

**EFFICACY OF FABRIC PANELS IMPREGNATED WITH *METARHIZIUM ANISOPLIAE* AGAINST ADULT *GLOSSINA FUSCIPES FUSCIPES* IN MANGA AND MAGARE ISLANDS, LAKE VICTORIA, KENYA**

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**A THESIS SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTER OF SCIENCE (MEDICAL ENTOMOLOGY) IN THE SCHOOL OF PURE AND APPLIED SCIENCES OF KENYATTA UNIVERSITY.**

**JUNE 2022**

**DECLARATION**

I hereby declare that this thesis is my original work and has not been presented for degree or other awards in any other University.

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**DEDICATION**

This work is dedicated to my parents Mr and Mrs Isaac Riithi and siblings Dickson, Alexander and Caroline, for their unwavering faith and belief in my academic success.

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## LIST OF ABBREVIATIONS AND ACRONYMS

<b>AAT</b>	Animal African Trypanosomiasis
<b>ANOVA</b>	Analysis of Variance
<b>APU</b>	Arthropod Pathology Unit
<b>ARU</b>	Arthropod Rearing Unit
<b>CI</b>	Confidence interval
<b>DNA</b>	Deoxyribonucleic acid
<b>E-nets</b>	Electrocuting nets
<b>EPF</b>	Entomopathogenic Fungus
<b>FAO</b>	Food Agricultural Organization
<b><i>gHAT</i></b>	Gambian Human African Trypanosomiasis

<b>GPS</b>	Global Positioning System
<b>HAT</b>	Human African Trypanosomiasis
<i>icipe</i>	International Centre for Insect Physiology and Ecology
<b>IGS</b>	Intergenic spacer
<b>IPM</b>	Integrated Pest Management
<b>ITOC</b>	<i>icipe</i> Thomas Odhiambo Campus
<b>KETRI</b>	Kenya Trypanosomiasis Research Institute
<b>OR</b>	Odds ratio
<b>PCR</b>	Polymerase Chain Reaction
<b>PVC</b>	Polyvinyl chloride
<b>rHAT</b>	Rhodesian Human African Trypanosomiasis
<b>RNA</b>	Ribonucleic acid
<b>rRNA</b>	Ribosomal RNA
<b>rpm</b>	revolutions per minute
<b>SDA</b>	Sabourand Dextrose Agar
<b>SIT</b>	Sterile Insect Technique
<b>µl</b>	Microlitre
<b>UV</b>	Ultraviolet Radiation
<b>WHO</b>	World Health Organization

**ABSTRACT**

Tsetse flies (*Glossina sp.*) transmit parasites of the genus *Trypanosoma* that cause Human African Trypanosomiasis (sleeping sickness) in man and Animal African trypanosomiasis in livestock and wildlife. African Trypanosomiasis is a devastating tropical disease that is endemic to 38 countries in sub-Saharan Africa. Integrated management of tsetse flies using pyrethroid insecticides has been the mainstay of trypanosomiasis control. Vector control and disease treatment have led to drastic declines in the incidence of sleeping sickness, hence, the World Health Organization set a goal to eliminate the disease by 2030. Despite this progress, African trypanosomiasis still impacts substantial health and economic burden in less fortunate communities in Sub-Saharan Africa. There is, therefore, a need for supplementary vector control strategies which are cost-effective and easy to maintain to fight this disease. The entomopathogen *Metarhizium anisopliae* has been effective in reducing tsetse flies longevity and overall vectorial capacity, while being cost-effective and easy to deploy. This study evaluated the efficacy of fabric panels impregnated with *M. anisopliae* against adults of *Glossina fuscipes fuscipes* in Manga and Magare islands of Lake Victoria in Kenya. Baseline studies were conducted to determine the existence of natural *M. anisopliae* using molecular analysis tools. For eight days, between 9.00 - 12.00 h, tsetse flies were sampled with four biconical traps and four targets with adhesives set 1m from the lakeshore at intervals of 200 m. Tsetse flies trapped were screened for naturally occurring *M. anisopliae* DNA. To compare the capacity of polyester, cotton and fleece fabrics to retain fungal conidia, panels were made by sewing 25 cm × 25 cm of each on pthalogen blue cotton fabric. Three replicates of each panel were treated with 0.1 g/cm<sup>2</sup> of conidia and suspended 10 cm from the ground for fifteen days. Every 24 hours, conidia were sampled on three random points of each fabric and quantified. Electrocuting nets were used to estimate the attraction and landing rate of tsetse flies on panels with conidia doses of 0.25 – 1.00 g. The effectiveness of the fabric panels to disseminate lethal dosages of *M. anisopliae* to tsetse flies were evaluated by setting five panels treated with conidia dosage of 0.25 g in Manga Island. Five untreated panels in Magare island served as control. Tsetse flies were collected using biconical traps on each island and maintained on rabbit blood in the tsetse fly insectary. Dead flies were placed on petri dishes with dump paper towels and observed for mycosis after 5 days. Counts of tsetse flies and conidia were analysed in R packages using the zero-inflated generalised linear models and mortality rates estimated with Kaplan-Meier models. A total of 2,415 *Glossina fuscipes fuscipes* were trapped with 3.7 times (95 % Confidence Interval (CI) 2.8 - 4.9 flies,  $p>0.01$ ) more tsetse flies were trapped in Manga Island than Magare island. There was zero detection of naturally occurring *M. anisopliae* DNA in the trapped tsetse flies. Fleece fabrics retained seven times (95 % CI: 5.6 - 8.8 times,  $p<0.01$ ) more conidia than polyester and 1.2 times (95 % CI: 1.0 – 1.5 times,  $p=0.04$ ) more than cotton fabrics. Incorporation of 1 g of conidia to panels reduced the landing rate of tsetse flies by 60 % (95 % CI: 30 – 70 %,  $p<0.01$ ). Significantly fewer tsetse flies attempted to land on panels with conidia doses of 0.5 g and 1.0 g ( $p<0.05$ ). Female tsetse flies were more affected by the colour change on the treated fabric panels. The prevalence of *M. anisopliae* on tsetse flies increased steadily to 24 % and 16.7 % in Manga and Magare islands respectively over three weeks. This study demonstrates that fabric panels made from fleece fabric and treated with 0.25 g of *M. anisopliae* conidia can effectively contaminate *G. f. fuscipes* in the field with the entomopathogen. With further optimization, the prototype can be developed into an effective large scale vector control tool.

## CHAPTER ONE

### INTRODUCTION

#### 1.1 Background information on tsetse flies and trypanosomiasis

Tsetse flies (*Glossina spp.*) are haematophagous dipterans found in sub-Saharan Africa and the primary vectors of African trypanosomiasis. They infest over 38 countries covering a total area of 9-11 million km<sup>2</sup> (Franco *et al.*, 2014; Simarro *et al.*, 2015). The habitats of different tsetse flies species extend from the rain forest to the savannah in sub-Saharan Africa (Cecchi *et al.*, 2008). Vegetation cover which is influenced by climatic changes and human activities affects the densities of tsetse flies (Bouyer *et al.*, 2015). Further, the distribution of tsetse flies is influenced by the presence of host sources of blood meal, prevailing temperatures and humidity levels (Dicko *et al.*, 2014; 2015). As a result of these reasons, the spread of tsetse fly species in infested countries is not uniform and varies from place to place over time.

Africa has more than 30 species and sub-species of tsetse flies (WHO, 2012; Holmes, 2014; 2015). Tsetse fly species are grouped into three sub-genera based on the differences in their habitats, distribution, behaviour and, molecular and morphological characteristics (Elsen *et al.*, 1990). The *morsitans* subgenus also known as savannah tsetse flies, the *fusca* group known as forest flies, and the *palpalis* subgenus commonly called riverine or lacustrine flies. The *morsitans* subgenus contains about 7 sub-species while *fusca* and *palpalis* have 15 and 9 subspecies, respectively (Abaru, 1985, D'Amico *et al.*, 1992; Mweempwa *et al.*, 2015).

African Trypanosomiasis is a devastating and neglected parasitic disease that impacts human and animal health. It is caused by parasites of the genus *Trypanosoma* when

these are passed onto susceptible hosts by tsetse flies and other biting flies. *Trypanosoma brucei* causes two distinct forms of Human African Trypanosomiasis also known as sleeping sickness; the Gambian kind caused by *Trypanosoma brucei gambiense* and the acute Rhodesian form caused by *Trypanosoma brucei rhodesiense* (WHO, 2020). Gambian HAT is widespread in West Africa and parts of Central Africa while Rhodesian HAT is historically more prevalent in East and Southern Africa (Katsidzira and Fana 2010; Simarro *et al.*, 2012). Uganda is the only country endemic for both forms of HAT with *gambiense* foci in the North-west region and *rhodesiense* foci in the South-East and central regions of the country (WHO, 2010). Gambian sleeping sickness accounts for up to 98 % of HAT cases in Africa. About 90 % of these cases are transmitted by the tsetse fly subspecies, *G. f. fuscipes* (Cattand *et al.*, 2001; Simarro *et al.*, 2011). On the other hand, Animal African Trypanosomiasis (AAT) affects livestock and many wild animal species. *Trypanosoma brucei brucei*, *T. congolense* and *T. vivax* cause nagana in cattle and small ruminants, *T. evansi* causes surra in camels, *T. equiperdum* causes dourine in horses and other equids and *T. simiae* and *T. suis* causes disease in pigs (Cattand *et al.*, 2001; Simarro *et al.*, 2008).

Significant progress has been made towards developing novel biorational technologies for controlling tsetse flies. A low-cost tsetse fly repellent that reduces infective bites and enables animals to thrive despite tsetse flies infestation has been reported for livestock (Saini and Hassanali 2007; Saini *et al.*, 2017). This tool offers personal protection to individual animals/herds and is adapted for the privatised African livestock industry where, in most cases, the costs of disease management is borne by farmers. For HAT, relatively small and cost-effective panels *tiny targets*

were developed by Tirados and others (Tirados *et al.*, 2015). These are easier to deploy and maintain compared to previously mentioned tools and are now used on large scale to control vectors of sleeping sickness (Shaw *et al.*, 2015). Additional and supplementary vector control strategies that are low-cost and easy to deploy could enhance tsetse flies' control and hasten progress made towards eliminating HAT (Kaaya and Darji 1989; Maniania and Ekesi 2013).

The Entomopathogenic fungus *Metarhizium anisopliae* offers an exciting option for tsetse flies control. It has been shown to increase mortality in tsetse flies in the laboratory (Dimbi *et al.*, 2013; Maniania *et al.*, 2013). Further, infections with *M. anisopliae* can inhibit host-seeking in the vector and limit the reproduction potential, reducing its vectorial competence (Wamiti *et al.*, 2018). In field trials, *M. anisopliae* applied on cheap applicators mounted on traps can suppress the tsetse fly population (Maniania, 1998; Maniania *et al.*, 2006). It is hypothesised that the potential of horizontal transfer of conidia from one fly to another provides an option for auto-disseminating this killing agent, allowing it to act as a persistent insecticide with no adverse environmental effects (Maniania, 1998; Maniania *et al.*, 2013). However, there is a need to develop more practical approaches in using *M. anisopliae* against tsetse flies in the wild.

This study investigated the potential of small target-like fabric panels impregnated with *Metarhizium anisopliae* against *Glossina fuscipes fuscipes*, the major vectors of sleeping sickness. This entailed evaluation of the fungal conidia retention power of different fabric panels. Further to this, the effect of conidia spores on the attractiveness of the fabric panels to tsetse flies was determined and lastly the efficacy

of the fabric panels impregnated with conidia to contaminate tsetse flies was investigated. These experiments were carried out in Manga and Magare islands in Lake Victoria, Western Kenya.

## **1.2 Statement of the problem**

Tsetse fly-borne Trypanosomiasis causes significant health and economic challenges in sub-Saharan Africa (Barrett 1999; WHO, 2010; Cattand *et al.*, 2001; Bukachi *et al.*, 2017). Despite years of interventions against the disease and investment in research for new tools to control it, about 10,000 people are still affected by Human African Trypanosomiasis annually (Cattand *et al.*, 2001; Simarro *et al.*, 2008). The majority of victims are from communities that live in rural Africa and depend on labour-intensive agriculture, fishing and animal husbandry for livelihood (Simarro *et al.*, 2011; 2015; Holmes, 2014). Public health and the substantial economic burden imposed by HAT leaves them vulnerable to food shortages and poverty.

Several tools and technologies have been deployed to control vectors of HAT which include the use of biconical traps, insecticide-treated livestock, insecticide-impregnated targets, SIT and the recently introduced tsetse fly repellents. The key challenge with the large-scale use of these control methods is they are costly to maintain, time consuming and labour-intensive. There is, therefore, a need to develop tools that are cost-effective and easy to deploy to supplement the already existing vector control methods. Current chemotherapy for Human African Trypanosomiasis involves the use of a significantly toxic and hard-to-administer regime of up to five different drugs. There is no vaccine yet for the disease nor any significant progress in research that could lead to this. Consequently, the control of HAT has relied heavily on the preventative suppression of disease vectors with insecticidal sprays and

visually attractive traps/panels made of fabric and laced with insecticides. Insecticide-treated blue-black fabric panels are a cost-effective option for controlling riverine tsetse flies; they are relatively cheap, and their deployment is non-labour intensive. Panels are therefore a good model for new tools for controlling tsetse flies.

### **1.3 Justification of the study**

The efficacy of the entomopathogen *Metarhizium anisopliae* against tsetse flies and the incubation of trypanosomes in tsetse flies has repeatedly demonstrated that this bioagent could act as a replacement for chemical insecticides in the control of tsetse flies and trypanosomiasis. Furthermore, it holds the potential for auto-dissemination with infected flies transmitting it to wild populations autonomously. There has, however, been a challenge with the practical application of this biopesticide in the field. Modifying panels into delivery tools for deploying *M. anisopliae* against tsetse flies could unlock an alternative tool to augment the existing arsenal against the disease.

This study focused on the use of locally available fabric panels as fungus disseminating tools in the control of adult tsetse flies. It has been shown that the entomopathogenic fungus *Metarhizium anisopliae* can be used as a biopesticide in control of tsetse fly populations. Additionally, it has shown great potential as a persistent insecticide and due to the horizontal transfer of conidia from one tsetse fly to another during mating, it has presented itself as an efficient control tool.

### **1.4 Research questions**

- (a) What dose of *Metarhizium anisopliae* conidia can polyester, cotton and fleece fabrics retain in ambient conditions?

- (b) What is the effect on the tsetse flies' attraction to fabric panels impregnated with various *Metarhizium anisopliae* conidia doses?
- (c) What is the effectiveness of using fabric panels impregnated with *Metarhizium anisopliae* to contaminate adult tsetse flies in the field?

### **1.5 Null hypotheses**

- (a) There is no difference in the conidia retention capacity of polyester, cotton and fleece fabrics in ambient conditions.
- (b) There is no effect on tsetse flies' attraction to fabric panels impregnated with different conidia dosages.
- (c) There is no contamination of tsetse flies by fabric panels impregnated with *Metarhizium anisopliae* in the field.

### **1.6 Objectives of the study**

#### **1.6.1 General objective**

To evaluate the efficacy of fabric panels impregnated with *Metarhizium anisopliae* against adult *Glossina fuscipes fuscipes* in Manga and Magare Islands, Homabay, Kenya.

