded the highest average knockdown of 37.4% and percentage knockdown of 28.9% and 0.8% was observed in Alphacypermethrin (Domenix® treated group) and Control (untreated group) , respectively (Figure 5).At week four, the highest percentage knockdown of 29.16% was observed in Sypertix® treated cattle whereas 27.22%. and 1.56% was observed in Domenix® treated cattle and Control (untreated group), respectively (Table 2 and figure 6).

Table 2: Weekly percentage knockdown means (±SD) of male *Glossina pallidipes* from week 0 to 4 after 10 minutes of exposure on untreated (Control) and treated cattle with Alphacypermethrin (10%EC% Sypertix® and 100EC% Domenix®) in the wet season.

Weeks	Percentage knockdown post exposure			F[1]	P=Value
	Treatments				
	Sypertix®	Domenix®	Control		
0	99.71±5.83[a]	86.12±0.83[b]	0.69±0.56[c]	1.49	0.199
1	87.36±14.3[a]	78.20±12.05[a]	1.1±0.65[b]	4.67	0.07
2	66.23±15.05[a]	42.23±9.61[b]	0.85±0.6[c]	5.94	0.122
3	37.4±6.56[a]	28.9±3.16[b]	0.83±0.56[c]	6.85	0.796
4	29.16±3.36[a]	27.22±3.4[a]	1.5±0.57[b]	1.56	0.175
	Degrees of freedom for each comparison are 6, 71				

Means followed by the same letter within rows are not significantly different (Tukey's HSD at P≤0.05). HSD-Honest Significant Difference

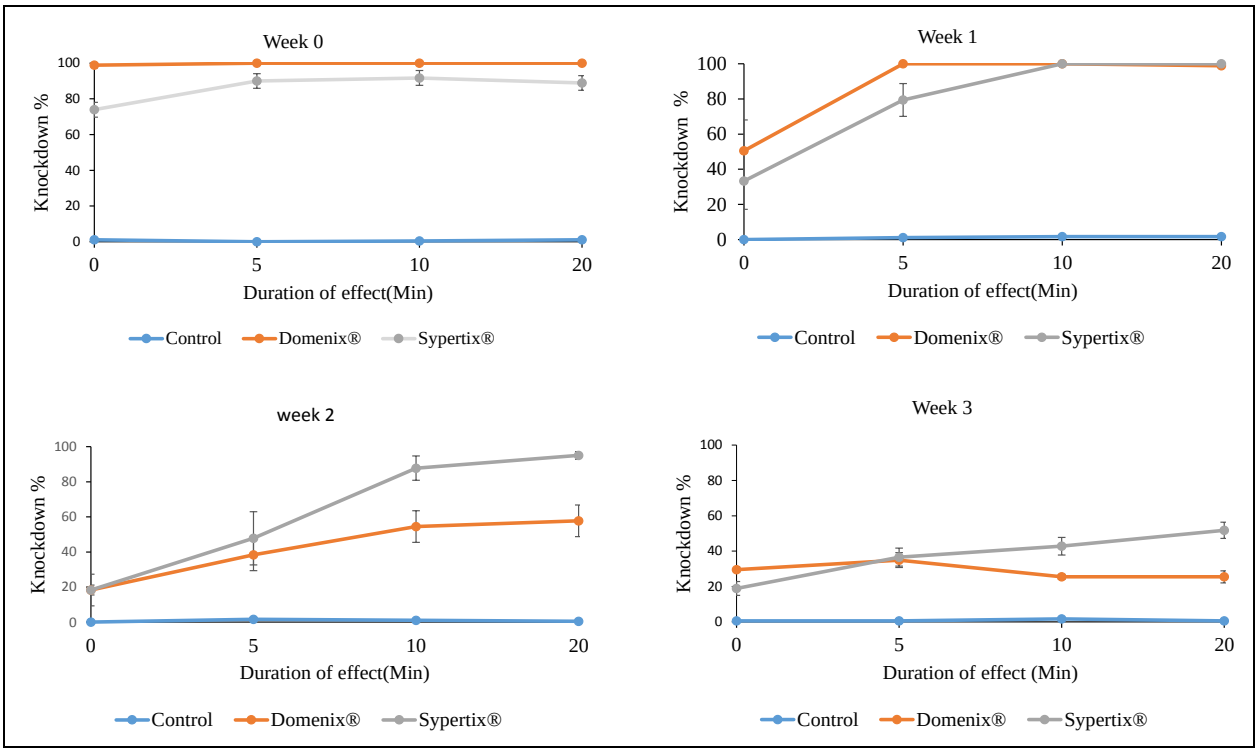


Figure 5: Percentage knockdown trends of male *Glossina Pallidipes* from week 0 to 3 at 0,5,10 and 20 minutes in treated cattle and untreated cattle in wet season

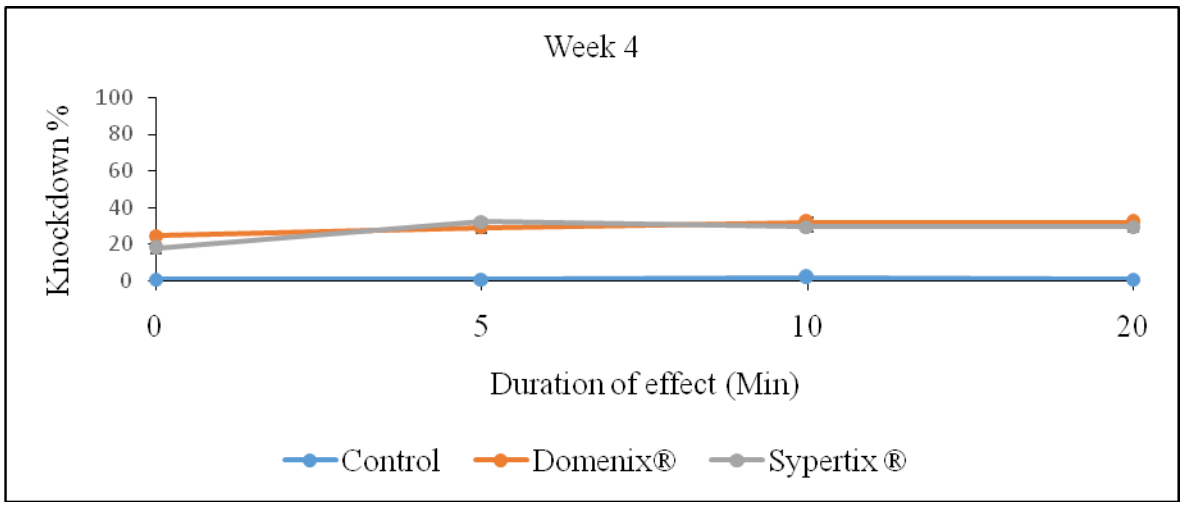


Figure 6: Percentage knockdown mean of male *Glossina pallidipes* in the 4th week after 10 minutes of exposure on treated cattle and control group (untreated) group in the wet season.

During the dry season the percentage knockdown across the three groups: Alphacypermethrin (Sypertix®), Alphacypermethrin (Domenix®) treated cattle and Control group had no significant variation (P=0.05) from week zero to the 4th week but varied significantly (P =0.05) between the

treated groups (Sypertix® and Domenix®) and the control (untreated) group (Table 3). At week zero of the dry season, 100% of the flies were knocked down in the Domenix® and Sypertix® treated groups. The percentage knockdown in the control (untreated) group was 0.4%. In the 1st week of the wet season, the percentage knockdown was similar in the treated cows with 95.4% and 94.5% in Sypertix® and Domenix® treated groups, respectively. The Control (untreated) group had a percentage knockdown of 1.7 %. The highest percentage knockdown of 68.2% was observed in Domenix® treated group followed closely by 67.6% in Sypertix® treated group and the least percentage knockdown of 1.4% was observed in the Control (untreated) group in week two of this study, in the dry season (Figure 7).

