2022-08

Efficacy and effect of entomopathogenic fungi (aspergillus oryzae) for control of ticks of major economic importance of cattle in Tanzania

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NM-AIST

https://doi.org/10.58694/20.500.12479/1508

EFFICACY AND EFFECT OF ENTOMOPATHOGENIC FUNGI (*Aspergillus oryzae*) FOR CONTROL OF TICKS OF MAJOR ECONOMIC IMPORTANCE OF CATTLE IN TANZANIA

Sylvia Samson Msangi

A Dissertation Submitted in Partial Fulfilment of the Requirements of the Degree of Master's in Life Sciences of the Nelson Mandela African Institution of Science and Technology

Arusha, Tanzania

August, 2022

ABSTRACT

Ticks are important ectoparasites that are responsible for severe economic losses. The use of chemical acaricides is the most common method used to control ticks in livestock. This study was conducted to determine the efficacy of *Aspergillus oryzae* in controlling ticks to enhance livestock productivity. The efficacy of *A. oryzae* at different concentrations was evaluated against different stages (larva and adult) of the life cycle of hard tick genera (*Rhipicephalus, Boophilus and Amblyomma*) using immersion test under laboratory conditions. Field trials were conducted in two cattle herds, which were purposively selected, and a spraying method was used to apply *A. oryzae* at a concentration of 1×10^6 conidial/mL on all tick infested areas. The results demonstrated a concentration-related increase in mortality in all tested tick genera. The mean mortality of larvae and engorged ticks was statistically significant at $p<0.05$ and $p<0.001$ respectively in all the tested genera. Egg production was found to decrease with increased *A. oryzae* concentration. Additionally, there was a statistically significant difference in egg production index and oviposition reduction ($p=0.009$) while there was no significant difference in egg hatching and product effectiveness at $p=0.089$ and $p=0.004$ respectively between the tested tick genera. Under field conditions, trials demonstrated a statistically significant tick reduction on all the treated cattle. This study concludes that *A. oryzae* has a good acaricidal activity against ticks and hence is one of the potential tick control methods for sustainable tick control schemes.

DECLARATION

I, Sylvia Samson Msangi, do hereby declare to the Senate of the Nelson Mandela African Institution of Science and Technology that this dissertation is my original work and that it has neither been submitted nor being concurrently submitted for consideration of a similar degree in any other University.

Sylvia Samson Msangi, Date

The Above declaration is confirmed by:

Prof. Gabriel Shirima

Date

Prof. Lughano Kusiluka Date

Dr. Esther Kimaro Date

Dr.Never Zekeya Date

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CERTIFICATION

The undersigned certify that have read and hereby recommend for acceptance by the Senate of the Nelson Mandela African Institution of Science and Technology, the dissertation titled *"Efficacy and effect of entomopathogenic fungi (Aspergillus oryzae) for control of ticks of major economic importance in cattle in Tanzania"* in Partial Fulfillment of the Requirements for the award of the degree of Master's in Life Sciences of the Nelson Mandela African Institution of Science and Technology.

Prof. Gabriel Shirima Date

Prof. Lughano Kusiluka Date

Dr. Esther Kimaro Date

Dr.Never Zekeya Date

ACKNOWLEDGEMENTS

First, I thank The Almighty God for his generous love and blessing on me through charitable protection, strength and courageous spirit to accomplish my Master's study.

I express my sincere gratitude to my supervisors Prof. Gabriel Shirima, Prof. Lughano Kusiluka, Dr. Never Zekeya and Dr. Esther G. Kimaro for their supervision, guidance, useful advice and support to accomplish this study.

It is my pleasure to acknowledge the African Development Bank (AFDB) for funding this study.

My special thanks are extended to the Management of Tanzania Biotech Products Limited (TBPL) for granting me a study leave to carry out this study.

I would like to express my thanks to Dr. Yakobo Petro, Ms. Grace Jayambo and Mr. Ibrahim Sungi from the Tanzania Plant health and Pesticides Authority (TPHPA), Arusha, Tanzania for their assistance in tick identification and laboratory assays. Dr. Yandu Marmo, -District Veterinary Officer (DVO), Mr. Respick Maunga and Senorina Shirima all from Monduli district are appreciated for their assistance in all logistics for the field trials. In addition, I would also like to extend my sincere thanks to the Maasai pastoralists of Meserani village in Monduli for allowing me to use their cattle for the study.

Special appreciation goes to my husband, Babere B. Babere and son, Jeremiah for their love, care, encouragement, patience and tolerance during my Master's study. Special acknowledgment also goes to my parents Mr. and Mrs. Samson Msangi, my sisters Neema and Irene for their prayers, encouragement, inspiration and support during the whole period of my study.

DEDICATION

I would like to dedicate this work to my parents Samson and Vicky Msangi, my husband Babere B. Babere and my lovely son, Jeremiah Babere for their love and inspiration in my academic life.