#### **1.6.2 Specific objectives**

- (a) To investigate the *Metarhizium anisopliae* conidia relative retention capacities of polyester, cotton and fleece fabrics in ambient conditions.
- (b) To determine how different dosages of *Metarhizium anisopliae* conidia affect the tsetse flies' attraction to the fabric panels.
- (c) To evaluate the effectiveness of using treated fabric panels to contaminate adult tsetse flies with *Metarhizium anisopliae* conidia in the field.

## 1.7 Significance of the study

Human African Trypanosomiasis is a neglected tropical disease that has been marked for elimination by WHO (WHO, 2010; Holmes, 2014). The control of tsetse vectors of the disease is a critical part of intervention towards the elimination of HAT. There is a need to find alternative and effective control approaches that can be integrated with existing ones to reduce tsetse fly populations. The development and use of biorational technologies reduce the potential risks of negatively impacting non-target insect species.

Entomopathogens have shown great potential in pest and vector control and they have been tested on a vast number of vectors and pests to determine the lethal dosages (Maniania *et al.*, 2002; Dimbi *et al.*, 2003; 2013). The use of the entomopathogenic fungus, *Metarhizium anisopliae* (ICIPE 30) is an efficient way of suppressing tsetse flies and easily penetrates areas that seem inaccessible to other control measures. Using easily available fabric panels as fungus disseminators is an efficient cost-effective control method. This device can be harnessed to improve the success of management programmes by integrating it to achieve total tsetse flies elimination. Therefore, this study investigated the persistence and delivery of *M. anisopliae* fungal spores in selected fabrics for efficient horizontal transfer of conidia to enhance *Glossina fuscipes fuscipes* reduction. Moreover, the study determined the effects of fabric panels impregnated with *M. anisopliae* on tsetse flies densities in Manga Island and the consequent effect on the adjacent control island (Magare island).

## CHAPTER TWO

### LITERATURE REVIEW

#### 2.1 African trypanosomiasis disease

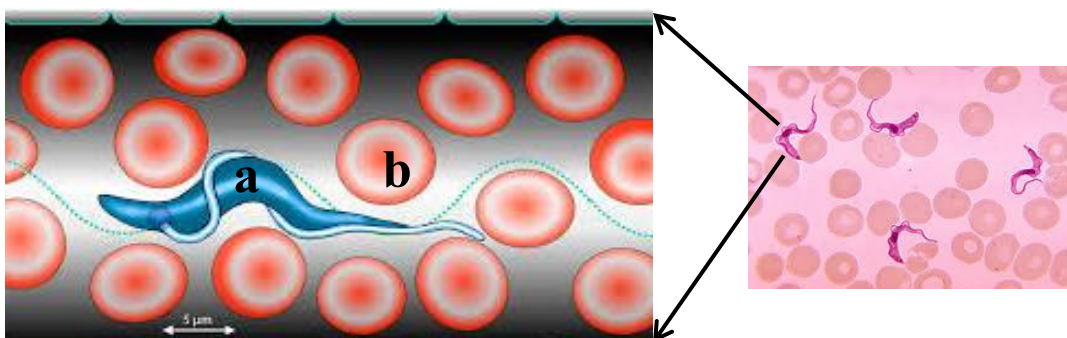
African trypanosomiasis is a neglected tropical disease that affects the less fortunate people in 38 Sub-Saharan African countries (Cecchi *et al.*, 2008; Bouyer *et al.*, 2015). The tsetse fly is the major vector of trypanosomes, the causative agent of trypanosomiasis (Rogers and Robinson 2004). This disease has a resounding negative impact on both the public health and economic sectors (WHO, 2010; 2013). The most severely affected people are those living in remote settings who have minimal or no access to proper health care systems and veterinary services, leading to economic loss and social stigmatization (Bukachi *et al.*, 2017).

Trypanosomiasis affects both humans and animals. Despite intensive research in both the disease and the vector, trypanosomiasis remains a threat to livestock in sub-Saharan Africa and a potentially fatal human disease in the endemic regions (Barrett, 1997; Geiger *et al.*, 2005; Bukachi *et al.*, 2017). There are no vaccines for Trypanosomiasis (Brandl, 1988; Barrett, 1997 and 1999; WHO, 2020). The control of both HAT and AAT depends on the suppression of vectors using different control strategies (Shaw *et al.*, 2007) and using chemotherapy to treat infected people and animals. Tsetse flies are highly susceptible to insecticides and are often controlled through aerial or ground spraying of insecticides, or using insecticides applied on fabric panels (targets/screens) or on livestock to kill landing flies. In addition, biorational interventions with odour-baited stationary tsetse fly traps and the sterile insect technique (SIT) can significantly impact tsetse flies densities (Rayaisse *et al.*, 2011; Shaw *et al.*, 2015). Despite significant success with these interventions in some

parts of Africa, infections with HAT is still rampant (WHO, 2010; Malele, 2011; WHO, 2013) in part due to the re-emergence and the spread of both vector and parasite resistance (Hargrove, 2003). Tsetse flies continuously re-invade areas where they have been previously effectively controlled (Feldmann, 2004; Vale and Torr 2005). Re-invasion increases the costs of interventions and hinders the sustained implementation of area-wide control programs (Shaw *et al.*, 2007).

### 2.1.1 Causative agent of Trypanosomiasis

Trypanosomiasis occurs when parasitic pathogens known as trypanosomes are introduced into a human or animal host by a bite from the vector (Abaru, 1985; Lai *et al.*, 2008). Trypanosomes are flagellated-unicellular protozoans of the genus *Trypanosoma* and Trypanosomatidae family in the kingdom Protista (Figure 2.1). Primary vectors of trypanosomiasis are tsetse flies (genus: *Glossina*) and other biting flies such as tabanids and stomoxys which transmit the disease mechanically when feeding on one host to the other.



**Figure 2.1:** The Trypanosome parasite, (a)*Trypanosoma sp.* (b) Red blood cells  
 Modified from: <https://www.mdpi.com/2072-666X/9/2/63>  
 and <https://www.veterinaryparasitology.com/trypanosoma.html>

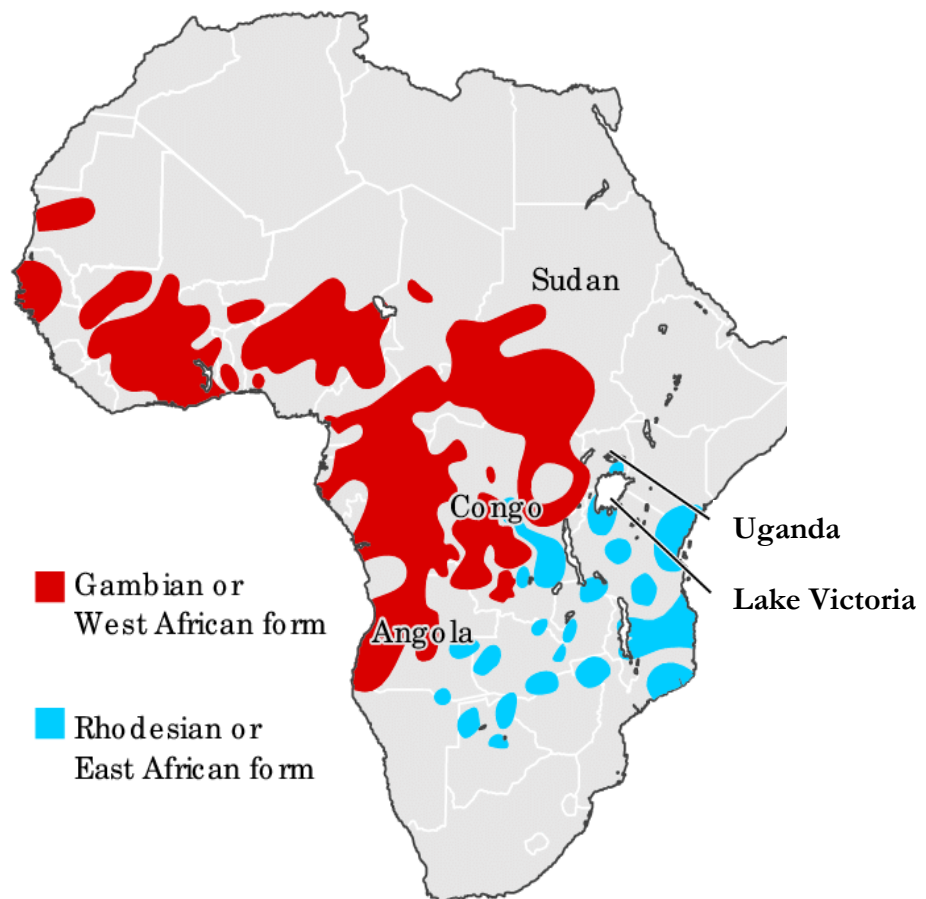
Trypanosomes continuously change their antigenically distinct variant surface glycoprotein coat in order to escape the mammalian host immune system, an adaptation that has invalidated the process of vaccine development. Hence, parasite control and the eventual disease eradication relies heavily on vector suppression (Holmes, 2014; 2015).

### **2.1.2 Types of African trypanosomiasis**

Sleeping sickness is a disease caused by protozoan parasites belonging to the genus *Trypanosoma*. These parasites are transmitted through tsetse fly bites, which acquire these infection(s) from infected human beings or animal reservoirs. There are two forms of sleeping sickness namely the Gambian HAT also called the West African HAT and Rhodesian HAT or East African HAT. Gambian HAT (gHAT) is a chronic form of sleeping sickness caused by *Trypanosoma brucei gambiense*. It has been recorded in 24 countries in Western and Central Africa and it accounts for 98 % of all reported cases (WHO, 2012).

Rhodesian HAT (rHAT) is an acute zoonotic form of sleeping sickness that is caused by *Trypanosoma brucei rhodesiense*. Rhodesian HAT is found in 13 countries in Eastern and Southern Africa. It accounts for less than 2 % of the reported cases of sleeping sickness. Uganda is the only distinct country that has reported both gHAT and rHAT. However, the location of these two forms of the disease indicates that they are spatially distinct with no report of convergence. The levels of sleeping sickness significantly dropped in the 1960s, but due to relaxed surveillance systems, there was an increased resurgence in the 1980s and 1990s (Hargrove *et al.*, 2000; Hargrove, 2003).

Sleeping sickness causes high morbidity and mortality in human populations, and more than 70 million people are at risk of HAT infection (WHO, 2013). There have been sporadic cases of tourists infected with the disease. This endangers the tourism industry, which is a significant economic activity in Africa (Jelinek *et al.*, 2002). In Kenya, a total human population of about 11 million are at risk of infection and most importantly inhabitants near the Lake Victoria basin shared with Tanzania and Uganda make this zone a hotspot for sleeping sickness outbreaks (Rogers and Robinson 2004; KETRI, 2008).



**Figure 2.2:** The distribution of Human African Trypanosomiasis (Sleeping sickness) in Africa.

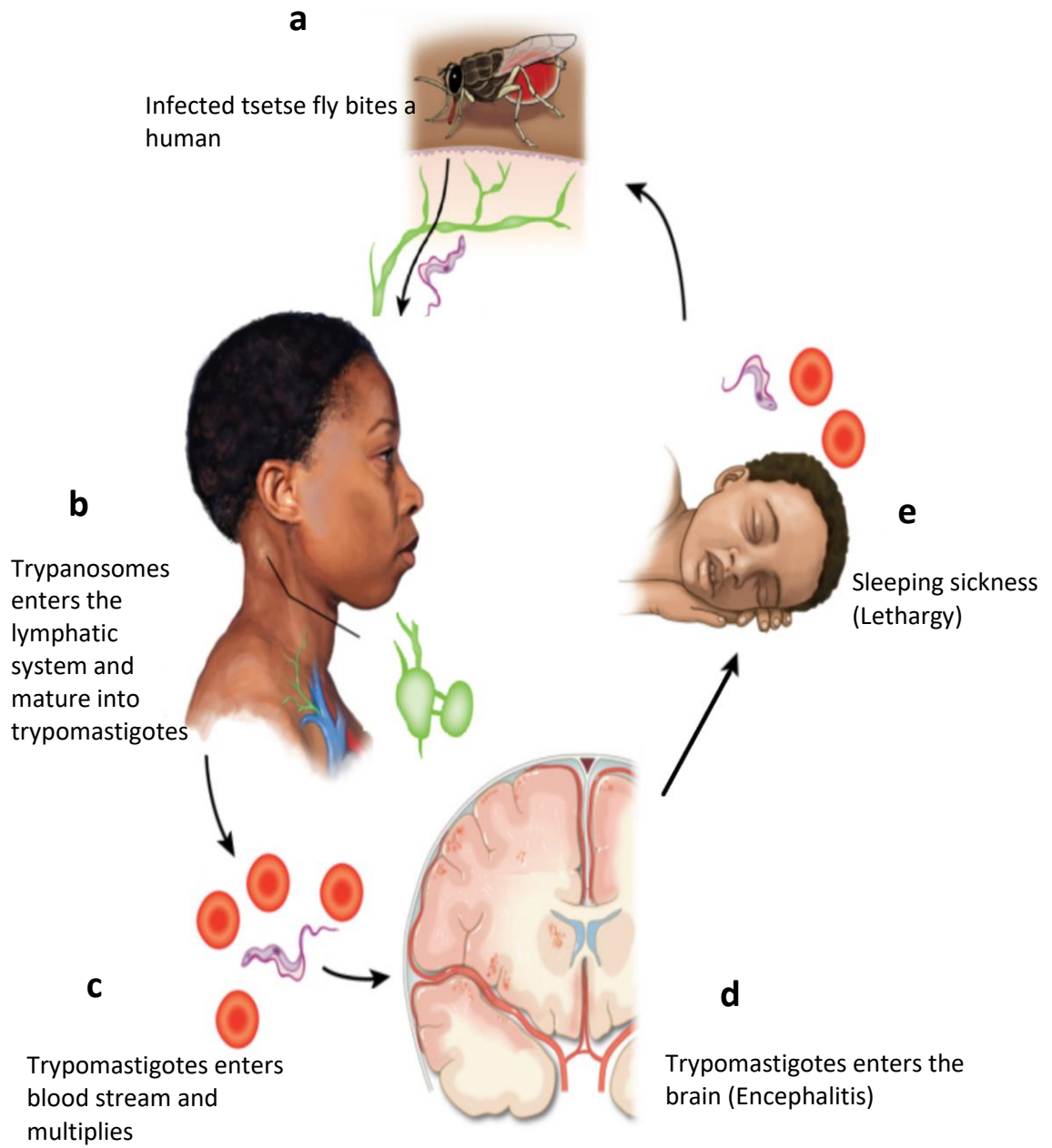
**Lundkvist 2004:** Why Trypanosomes cause sleeping sickness

Animal African trypanosomiasis (Nagana) is also a result of infection by protozoan parasites belonging to the genus *Trypanosoma*. The following species are responsible, including *Trypanosoma brucei*, *T. congolense*, *T. evansi*, *T. vivax* cause nagana in cattle and small ruminants, *T. evansi* causes surra in camels, *T. equiperdum* causes dourine in horses and other equids and *T. simiae* and *T. suis* causes disease in pigs (Cattand *et al.*, 2001). Animal African Trypanosomiasis is widespread across most of the 38 countries in sub-Saharan Africa that are considered endemic for tsetse flies, therefore, becoming a major limiting factor to agricultural production. Nagana limits livestock productivity in Africa. Approximately 3 million cattle die every year due to AAT (Kristjanson *et al.*, 1999; Budd, 2000). Farmers administer about 35 million dosages of trypanocidal drugs to treat AAT. Ultimately, the disease causes agricultural losses amounting to 4.75 billion dollars (Simarro *et al.*, 2012; Shaw *et al.*, 2013).