In week three of this study, the percentage knockdown in Sypertix® and Domenix® treated groups were 50.8% and 32.9%, respectively. The control group had the least knockdown of 1.3%. In week four, the percentage knockdown of 32.9% was observed in Domenix® treated group, 21.1% in Sypertix® treated group and 0.55% in Control (untreated) group (Figure 8).

Table 3: Weekly percentage knockdown means (±SD) of male *Glossina pallidipes* from week 0 to 4 after 10 minutes of exposure on treated cattle with Alphacypermethrin (10%EC% Sypertix® and 100EC% Domenix®) and Control (untreated) group in the dry season.

	Percentage knockdown (Post exposure)				
	Treatments				
Weeks	Sypertix®	Domenix®	Control	F[1]	P-Value
0	100±0[a]	100±0[a]	0.4±0.22[b]	1.49	0.197
1	95.4±2.14[a]	94.9±3.14[a]	1.7±0.49[c]	1.77	0.312
2	67.6±3.80[a]	68.2±5.68[a]	1.4±0.33[b]	2.40	0.400
3	50.84±2.14[a]	32.91±2.0[ab]	1.27±0.45[b]	0.84	0.544
4	21.1±2.71[b]	32.91.6±2.0[a]	0.55±0.25[c]	0.52	0.794
	Degree of freedom for each comparison are 6,71				

Means followed by the same letter within rows are not significantly different (Tukey's HSD at P≤0.05). HSD-Honest Significant Difference

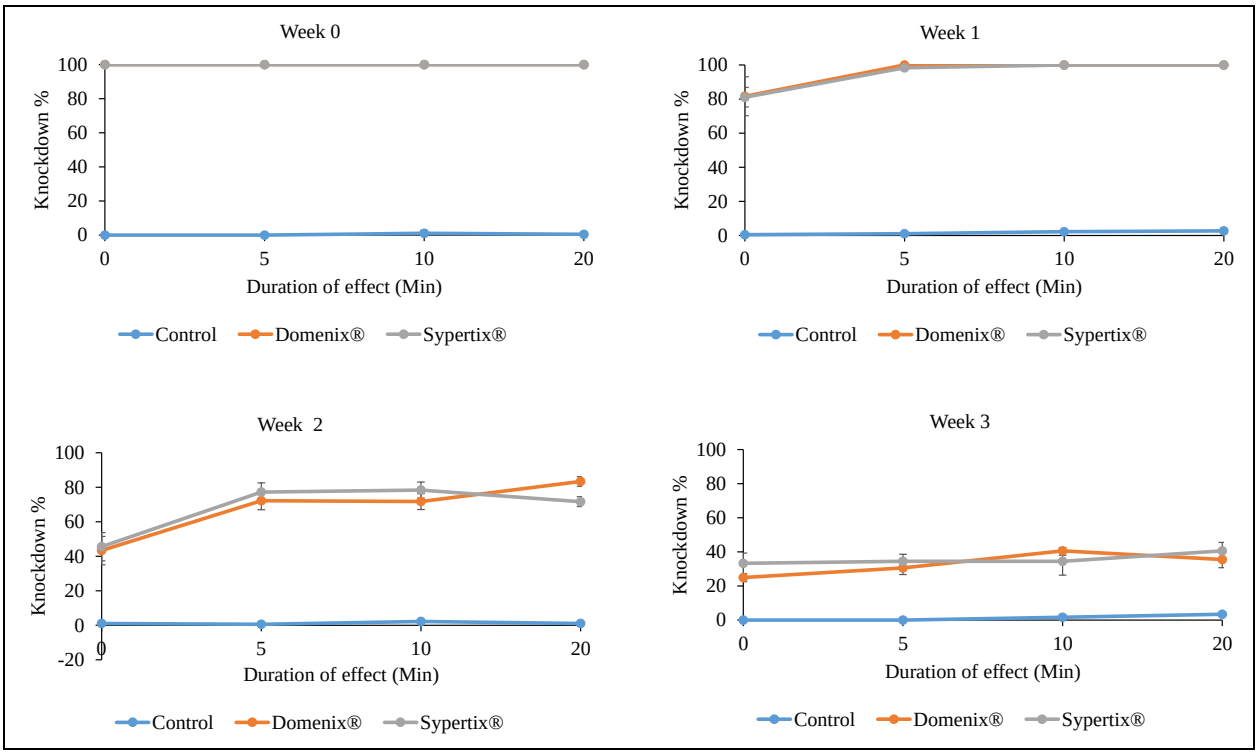


Figure 7: Average percentage knockdown of male *Glossina Pallidipes* from week 0 to the 3rd at 0,5,10 and 20 minutes on treated cattle with Alphacypermethrin (10%EC Sypertix®and Alphacypermethrin 100%EC Domenix®) and control group in the dry season.

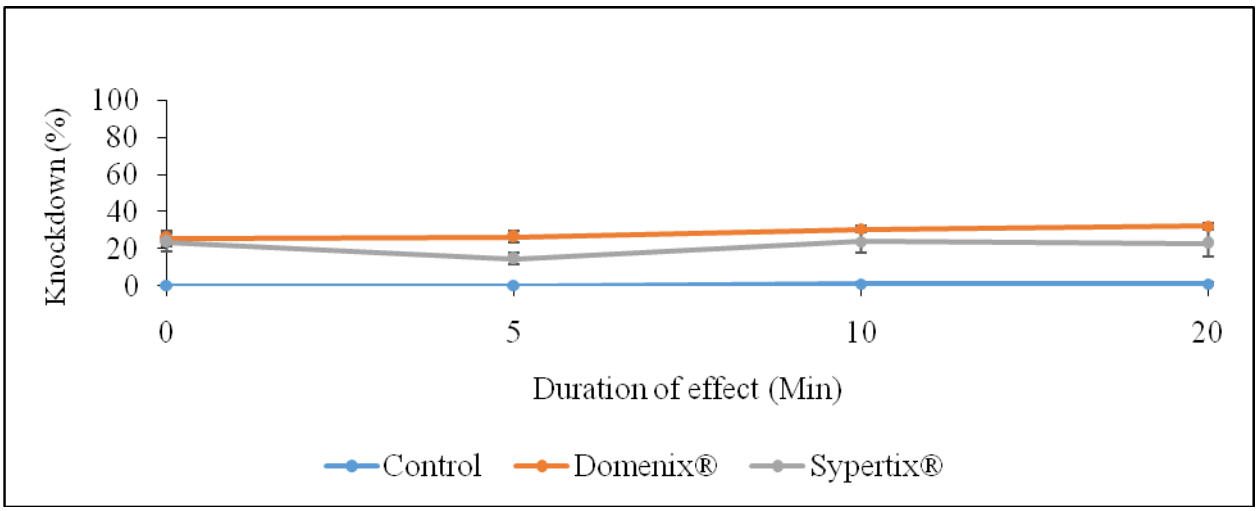


Figure 8: Percentage knockdown mean of male *Glossina pallidipes* in the 4th week after 10 minutes of exposure on treated cattle and control group (Untreated group) in the dry season.

4.3 Mortality rates of tsetse flies exposed to insecticide treated cattle

The mortality rates of tsetse flies exposed to treated groups (Sypertix® and Domenix®) and Control (untreated) group was evaluated at the 24th hour post exposure in every week of the bioassay in both wet and dry seasons. The results of 10%EC Sypertix® -the trial product-, 100%EC Domenix® -the Positive reference- and the Control (untreated) group were compared for efficacy. Higher mortality rates (64 %) were observed in tsetse flies collected from the treated cattle as compared to untreated cattle (20%). There was no significant difference (P =0.05) in mortality rates of tsetse flies between cattle treated with Sypertix® and Domenix® in dry and wet seasons. However, higher mortality numbers in tsetse flies were recorded in the Sypertix® treated cattle (70%) as compared to in Domenix® treated cattle (65%) in the wet season.