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LIST OF ABBREVIATIONS AND SYMBOLS

BOD	Biological Oxygen Demand
ECF	East Coast Fever
ITM	Infection and Treatment Method
LHDV	Livestock and Human Disease Vector
TPHPA	Tanzania Plant Health and Pesticide Authority

CHAPTER ONE

INTRODUCTION

1.1 Background of the Problem

Ticks are the most important ectoparasites of livestock in tropical and sub-tropical areas and are responsible for severe economic losses both through the direct effect of blood-sucking and indirectly as vectors of pathogens (Almazan *et al.*, 2018; Jongejan & Uilenberg, 1994; Jongejan & Uilenberg, 2004; Rodriguez *et al.*, 2018). Ticks are small arachnids of 3 to 5 mm long classified with mites in the subclass *Acari*. They exist in two families namely, the *Argasidae* (soft ticks) and the *Ixodidae* (hard ticks) with about 840 species, of which 80% belong to the family *Ixodidae* and have the potential for adverse socio-economic implications in livestock production (Jongejan & Uilenberg, 1994; Lew-Tabor & Valle, 2016).

Ticks are vectors for tick-borne diseases, transmitting protozoan, rickettsial and viral diseases of livestock and humans with adverse socio-economic impacts (Almazan *et al.*, 2018; Benelli *et al.*, 2016; de la Fuente, 2018; Jongejan & Uilenberg, 2004; Lynen *et al.*, 2007). In the livestock industry, tick-borne diseases are important as they cause great losses particularly in the production of ruminants and their products such as meat and milk. They also affect the quality of animal by-products especially hides and skins in the processing industries (Adehan *et al.*, 2018; Jongejan & Uilenberg, 1994; Lew-Tabor & Valle, 2016; Nagagi *et al.*, 2020). Ticks of the genera *Rhipicephalus, Boophilus, Hyalomma and Amblyomma* are the major causes of tick-borne diseases in cattle-keeping areas (Lynen *et al.*, 2007). In Tanzania, five species, namely *Rhipicephalus appendiculatus, Rhipicephalus evertis, Rhipicephalus microplus, Amblyomma variegatum* and *Boophilus decoloratus,* are the main vectors of the most economically important tick-borne diseases (Mamiro *et al.*, 2016). Four common tick-borne diseases affecting cattle in Tanzania (Adrian, 2012; Nagagi *et al.*, 2020), includes two protozoan diseases (Babesiosis and Theileriosis or East Coast fever) and two rickettsial diseases (Anaplasmosis and Heartwater or cowdriosis) (Bram, 1983; Jongejan & Uilenberg, 1994; Kerario *et al.*, 2018; Rajput *et al.*, 2006).

In Tanzania, the socio-economic impact of ticks and tick-borne diseases in cattle production is due to high mortalities, decreased production, and treatment and control costs. A study carried out by Kivaria (2006) showed that Tanzania incurs an estimated direct cost of up to 364 million USD due to tick-borne diseases annually with East Coast fever (ECF) accounting for 68%, babesiosis and anaplasmosis each accounting for 13% and heartwater for 6%. In addition, approximately, 71.4%

of all cattle mortalities are due to tick-borne diseases with high mortality in calves especially in pastoral and agro-pastoral communities (Kerario *et al.*, 2018; Kivaria, 2006; Laisser *et al.*, 2017).

For decades, the management of ticks and tick-borne diseases in Tanzania has involved the use of therapeutics and chemical acaricides through dipping, spraying or pour-on application once or twice per week (Adrian, 2012; Kerario *et al.*, 2018; Laisser *et al.*, 2017; Nagagi *et al.*, 2020). However, these chemicals are environmentally unpleasant and economically unaffordable by majority of livestock keepers as well as leaving toxic residues in meat and milk (Almazan *et al.*, 2018; Drummond, 1976; Jongejan, 1999; Kaaya & Hassan, 2000; Lew-Tabor & Valle, 2016). Furthermore, constant use of acaricides has led to tick resistance, posing challenges in the management of tick-borne diseases (Almazan *et al.*, 2018; de la Fuente *et al.*, 2016; Kaaya & Hassan, 2000; Laisser *et al.*, 2017; Wharton, 1983). Although therapeutic drugs are available for the treatment of tick-borne diseases such as East Coast fever, they are only effective during the early stages of the disease and require repeated doses that are too costly to most livestock keepers in Tanzania. Again, these drugs are not readily available in local settings (Jongejan, 1999; Kerario *et al.*, 2018). Vaccination based on Infection and Treatment Method (ITM) (Magulu *et al.*, 2019; McKeever, 2007) is also used but it is only effective against ECF. Moreover, ITM is hardly afforded by the majority of livestock keepers. The above challenges have prompted the need to explore other interventions such as the use of bio-acaricides, which are cost-effective, environmentally friendly and effective with less deleterious effects on non-targeted organisms as an alternative for the control of tick and tick-borne diseases in Tanzania.

Entomopathogenic fungi have been reported to be effective against ticks and appeared to be more promising than other potential biological control agents (Stafford & Allan, 2014). Entomopathogenic fungi can infect ticks, by the fungal conidia attaching and penetrating through the tick cuticle leading to death (Fernandes *et al.*, 2012; Ghany, 2015; Jiang *et al.*, 2020; Perinotto *et al.*, 2012). For instance, fungi belonging to species *Beauvena bassiana* and *Metarhizium anisopilevae* are the most used entomopathogenic fungi for biological control of ticks (Bittencourt, 2008; Fernandes *et al.*, 2012; Ghany, 2015; Kaaya & Hassan, 2000; Kalsbeek *et al.*, 1995; Perinotto *et al.*, 2012). The use of entomopathogenic fungi may reduce the frequency of acaricide application and the use of curative drugs or vaccines for tick-borne diseases hence, reducing the cost of tick control as well as the development of tick resistance to acaricides (Jiang *et al.*, 2020; Kaaya & Hassan, 2000; Murigu *et al.*, 2016).