### **2.1.3 The transmission cycle of sleeping sickness**

The disease transmission cycle of Gambian sleeping sickness is commonly considered to be a human-fly-human route (Figure 2.2). During feeding, infected tsetse flies subcutaneously inject metacyclic trypomastigotes (trypanosomes) into mammalian hosts. The parasites make their way into the lymphatic system and later pass into the bloodstream (WHO, 2010). These transform into bloodstream trypomastigotes and they are transported to other body fluids including the lymph fluid and the spinal fluid, where they continue to reproduce asexually via binary fission (Dargie, 2008). Tsetse flies get infected with bloodstream trypomastigotes when taking a blood meal from an infected mammalian host. The fly's midgut allows the parasite to transform into procyclic trypomastigotes where they multiply by binary fission. They later leave

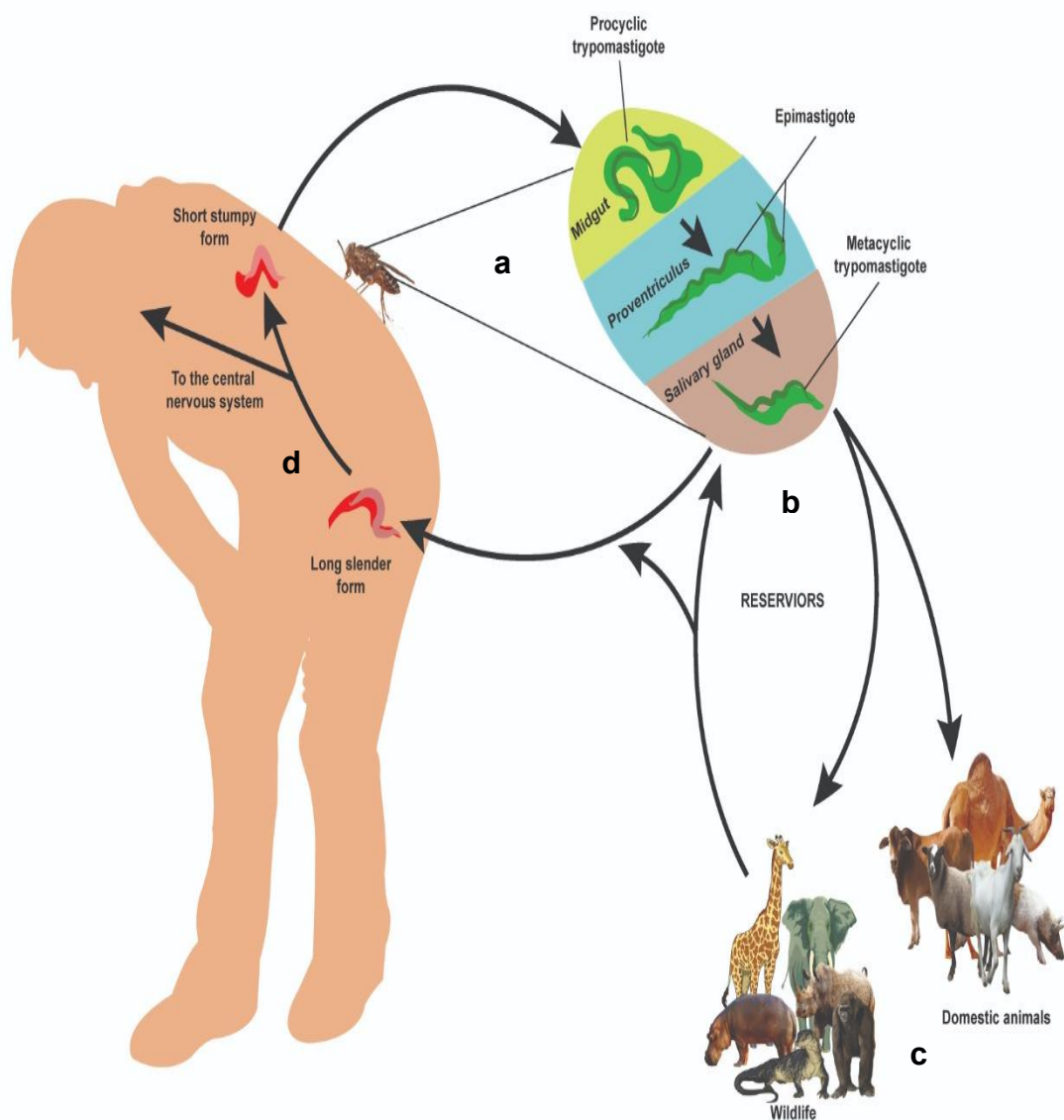
the midgut and transform into epimastigotes which migrate to the fly's salivary glands and continue to replicate through binary fission (MacGregor and Matthews, 2010).



**Figure 2.3:** The transmission cycle of Gambian sleeping sickness

Modified from: <https://www.netterimages.com/trypanosomiasis-african-sleeping-sickness-unlabeled-neurology-anita-impagliazzo-57765.html>

Rhodesian sleeping sickness transmission relies on the presence of vertebrates that act as *Trypanosoma* reservoirs; this comprises both domestic and wild animals (Wamwiri *et al.*, 2007; Wamwiri and Changasi, 2016). *Rhodesiense* transmission cycle can be described as animal-tsetse fly-animal/human (Figure 2.3). In Eastern and Southern Africa, the wildlife conservation areas harbour diverse wildlife species that serve as disease reservoirs (Simarro *et al.*, 2015).



**Figure 2.4:** The transmission cycle of Rhodesian sleeping sickness  
Created using Adobe spark

This existing human-wildlife interface allows continuous circulation of *r*HAT in and around the game parks and reserves (Namangala *et al.*, 2012). In low endemic areas, livestock have been shown to exhibit low incidences of *T. b. rhodesiense* which if not managed could translate into an outbreak. A case in point is the emergence of HAT in northern Uganda linked to the introduction of infected cattle from endemic areas in the southern region (Fèvre *et al.*, 2001).

#### **2.1.4 Mechanical transmission of animal trypanosomiasis**

In addition to the canonical transmission of AAT by tsetse flies, biting flies like stomoxys and tabanids have been known to mechanically transmit *Trypanosoma evansi* to camels, livestock, and other large wild animals. Mechanical transmission takes place when a biting insect commences taking a blood meal on an infected host and while feeding it is interrupted by the host's defensive movements (Dicko *et al.*, 2015). The biting fly then moves to another animal to initiate another blood meal feeding process. The residual blood from the infected animal is inoculated into the naïve host during the next blood meal when the insect is injecting the anticoagulant into the saliva. In this case, trypanosomes do not survive for very long in biting flies as they are not competent vectors for parasite development. The survival of trypanosomes is estimated to be about 30 minutes which allows efficient transmission during the transient period between two interrupted blood meals (Hargrove, 2003; Vreysen *et al.*, 2013). Alternatively, mechanical transmission occurs when blood from the biting fly's gut is regurgitated during the early stages of the blood-feeding process and the parasites are still pathogenic (Kidambasi *et al.*, 2019).

### **2.1.5 Treatment of sleeping sickness**

The successful treatment of trypanosomiasis infection is dependent on the disease stage. The drugs administered during the early stages of infection are easier and safer to administer compared to those administered during advanced/secondary stages. The success of treatment in the secondary stage is dependent on the ability of the drugs to break the blood-brain barrier, in order to reach the parasite. Such drugs are very complicated to administer and are equally toxic (Cattand *et al.*, 2001; Simarro *et al.*, 2012). There are five different drugs donated by the World Health Organization that are used for treating sleeping sickness across endemic regions (WHO, 2010).

In the early stages of Rhodesian infection, suramin discovered in 1920 is administered intravenously while pentamidine discovered in 1940 is administered intramuscularly for early infections by Gambian sleeping sickness. Despite the undesirable toxic effects, these drugs are tolerable (Barrett, 1999). Melarsoprol, a drug discovered in 1949, has been used for the second stage treatment of both Gambian and Rhodesian infections. It is an arsenic derivative associated with many undesirable side effects including encephalopathy which can be fatal. Eflornithine is a molecule that was registered in 1990 and with less toxicity compared to melarsoprol. It is known to be effective against Gambian infections, although, it is difficult to apply. Another drug is a combination treatment of eflornithine and nifurtimox to make nifurtimox-eflornithine therapy (NECT). This drug was introduced in 2009 (Kennedy, 2013). This has largely been studied and registered for American trypanosomiasis and has since been recommended as the first line of treatment for Gambian. New drugs have been discovered as a comprehensive oral treatment for Gambian sleeping sickness

(Maxmen, 2017). An example is fexinidazole which recently received positive scientific opinion (Pollastri, 2018).

## **2.2 Morphology of tsetse flies**

The word “*tsetse*” means “fly” in Tswana, in general, it refers to all the 31 tsetse fly species of the genus *Glossina*. The morphological structure of tsetse flies resembles that of the common house fly but can be distinguished by two anatomical characteristics which include the long proboscis and the wings position while resting. Among the tsetse fly species, only two subgenera are incriminated in the transmission of the two pathogenic human parasites. These include; the subgenus *palpalis* group and subgenus *morsitans* group each with six and two subspecies respectively (Hargrove, 2003; Wamwiri and Changasi 2016).

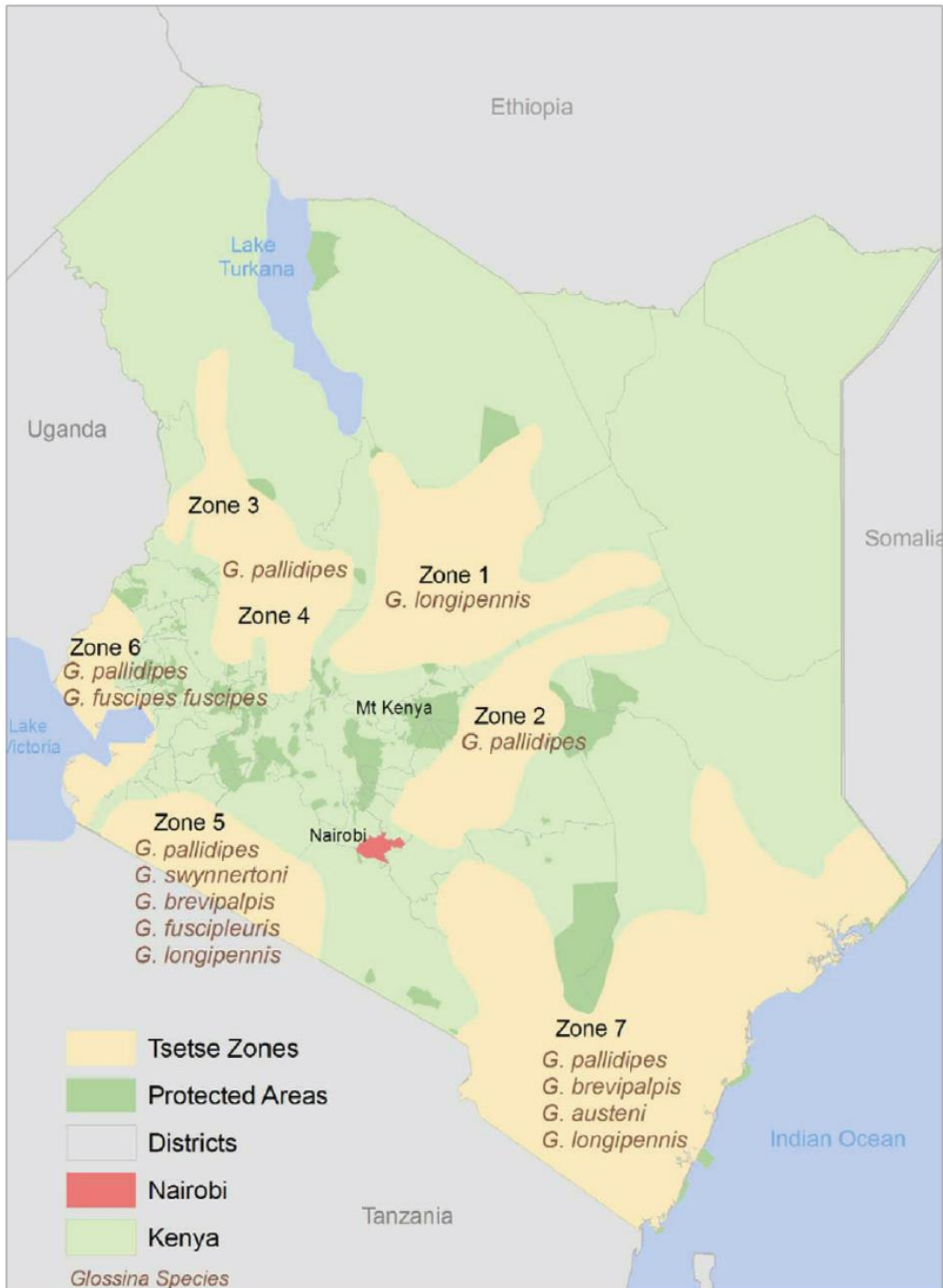
### **2.2.1 Systematics and biology of tsetse flies**

Tsetse flies belong to the order Diptera in the superfamily Hippoboscoidea in which the tsetse fly family Glossinidae is one of the four families of blood-feeding obligate parasites. Glossinidae is made up of a single genus *Glossina* which is composed of 23 species, which are further divided into 14 subspecies (Namangala, 2012). These species are classified into three subgenera that correspond with groups of species found in different ecological settings, these include: - the *fusca* group (*Austenina*), the *palpalis* group (*Nemorhina*) and the *morsitans* group (Savanna). There are six major *Glossina* species namely; *Glossina palpalis*, *Glossina tachinoides*, *Glossina fuscipes*, *Glossina morsitans*, *Glossina swynnertoni* and *Glossina pallidipes* (Austen, 1903).

Tsetse flies are found in the tropical and subtropical parts of sub-Saharan Africa. The *morsitans* subgenus occurs in the open country and they are popular in thickets,

scrubs, and savannah woodlands with the exception of the forest-dwelling *G. austeni*. *Glossina morsitans* is the most widespread tsetse fly species extending through much of the African Savannah (Rogers and Robinson 2004). The *palpalis* subgenus species are distributed in vegetations around watercourses in Central and Western Africa while the *fusca* subgenus is mostly found in forested habitats such as in the rainy mangrove forests overlapping with the *palpalis* subgenus. The land cover and tsetse fly habitat distribution of *fusca* and *palpalis* subgenus correlate while that of *morsitans* has a weak correlation. This is evident in their numerous numbers despite their unique reproduction (Cecchi *et al.*, 2008).