During the wet season there was a significant difference (P =0.05) in the mortality rate of tsetse flies at weeks zero, 2 and 3 among the three experimental groups but no significant difference (P=0.05) in the 1st and the 4th week between the two treated groups (Sypertix® and Domenix®). There was no significance difference (P=0.05) in mortalities among the three groups in week 4 (Table 4). In week zero, 1 and 2 of the dry season, there was no significant difference (P =0.05) in the mortality rates of tsetse flies between the treated groups (Sypertix® and Domenix®) and the Control (untreated) group (Table 5). There was however a significant difference (P=0.05) between the treated groups (Sypertix® and Domenix®) and Control (untreated) group in week 3 and 4 in the dry season. The 10%EC Sypertix® produced high tsetse fly mortalities of 80%within the first two weeks in both dry and wet season (Table 5).

Table 4: Mean percentage on Observed and Controlled mortality (%) (±SD) of male *Glossina Pallidipes* exposed to untreated and treated cattle with formulations of Alphacypermethrin (100%EC Domenix®) and Alphacypermethrin (10%EC Sypertix®) in the wet season.

%Mortality	Treatments	Weeks (post exposure)				
		0	1	2	3	4
Observed	Sypertix®	94.4±5.55^{abc}	97.78±1.113^{abc}	82.10±5.3^{bc}	57.78±7.074^{d}	37.22±5.397^{e}
	Domenix®	100.00±0^{a}	99.45± 0.55^{ab}	80.02±3.101^{c}	42.20±3.408^{de}	30.57±3.375^{ef}
	Control	9.45±1.014^{g}	11.12±2.677^{g}	15.02±3.528^{fg}	12.23±1.650^{g}	13.88±1.594^{fg}
	F=26.36; df=8,89; P=0.05					
Controlled	Sypertix®	94.± 6^{abc}	97.42± 1.287^{a}	78.23± 6.30^{bc}	52.80±7.710^{d}	26.77± 6.644^{ef}
	Domenix®	100±0^{a}	99.33± 0.667^{a}	75.72± 3.978^{c}	30.05±4.358^{e}	19.42± 4.358^{ef}
	Control	9.45±1.014^{f}	11.12±2.677^{g}	15.02±3.528^{fg}	12.23±1.650^{ef}	13.88±1.594^{ef}
	F=27.09; df=8,89; P=0.05					

Means followed by the same letter within columns are not significantly different (Tukey's HSD at P ≤0.05). HSD-Honest Significant Difference

Table 5: Mean percentage on Observed and Corrected mortality (%) (±SD) of male *Glossina Pallidipes* exposed to untreated (Control) and treated cattle with formulations of Alphacypermethrin (100%EC Domenix®) and Alphacypermethrin (10%EC Sypertix®) in the dry season.

%Mortality	Treatments	Weeks (Post Exposure)				
		0	1	2	3	4
Observed	Sypertix®	100 ± 0^{a}	100 ± 0^{a}	83.35 ± 3.648^{b}	35.17 ± 1.643^{c}	27.23 ± 1.807^{cde}
	Domenix®	100 ± 0^{a}	100 ± 0^{a}	79.45 ± 7.324^{b}	38.90 ± 2.807^{c}	28.90 ± 2.383^{cd}
	Control	16.67 ± 4.038^{def}	13.88 ± 2.677^{ef}	16.65 ± 1.498^{def}	8.87 ± 3.893^{f}	11.67 ± 2.402^{f}
	F=31.23;df= 8,89; P=0.05					
Controlled	Sypertix®	100 ± 0^{a}	100 ± 0^{a}	79.80 ± 4.705^{b}	27.984 ± 4.724^{cd}	12.43 ± 3.875^{d}
	Domenix®	100 ± 0^{a}	100 ± 0^{a}	74.68 ± 9.134^{b}	32.52 ± 3.397^{c}	$21.62\pm4.233cde$
	Control	16.67 ± 4.038^{cde}	13.88 ± 2.677^{cde}	16.65 ± 1.498^{cde}	8.87 ± 3.893^{e}	11.67 ± 2.402^{de}
	F=31.24;df= 8,89; P=0.05					

Means followed by the same letter within columns are not significantly different (Tukey's HSD at ($P \leq 0.05$). HSD-Honest Significant Difference

CHAPTER FIVE: DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1Feeding success of tsetse flies exposed to Alphacypermethrin 10%EC (Sypertix®), Alphacypermethrin 100%EC (Domenix®) and Control treatments.

5.1.1 Feeding success

The bio-efficacy results indicate a reduction in the proportion of tsetse flies that fed on treated cattle in both dry and wet seasons. The blood feeding inhibition of tse tse flies on treated groups (10%EC% Sypertix® and 100%EC Domenix®), declined with increase in time. This could be perceived as irritancy effects of the insecticides. These findings are similar to that of Waltisbuhl *et al*., (2005) who reported that Pyrethroid insecticides applied on cattle have been implicated to irritate alighting insects and prevent them from feeding. Studies by Ricardo *Molina et al.,* (2001) showed that sand flies did not feed immediately after landing on the hair of the dogs treated with 65% permethrin pour on insecticide which has a cyano group like Alphacypermethrin used in this study but flew away without feeding.

The feeding success had a significant variation (P= 0.05) between the treated groups (Sypertix® and Domenix®) and the control (untreated) group from week 0 to the 3rd week ,with >90% of tsetse flies feeding on the Control (untreated) group. The negligible blood feeding inhibition recorded in the control group was thus attributed to absence of insecticide spraying in the group. These results are in line with findings by (Vatandoost *et al.,* 2013), who showed that inhibition of blood feeding of mosquitoes on Alphacypermethrin treated nets was higher in the treated group compared to the control group. Vatandoost *et al.,* (2013), also reported that low percentages of mosquitoes fed on Alphacypermethrin treated cattle in week zero while higher numbers fed on the control group. Similar findings have also been reported by Fankhauser *et al.,*

(2015) who found a significance variation between the populations mean of mosquitoes on combination of repellent and insecticidal efficacy of Fipronil and Permethrin insecticides against treated and untreated groups of dogs, a phenomenon also observed from week 0 to the 3rd of this study.

Contrary to findings from this study, Fipronil, another synthetic pyrethroid did not prevent tsetse flies from feeding (Bauer and Baumann, 2015). Other studies by Bauer and Bauman (2015) showed a high feeding success associated with high mortalities contrary to findings in this study, where high feeding success was linked to low mortalities.

5.2 Knock down effects of pyrethroids and variations of effects in dry and wet seasons.

A 90% knockdown occurred in the insecticide treated group within 5 minutes post treatment up to the second week in both dry and wet seasons. A higher number of knockdown which declined with increase in time was observed in treated cattle (Sypertix® Domenix®) ($P = 0.05$). However, there was significant variation between knockdown observed in treated group (Sypertix® and Domenix®) compared to the control (Untreated) group. No significant difference in the knockdown rates of the insecticides (Sypertix® and Domenix®) was observed in this study from 0 to the 2rd week and the 4th week in the dry and wet seasons. Similar findings have been reported with Deltamethrin (Torr *et al*., 2007) where a significant difference between knockdown of wild tsetse flies on treated and untreated cattle was reported.

Studies by Harris *et al.*, (1992) showed that targets impregnated with 3.2% Deltamethrin suspension concentrate, a pyrethroid insecticide like Alphacypermethrin produced 100% kill within 10 seconds producing significant knockdown at much lower levels although in all concentrations 100% knockdown occurred within 15 minutes. The kills after 20s and 40s showed

that time has little effect on the knockdown rates but landings and amount of movement are more effective than the time spent on the impregnated material. Similar reports (Maia, 2009 ; Hougard *et al.*, 2002), recorded that Bifiethrin, a pyrethroid insecticide had the highest immediate knockdown on caged 2-5 day old mosquitoes between 2-10 minutes of exposure, in line with the results of this study.