This study aimed at investigating the activity of an entomopathogenic fungus namely *Aspergillus oryzae* which has shown high efficacy in the control of tomato leaf miner (*Tuta absoluta* Meyrick)

(Zekeya, 2019; Zekeya *et al.*, 2019) and tick species in the laboratory experiment (Zekeya *et al.*, 2020). The entomopathogenic effect of the fungi on tomato and tick species has aroused some interest to test the potential effects of *Aspergillus oryzae* on cattle ticks under field conditions.

1.2 Statement of the Problem

Livestock production contributes to the national economy, poverty alleviation at the household level and supply animal proteins to humans. Diseases adversely affect livestock productivity. Ticks and tick-borne diseases cause high mortality in cattle, hence reducing the productivity of the animals (Adehan *et al.*, 2018; Kerario *et al.*, 2018; Kivaria, 2006; Laisser *et al.*, 2017). Ticks are routinely controlled by the use of chemical acaricides (Adrian, 2012; de la Fuente *et al.*, 2016). Although acaricides have been used for decades, a number of shortfalls have been observed in the course of their use. Chemical acaricides are expensive, lead to residues in meat and milk, development of tick resistance to acaricides and environmental contamination (Adehan *et al.*, 2018; Drummond, 1976; Jongejan, 1999; Kaaya & Hassan, 2000; Lew-Tabor & Valle, 2016; Maniania *et al.*, 2007). Also, the current ECF vaccine applied through ITM method does not produce cross-immunity for other tick-borne diseases (McKeever, 2007).

Entomopathogenic fungi have been reported to be effective against ticks and appeared to be more promising than other potential biological control agents (Stafford & Allan, 2014). Some species of fungi such as *Beauvenia bassiana* and *Metarhizium anasopilevae* have been used as bio-acaricides for the biological control of ticks elsewhere (Fernandes *et al.*, 2012; Ghany, 2015; Kaaya & Hassan, 2000; Perinotto *et al.*, 2012). Recently, Zekeya *et al*. (2019), identified a new species of fungi, namely, *Aspergillus oryzae* with insecticidal activity and which is considered to have bio-acaricidal activity under a controlled environment. This study investigated the effect of *Aspergillus oryzae* for the control of ticks of major economic importance in cattle under field conditions in Monduli district, Tanzania.

1.3 Rational of the Study

Tick cause high economic losses as vectors for tick-borne diseases. Chemical acaricides have been used for decades in controlling ticks. However, they are expensive, leads to toxic residual in meat and milk, leads to acaricidal resistances and environmental contamination. A safer and more officious bio-acaricidal product (entomopathogenic fungi) is expected to address some of these challenges in control of ticks in Tanzania. Therefore, this study aims to determine the bio-efficacy of *A. oryzae* as innovative intervention for controlling ticks infestation in Tanzania.

1.4 Research Objectives

1.4.1 General Objective

To determine the efficacy of *Aspergillus oryzae* in controlling ticks in cattle under field conditions to enhance livestock productivity in Tanzania.

1.4.2 Specific Objectives

(i) To determine the acaricidal effect of *Aspergillus oryzae* in the different life cycles of hard tick genera (*Rhipicephalus, Boophilus and Amblyomma*) of economic importance in Tanzania.

(ii) To determine the residual effect of the bio-acaricide (*Aspergillus oryzae*) on cattle under field conditions.

1.5 Research Questions

(i) What is the acaricidal effect of A. oryzae in the different life cycle of common cattle ticks?

(ii) What is the bio-efficacy of A. oryzae against cattle ticks under field conditions?

1.6 Hypothesis

Application of an entomopathogenic fungus (*Aspergillus oryzae*) as a bio-acaricide will be effective in controlling ticks in cattle in Tanzania.

1.7 Significance of the Study

Aspergillus oryzae has shown high efficacy in tick control in the laboratory (Zekeya *et al.*, 2020). This has formed the basis for an efficacy study of this fungus under field conditions in Tanzania. As a bio-acaricide *A. oryzae* is considered as an eco-friendly and cheaper option which could contribute to overcoming the tick challenge in the livestock sector in Tanzania by reducing the use of chemical acaricides in animals and the environment. Additionally, it may address the challenge of acaricide resistance, improve animal health and production. Upscaling this technology has the potential to reduce the use of chemical acaricides, safeguard animal health and alleviate poverty among livestock-dependent communities.

1.8 Delineation of the Study

This study focused on evaluate the efficacy and effect of *A.oryzae* for the control of cattle ticks in Tanzania. Effective evaluation was done on eggs, larvae and female engorged ticks under controlled laboratory conditions and the application to rabbits as laboratory animals and cattle herds of Maasai pastoralists.