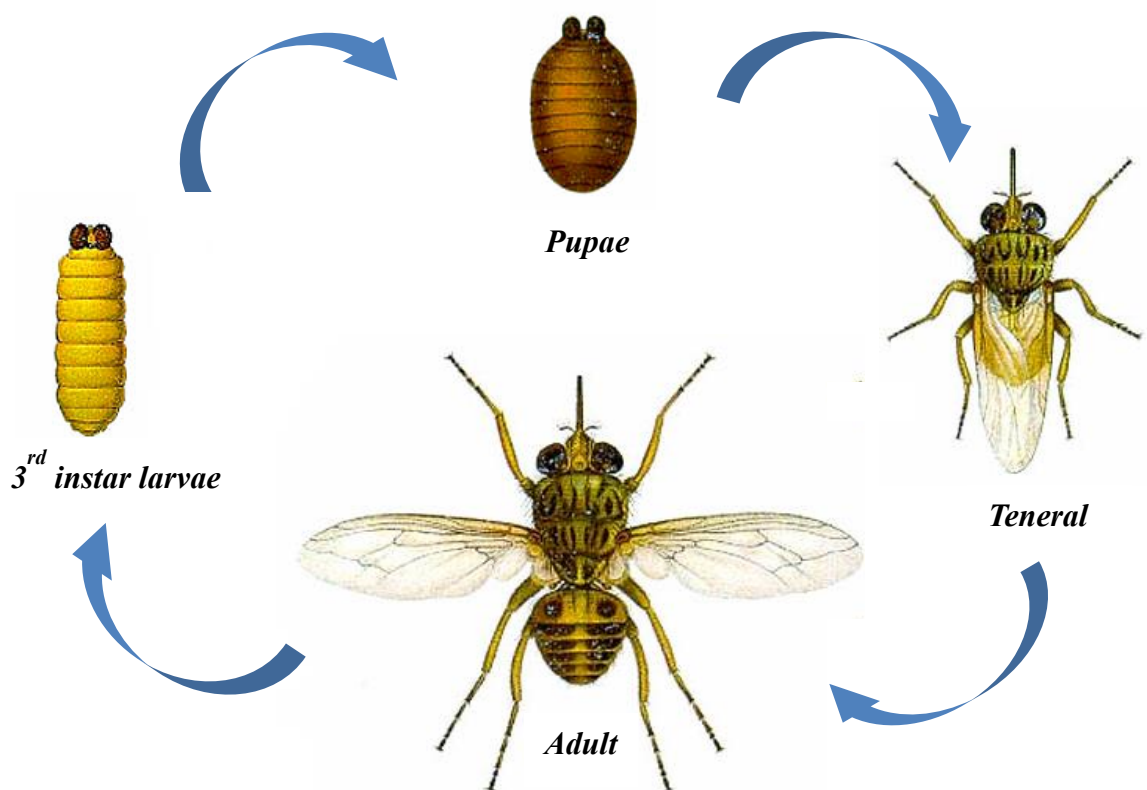
In Kenya, there are eight tsetse fly species which are distributed in seven distinct zones (Figure 2.4). These include *Glossina pallidipes* which is common in all the tsetse fly belts in Kenya, while the *G. brevipalpis*, *G. longipennis* and *G. austeni* are commonly found in the coastal belt, which extends from Somalia to Tanzania and some species on the Kenyan-Ethiopian border on the eastern shores of Lake Turkana. *Glossina fuscipleuris* and *G. swynnertoni* are found in South-Western Kenya in Narok and Trans-mara areas. *Glossina fuscipes fuscipes* is found strictly around the Lake Victoria basin and surrounding river systems and extends into Tanzania and Uganda (KETRI, 2008; McCord *et al.*, 2012).



**Figure 2.5:** Spatial distribution of tsetse flies in Kenya by KETRI 2008

### 2.2.2 The life cycle of tsetse flies

Tsetse flies undergo a complete metamorphosis which consists of the following developmental stages: the egg, three larval stages, a pupal stage, and the adult (Figure 2.5). The females mate only once in their lifetime and retain the sperms in the spermathecae (WHO, 2020). At any one point, a single egg is fertilized and retained in the uterus to develop internally during the first three larval stages, a reproductive mode is known as adenotrophic viviparity (Leak *et al.*, 1993). The developing offspring is nourished by specialized accessory glands termed as milk glands that empty their secretions in the uterus. Following larviposition of a third instar, the larva burrows into the soil and pupates. It takes approximately 30 days for the adults to emerge after pupation (Potts, 1973; Hargrove, 2003).



**Figure 2.6:** The four visible stages that characterize the life cycle of tsetse flies

Modified from: [https://commons.wikimedia.org/wiki/File:Glossina\\_palpalis\\_morsitans.jpg](https://commons.wikimedia.org/wiki/File:Glossina_palpalis_morsitans.jpg)

### **2.3 Control of tsetse flies**

Tsetse flies' control has constantly changed over centuries due to the vector's biology and its interaction with the surrounding environment. This dynamic nature of their co-existence has necessitated the development of better control strategies (Maniania, 1994; 1998). Before the era of insecticides, the control measures mainly involved; the destruction of tsetse flies habitats by removal of woody vegetation and elimination of wild games which serve as reservoirs for the pathogen (Hocking *et al.*, 1963). Control measures have primarily focused on three broad areas namely: the use of chemical products, non-chemical products and biological control agents (Potts, 1973; Gouteux *et al.*, 1986).

#### **2.3.1 Chemical control strategies**

The use of chemicals in tsetse fly control was the commonly used strategy in the suppression of tsetse fly populations. For many decades, the blanket application of insecticides by aircrafts and aerosol ground spraying gave rapid and effective results. However, after extensive research on vector behaviour and habits, the targeted approach of insecticide application was found even more economical and efficient.

##### **2.3.1.1 Use of insecticides**

The use of insecticides such as DDT has been the classical vector control method. Aerial sprays and ground spraying were commonly used (Hargrove *et al.*, 2000). The spraying of DDT was widely used on vast acres of land at the beginning of the twentieth century and this was done in collaboration with different countries on an area-wide control programme (Allsopp *et al.*, 2004; Torr *et al.*, 2005). However, these techniques have caused high monetary efflux in poor communities and

environmental challenges such as the elimination of non-target insects of importance (Rayaisse *et al.*, 2011; Shaw *et al.*, 2015). Insecticides over the years supplemented the existing management methods even though their efficiency was faced by resistance (Rowlands *et al.*, 2001).

### **2.3.1.2 Insecticide-treated targets**

Insecticide-treated targets are made up of blue and black cloth impregnated with pyrethroids such as deltamethrin. Application of pyrethroid insecticide formulations in artificial attractive devices has shown success in controlling *G. f. fuscipes* (Esterhuizen *et al.*, 2011). In another study, they reported that tsetse fly populations were reduced by over 90 % in Uganda and Manga Island in Kenya after targets were impregnated with 300 mg/m<sup>2</sup> deltamethrin for 12 months (Esterhuizen *et al.*, 2011; Tirados *et al.*, 2015). Targets have been found to be more economical and effective in comparison to the conventional tsetse fly traps. This is attributed to their small size and simplicity in design, the cost implication and delivery efficiency, these insecticide-treated targets are more cost-effective and impressive (Vale *et al.*, 1986).

### **2.3.1.3 Insecticide-treated livestock**

African animal trypanosomiasis and tick-borne diseases are the main challenges to livestock production and sustainability. To counter this, livestock farmers have been protecting their cattle by using acaricides such as amitraz, permethrin, decamethrin and deltamethrin and cattle pour-on. There are two-universal methods of applying insecticide on cattle: - the use of hand sprayers and cattle dipping in acaricides baths. Livestock treatment is not only advantageous in respect to reduced tick infestation but

also tsetse flies biting patterns and mosquito bites (Hargrove *et al.*, 2000; Gimonneau *et al.*, 2016).

#### **2.3.1.4 Waterbuck repellent**

Tsetse flies are predominantly blood-feeders, their host range is wide and diverse and for this reason, they serve as effective vectors for various zoonotic diseases (Dargie, 2008). Studies aimed at understanding the blood-feeding patterns of tsetse flies reported some characteristic host preferences across different species (Saini and Hassanali 2007). Researchers at *icipe* developed repellent chemicals that mimicked natural odours emanating from non-host bovid to affect the olfactory signals of tsetse flies hence changing their behaviour (Saini and Hassanali 2007; Wachira *et al.*, 2016). Findings from these studies led to the invention of cattle collars which release a potent waterbuck repellent from the prototype periodically protecting cattle from tsetse fly bites (Saini *et al.*, 2017). More studies on olfactory genes may result in the development of more potent tsetse fly attractants and repellents (Mbewe *et al.*, 2019; Olaide *et al.*, 2019). This will offer an effective integrated disease and vector management strategy which will be able to capitalise on all possible vulnerability spots of the vector and offer synergies for tsetse fly control (Saini *et al.*, 2017).

#### **2.3.2 Non-chemical-based control strategies**

Non-chemical-based control strategies ensure tsetse flies retrieval during field experiments for the purposes of monitoring tsetse flies' diversity, population density and distribution over time. Most of these techniques are used to obtain tsetse flies samples for laboratory analysis and colony rearing. They range from conventional tsetse fly traps, baited traps, sticky panels and electrocuting grids.

### 2.3.2.1 Tsetse fly traps

Trapping technologies are vastly applied in endemic areas to suppress tsetse fly populations significantly. The most commonly used is the biconical trap, which comes in two forms: - the Biconical Challier-Laveissière trap, and its simplified derivative, the monoconical Vavoua trap (Challier *et al.*, 1977). These are fitted with a collection cage, thus preserving live flies (Laveissière and Grébaut 1990). Enhanced traps with odours from humans, cattle or pigs have also shown superior attraction as tsetse flies' baits. In a study by Rayaisse *et al.* (2010) natural cues from domesticated animals and humans' odours were shown to intensify tsetse flies responses by either using them as baits on biconical traps or electrocuting nets (Rayaisse *et al.*, 2010). The Ngu trap baited with acetone and cow urine caused a dramatic reduction of *G. pallidipes* and *G. longipennis* in Nguruman, South-West Kenya (Brightwell *et al.*, 1987; 1991; 1997).



**Plate 2.1:** A pictorial view showing types of tsetse traps. a) Biconical trap and b) Ngu trap

### **2.3.2.2 Sticky panels**

Sticky panels comprise adhesive materials that are used for easy retrieval of trapped tsetse flies. These panels have been extensively used hand in hand with electrocuting nets and tsetse fly attractive surfaces. In a mass sterile male release experiment, sticky panels were used in monitoring the daily catches of *G. austeni* in Unguja Island, Zanzibar (Vreysen and Saleh 2001). Besides this, Mbewe *et al.* demonstrated that these sticky materials mounted on 0.25 m × 0.25 m blue-black panels make a very effective sampling tool in low tsetse flies density areas (Mbewe *et al.*, 2018b).

### **2.3.2.3 Electrocuting grids**

Experimental designs in tsetse vector control have been informative on tsetse flies' behaviour. Considering this, electrocuting panels have clarified how tsetse flies approach a trap or a target, where they first hover around it and then land on it. Furthermore, their attraction to different fabric colours has also been made possible (Lindh *et al.*, 2012; Vale *et al.*, 2015). Electrocuting targets measuring 0.25 m × 0.5 m have been instrumental in modelling tsetse flies landing responses that infiltrate them before they land on a host (Lindh *et al.*, 2009). Vale (1974) and Torr (1994) determined tsetse flies host preference by using an electric ring of e-nets, where different hosts were placed at the centre of five 1 m × 1 m electrocuting nets and assessed their attraction across a 20 % perimeter. These showed varying luring power depending on the host (Vale, 1974; Torr, 1994).

## **2.3.3 Biological control strategies**

Living micro-organisms have been used as biopesticides in vector control. The shortcomings of chemical use have prompted researchers to evaluate the use of eco-

friendly agents. This has led to the development of technologies like Sterile insect techniques, paratransgenesis and the use of entomopathogens like fungi (Maniania, *et al.*, 2002; Medlock *et al.*, 2013).

### **2.3.3.1 Sterile Insect Technique (SIT)**

The Sterile Insect Technique is a form of vector/pest control method that uses ionizing radiation to sterilize male flies that are mass-produced in special rearing facilities. The sterile males are systematically released from the ground or by air in tsetse fly-infested areas, where they mate with wild females which do not subsequently produce off-spring (Guz *et al.*, 2007). Tsetse fly's female mating behaviour indicates that they only mate once in their lifetime, where the sperms are stored in the spermatheca. Consequently, the male's sterility cripples the future generations leading to tsetse flies population density reduction (Abd-Alla *et al.*, 2013).

Sterile Insect Technique is an environmentally friendly control tool and has been instrumental in tsetse vector control. Ionizing radiation which involves the use of gamma-rays or X-rays are used in special rearing units to achieve male sterility (Abd-Alla *et al.*, 2013; Vreysen and Saleh 2001). In 1994 to 1997 a mass release of sterile male flies showed great success in eradicating *Glossina austeni* in Zanzibar (Vreysen and Saleh 2001). This was integrated with insecticides which suppressed the tsetse fly population to some level and later eliminated them using SIT. In this account, SIT can be more effective in removing the last remnants of tsetse flies after an area-wide mass vector control (Vreysen *et al.*, 2014).

### **2.3.3.2 Paratransgenetic control**

Tsetse fly symbionts control takes advantage of its associations with microbes like *Sodalis*, *Wolbachia* and *Wigglesworthia* (O'Neill *et al.*, 1997). These are symbiotic bacterium that can be vertically transmitted through the maternal milk gland secretions to the larval stage. *Wigglesworthia* has been traced in laboratory-reared and field captured tsetse flies and their symbiotic relations is ancient. They contribute to the larval development in the female uterus where control measures can be put to infiltrate the larval oviposition process and use it as a management point ( Medlock *et al.*, 2013). According to Abd-alla *et al.*, these endosymbionts can be targeted and used to manage vector populations (Abd-Alla *et al.*, 2011; 2013). These microbes can act by (i) causing pathogenic effects in the host, (ii) interfering with the host's reproduction, (iii) reducing the vector competence and (iv) interfering with oogenesis and embryogenesis.

## **2.4 Entomopathogenic fungus (EPF)**

Naturally, tsetse flies have many entomopathogens, which range from different types of viruses, bacteria and fungi (Kaaya and Darji 1989; Maniania *et al.*, 2006). Entomopathogenic fungus is among the new alternative active ingredients being included in Integrated Pest Management (IPM). Worldwide, over 750 different entomopathogenic fungal species have been identified and isolated from numerous habitats and infected insects (Scheepmaker and Butt, 2010). Despite the high vector and pest susceptibility to these biopesticides, their use in control is limited to personal knowledge of their ability and effectiveness (Maniania, *et al.*, 2002; Mohammadyani *et al.*, 2016; Litwin *et al.*, 2020).

The use of EPF has brought an alternative to using synthetic chemical insecticides, with several species being commercial. This include *M. anisopliae*, *Beauveria bassiana*, *Beauveria brongniartii*, *Lecanicillium spp.* and *Paecilomyces fumosoroseus* (Butt *et al.*, 2001). They have shown promising results in reducing pest populations. Some of the most widely used are *Beauveria bassiana* and *Metarhizium anisopliae*. *Metarhizium anisopliae* is a fungus complex with different strains that have been shown to be infectious to different vectors and pests (Fernandes *et al.*, 2010). The study by Dimbi *et al* reported that 4 days post-infection, different *B. bassiana* and *M. anisopliae* strains caused 72 - 78 % mortality rates to fruit flies (Dimbi *et al.*, 2013). In another study, *M. anisopliae* strain (ICIPE 30) was infectious to *Glossina* species causing high mortality that led to population decrease (Maniania, 1994).