Findings by (Maia, 2009) showed that 80% of the flies exposed to the treated nets were paralyzed after 10 minutes of exposure. This killed most of the muscids that tried to enter the pen reducing most of the flies in the surrounding area.

In this study in both dry and wet seasons, the knockdown rates varied significantly between the treated group (Domenix® and Sypertix®) and the Control (untreated group). There was however, no significance difference between cattle treated with Alphacypermethrin (Domenix®) and Alphacypermethrin (Sypertix®) during the wet season in the 4^{th} week of this study. This was attributed to lack of degradation. This observation is similar to reports of Vale *et al.*, (2015). In the 3^{rd} and the 4^{th} week of this study the knockdown percentage of Alphacypermethrin (Domenix®) treated cattle was similar to the findings by Vale *et al.*, (2015).

Tsetse flies exposed to Sypertix® treated cattle demonstrated 50% percentage knockdown up to the 3^{rd} week in the dry season and 62 % up to the 2^{nd} week in the wet season. This conforms to studies proposed by Mahande *et al.*, (2007) on contact bioassay of cattle treated with pyrethroid acaricide (Deltamethrin), which showed a knockdown effect of 50% within 21 days on mosquitoes feeding on treated grazing cattle. Reports by Torr *et al* (2007),showed that Deltamethrin (Decatix®) persisted for 20 days on the entire animal body in the dry season and 16 days in the wet season , a similar pattern of persistence of Sypertix® seen in this study.

5.3 Efficacy of Alphacypermethrin (Domenix®) and Alphacypermethrin (Sypertix®) during wet and dry seasons.

The efficacy of the insecticides was based on the mortalities of the dead tsetse flies after 24thhour of post exposure in both wet and dry seasons. The results of this study showed that tsetse flies were successfully controlled using 10%EC Sypertix® and 100%EC Domenix®. At the onset of the experiment both insecticides (10%EC Sypertix® and 100%EC Domenix®) gave the efficacy of 100% -80 % in week 0-2 during wet and dry seasons. These findings revealed that the mortality caused by both insecticides on the 24th hour post treatment was efficacious at 80%, the cut off point for rating insecticides. The mortalities results obtained were comparable but not significantly different (P=0.05) in the insecticide treated group (Sypertix® and Domenix®). Similar studies by Uragayala *et al.*, (2015) using Alphacypermethrin on treated surfaces with slightly higher concentrations of 30% and 20% respectively, led to mortality of 80% on *Anopheles* species up to 16 and 15 weeks respectively. Faraj *et al.*, (2013) using Alphacypermethrin on mosquitoes reported 100% mortality from week 0-1st although the efficacy decreased progressively with weeks in the treated groups, a feature also noted in this study.

The findings by Courtenay *et al.,* (2009) showed that formulations of Deltamethrin on san d flies had similar efficacy at the time of initial application similar to this study. Studies by Oyewusi *et al.,* (2016), using1% Flumethrin (Bayticol® pour on) on ticks fed on treated cattle also recorded 100% mortality in the 7 days post treatment similar to findings of this study where 100% mortality of tsetse was found from week 0- to the 1st. Studies by Rothwell *et al.,* (1998) indicated efficacious control of *Haematobia irritans exigua,* Diptera like tsetse flies. In their study, cattle treated with Deltamethrin pour-on had similar efficacy to Zeta- cypermethrin pour

on for 14 days but efficacy decreased more rapidly and by 28 days it was ineffective. Field evaluation of Alphacypermethrin 10% SC in indoor residual spraying by Faraj *et al.,* (2013) on leshmaniasis vectors showed mortality rates of 89.3% one day after spraying till the loss of efficacy efficiency to (70%) after two weeks similar to this study where Sypertix® was efficacious up to 80% for two weeks in both wet and dry seasons.

The efficacy of insecticides varied seasonally (P =0.05) between the tsetse flies exposed to the treated cattle in both seasons with a longer efficacy observed in the dry season and a shorter efficacy in the wet season. The shortest persistence experienced in wet season led to shortest periods of efficacy as observed in this study in Alphacypermethrin Domenix® treated cattle with 30% efficacy in comparison to 53% in Alphacypermethrin Sypertix® treated cattle in the 3rd week. Similar Studies have shown that time of exposure and death of midges in contact with Deltamethrin treated cattle was shorter in wet steers than in dry cattle although in some cases the results were similar (Schmahl *et al.*, 2009). Torr *et al*., (2007) observed that there was seasonal effect of Deltamethrin in the control of tsetse flies with persistence lasting 1 week in the wet season and 4 weeks in the dry season due to fluctuation in temperature and rainfall. Torr *et al*., (2007) observed no significance variation in the persistence of Deltamethrin (Decatix) and Deltamethrin Spot on during the efficacy to control tsetse flies. Similarly, in the 4th week, in this study, Alphacypermethrin Domenix® and Alphacypermethrin Sypertix® had no significance variation ($P \geq 0.05$) in the persistence in the wet season.

Studies by Mekonnen, (2014) showed a significant reduction of ticks on cattle treated with 1% Deltamethrin which showed 100% reduction of ticks up to the 14th day similar to this study from week 0 to week 2nd in both wet and dry seasons. In this study, tsetse flies were observed in treated cattle from the 2nd week to the 4th week in both dry and wet seasons. These could be

attributed to the decrease in the efficacy and longevity of the insecticide with increase in time. The decrease in mortalities as the time increased was due to a decrease in residual activity of the insecticides in both dry and wet seasons.

The results obtained on the persistence of treated groups (Sypertix® and Domenix®) on the 4th week of the wet season had no significant variation (P =0.05) to the Control (untreated) group. This could be perceived as a lack of weathering during the wash off by rains. This concord with the previous studies on related synthetic pyrethroids by Hogsette *et al*.,(2008) who showed similar findings using 0.1% λ Cyhalothrin and 0.1% ᶻ Cypermethrin on *Stomoxys calcitrans*. Following the previous published work in Zimbabwe (Toll *et al*., 2007), it has commonly been accepted that no significance variation in the persistence of Deltamethrin (Decatix) and Deltamethrin (Spot on) during the efficacy to control tsetse flies. Present work demonstrated similar observation between Sypertix®and Domenix® in the 4th week of the wet season.

Studies using Deltamethrin in Cameroon indicated that there was no significant difference in the mean catch of male *Glossina mortisans* at the end of the rain season in 2012 and 2013 but remained significantly different at the beginning of the rainy season in different trials of impregnated screens with Deltamethrin treated odor baited targets (Abdoulmoumini *et al*., 2016). Other studies by Schmahl *et al.,* (2009) reported that cattle and sheep treated with Deltamethrin (Butox® 7.5, Versatrine®) which is also a synthetic pyrethroid like Alphacypermethrin remained active and protected the cattle and sheep from biting midges up to the 4th week similar to findings of this study where the bio-efficacy was up to the 4thweek in both wet and dry seasons.

Consequently, the observation of tsetse flies on treated cattle from the 2nd week to the 4th week in both dry and wet seasons in the present study could be attributed to the decrease in the efficacy and longevity of the insecticide with increase in time. These findings are similar to a study

carried out by Oyewusi *et al.*, (2016), that observed ticks after 21 days' post treatment on Flumethrin treated cattle.

This is the first report to describe the efficacy of Sypertix® to control tsetse flies in Kenya. Our results indicate that Sypertix® at an application rate of approximately 10. % w/v. is an effective alternative to Domenix® for tsetse control on cattle. This observation is in accordance with the findings in Uganda that Sypertix® applied on the same rate provided effective control of ticks on cattle (Bardosh *et al.*, 2013b).