CHAPTER TWO

LITERATURE REVIEW

2.1 Tick and Tick-Borne Diseases

Many societies in Africa depend on livestock-keeping as a source of food and income (Gonzo *et al.*, 2014), however, livestock diseases have been among the challenges that affects the livelihood of livestock-keeping communities (Adrian, 2012; Byaruhanga *et al.*, 2015; Laisser *et al.*, 2017). In Africa, livestock have always suffered from a great range of diseases such as arthropod-borne diseases that have had negative impacts on animal health and productivity (Gilioli *et al.*, 2009; Tomley & Shirley, 2009). In the tropical regions, humidity, temperature and precipitation provide favorable conditions for the survival of vectors and transmission of infectious livestock diseases (Jabbar *et al.*, 2015).

Ticks are important ectoparasites of livestock in tropical and sub-tropical regions, responsible for causing great economic losses due to their ability to transmit protozoan, rickettsial and viral diseases of livestock (Jongejan & Uilenberg, 2004; Lynen *et al.*, 2007; Rajput *et al.*, 2006). Ticks are responsible for severe economic losses both through direct effects of blood-sucking and indirectly as the vector of pathogens due to the toxins they produce; transmitting diseases, causing tick paralysis, physical damage and death of livestock (Adehan *et al.*, 2018; Kivaria, 2006; Lew-Tabor & Valle, 2016; Rajput *et al.*, 2006). The major tick-borne diseases of cattle encountered in several African countries including Tanzania are East Coast fever (Theileriosis) caused by *Theileria parva* and transmitted by *R. appendiculatus*, babesiosis caused by *Babesia bovis* and transmitted by *B. decolaratus*, anaplasmosis causative by *Anaplasma marginale* and transmitted by *R. microplus and R. evertis.* In addition, *Amblyomma variegatum* transmits heartwater (cowdriosis) which is caused by *Rickettsia ruminantium* (Jongejan & Uilenberg, 1994; Kerario *et al.*, 2018; Rajput *et al.*, 2006).

Although eight tick genera with 60 species have been reported to occur in Tanzania, only four genera with nine species were characterized as cattle ticks (Lynen *et al.*, 2007; Mamiro *et al.*, 2016; Yeomen & Walker, 1967). These are *Boophilus decoloratus, Rhipicephalus appendiculatus, Amblyomma variegatum, Rhipicephalus evertsi, Rhipicephalus microplus, Amblyomma lepidum, Rhipicephalus pravus, Amblyomma gemma,* and *Hyalomma albiparmatum.* Five of the nine tick species are the principal vectors of the most common tick-borne diseases of cattle. These include *Boophilus decoloratus, Rhipicephalus appendiculatus, Amblyomma variegatum, Rhipicephalus evertsi* and *Rhipicephalus microplus* (Mamiro *et al.*, 2016). East Coast fever is the major tick-borne disease in Tanzania, accounting for about 40-80% of calf-mortality in cattle and which

translates to approximately USD 43 million annual losses in pastoral areas (Kerario *et al.*, 2018; Kioko *et al.*, 2015; Laisser *et al.*, 2017; McLeod & Kristjanson, 1999; Mtei & Msami, 1996).

2.2 Distribution of Ticks in Tanzania

Tick and tick-borne diseases are prevalent in almost all cattle-keeping areas in Tanzania. Precipitation is one of the factors affecting the distribution of different tick species (Lynen *et al.*, 2007, 2008; Yeomen & Walker, 1967). The main tick species found in Tanzania are *Rhipicephalus appendiculatus, Amblyomma* spp. and *Boophilus* spp. *Rhipicephalus appendiculatus,* are more prevalent in areas with moderate mean temperature (less than 24°C) and are mainly found in the Lake Victoria basin with a distinct southward expansion to Shinyanga region and northern regions (Arusha and Manyara), Mtwara and Rukwa regions (Lynen *et al.*, 2007; Tatchell & Easton, 1986; Yeomen & Walker, 1967). *Amblyomma* species, (*A. variegatum and A. lepidum)* are also common in Tanzania (Lynen *et al.*, 2007). *Amblyomma variegatum* is the most common and widely distributed specie in the country, covering the sub-humid and low to high altitudes (Kerario *et al.*, 2017; Lynen *et al.*, 2007). The two *Boophilus* spp. (*B. microplus* and *B. decoloratus)* are also distributed differently across Tanzania, whereby, *B. microplus* is more common in the northern regions of Tanzania while *B. decoloratus* is in the highlands in the north central part of the country as well as other high altitude regions (Lynen *et al.*, 2008).

2.3 Life Cycle of Important Ticks

There are four stages in the life cycle of ixodid ticks namely; the egg, larva, nymph and adult. The larva, nymph and adult are the active stages and requires a blood meal to survive in the next stage or to enable a female to produce eggs. Ticks are referred to as one- host (e.g. *Boophilus*), two-host (e.g. *R. evertis*) or three-host (e.g. *Amblyomma*) depending on how many hosts they must parasitize to complete their life cycle (Jongejan & Uilenberg, 1994).