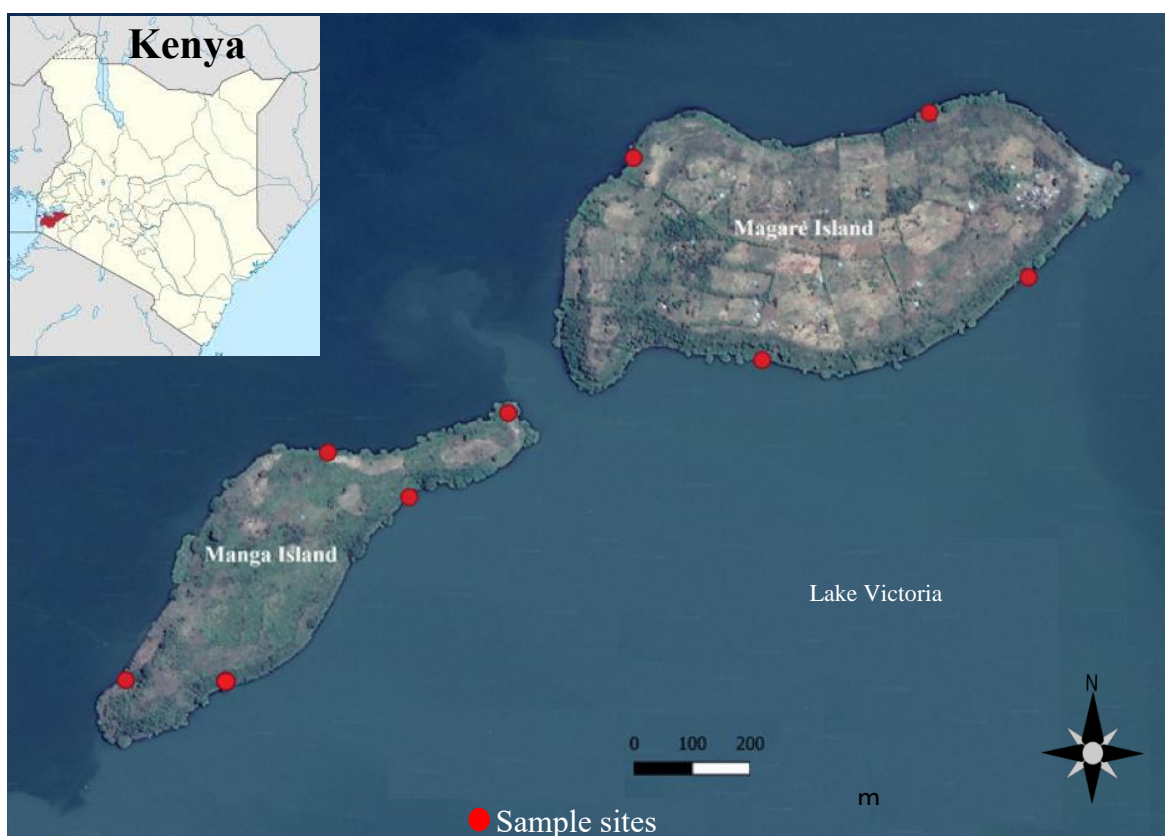
Hence, the use of tsetse fly attractive fabric panels impregnated with the fungal conidia would form an effective control tool (Dimbi *et al.*, 2013; Maniania *et al.*, 2013; Wamiti *et al.*, 2018). Besides this, biological control tools like this fungus have not registered any resistance over the years. There is, therefore, a strong need to develop affordable control tools to help suppress the transmission of HAT. Tools that are easy to deploy in hard-to-reach places are ideal.

## CHAPTER THREE

### MATERIALS AND METHODS

#### 3.1 The study sites

The study was carried out in Manga (-0° 21' 10.8"S; 34° 15' 10.8"E) and Magare Islands (-0° 20' 52.8"S; 34° 15' 43.2"E) in Homabay County, Western Kenya (Figure 3.1). Manga has a surface area of 1.0 km<sup>2</sup> and a circumference of approximately 2.0 km. Magare is relatively larger with a surface area of 2.0 km<sup>2</sup> and a circumference of about 2.5 km. The two islands are 1143 - 1500 m above sea level and receive an annual average rainfall of 500 - 1000 mm. The average annual temperature in the area is 25 - 30 °C. The distance between the two islands is approximately 100m.



**Figure 3.1:** Satellite map of the study sites, Manga and Magare Islands.  
Created using Google Earth and Q-GIS mapping tools 2019.

About three hundred fisher folks, many of whom also keep livestock and practice small scale subsistence maize farming live in Manga and Magare. Magare has lower vegetation cover due to the anthropogenic activities compared to Manga which has low population of human inhabitants. The terrain is rocky and characterised by high nutrient black cotton soil. The vegetation cover consists of freshwater mangrove trees (*Aechynomene eraphroxylon*: Family: Fabaceae), tropical Hydrangea (*Dombeya spp.* : Family: Malvaceae) and Tick-berry (*Lantana camara*: Family: Verbenaceae ) strands (Tirados *et al.*, 2015). The potential tsetse fly host range in the area include humans, cattle, Nile monitor lizards (*Varanus niloticus*) and hippopotamus (*Hippopotamus amphibious*).

### **3.2 Baseline survey for tsetse flies densities and presence of wild *Metarhizium anisopliae* in Manga and Magare Islands**

Prior to initiating the planned research experiments, cross-sectional studies were conducted to determine the presence, density, diversity and distribution of tsetse flies in the two islands. Additionally, the prevalence of *Metarhizium anisopliae* in the wild tsetse fly population in the study area was evaluated. Four biconical traps and four sticky small targets were deployed on each island daily between 9:00 and 12:00 hours for 8 days. The traps and targets were set 1 m from the shoreline of Lake Victoria and alternated leaving a minimum of 150 - 200 m between adjacent devices. Trapping positions were marked using Global Positioning System units (Etrex 20X, Garmin, US). Tsetse flies trapped were collected and identified using morphological keys. Each tsetse fly was transferred to a 1.5 ml Eppendorf tube for preservation with 70 % ethanol to preserve the DNA integrity and later transferred to a -80 °C fridge for

storage, awaiting further molecular analysis at Molecular Biology and Bio-informatics Unit, *icipe* Nairobi.

### **3.2.1 Investigating the prevalence of *Metarhizium anisopliae* in Manga and Magare Islands using molecular analysis**

Two hundred flies were screened for *Metarhizium anisopliae* using DNA analyses. The whole tsetse fly was crushed in liquid nitrogen using a motor, to achieve a homogenate. The Genomic DNA was extracted as described in the Isolate II Genomic kit protocol (BIO-52067, Bioline, UK). Polymerase Chain Reactions (PCR) were carried out using specific F3 (5'-GGGTATATGAGAGGGAGGGC-3') and B3 (5'-GGTTCCTGGTCGGGACTT-3') primers which amplify fragments of a gene in the intergenic spacer region of Ribosomal RNA (*rRNA*) of *M. anisopliae*.

A volume of 10 µl was used for the PCR containing 5 µl Dreamtaq Green PCR master mix (Thermo Fisher Scientific, USA), 3 µl PCR water, 0.5 µl forward and reverse primers and 1 µl DNA template. This was analysed using Proflex thermocycler (Applied Biosystems, USA) for 35 cycles in the following conditions: initial denaturation at 94 °C for 30 seconds, final denaturation at 94 °C for 30 seconds, annealing temperature at 60 °C for 30 seconds, extension temperature at 72 °C for 30 seconds and a final denaturation at 72 °C for 10 minutes. Polymerase Chain Reaction products were further analysed using gel electrophoresis in 1.2 % agarose gel in Bio-Rad model 200/2.0 power supply for 1 hour. The PCR products were visualized under ultraviolet light (MD-25/HD-25 UV Transilluminator, Wealtec Corp, US) and the images were taken with KODAK Gel Logic 200 Imaging System (Raytest GmbH, Straubenhardt, Germany).

### 3.3 Mass production and validation of *Metarhizium anisopliae*

The entomopathogen *Metarhizium anisopliae* ICIPE 30 strain was used throughout the study. Mass production of fungal conidia was done following standard operating procedures established in the Arthropod Pathology Unit of *icipe*, Kenya. A liquid broth was constituted by adding 15 g peptone, 30 g yeast and 30 g glucose in 1 L of distilled water. The mixture was covered with aluminium foil and autoclaved at 121 °C for 30 minutes, then left to cool to room temperature at 25 °C. Using a sterile inoculating loop, *M. anisopliae* spores from the master mix were inoculated in the 50 ml of liquid broth in a 250 ml flask. To prevent bacterial contamination, 5 µg/ml of chlortetracycline, 500 µ/ml of chloramphenicol and 250 µ/ml of streptomycin/penicillin were added to the broth and incubated at 25 - 27 °C for three days in an incubator shaker (New Brunswick Scientific, innova®44, UK), 100 revolutions per minute. Two kilograms of rice washed with distilled water was placed in a Milner bag (45.72 × 40.64 cm, 2 millimetres thick, 3.5 Gallon size, Shiny select, US), autoclaved for 40 minutes at 121 °C and left to cool at room temperature.

The previously constituted liquid broth was added into the Milner bags and incubated at 25 - 27 °C. The bag was massaged daily to ensure uniform fungal germination. After 21 days, contents of the Milner bag were emptied into an open plastic basin (Internal diameter (ID) 15.24 cm, H100XDia248, Capacity 3 Litres, KENPOLY, KE) and air-dried for a week. Conidia were extracted by carefully agitating the dry rice substrate in a metallic fine mesh sieve (ID 20.32 cm, 35 mesh (500 µm), Field master® Wildco, US) mounted on conidia collecting trays. Extracted conidia were dried for 72 hours in trays, then transferred into Ziploc bags (29 × 23.6 × 15.7 cm, Ziploc B01J54T50M, ASIN, US) for storage in a dark room.

Conidia viability was determined by adding 0.1 g of conidia into 10 ml of triton water (Diluted Triton <sup>TM</sup> X - 100, Sigma - Aldrich, MERCK, US). The mixture was inoculated using a sterile wire loop onto petri dishes (10 × 1.5 cm) with Sabouraud Dextrose Agar (CM 0041, Oxoid limited, UK) media and incubated at 25 °C for 16 - 18 hours. Four cover slips (2.2 × 2.2 cm) were placed on the media and inspected under a compound microscope. The proportion of germinated conidia under each cover slip were counted. Proportions of germinated conidia in the four cover slips were averaged to give an estimate of viability. Only conidia batches with a viability of at least 85 % were used for experiments.

### **3.4 Test fabric panels**

Three fabrics: - fleece, cotton and polyester were subjectively chosen for evaluation as potential panels for dispensing *M. anisopliae* conidia in the field. Polyester is the standard fabric for commercially available insecticide-treated targets manufactured by Vestergaard Frandsen (Sweden). The fleece and cotton fabrics imported from India were bought from a fabric shop in Nairobi, Kenya.

### **3.5 Evaluating retention capacity of *Metarhizium anisopliae* conidia by polyester, cotton and fleece fabrics**

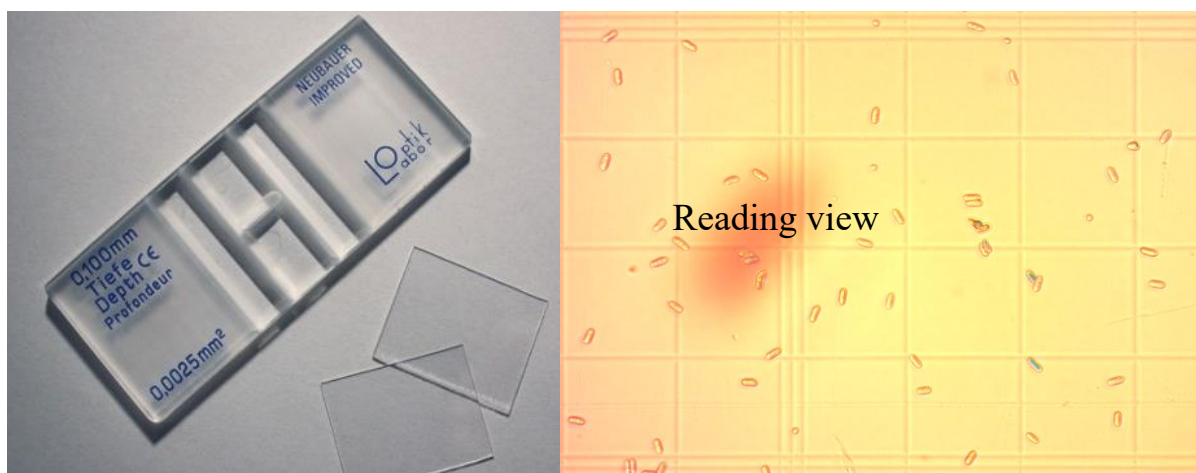
To determine the conidia retention capacity of the different fabric panels, three replicates of fabric panels made of polyester, fleece and cotton were used. The panels are comprised of blue and black fabric panels. The blue panel made of pthalogen blue cloth (25 × 25 cm) was attached to a black panel of the same size (25 × 25 cm) made of polyester netting material, cotton fabric or fleece heavy density fabric (Plate 3.1). These were dusted with *M. anisopliae* conidia at a concentration of 1mg/cm<sup>2</sup>.



**Plate 3.1:** A pictorial view showing a fungus impregnated fabric panel prototype

In the field, the three fabrics were positioned one meter apart: with each set not less than 20 meters apart. These were monitored daily for two weeks, where the conidia retention per fabric was determined over time. The proportions of the retained conidia per panel were collected using adhesive material (Luminous 4, Adhesive Rolls [UNGRIDDED] Stock code: FE217). On each fabric, three random spots measuring 19 mm × 13 mm were selected as the sampling area. The conidia were washed off the adhesive material using 10 ml sterile Triton water. With the aid of glass beads, the solution was vortexed vigorously for uniformity. Fifty microlitres of the solution were

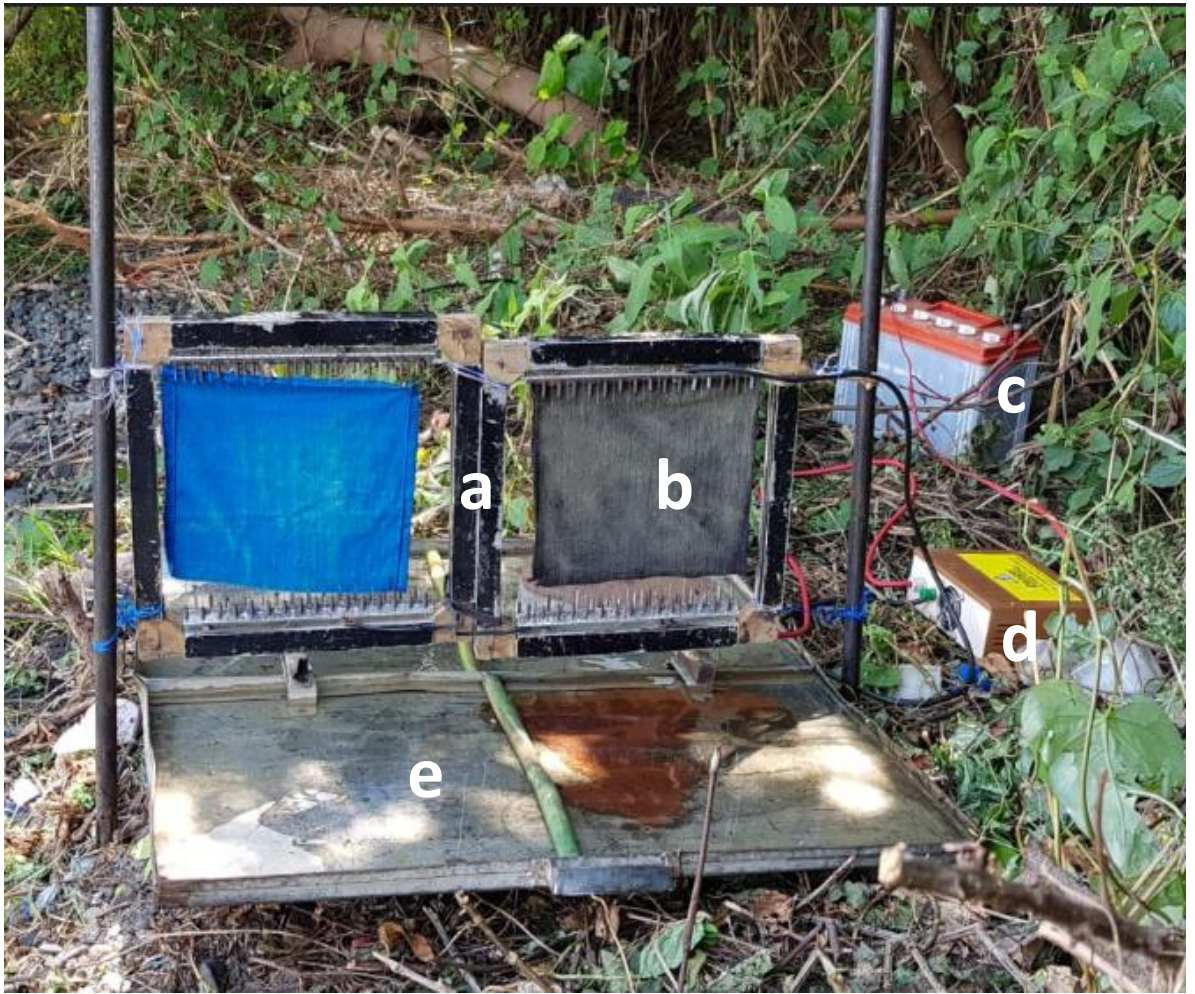
placed on a Neubauer haemocytometer chamber (Plate 3.2) for quantifying fungal spore/conidia. Counting of the conidia was done under a compound microscope per fabric across all replicates at *icipe* Thomas Odhiambo Mbita Campus (ITOC) field laboratory.