5.4 Conclusions

1. The Sypertix® blood feeding inhibition and irritant effects is a positive impact that reduces tsetse flies and other biting flies from contacting the treated cattle. This decrease the re-invasion of tsetse flies and prevalence of trypanosomiasis.
2. The instant 90% knockdown effect of Sypertix® on tsetse flies within 5 minutes post treatment, and its persistence in the wet season during wash off by rains, is a novel quality indicative that it can be used as an effective alternative insecticide for control of tsetse flies and prevent incidence of trypanosomiasis.
3. Sypertix® was efficacious up to 80% 24 hours post exposure, for a period of two weeks after the initial application in both dry and wet seasons, and at a lower concentration in comparison to Domenix® and also cheaper. Therefore it can be used as a suitable and effective alternative Alphacypermethrin for both tse tse and tick control.

5.5 Recommendations.

1. Farmers at Kiboko Makueni County are advised to treat cattle with Sypertix® as there is no successful feeding of tsetse flies on Sypertix® treated cattle, a factor that contributes to its efficacy.

2. Sypertix® is recommended as a drug of choice due to its rapid knock down effect, a feature that enhances its efficacy.

3. Sypertix® is recommended as a drug of choice as it is efficacious in dry and wet seasons and its more persistence than Domenix® in the wet season.

4. Local Farmers at Kiboko Makueni County should apply the insecticide to prevent re-invasion of the flies after two weeks in the dry and wet seasons to maintain 80 % efficacy of the drug in order to reduce the re-invasion of tsetse flies and spread of trypanosomiasis in Kiboko and adjacent areas.

5.6 Future research

1. Advanced research in production and formulation of synthetic pyrethroids which are cheaper, environmental friendly and more effective in lower concentrations.
2. Development of cost-effective efficacy trials for evaluating the effective life of various insecticides which need to be registered by Pest Control Product Board for use in the agricultural sector.

REFERENCES

AbdoulmouminI, M., Lendzele, S. S., Feussom, J. M., and Mfewou, A. (2016). Deltamethrin 10%-Impregnated Screens Pitched in a Tsetse Endemic Area in the Sudano-Sahelian Region of Cameroon Reduce Tsetse Fly Density and Trypanosomosis Incidence. *Walailak Journal of Science and Technology (WJST), 14*(11), pp.893-909.

.Adrian, M. (2012). Tick infestations in extensively grazed cattle and efficacy trial of high-cis cypermethrin pour-on preparation for control of ticks in Mvomero district in Tanzania. *BMC veterinary research, 8*(1), p.224.

Affected, S. (2009). African Animal Trypanosomiasis. Retrieved online 3/9/16 :*www.cfsph.iastate.edu/Factsheets/pdfs/trypanosomiasis_african.pdf.*

Aksoy, S. (2003). Control of tsetse flies and trypanosomes using molecular genetics. *Veterinary parasitology, 115*(2), pp125-145.

Aksoy, S., Pourhosseini, A., and Chow, A. (1995). Mycetome endosymbionts of tsetse flies constitute a distinct lineage related to Enterobacteriaceae. *Insect molecular biology, 4*(1), pp.15-22.

Aksoy, S., and Rio, R. V. (2005). Interactions among multiple genomes: tsetse, its symbionts and trypanosomes. *Insect biochemistry and molecular biology, 35*(7), 691-698.

Albert, M., Wardrop, N. A., Atkinson, P. M., Torr, S. J., and Welburn, S. C. (2015). Tsetse Fly (G. f. fuscipes) Distribution in the Lake Victoria Basin of Uganda. *PLoS Negl Trop Dis, 9*(4), e0003705.

Alemu Aylate Ayano1, T. O. a. B. K. (2013). Experimental Study on the Efficacy and Persistence of

Deltamethrin1%, Pour-on (Smash) for the Control of Tsetse Flies

in the Southern Rift Valley of Ethiopia. *Research Journal of Chemical and Environmental Sciences, 1*(5), pp.89-98.

Alliance, R., Barat, J. T., Plaza, S., Chambers, L. (2015). Alpha-Cypermethrin Derogation Application.

Arora, R. (2000). *Effect of some synthetic insecticides and biopesticides on the survival and acetylcholinesterase activity of little honey bee (Apis florea F.).* Chaudhary Charan Singh Haryana Agricultural University; Hisar.

Banks, S., Murray, N., Wilder-Smith, A., and Logan, J. (2014). Insecticide-treated clothes for the control of vector-borne diseases: a review on effectiveness and safety. *Medical and veterinary entomology, 28*(S1), pp.14-25.

Bardosh, K., Waiswa, C., and Welburn, S. C. (2013). Conflict of interest: use of pyrethroids and amidines against tsetse and ticks in zoonotic sleeping sickness endemic areas of Uganda. *Parasit Vectors, 6*, p.204.

Barnard, D. (2000). Repellents and toxicants for personal protection. *World Health Organization, Department of Control, Prevention and Eradication, Programme on Communicable Diseases, WHO Pesticide Evaluation Scheme (WHOPES), WHO/CDS/WHOPES/GCDPP/2000.5, WHO, Geneva, Switzerland*.

Barrett, M. P., Burchmore, R. J., Stich, A., Lazzari, J. O., Frasch, A. C., Cazzulo, J. J., Krishna, S. (2003). The trypanosomiases. *The Lancet*, *362*(9394), pp 1469-1480.

Bauer, B., and Baumann, M. (2015). Laboratory evaluation of efficacy and persistence of a 1% w/w fipronil pour-on formulation (Topline®) against Glossina palpalis gambiensis, Diptera: Glossinidae. *Parasitology research*, *114*(8), pp.2919-2923.

Bauer, B., and Baumann, M. P. (2015). Laboratory evaluation of efficacy and persistence of a 1 % w/w fipronil pour-on formulation (Topline) against Glossina palpalis gambiensis, Diptera: Glossinidae. *Parasitol Res*. doi: 10.1007/s00436-015-4493-0.

Bett, B., Gathuma, J. (2008). *Evaluating the impact of tsetse-repellent technology on the incidence of trypanosomosis in cattle managed under pastoral production systems in selected site in kajiado and Narok districts, Kenya.* University of Nairobi, faculty of Veterinary Medicine.

Bourtzis, K., Lees, R. S., Hendrichs, J., Vreysen, M. J. (2016). More than one rabbit out of the hat: Radiation, transgenic and symbiont-based approaches for sustainable management of mosquito and tsetse fly populations. *Acta tropica, 157*,pp 115-130.

Bekele, J., Asmare, K., Abebe, G., Ayelet, G., and Gelaye, E. (2010). Evaluation of Deltamethrin applications in the control of tsetse and trypanosomosis in the southern rift valley areas of Ethiopia. *Veterinary Parasitology*, *168*(3), pp.177-184.

Bouwman, B. Sereda and H.M Meinhardt(2006). Simultaneous presence of DDT and pyrethroid residues in human breast milk from malaria endemic area in South Africa. environmental Pollution 144(3) : 90-917: doi:10.1016/j.envpol.2006.02.002. PMID 16564119(NPTN, 1998)(http://ace.orst.edu/info/extonet/retreaved online from 15 Jan /2018).

Cecchi, G., Mattioli, R., Slingenbergh, J., and De La Rocque, S. (2008). Land cover and tsetse fly distributions in sub-Saharan Africa. *Medical and veterinary Entomology, 22*(4), pp.364-373.

Cisak, E., Wójcik-Fatla, A., Zajac, V., Dutkiewicz, J. (2012). Repellents and acaricides as personal protection measures in the prevention of tick-borne diseases. *Annals of Agricultural and Environmental Medicine, 19*(4).

Committee, I. R. A. (2011). Prevention and management of insecticide resistance in vectors of public health importance. *CropLife International*, 70.

Courtenay, O., Kovacic, V., Gomes, P., Garcez, L., and Quinnell, R. J. (2009). A long-lasting topical deltamethrin treatment to protect dogs against visceral leishmaniasis. *Medical and veterinary entomology, 23*(3), pp.245-256.