Most hard ticks are three-host, requiring three (3) hosts to complete their life cycle, where they detach on completion of feeding, drop from host, moult and wait for another host. After female ticks drop from the host, they seek shelter for oviposition where they lay thousands of eggs (2 000-18 000) and then die while the male tick stay longer on the host and mate repeatedly (Alonso-Díaz & Fernández-Salas, 2021; Jongejan & Uilenberg, 1994; Tuppurainen, 2015). For one-host ticks, the nymph remains on the same host and continues feeding to adult while for two host-ticks the engorged larvae remain on the same host where they molt into a nymph and drop as an engorged nymph.

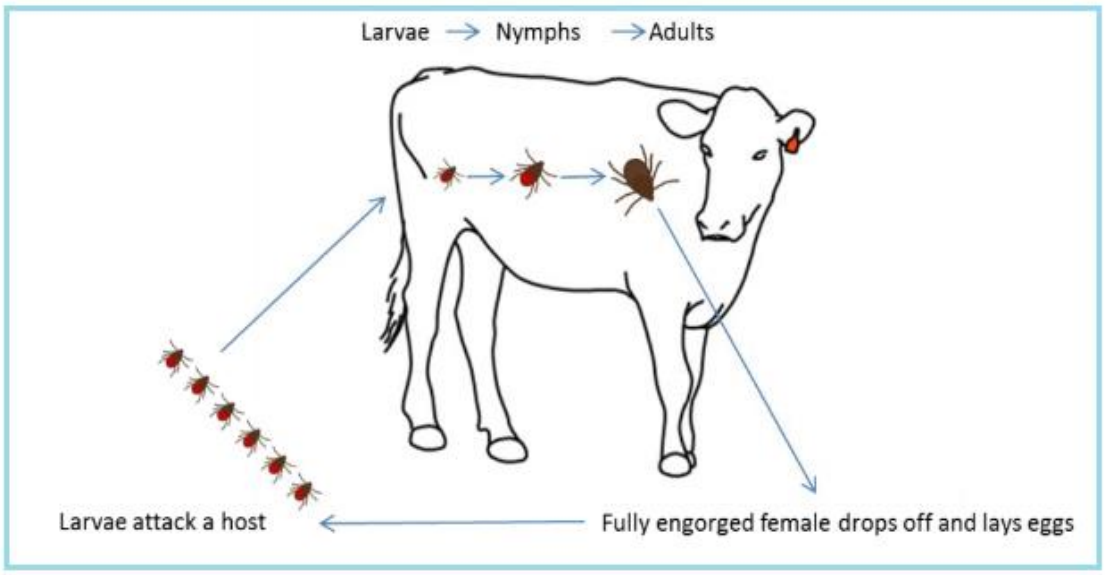

Figure 1: Life cycle of one-host tick (Tuppurainen, 2015)

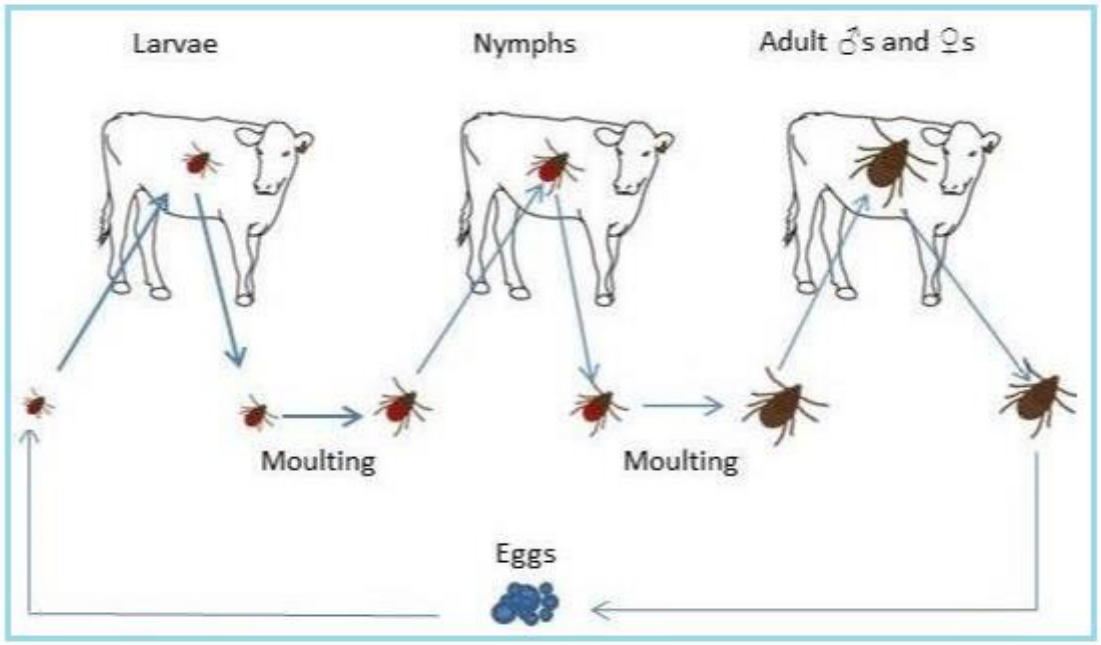

Figure 2: Life cycle of three-host tick (Tuppurainen, 2015)