**Plate 3.2:** A presentation of the Neubauer haemocytometer chamber reading view  
Modified from Trade21 Pte.ltd <https://www.trade21.com.sg/counting-chamber-neubauer-improved-dark-line-petroff/>

### **3.6 Estimating tsetse flies landing responses (attraction) on fabric panels impregnated with *Metarhizium anisopliae***

The efficacy of fabric panels dusted with *M. anisopliae* conidia were compared against control panels that were not dusted with fungus. These fabric panels were mounted on 25 × 25 cm electrocuting nets (Plate 3.3). The electrocuting nets (hereafter, e-nets) were powered using batteries each connected to a spark box. The e-nets were mounted on metallic trays and filled with soapy water. These panels were set around Manga Island, where they were monitored for 3 hours between 09:00 - 12:00 hours. They were placed at an interval of 200 - 500 m, a meter from the lake. Tsetse flies that landed on the fabric panels were electrocuted and collected in the trays. These flies were stored in 15 ml falcon tubes filled with 70 % ethanol. In the laboratory, the flies were sexed, counted and the numbers were scored daily per site.



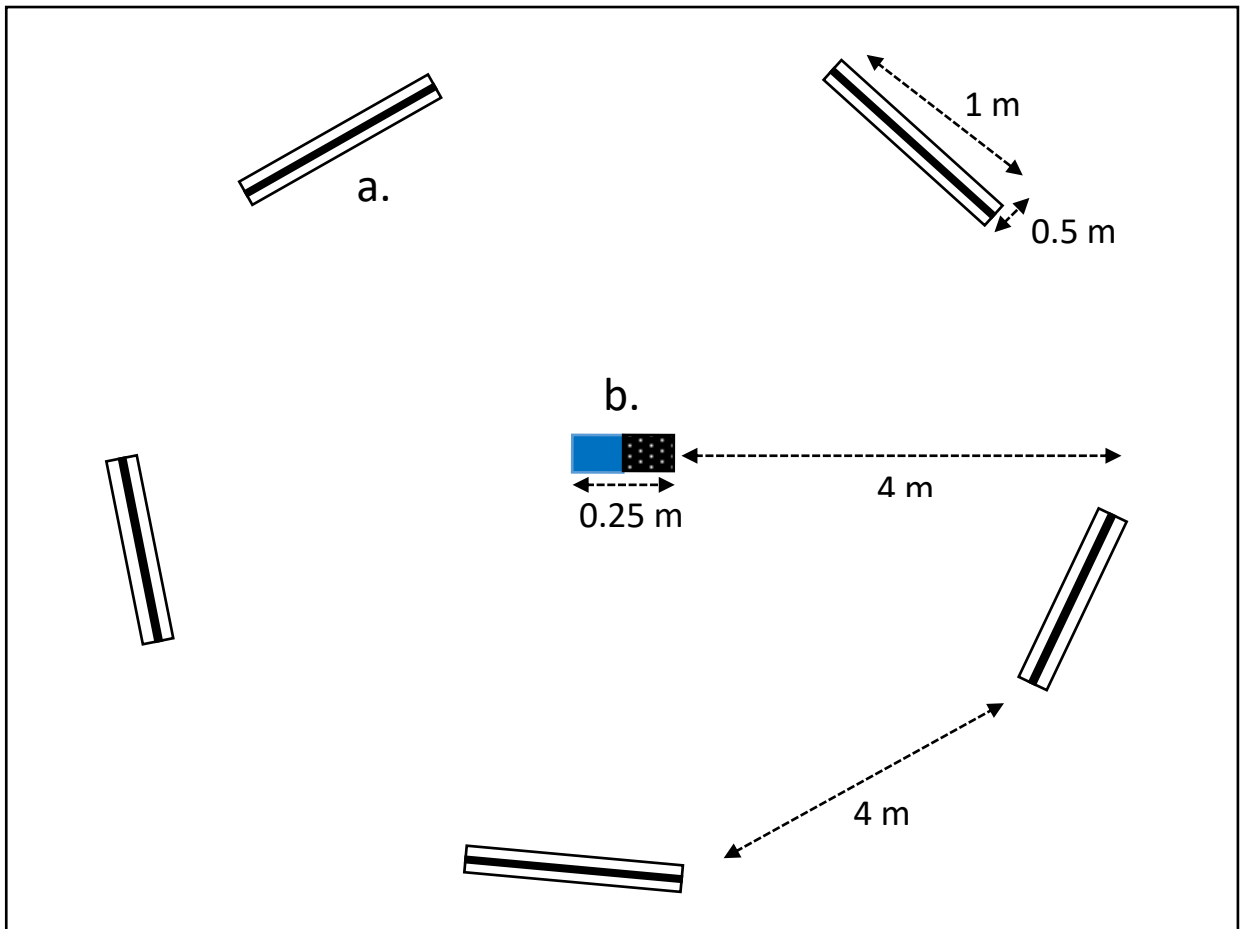
**Plate 3.3:** Experimental set up of panels impregnated with fungus flanked with electrocuting nets.

**a** electrocuting net, **b** fungus-impregnated panel, **c** battery, **d** spark box and **e** collecting tray with soapy water

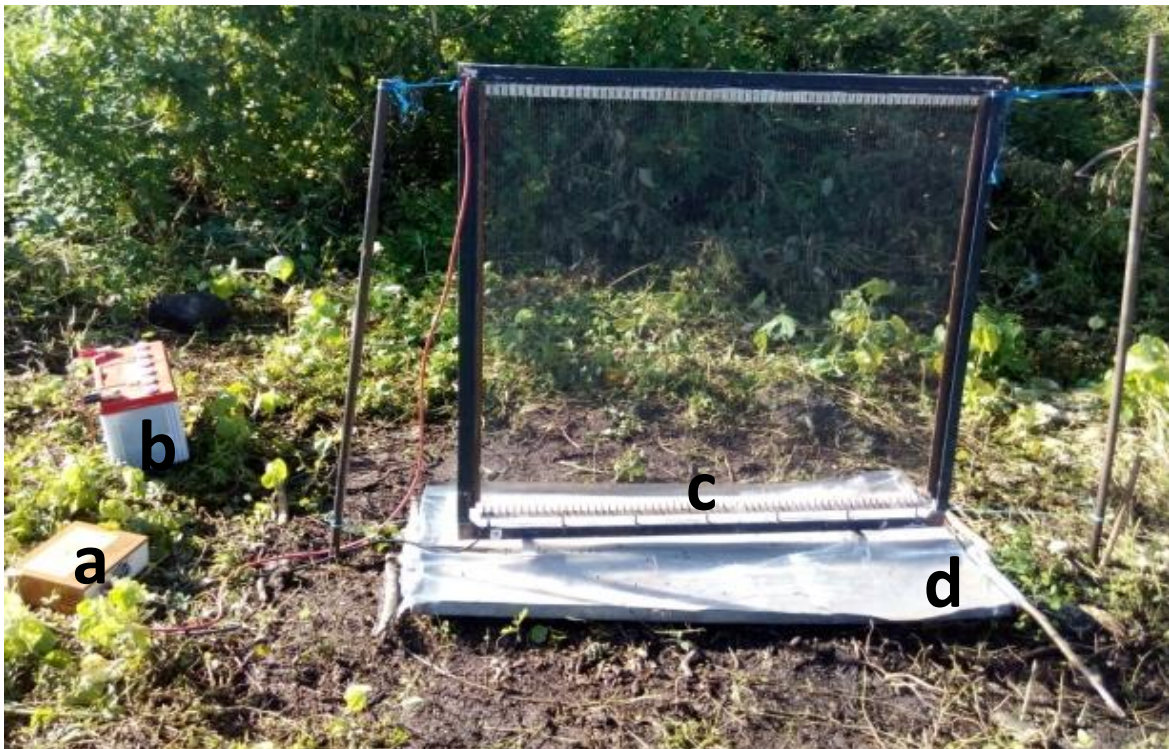
### 3.7 Evaluating the fabric panel's conidia dispensing capacity

The conidia dispensing capacity was determined on only the fabric panel with the highest retention capacity. Therefore, three fleece fabric panels were dusted with different conidia concentrations: - 0.25 g, 0.50 g and 1.00 g respectively. This was carried out by placing the dusted fabric panels in the middle of an incomplete ring of 5 electrocuting nets. The 1 m × 1 m e-nets were placed at intervals of 4 m in a circle

with a diameter of approximately 8 m (Figure 3.2 and Plate 3.4). They were powered using batteries and regulated using spark boxes. The e-nets were mounted on metallic trays and instead of soapy water, an adhesive material was placed on it to collect the killed or stunned tsetse flies. This was done to ensure that fungal spores attached to the tsetse fly body surface were not lost. Using sterilized forceps, the electrocuted tsetse flies were picked from the adhesive material, and each was placed in a sterile Eppendorf tube filled with 1 ml Triton water. In the laboratory, the tubes were vortexed vigorously to wash off the conidia. Fifty microliters of the solution were placed on the Neubauer haemocytometer chamber, and the conidia spores were counted under a compound microscope.



**Figure 3.2:** A schematic drawing of the incomplete ring of electrocuting net set-up for investigating the efficacy of fungus impregnated panels



**Plate 3.4:** Experimental set-up of 1 m × 1 m electrocuting net. **a** spark box, **b** battery, **c** 1 m × 1 m e-net and **d** collecting tray covered with an adhesive material

### **3.8 Evaluating the effectiveness of fabric panels impregnated with *Metarhizium anisopliae* against tsetse flies**

Five fleece fabric panels dusted with *M. anisopliae* conidia were deployed in five sites on Manga Island, and four monitoring sites were set on Magare Island (which was set as the control island). Conidia presence and abundance on tsetse flies was monitored once a week between 09:00 - 12:00 hours using biconical traps. In the laboratory, the flies captured were scored and sexed separately according to site and island. They were transferred to separate PVC tsetse fly rearing cages (17 × 7 × 5 cm) and kept under a temperature of 20 - 26 °C and relative humidity of 50 - 70 % with rabbits being the ad libitum blood-meal scheduled three times a week. Tsetse fly mortality rates were recorded after every two days. Dead flies were placed in plastic petri dishes lined up with damp filter papers to allow mycosis. Mortality due to *Metarhizium anisopliae* infection was confirmed under a dissecting microscope and scored as a percentage count of total captured flies per island.

### **3.9 Data analysis**

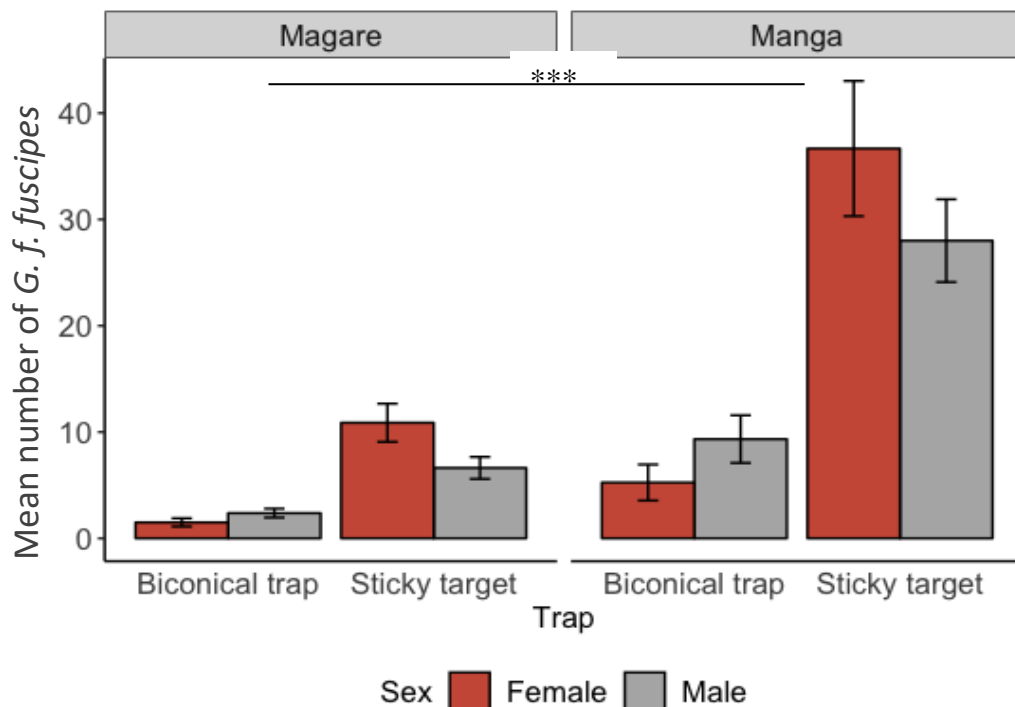
Data analysis and graphs design was carried out using R version 3.5.2 packages (Team, 2018). Quantified conidia were analysed using the Zero-inflated negative binomial. Fly catches were analysed using the generalized linear model's procedure while the tsetse fly attraction and landing responses were analysed using ANOVA. The rate of tsetse fly mortality after the introduction of the *M. anisopliae* in Manga and Magare island was analysed using the Kaplan-Meier model.