Davis, S., Aksoy, S., and Galvani, A. (2011). A global sensitivity analysis for African sleeping sickness. *Parasitology, 138*(04), pp,516-526.

De Meneghi, D., Stachurski, F., Adakal, H. (2016). Experiences in tick control by Acaricide in the traditional cattle sector in Zambia and Burkina Faso: possible environmental and public health implications. *Frontiers in public health, 4*,p 239.

Devisser, M., Messina, J. P., Moore, N. J., Lusch, D. P., and Maitima, J. (2010). A dynamic species distribution model of Glossina subgenus Morsitans: The identification of tsetse reservoirs and refugia. *Ecosphere, 1*(1),pp, 1-21.

Dumesa, T., and Demessie, Y. (2015). Review on Tsetse Transmitted Bovine Trypanosomosis in Ethiopia. *European Journal of Applied Sciences, 7*(6), pp.255-267.

Ebrahim, A. Y. (2015). Biological Control of vector born Protozoan Parasites of Veterinary Importance.

Eisler, M. C., Torr, S. J., Coleman, P. G., Machila, N., Morton, J. F. (2003). Integrated control of vector-borne diseases of livestock–pyrethroids: panacea or poison? *Trends in parasitology, 19*(8), pp 341-345

Fankhauser, B., Dumont, P., Hunter, J. S., McCall, J. W., Kaufmann, C., Mathis, A., Chester, S. T. (2015). Repellent and insecticidal efficacy of a new combination of Fipronil and 63

permethrin against three mosquito species (Aedes albopictus, Aedes aegypti and Culex pipiens) on dogs. *Parasites & vectors, 8*(1), p 64.

Faraj, C., Adlaoui, E. B., Ouahabi, S., Elkohli, M., Elrhazi, M., Laqraa, L., and Ameur, B. (2013). Field evaluation of alphacypermethrin in indoor residual spraying for leishmaniasis control in an endemic area, northern Morocco. *Parasites & vectors, 6*(1), p.354.

Ford, J. (1971). The role of the trypanosomiases in African ecology. A study of the tsetse fly problem. *The role of the trypanosomiases in African ecology. A study of the tsetse fly problem.*

Franco, J. R., Simarro, P. P., Diarra, A., Jannin, J. G., and Organization, W. H. (2015). Epidemiology of human African trypanosomiasis.

Geiger, A., Ponton, F., and Simo, G. (2015). Adult blood-feeding tsetse flies, trypanosomes, microbiota and the fluctuating environment in sub-Saharan Africa. *The ISME journal, 9*(7), pp.1496-1507.

Gimonneau, G., Alioum, Y., Abdoulmoumini, M., Zoli, A., Cene, B., Adakal, H., Bouyer, J. (2016). Insecticide and repellent mixture pour-on protects cattle against animal trypanosomosis. *PLoS neglected tropical diseases, 10*(12), e0005248.

Glasgow, J. P. (1963). The Distribution and Abundance of Tsetse. *The Distribution and Abundance of Tsetse.*

Gooding, R., and Krafsur, E. (2005). Tsetse genetics: contributions to biology, systematics, and control of tsetse flies. *Annual review of entomology, 50*, p.101.

Grady, S.C., Messina, J.P. and McCord, P.F., 2011. Population vulnerability and disability in Kenya's tsetse fly habitats. *PLoS neglected tropical diseases, 5*(2), p.e957.

Grant, I. F. (2001). Insecticides for tsetse and trypanosomiasis control: is the environmental risk acceptable? *Trends in parasitology, 17*(1), pp 10-14.

Hargrove, J. (2003). *Tsetse eradication: sufficiency, necessity and desirability*: DFID Animal Health Programme, Centre for Tropical Veterinary Medicine, University of Edinburgh United Kingdom.

Harris, E., Brown, M., and Smith, S. (1992). Laboratory investigations of insecticide impregnated materials for the control of New World screwworm flies.

Hartnik, T., Sverdrup, L. E., and Jensen, J. (2008). Toxicity of the pesticide alpha-cypermethrin to four soil nontarget invertebrates and implications for risk assessment. *Environmental toxicology and chemistry, 27*(6), pp.1408-1415.

Hendrick, G., de La Rocque, S., and Mattioli, R. C. (2004). *Long-term tsetse and trypanosomiasis management options in West Africa* (Vol. 6): Food and Agriculture Org.

Hertz, J. C. (2007). *Potential of insecticide-treated cords and sprayable baits for control of house flies (Diptera: Muscidae).* University of Florida.

Hewitt, S., & Rowland, M. (1999). Control of zoophilic malaria vectors by applying pyrethroid insecticides to cattle. *Tropical medicine & international health, 4*(7), pp 481-486.

Hocking, K., Lamerton, J., and Lewis, E. (1963). Tsetse-fly control and eradication. *Bulletin of the World Health Organization, 28*(5-6), p.811.

Hogsette, J. A., Nalli, A., and Foil, L. D. (2008). Evaluation of different insecticides and fabric types for development of treated targets for stable fly (Diptera: Muscidae) control. *Journal of economic entomology, 101*(3), pp.1034-1038.

Hossain, M. M. (2015). *Studies of purity of some pesticides applied in vegetables and fishes.* University of Dhaka.

Hougard, J.-M., Duchon, S., Zaim, M., Guillet, P. (2002). Bifenthrin: a useful pyrethroid insecticide for treatment of mosquito nets. *Journal of medical entomology, 39*(3), pp 526-533.

Ilemobade, A. (2009). Tsetse and trypanosomosis in Africa: the challenges, the opportunities. *Onderstepoort J Vet Res, 76*(1), pp.35-40.

Kagbadouno, M. S., Camara, M., Bouyer, J., Courtin, F., Onikoyamou, M. F., Schofield, C. J., and Solano, P. (2011). Progress towards the eradication of Tsetse from the Loos islands, Guinea. *Parasit Vectors, 4*(1), p.18.

Kariithi, H. M., van Oers, M. M., Vlak, J. M., Vreysen, M. J., Parker, A. G., and Abd-Alla, A. M. (2013). Virology, epidemiology and pathology of Glossina hytrosavirus, and its control prospects in laboratory colonies of the tsetse fly, Glossina pallidipes (Diptera; Glossinidae). *Insects, 4*(3), pp.287-319.

Kennedy, P. G. (2004). Human African trypanosomiasis of the CNS: current issues and challenges. *The Journal of clinical investigation, 113*(4), pp.496-504.

Kenya agricultural value chain enterprises project (kaves) pesticide evaluation report and safer use Action plan (persuap) https://www.rti.org/sites/default/files/kaves_persuap.pdf 20th/Feb/2018.

Kolaczinski, J., Curtis, C. (2004). Chronic illness as a result of low-level exposure to synthetic pyrethroid insecticides: a review of the debate. *Food and Chemical Toxicology, 42*(5) pp , 697-706.

Kotlyar, S. (2010). Recommendations for control of East African sleeping sickness in Uganda. *Journal of global infectious diseases, 2*(1), p.43.

Krafsur, E. (2009). Tsetse flies: genetics, evolution, and role as vectors. *Infection, Genetics and Evolution, 9*(1), pp.124-141.

Kuzoe, F., and Schofield, C. (2004). Strategic review of traps and targets for tsetse and african trypanosomiasis control. *Strategic review of traps and targets for tsetse and African trypanosomiasis control*.

Leak, S. G. (1999). *Tsetse biology and ecology: their role in the epidemiology and control of trypanosomosis*: CABI publishing New York.

Lukaw, Y. S., Abdelrahman, M. M., Mohammed, Y. O., Ochi, E. B., and Elrayah, I. E. (2014). Factors Influencing Seasonal Abundance of" Glossina fuscipes fuscipes"(" Glossina: Glossinidae") in Kajo-Keji County, South Sudan. *Current Research Journal of Biological Sciences, 6*(6), pp.222-228.