2.4 Management of Ticks and Tick-borne Diseases

2.4.1 Use of Chemical Acaricide to Control Ticks

Chemical acaricides have, for decades been used with promising results in the control of ticks (Adrian, 2012; de la Fuente *et al.*, 2016; Lew-Tabor & Valle, 2016; Nejash, 2016). The common commercial chemical acaricides are organophosphates, carbamates and pyrethroids (George *et al.*,

2004; Rajput *et al.*, 2006; Ravindran *et al.*, 2011). However, continuous and indiscriminate use of these acaricides has led to resistance of ticks against these chemicals and hence, reducing the control ability that in turn, results in tick-borne diseases (Abbas *et al.*, 2014; de la Fuente *et al.*, 2016; Kaaya & Hassan, 2000; Raynal *et al.*, 2013). In addition, environmental contamination, toxicity to non-target organisms and residues in animal products have been reported as untoward consequences of using chemical acaricides (Abbas *et al.*, 2014; Drummond, 1976; George *et al.*, 2004; Jongejan, 1999; Kaaya & Hassan, 2000; Kerario *et al.*, 2018; Maniania *et al.*, 2007; Rajput *et al.*, 2006; Wharton, 1983).

2.4.2 Chemotherapy

Chemotherapy against tick-borne diseases is available but some of the drugs used require daily treatment up-to 28 days while others require up to three (3) months of daily treatment (Jongejan, 1999; Young *et al.*, 1988). In addition, the drugs are only effective when given in the early stages of the disease (Jongejan, 1999) especially for ECF. Some of the drugs used in the treatment of tick-borne diseases are naphthoquinones (e.g. parvaquone and buparvaquone), tetracycline and monoclone, which are expensive and their efficacy depends on early diagnosis of the disease (Ganga *et al.*, 2010; Magulu *et al.*, 2019; Maharana *et al.*, 2016).

2.4.3 Immunization

Vaccination against East Coast fever based on infection and treatment method (Jongejan & Uilenberg, 1994; Magulu *et al.*, 2019; McKeever, 2007; Young *et al.*, 1988) has been used for over 20 years in Tanzania. This method involves infecting healthy cattle with live *T. parva* sporozoites and simultaneously treating them with a single dose of long-acting formulation of oxytetracycline, a broad-spectrum antibiotic to moderate the infection resulting in a life-long immunity to similar or related parasites (Di Giulio *et al.*, 2009; Magulu *et al.*, 2019; Mbassa *et al.*, 2009; McKeever, 2007). Cross-immunity has not been reported against other tick-borne diseases. In addition, ITM is too expensive for rural farmers with large herds of cattle.

2.4.4 Use of Entomopathogenic Fungi

Entomopathogenic fungi are microbes that specifically infect and often kill insects and other anthropods (Ghany, 2015; Skinner *et al.*, 2014). They are host-specific and hence, are nonpathogenic to plants and leaves no toxic residue in crops (Jiang *et al.*, 2020; Skinner *et al.*, 2014). Additionally, they are non-toxic to animals and humans and are environmentally friendly as compared to chemicals (Skinner *et al.*, 2014). Entomopathogenic fungi are found in a wide range of environmental conditions and can infect a wide range of insects (Jiang *et al.*, 2020;

Skinner *et al.*, 2014). Entomopathogens have been used as control agents for insect pests for over a century and appear to be a more promising agent for the control of ticks than other potential biological control agents (Ekesi & Maniania, 2007; Maniania *et al.*, 2007; Stafford & Allan, 2014). These fungi are present within the natural insect population and are considered as effective microbial control agents in integrated pest management (Jiang *et al.*, 2020; Skinner *et al.*, 2014). Entomopathogenic fungi enter the host through the cuticle or openings on the host's body where they multiply and feed on the host's internal content causing the death of the host either by nutritional deficiency, tissue destruction, disruption of normal biological functions or toxic substances from the fungus (Jiang *et al.*, 2020; Narladkar, 2018; Skinner *et al.*, 2014). The use of entomopathogens will contribute to the reduction or abolition of the use of chemical products because they are safe for humans and other non-target organisms due to their high specificity to target organisms. Entomopathogenic fungi also reduce chemical residues in foods, are cheap and have high efficacy (Ghany, 2015; Kaaya & Hassan, 2000). The fungal species *Metarhizium anisoplie* and *Beauveria bassiana* have been reported to exhibit high virulence and hence, more widely used in the control of ticks worldwide (Bittencourt, 2008; Fernandes *et al.*, 2012; Narladkar, 2018; Perinotto *et al.*, 2012; Pirali-Kheirabadi *et al.*, 2007). *Metarhizium anisoplie* and *Beauveria bassiana* have been used for the control of ticks in Africa and south America (Ekesi & Maniania, 2007). Various researches have been done on the pathogenicity of entomopathogenic fungi on different tick stages such as adult, larvae and eggs (Ghany, 2015; Kaaya & Hassan, 2000; Kalsbeek *et al.*, 1995). However, there is limited information regarding the entomopathogenic activity of recently identified fungi *Aspergillus oryzae* in Tanzania. To the best of our knowledge, this study is intended to document for the first time, the acaricidal activity of *A. oryzae* on selected cattle ticks under field conditions in Tanzania.