## CHAPTER FOUR

### RESULTS

#### 4.1 Baseline survey evaluating the tsetse flies density in Manga and Magare islands

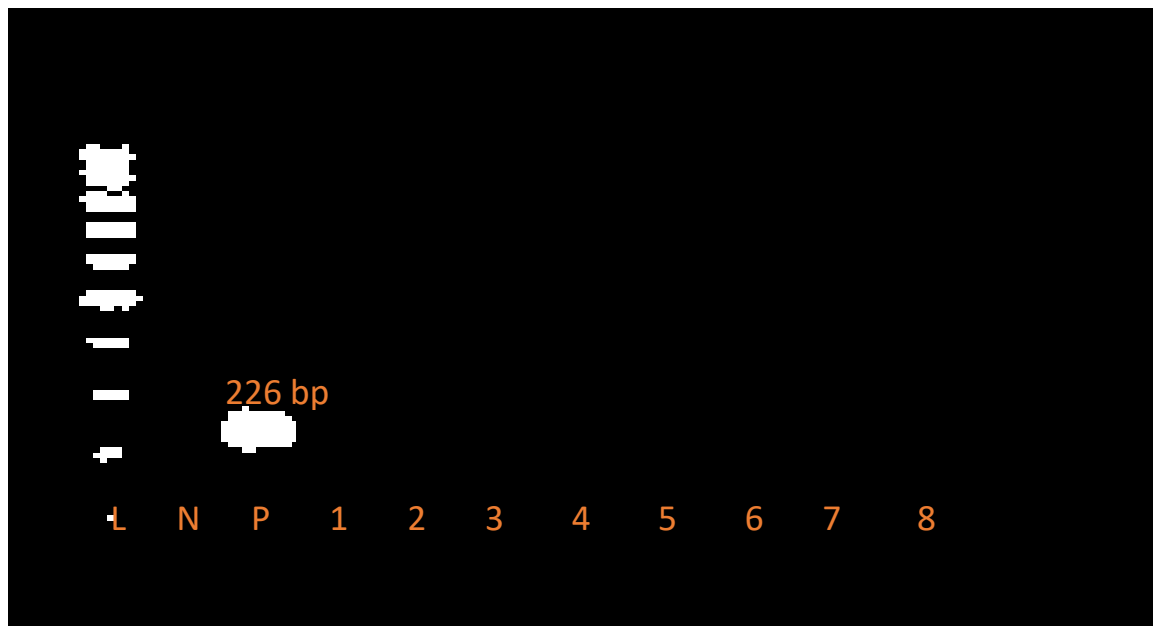
A total of 2,415 tsetse flies were trapped using sticky small targets and biconical traps. All tsetse trapped were *Glossina fuscipes fuscipes*. Sticky small targets trapped four times as many tsetse flies compared to biconical traps (OR=4.4, 95 % CI=3.4 - 5.9,  $p<0.01$ ). Magare island had significantly fewer tsetse flies as compared to Manga: 3.7 (95 % CI 2.8 - 4.9,  $p<0.01$ ) (Figure 4.1). Out of 1902 flies trapped in Manga, biconical traps collected 350 (18.4 %) tsetse flies compared to 1,552 (81.6 %) trapped on sticky small targets. In Magare, biconical traps collected 93 flies (18.1 %) of the total 513 compared to 420 (81.9%) flies trapped by sticky small targets.



**Figure 4.1:** Mean number of *Glossina fuscipes fuscipes* captured on Manga and Magare islands using biconical traps and sticky small targets

#### 4.1.1 *Metarhizium anisopliae* presence and prevalence in wild tsetse flies

There was no detection of any wild *M. anisopliae* DNA in the tsetse flies trapped in both Islands (Plate 4.1). Out of the 200 tsetse fly samples that were screened using molecular analysis techniques none of them had any traces of the fungus infection or contamination.

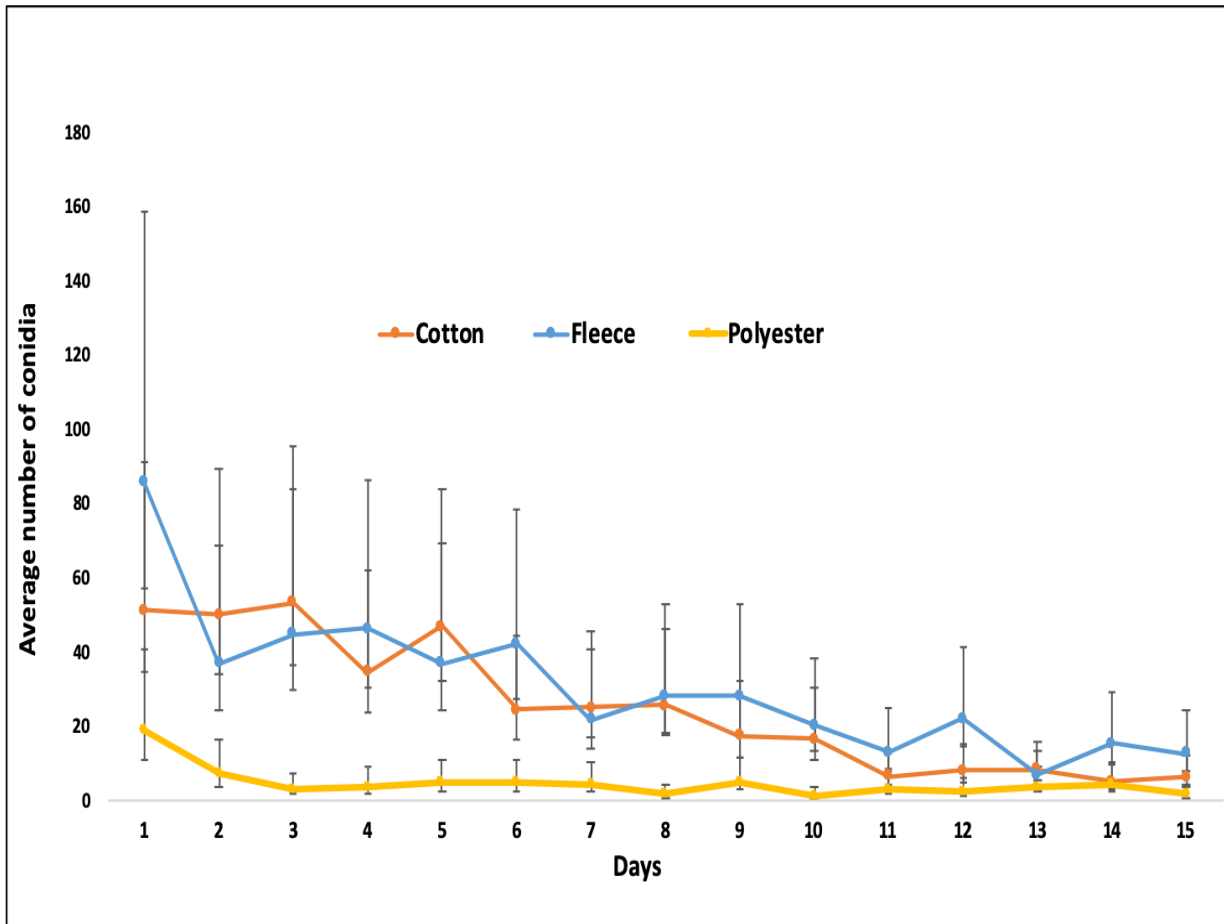


**Plate 4.1:** A gel photo indicating zero *Metarhizium anisopliae* on wild *Glossina fuscipes fuscipes*. L ladder, N negative, P positive and 1-8 samples screened

#### 4.2 Conidia retention capacity on polyester, cotton, and fleece fabrics

The initial conidia viability after mass production was at 94 %. Cotton fabric retained six times as much conidia as polyester (OR=5.7, 95 % CI: 4.6 - 7.2 times,  $p<0.01$ ) while fleece fabric retained 7 times as much conidia as polyester (OR=7, 95 % CI: 5.6 - 8.8 times,  $p<0.01$ ) (Figure 4.2). There was a significant difference in conidia retention capacity between fleece and cotton fabric panels. Fleece fabrics retained 1.2

times more conidia as cotton (OR= 1.2, 95 % CI: 1.0 - 1.5 times,  $p=0.04$ ). The average spore retention on polyester, cotton and fleece was  $1.2 \times 10^6$  spores/ml,  $7.5 \times 10^6$  spores/ml and  $8.1 \times 10^6$  spores/ml respectively.

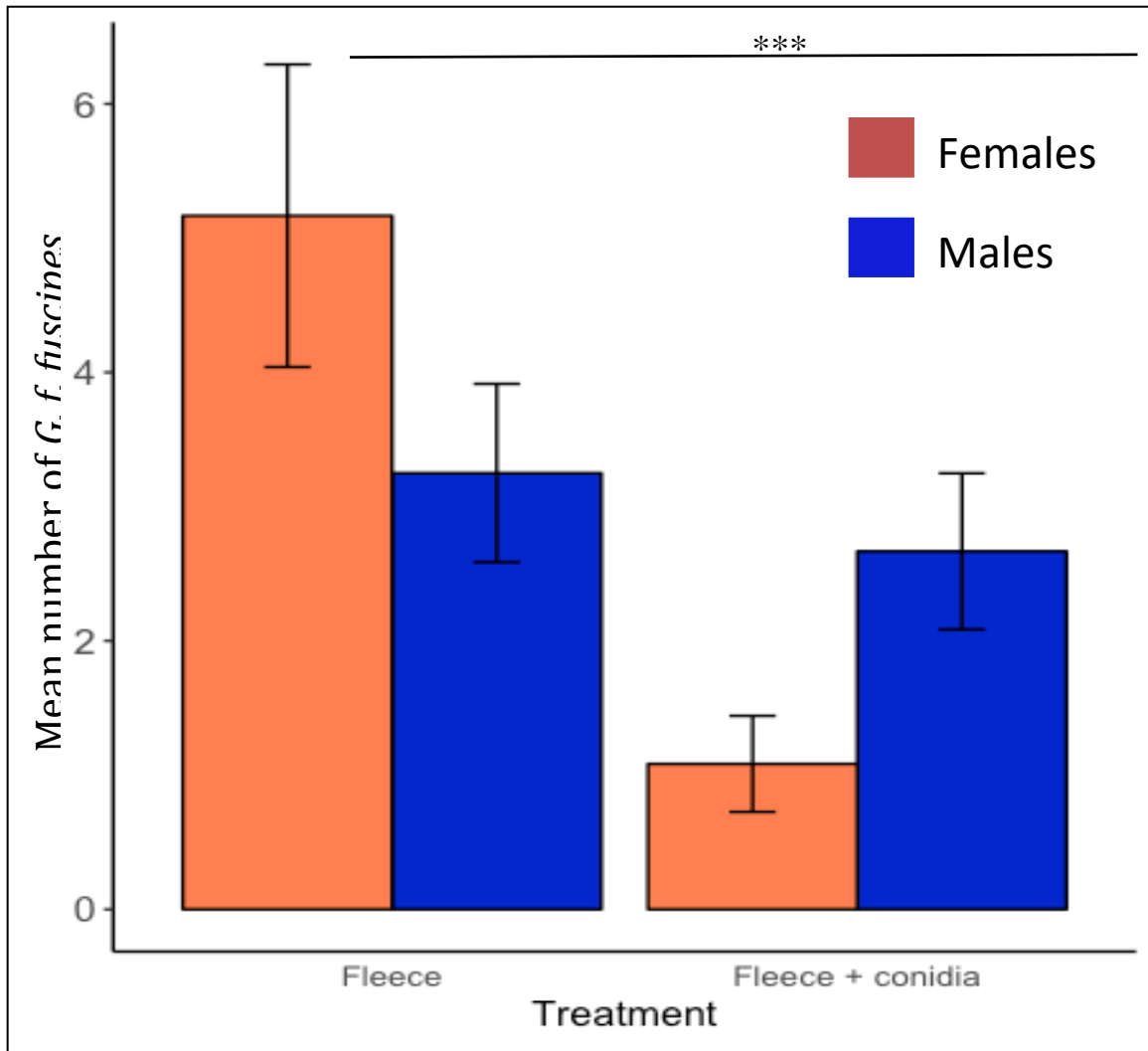


**Figure 4.2:** Relative retention capacity of *Metarhizium anisopliae* conidia on cotton, polyester netting and fleece fabrics overtime

#### 4.3 The attraction of tsetse flies on fabrics treated with *Metarhizium anisopliae*

A total of 146 tsetse flies were collected on the metallic trays. A significant number of tsetse flies attempted to land on untreated fleece fabrics as compared to conidia treated fleece fabric (0.4, 95 % CI: 0.3 - 0.7,  $p<0.01$ ) (Figure 4.3). The proportion of

adult males against females in the treated fleece fabric was significant (71.1% versus



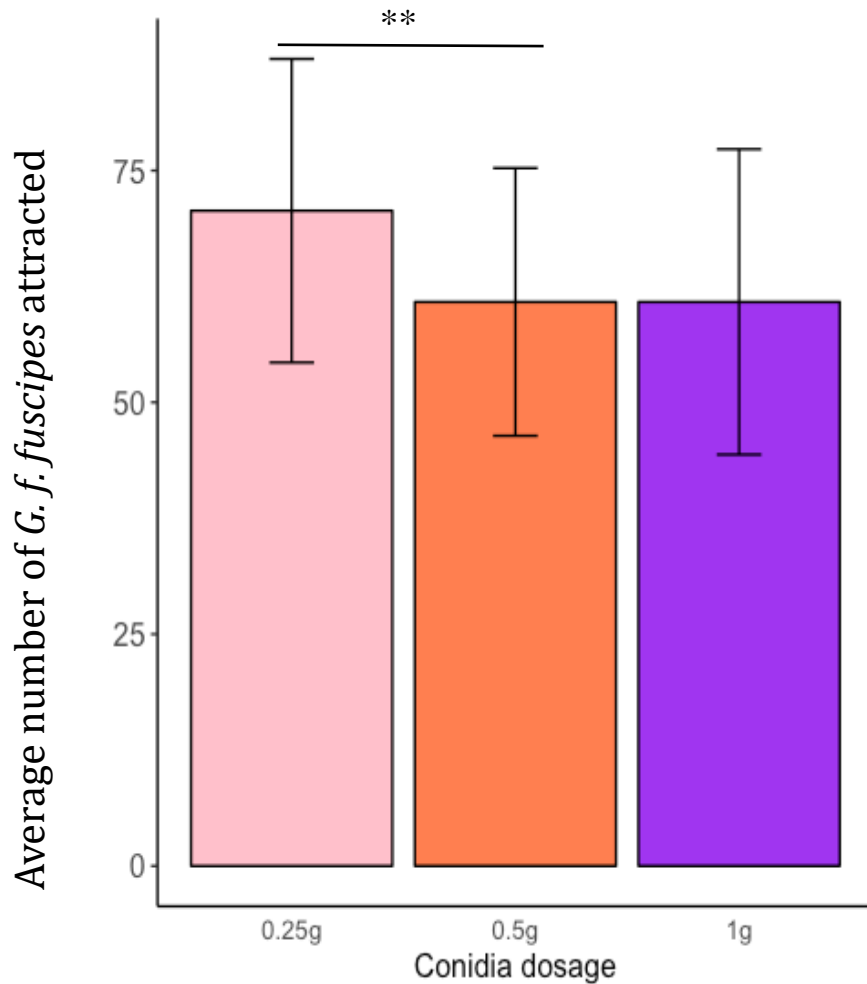
**Figure 4.3:** Mean number of tsetse flies attracted to fleece fabrics impregnated with  $1\text{mg}/\text{cm}^2$  of *Metarhizium anisopliae* conidia

28.9 %,  $p < 0.01$ ) compared with fleece untreated (38.6 % versus 61.4 %,  $p = 0.02$ ).