Mahande, A. M., Mosha, F. W., Mahande, J. M., and Kweka, E. J. (2007). Role of cattle treated with deltamethrine in areas with a high population of Anopheles arabiensis in Moshi, Northern Tanzania. *Malaria Journal, 6*(1), p.109.

Maia, M. F. (2009). *Impact of insecticide-treated nets protecting cattle in zero-grazing units on nuisance and biting insects in the forest region of Kumasi, Ghana.* Freie Universität Berlin.

Majekodunmi, A. O. (2012). Pastoral livelihoods and the epidemiology of emergent trypanosomiasis on the Jos Plateau, Nigeria.

Mangwiro, T., Torr, S., Cox, J., Holloway, M. (1999). The efficacy of various pyrethroid insecticides for use on odour-baited targets to control tsetse. *Medical and veterinary entomology, 13*(3),pp 315-323.

Maniania, N. K., and Takasu, K. (2006). Development of microbial control agents at the international centre of insect physiology and ecology. *Bulletin of the Institute of Tropical Agriculture, Kyushu University, 29*(1), pp.1-9.

McCord, P. F., Messina, J. P., Campbell, D. J., and Grady, S. C. (2012). Tsetse fly control in Kenya's spatially and temporally dynamic control reservoirs: A cost analysis. *Applied Geography, 34*, pp.189-204.

Mekonnen, S. (2014). Assessment of the efficacy of Deltamethrin 1% pourson (Smash) and Deltamethrin 1% SC (Pali) and comparison of their mode of applications against ticks in Ethiopia. *Ethiopian Veterinary Journal, 18*(1), pp.17-27.

Miller, L. D. (2017). Farming practices and Trypanosomiasis in Northern Uganda: an assessment of Trypanosomiasis prevalence and the ongoing management of vector borne infections.

Molina, R., Espinosa-Góngora, C., Gálvez, R., Montoya, A., Descalzo, M., Jiménez, M. Miró, G. (2012). Efficacy of 65% permethrin applied to dogs as a spot-on against Phlebotomus perniciosus. *Veterinary parasitology, 187*(3-4), pp 529-533.

Molina, R., Lohse, J.-M., and Nieto, J. (2001). Evaluation of a topical solution containing 65% permethrin against the sandfly (Phlebotomus perniciosus) in dogs. *Vet Ther, 2*(3), pp.261-267.

Moore, N., and Messina, J. (2010). A landscape and climate data logistic model of tsetse distribution in Kenya. *PLoS One, 5*(7), pp.1-10.

Munang'andu, H. M., Siamudaala, V., Munyeme, M., and Nalubamba, K. S. (2012). A review of ecological factors associated with the epidemiology of wildlife trypanosomiasis in the Luangwa and Zambezi valley ecosystems of Zambia. *Interdisciplinary perspectives on infectious diseases, 2012*.

Ngaruiya, P. N. (2003). Overview of Registration of pesticides in Kenya. *Registration for Biocontrol Agents in Kenya*, 79.

Nnko, H. J., Ngonyoka, A., Salekwa, L., Estes, A. B., Hudson, P. J., Gwakisa, P. S., Cattadori, I. M. (2017). Seasonal variation of tsetse fly species abundance and prevalence of trypanosomes in the Maasai Steppe, Tanzania. *Journal of Vector Ecology*, *42*(1), pp 24-33.

Norval, R., Sonenshine, D. E., Allan, S. A., Burridge, M. J. (1996). Efficacy of pheromone-acaricide-impregnated tail-tag decoys for controlling the bont tick, Amblyomma hebraeum (Acari: Ixodidae), on cattle in Zimbabwe. *Experimental and applied acarology*, *20*(1), pp 31-46.

Nthiwa, D. M., Odongo, D. O., Ochanda, H., Khamadi, S., and Gichimu, B. M. (2015). Trypanosoma Infection Rates in Glossina Species in Mtito Andei Division, Makueni County, Kenya. *Journal of parasitology research*, *2015*.

Obiero, J., Mutai, E., and Toromo, A. (2014). Evaluation of Land use Change in Kiboko

Okeyo, W.A., Saarman, N.P., Mengual, M., Dion, K., Bateta, R., Mireji, P.O., Okoth, S., Ouma, J.O., Ouma, C., Ochieng, J. and Murilla, G., 2017. Temporal genetic differentiation in Glossina pallidipes tsetse fly populations in Kenya. *Parasites & vectors*, *10*(1), p.471 Watershed, Kenya. *African Journal of Education Science and Technology*, *2*(1), p.17.

Ouma, J.O., Marquez, J.G. and Krafsur, E.S., 2005. Macrogeographic population structure of the tsetse fly, Glossina pallidipes (Diptera: Glossinidae). *Bulletin of entomological research*, *95*(5), pp.437-447.

Ouma, E. M. (2010). *Management of trypanocidal drug resistance in cattle in identified chemoresistance hot spots in the administrative District of Sikasso, south-east Mali.* Freie Universität Berlin.

Oyewusi, J., OtesilE, E., and Sadiq, N. (2016). Efficacy of flumethrin pour-on against ticks on nâ€™ dama/brown cross cattle under field conditions. *Journal of Natural Sciences Engineering and Technology, 10*(2), pp.105-110.

Products Registered for use on Animal health;Pest Control Products Board 10th version 2016.(www.pcpb.or.ke/animalhealthviewform.php 5th /Jan/2018)

Pais, R., Lohs, C., Wu, Y., Wang, J., and Aksoy, S. (2008). The obligate mutualist Wigglesworthia glossinidia influences reproduction, digestion, and immunity processes of its host, the tsetse fly. *Applied and envi ronmental microbiology, 74*(19), pp.5965-5974.

Pendleton, F. N. (2003). *The Effects of Spraying Deltamethrin Against Tsetse Flies on Insectivorous Birds in the Okavango Delta, Botswana.*Doctoral dissertation university of Maryland.

Pollock, J. (1982). Training manual for tsetse control personnel. Volume II. Ecology and behaviour of tsetse. *Training manual for tsetse control personnel. Volume II. Ecology and behaviour of tsetse.*

Quarcoo, F. Y., Appel, A. G., and Hu, X. P. (2010). Descriptive study of non-repellent insecticide-induced behaviors in Reticulitermes flavipes (Isoptera: Rhinotermitidae). *Sociobiology, 55*(1), p.217.

Regasa, T., Bedada, M., Workine, M., Beyera, M., Terefe, S., and Tsegay, A. K. (2015). Study on Spatial Distribution of Tsetse Fly and Prevalence of Bovine Trypanosomosis and Other Risk Factors: Case Study in Bedele Woreda, Ilu Aba Bora Zone, South Western Ethiopia.

Rehman, H., Aziz, A. T., Saggu, S., Abbas, Z., Mohan, A., and Ansari, A. (2014). Systematic review on pyrethroid toxicity with special reference to deltamethrin. *Journal of Entomology and Zoology Studies, 2*(6), pp.60-70.

Reichard, R. (2002). Area-wide biological control of disease vectors and agents affecting wildlife. *Rev Sci Tech, 21*(1), pp.179-185.

Reid, R. S., Kruska, R. L., Deichmann, U., Thornton, P. K., and Leak, S. G. (2000). Human population growth and the extinction of the tsetse fly. *Agriculture, ecosystems & environment, 77*(3), pp.227-236.

Rock, K. S. (2014). *How much do we care about biting insects?: modelling the dynamics of vector-borne diseases.*Doctoral dissertation University of Warwick.

Rogers, D. J., and Randolph, S. E. (1986). Distribution and abundance of tsetse flies (Glossina spp.). *The Journal of Animal Ecology*, pp.1007-1025.

Rothwell, J., Hacket, K., Friend, M., Farnsworth, W., and Lowe, L. (1998). Efficacy of zeta-cypermethrin as pour-on or spray formulations for the control of buffalo fly (Haematobia irritans exigua) in cattle. *Australian veterinary journal, 76*(9), pp. 610-612.