2.4.5 Aspergillus

The fungus *Aspergillus* belongs to class *Deuteromycetes* which are characterized by reproducing by conidia, that are generally formed aerially on conidiophores arising from the substrate (Bennett, 2010; Narladkar, 2018). *Aspergillus* are found naturally in many environments and distributed in food remains, soil, textile products and leather (Beguin & Nolard, 1994; Bennett, 2010; Hubka *et al.*, 2013; Zekeya *et al.*, 2019)

Aspergillus fungi have been reported to be very effective against numerous insect species and have the potential as biocontrol agents (Dahliz *et al.*, 2013; Lakhdari *et al.*, 2016; Wang *et al.*, 2019; Zhang *et al.*, 2015). Additionally, they has been used in the food fermentation process for over

2000 years and other industrial processes such as enzymes and commodity chemicals (Bennett, 2010; Wang *et al.*, 2019; Zekeya *et al.*, 2019; Zhang *et al.*, 2015).

2.5 Infection Mechanism of Entomopathogenic Fungi

Entomopathogenic fungi use different mechanisms in infecting, colonizing and killing ticks. They use mechanical invasion, enzymatic and toxicological systems to target all stages of the tick's life cycle. The infection mechanism of entomopathogenic fungi occurs as:

(i) Recognition of susceptible host.

(ii) Adhesion of conidia and germination on host cuticle

(iii) Development of specific structures (germ tube and aspersorium).

(iv) Penetration through the host's cuticle

(v) Intense fungal growth and death of the host

(vi) Production of conidia after hyphae emergence through the host cuticle.

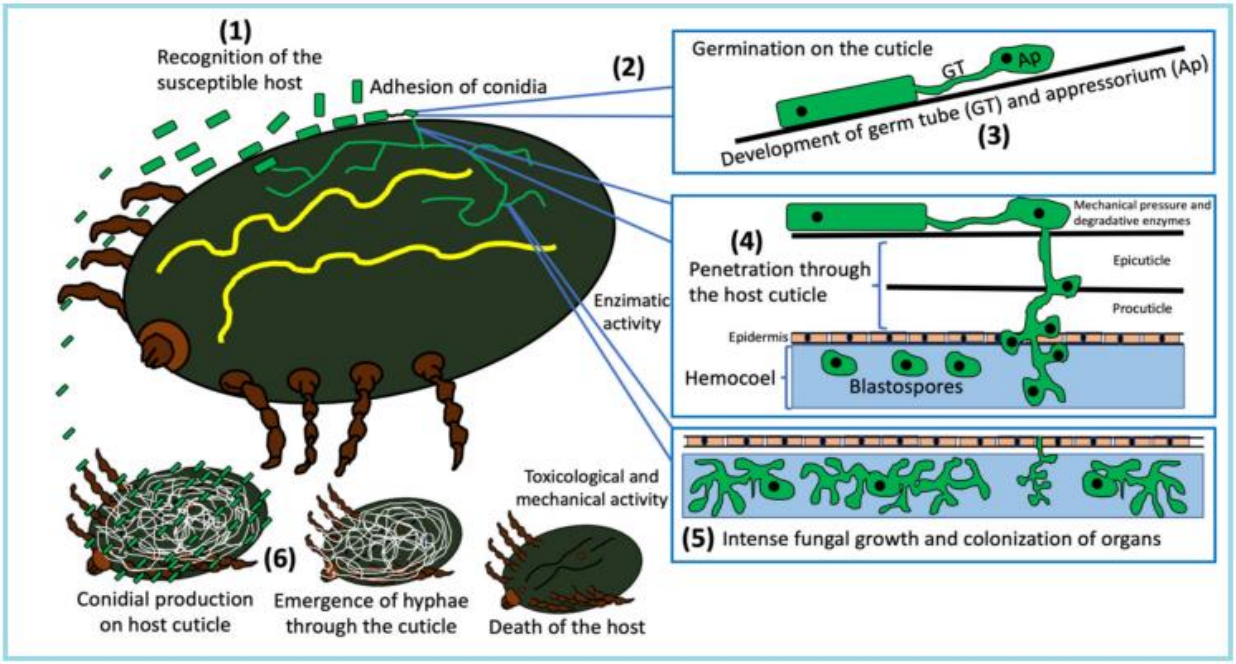

Figure 3: Entomopathogenic Fungi Infection mechanism. source (Alonso-Díaz & Fernández-Salas, 2021)

2.5.1 Recognition of a Susceptible Host, Adhesion of Conidia and Germination on the Host Cuticle

Adhesion of fungal conidia to the host's cuticle is facilitated by hydrophobic mechanisms(Alonso-Díaz & Fernández-Salas, 2021; Ortiz-Urquiza & Keyhani, 2013) mediated by conidia surface

proteins hydrophobins (Fig. 3) (Leger *et al.*, 1992; Skinner *et al.*, 2014) and adhesions (Valero-Jiménez *et al.*, 2016; Wang & St Leger, 2007).

Once the conidia have been attached (Fig.3), under optimal humidity and temperature conditions they germinate and produce a germination tube followed by a peg or appressorium for penetration into the host's cuticle (Alonso-Díaz & Fernández-Salas, 2021; Brunner-Mendoza *et al.*, 2019; Skinner *et al.*, 2014). The penetration process is aided by the production hydrolytic cuticular enzymes such as lipases, proteases and chitinases, and the mechanical pressure exerted by the appressorium (Alonso-Díaz & Fernández-Salas, 2021; Brunner-Mendoza *et al.*, 2019).