#### 4.4 Selection of an optimal conidia dosage

A total of 1,154 tsetse flies were retrieved from the electrocuting nets and the adhesive material. Conidia dosage of 0.25 g affected the panel attraction least (0.3, 95 % CI: 0.1-0.7,  $p < 0.05$ ) as compared to 0.50 g and 1.00 g (0.8, 95 % CI: 0.3 - 1.9,  $p = 0.60$ ) (Figure 4.4). A proportion of 79.5 % (918) were females and 20.5 % (236) were males. Among this 57.5 % (663 tsetse flies) were caught inside the ring and 80.7

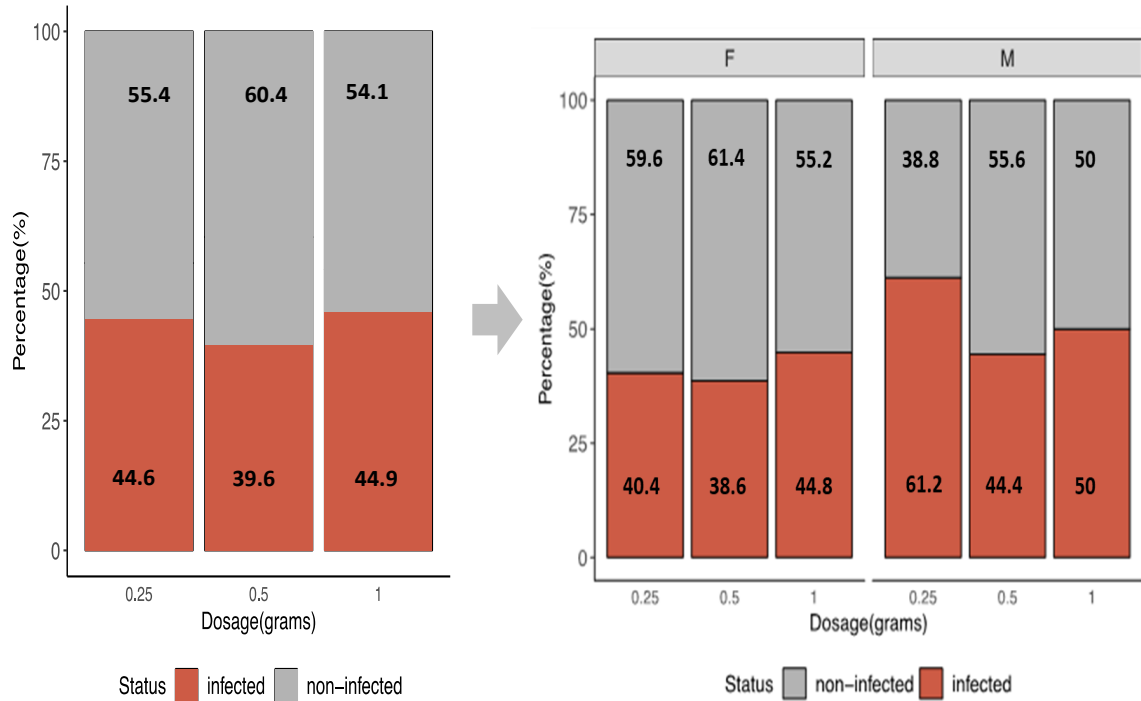
% (535) were females. Out of the 491 flies electrocuted outside the ring, 383 (78 %) were females.



**Figure 4.4:** Relative attraction of tsetse flies to fleece fabrics dusted with different doses (0.25 g, 0.50 g and 1.00 g) of *Metarhizium anisopliae* conidia

Conidia concentration between  $2.5 - 27.5 \times 10^5$  spores/ml was observed on tsetse flies that landed on the fungus panels. There was no significant difference in the number of

flies that had conidia spores when exposed to the three dosages (0.25,0.50 and 1.00 g) ( $p<0.05$ ) (Figure 4.5).



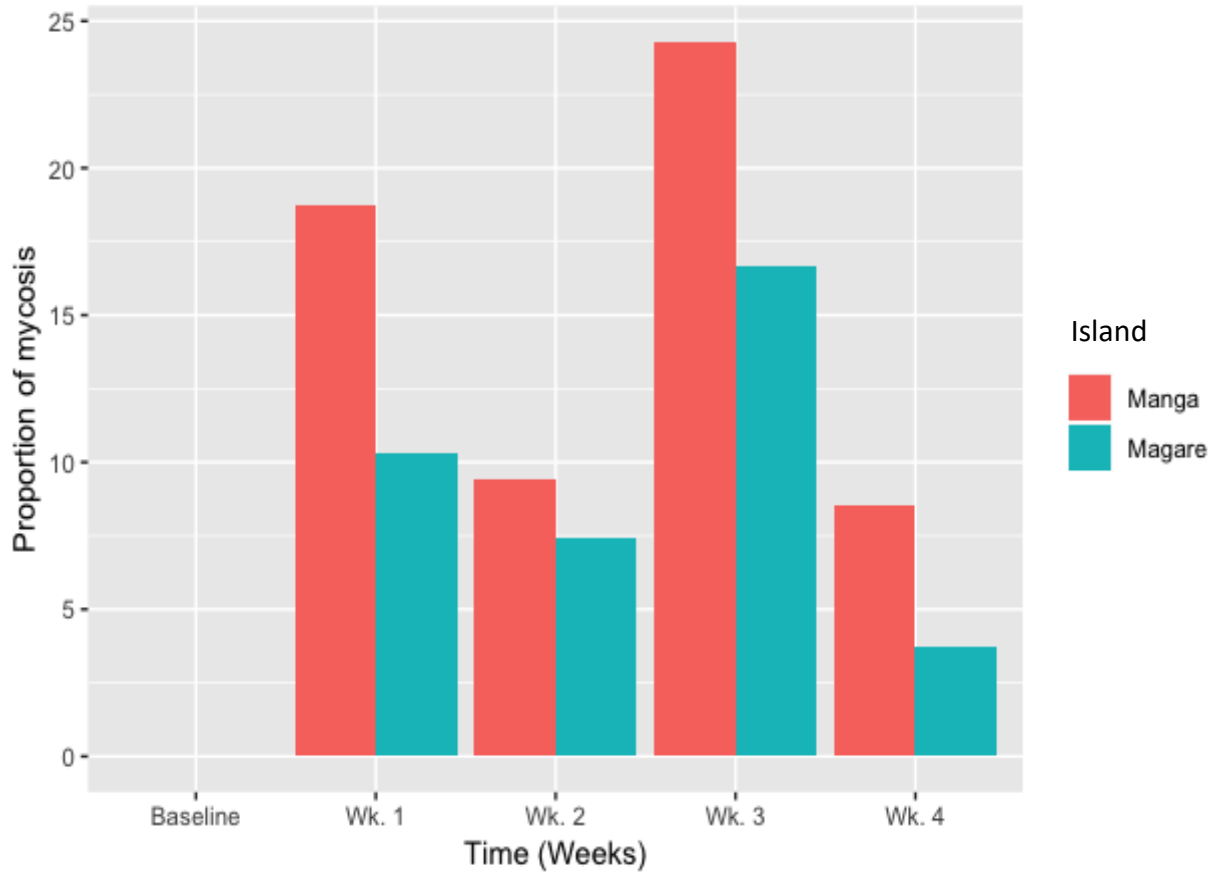
**Figure 4.5:** Proportions of tsetse flies contaminated after landing on fleece fabrics with different doses of *Metarhizium anisopliae* conidia

#### 4.5 Efficacy of fleece fabric panels impregnated with *Metarhizium anisopliae*

The prevalence of *Metarhizium anisopliae* in Manga Island increased steadily to 18.7 %, 9.4 % and 24.3 % over one, two and three weeks but declined to 8.5 % on the fourth week (Table 4.1 and Figure 4.6). The same trend was observed in Magare island (10.3 %,7.4 %,16.7 % and 3.7 %).

**Table 4.1: Proportions of fungal mycosis on tsetse flies overtime**

	MANGA ISLAND				MAGARE ISLAND			
	Replicate 1	Replicate 2	Replicate 3	% Mycosis	Replicate 1	Replicate 2	Replicate 3	% Mycosis
Week 1	13.2	33.7	9.2	18.7	0	20.5	10.4	10.3
Week 2	17.1	3.7	7.5	9.4	0	14.2	8.1	7.4
Week 3	31.3	16.0	25.5	24.3	29.4	7.8	13.0	16.7
Week 4	19.3	0	6.3	8.5	0	0	11.1	3.7

**Figure 4.6:** Weekly rates of mortality and mycosis due to *Metarhizium anisopliae* in tsetse flies recovered for the test (Manga) and control (Magare) islands

## CHAPTER FIVE

### DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

#### 5.1 Discussion

Only *Glossina fuscipes fuscipes* were trapped in surveillances and all experiments conducted in Manga and Magare islands. This is consistent with recent studies implemented in the islands (Mbewe *et al.*, 2018a; 2018b; 2019). In Kenya, it has been reported that the riverine tsetse fly species are mainly found in the Lake Victoria region and the surrounding islands, extending to Uganda and Tanzania (KETRI, 2008; Tirados *et al.*, 2015). A significantly higher number of tsetse flies were trapped in Manga Island compared to Magare island. Manga has a dense vegetation cover and is inhabited by moderately few humans and consequently characterised by relatively high numbers of monitor lizards; a preferred host of *G. f. fuscipes* (Omolo *et al.*, 2009). Anthropogenic activities in Magare have led to lower vegetation cover. Land use by humans can modify the ecosystem structure leading to fragmentation of tsetse fly habitats causing a reduction in numbers (Ducheyne *et al.*, 2009; Mweempwa *et al.*, 2015).

In this study, none of the field-collected tsetse flies were infected with *Metarhizium anisopliae*. Previous studies by Maniania and others with *M. anisopliae* in the islands of Lake Victoria showed that the fungus can be horizontally transmitted in nature (Maniania, *et al.*, 2002; Maniania *et al.*, 2013). Therefore, it was anticipated that the low prevalence of the entomopathogens might have persisted in the population of flies sampled making it difficult to implement planned field studies. However, even with highly sensitive molecular tools that target DNA and could have detected previous exposure, infection, or contamination with *M. anisopliae*, there was zero prevalence

of the fungus. Infections with *M. anisopliae* reported for field-collected tsetse flies in this study are an unequivocal effect of the numbers evaluated. The tiny targets used for sampling flies trapped four times more tsetse flies compared to the biconical traps. Previous studies by Mbewe *et al.* (2018) demonstrated that the tiny targets covered with sticky film caught 13.5 and 3.6 times more female and male tsetse flies than biconical traps (Mbewe *et al.*, 2018b). Tiny targets are cheaper than the biconical traps used by Maniania and others to contaminate tsetse flies with fungus (Maniania, 1998; Maniania *et al.*, 2006). One tiny target cost approximately USD 1 (KES 100) compared with biconical traps which each cost USD 13.5 (KES 1,350). Previous studies in Kenya and Uganda showed that it is 2-3 fold more cost-efficient to use tiny targets for tsetse flies control compared to traps (Lindh *et al.*, 2009; Rayaisse *et al.*, 2011; Shaw *et al.*, 2015). For these reasons, the fabric panel prototypes of the tiny targets were further evaluated as potential alternative tools for contaminating tsetse flies with fungus in the field.

This study showed that fleece fabrics treated with the same dose of conidia consistently retained more conidia than cotton and polyester, the fabrics used for commercially available panels and traps for tsetse flies (Rayaisse *et al.*, 2011; Torr *et al.*, 2011). The fabric was therefore selected for making panels evaluated in this study. Fleece fabrics are made by loosely weaving and brushing polyester fabrics (Dubrovski, 2010). This increases the relative surface of the fabric and could explain the initial higher retention. The differences in conidia retention of fleece and cotton fabrics to the initially applied conidia dose are significantly different, however, it is lost within the first 24 hours in the field suggesting that cotton could be an optional material for making the panels. On the contrary, it was found that the polyester mesh

commonly used to construct tiny target panels retained less than 10 % of conidia lost all in 15 days and is unsuitable for applying fungus against tsetse flies. Adding *M. anisopliae* conidia to fabric panels reduced the number of *G. fuscipes fuscipes* attracted to the panel and their landing rate. The effect was more prominent in female flies. Colour is an important cue for tsetse fly attraction and the black colour of panels is known to stimulate landing responses (Rayaisse *et al.*, 2011; Torr *et al.*, 2011). Visually, adding conidia on panels caused a shift from black to a green shade while higher doses intensified the shift. Conidia of *M. anisopliae* are generally shades of green (Fernandes *et al.*, 2010; Santer, 2014), a colour that has been negatively correlated with the attraction of this species of tsetse flies (Lindh *et al.*, 2012; Santer, 2017). The change in colour caused by the addition of conidia likely led to the reduced attraction and landing rate of tsetse flies on the treated panel. Previous studies with fabrics of different colours have demonstrated that females of *G. fuscipes* are relatively more sensitive to spectral changes. This results in lesser attraction when other colours besides the blue polyester are used. Consequently, fewer female flies are attracted when with other colours compared to blue polyester (Santer *et al.*, 2019). Therefore, it is plausible that the reduced response of female flies to fungus-treated targets could have been because of colour change.

The prevalence of *M. anisopliae* in tsetse flies in both Manga and Magare islands increased when fabric panels impregnated with fungus were deployed in Manga. This is evident when the fabric panel tool effectively contaminated *G. f. fuscipes* with fungal conidia. Maniania *et al.* (2016) reported that contamination devices deployed in the neighbouring Mfangano island gradually infected and killed tsetse flies, as a consequence, the wild tsetse fly population was reduced by 88 % in 49 weeks

(Maniania *et al.*, 2006). Unexpectedly, tsetse flies contaminated with fungus were also found in relatively fewer numbers in Magare island, initially assigned as a control site. It has been reported that tsetse flies can travel up to 1 km each day through random diffusion in search of hosts (Gibson *et al.*, 1991; Hargrove, 2000). In retrospect, the distance between Manga and Magare islands of approximately 100 m was not sufficient to prevent cross-contamination between experimental sites. Nonetheless, the number of contaminated flies increasing in Magare supports the efficacy of the novel tool.

The tool developed in this study offers comparative efficacy in the field and could be an alternative to Maniania's contamination device (CD) (Maniania *et al.*, 2002). It offers a relatively cheaper option since costs associated with the more expensive and harder to deploy biconical traps are avoided. However, unlike the fabric panels developed in this study, Maniania's CD is designed to protect conidia from UV light and precipitations (Maniania, 1998; Maniania *et al.*, 2006). High temperature, UV and moisture rapidly reduce the viability of *M. anisopliae* conidia in the field (Inglis *et al.*, 2001). This reduces the persistence of fungus and poses a challenge to the deployment of the biopesticide. It is anticipated that the fabric panels impregnated with fungus will require more frequent servicing thereby attracting higher operational costs than the contamination device.

## 5.2 Conclusions

1. Fleece fabrics retained more *Metarhizium anisopliae* conidia than cotton and polyester and are relatively more suitable for fabricating fungus impregnated panels for tsetse fly control.

2. Treating fabric with *M. anisopliae* reduced the attraction and landing rate of *Glossina fuscipes fuscipes* to the panels. The effect was greater in female flies compared to males.
3. Fabric panels made from fleece and treated with 0.25 g of *M. anisopliae* conidia effectively contaminated *G. f. fuscipes* in the field with lethal doses of the entomopathogen.

### **5.3 Recommendations**

1. Large scale field trials should be implemented to evaluate the effectiveness of fabric panels impregnated with *Metarhizium anisopliae* conidia against tsetse flies.
2. For improved effectiveness, further studies should be implemented to understand the colour response of tsetse flies to these and optimise the application to maintain the efficacy of the panels.
3. With further optimization, the prototype can be developed into an effective vector control tool.

### **5.4 Suggestions for further research**

1. Studies should be implemented to understand the reduced response of *Glossina fuscipes* to fabric panels with different doses of *Metarhizium anisopliae*.

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