Rowlands, G., Leak, S., Mulatu, W., Nagda, S., Wilson, A., and d'Ieteren, G. (2001). Use of deltamethrin 'pour-on'insecticide for the control of cattle trypanosomosis in the presence of high tsetse invasion. *Medical and veterinary entomology, 15*(1),pp, 87-96.

Schmahl, G., Mehlhorn, H., Abdel-Ghaffar, F., Al-Rasheid, K., Schumacher, B., Jatzlau, A., and Pohle, H. (2009). Does rain reduce the efficacy of Butox 7.5 pour on (deltamethrin) against biting midges (Culicoides specimens)? *Parasitology research, 105*(6), p.1763.

Simmonds, M. C., Higginsa, J. P., Stewartb, L. A., Tierneyb, J. F., Clarke, M. J., and Thompson, S. G. (2005). Meta-analysis of individual patient data from randomized trials: a review of methods used in practice. *Clinical Trials, 2*(3), pp.209-217.

Syed, Z., Guerin, P. (2004). Tsetse flies are attracted to the invasive plant Lantana camara. *Journal of insect physiology, 50*(1), pp 43-50.

Terblanche, J. S., Clusella-Trullas, S., Deere, J. A., and Chown, S. L. (2008). Thermal tolerance in a south-east African population of the tsetse fly Glossina pallidipes (Diptera, Glossinidae): implications for forecasting climate change impacts. *Journal of Insect Physiology, 54*(1), pp.114-127.

Torr, S., and Mangwiro, T. (2000). Interactions between cattle and biting flies: effects on the feeding rate of tsetse. *Medical and veterinary entomology, 14*(4), pp.400-409.

Torr, S., Mangwiro, T., and Hall, D. (2011). Shoo fly, don't bother me! Efficacy of traditional methods of protecting cattle from tsetse. *Medical and veterinary entomology, 25*(2), pp.192-201.

Torr, S., Maudlin, I., and Vale, G. (2007). Less is more: restricted application of insecticide to cattle to improve the cost and efficacy of tsetse control. *Medical and veterinary entomology, 21*(1), pp.53-64.

Torr, S., and Vale, G. (2005). Tsetse muse: an interactive programme to assess the impact of control operations on tsetse populations. Final technical report.

Torr, S., Vale, G., Hargrove, J., Morton, J., Mangwiro, T., van Munster, B., Habtewold, J. (2009). Final Technical Report. Message in a Bottle: Disseminating Tsetse Control Technologies.

Toynton, K.; Luukinen, B.; Buhl, K.; Stone, D. 2009. *Permethirn General Fact Sheet*; National Pesticide Information Center, Oregon State University Extension Services. http://npic.orst.edu/factsheets/PermGen.html/9th/Feb/2018.

Uragayala, S., Kamaraju, R., Tiwari, S., Ghosh, S. K., and Valecha, N. (2015). Small-scale evaluation of the efficacy and residual activity of alpha-cypermethrin WG (250 g AI/kg) for indoor spraying in comparison with alpha-cypermethrin WP (50 g AI/kg) in India. *Malaria Journal, 14*(1), p.223.

Vale, G. A., Hargrove, J. W., Chamisa, A., Grant, I. F., and Torr, S. J. (2015). Pyrethroid treatment of cattle for tsetse control: reducing its impact on dung fauna. *PLoS neglected tropical diseases, 9*(3), e0003560.

Van der Vloedt, A. (1992). The integration of biological techniques into tsetse control programmes. *FAO Animal Production and Health Paper (FAO)*.

Van Nieuwenhove, S., Betu-Ku-Mesu, V. K., Diabakana, P. M., Declercq, J., and Bilenge, C. M. M. (2001). Sleeping sickness resurgence in the DRC: the past decade. *Tropical Medicine and International Health, 6*(5), pp.335-341.

Vatandoost, H., Mamivandpoor, H., Abai, M. R., Shayeghi, M., Rafi, F., Raeisi, A., and Nikpoor, F. (2013). Wash Resistance and Bioefficacy of Alpha-cypermethrin Long Lasting Impregnated Nets (LLIN-Interceptor®) against Anopheles stephensi using Tunnel Test. *Journal of arthropod-borne diseases, 7*(1), p.31.

Vreysen, M. (2001). Principles of area-wide integrated tsetse fly control using the sterile insect technique. *Médecine tropicale, 61*(4-5), pp.397-410.

Vreysen, M. J., and Marc, J. (2006). Prospects for area-wide integrated control of tsetse flies (Diptera: Glossinidae) and trypanosomosis in sub-Saharan Africa. *Rev Soc Entomol Argent, 65*,pp. 1-21.

Vreysen, M. J., Seck, M. T., Sall, B., and Bouyer, J. (2013). Tsetse flies: their biology and control using area-wide integrated pest management approaches. *Journal of invertebrate pathology, 112,* S15-S25.

Waltisbuhl, D., Farnsworth, B., and Kemp, D. (2005). Pesticides used against ectoparasites (ticks, flies, lice) of cattle in Australia. *Ectoparasiticide use in contemporary Australian livestock production*, 45. .

World Health Organization, 2012. *Global plan for insecticide resistance management in malaria vectors: executive summary* (No. WHO/HTM/GMP/2012.5).

World Health Organization (2009). Guidelines for efficacy testing of insecticides for indoor and outdoor ground-applied space spray applications.

Yu, J., Xin, B., Shen, C., Liu, Y. (2016). reparation and characterization of anti-mosquito polyester nets finished by be ndiocarb/alphacypermethrin. *The Journal of The Textile Institute, 107*(11), pp 1369-1374.

APPENDICES

Appendices1: Field data recording sheet

FIELD TRIAL DATA RECORDING SHEET

Village

Insecticide **Species**

Diagnostic dose: **Diagnostic Time:**

Temperature **Season**

Humidity

Date			Minutes				Hours				
	Treatment	Animal Fed	0	5	10	20	1	2	4	8	24

Appendices 2: Meteorological data

KIBOKO- RAIN FALL MARCH-OCTOBER 2015

MONTH	MARCH	APRIL	MAY	JUNE	JULY	AUG.	SEPT.	OCTOBER
AMOUNT (MM)	44.8	71.4	70.6	1.0	1.2	TR	NIL	0.1

MEAN MAXIMUM&MINIMUM TEMPERATURES DEG. C

MONTH	MARCH	APRIL	MAY	JUNE	JULY	AUG.	SEPT.	OCTOBER
MEAN MAX TEMP.	32.0	30.7	28.9	28.4	27.7	27.9	30.3	31.4
MEAN MIN.TEMP.	19.0	19.6	17.9	15.8	15.3	15.7	15.7	18.9

MEAN RELATIVE HUMIDITY (%)

MONTH	MARCH	APRIL	MAY	JUNE	JULY	AUG.	SEPT.	OCTOBER
0600Z	72	81	80	73	76	75	68	69
1200Z	38	49	53	46	43	40	32	37

MEAN PRESSURE (HECTO PASCALS)

MONTH	MARCH	APRIL	MAY	JUNE	JULY	AUG.	SEPT.	OCTOBER
0600Z	903.5	903.5	904.6	905.5	906.6	906.5	905.9	904.8
1200Z	899.8	899.6	901.3	902.0	903.1	903.9	901.3	900.2

MEAN WIND RUN KM/DAY

MONTH	MARCH	APRIL	MAY	JUNE	JULY	AUG.	SEPT.	OCTOBER
WINDRUN	142.7	106.6	84.9	93.6	119.3	147.4	168.6	177.9

TOTAL MONTHLY EVAPORATION (MM)

MONTH	MARCH	APRIL	MAY	JUNE	JULY	AUG.	SEPT.	OCTOBER
EVAPORATION	191.8	146.2	126.1	128.0	135.0	150.5	206.0	177.9