2.5.2 Fungal Growth and Death of Host

Once inside of the host, fungi develop hypha bodies and blastopores that multiply and using circulation as means of transport for colonization and nutrient absorption (Brunner-Mendoza *et al.*, 2019; Valero-Jiménez *et al.*, 2016) they disseminate through the hemolymph and invade adverse different tissue (Alonso-Díaz & Fernández-Salas, 2021; Beys-da-Silva *et al.*, 2020; Maina *et al.*, 2018). During this process different virulent factors such as fungal toxins act on the host colonization to spread inside the host causing its death. These toxins cause flaccid paralysis, cellular alteration and inhibit the normal functioning of muscle tissues, the middle intestine and the Malpighian tube (Alonso-Díaz & Fernández-Salas, 2021; Mora *et al.*, 2018).

Once all the nutrients have been consumed and death of the host , the fungus initiates conidia dispersal by opening the integument, forming aerial mycelia and carry out sporulation on the corpse as shown in Fig. 3 (Alonso-Díaz & Fernández-Salas, 2021; Valero-Jiménez *et al.*, 2016). Under suitable abiotic conditions conidia are produced which are then dispersed by wing to attach to new susceptible host.

CHAPTER THREE

MATERIALS AND METHODOLOGY

3.1 Study Site

Laboratory experiments were carried out at the Tanzania Plant Health, and Pesticides Authority (TPHPA) located in Arusha region, Tanzania. Apart from having a bio-efficacy laboratory for livestock vector controlling under the Division of Livestock and Human Disease Vector Control (LHDV), TPHPA has many facilities for rearing ticks and thus, it was selected as the most convenient laboratory facility for this study.

The fieldwork for the present study was undertaken in Monduli district northern Tanzania. Maasai pastoralists who practice extensive grazing systems with large herds/flocks of cattle and small ruminants predominantly occupy this district. Additionally, dipping and spraying using chemical acaricides is the most predominant method for tick control in the district. Community dip tanks that have been revived recently in the area use government-subsidized acaricides with a minimal fee charge per head of cattle dipped. This encourages the continued use of chemical acaricides because an alternative is lacking.

3.2 Tick Collection and Managing Laboratory Bioassay

Female engorged ticks of the three genera of economic importance (*Rhipicephalus, Amblyomma* and *Boophilus*) were randomly collected from six cattle herds at Meserani Chini village in Monduli district. During collection, they were gently removed from cattle by bending the tick upward and forward and then exerting a steady pull. They were then placed in ventilated tubes with absorbent paper. Once in the laboratory they were incubated for 20 days to lay eggs at 28±1℃ and 80% relative humidity. The eggs were kept in 2.5 cm diameter x 8 cm long test tubes, sealed with cotton wool and gauze plugs and left to hatch into larvae at 28±1℃ and 80% relative humidity (Shyma *et al.,* 2019).

3.3 Bio-acaricide Preparation

(i) Source of Fungal Isolate (Vector Biocide)

Aspergillus oryzae with accession MG938642 in the Gene Bank that was later patented with reference number: TZ/P/2020/000119 was offered by Plant Bio-defender Limited Moshi-Tanzania. *A. oryzae* MG938642 was previously isolated from industrial waste water at Kilimanjaro International Leather Industry in Kilimanjaro region, Tanzania and cultured on potato dextrose agar in glass petri dishes (Zekeya *et al.,* 2019). Morphological features and molecular

characterization were used to identify the isolates *A. oryzae* (Zekeya *et al.*, 2019; Zhang *et al.*, 2015).

The aqueous suspension of *A. oryzae* with a concentration of 1.0 x 10^8 conidial/mL was diluted using sterile distilled water to make a working solution of 2.0 x 10^6 conidial/mL. The working solution was further serially diluted to make test concentration as follows: 1.0 x 10^6 conidial/mL, 5.0 x 10^5 conidial/mL, 2.5 x 10^5 conidial/mL, 1.25 x 10^5 conidial/mL, 6.25 x 10^4 conidial/mL, 3.125 x 10^4 conidial/mL and 1.5625 x 10^4conidial/mL for efficacy testing against larva and adult stages of tick life cycle.

3.4 Application of Bio-acaridae to Different Stages of Ticks

3.4.1 Larval Sensitivity Test

The sensitivity test procedures were carried out as described in the larvae immersion test but with minor modifications (De Sousa *et al.*, 2020). Sterile distilled water was used as a control during the experiment. Prior to conducting each test, the bio-acaricide in the test concentration was agitated for two minutes using a vortex at 3000 rpm for uniformity. Thereafter, 10 mL of the bio-acaricide of each test concentration were used for testing its efficacy on larvae as follows: 3 mL of the bio-acaricide was drawn using a 10 mL pipette and transferred to a 15 cm petri dish, then a 11cm Whatman filter paper was placed above, and using number 3 painting brush, larvae of 14-21 days old of each genus were removed from the rim of the specimen tube and were distributed evenly on the Whatman filter paper in the petri dish. An aliquot of 4 mL of the bio-acaricide was then added over the larvae covered by another Whatman filter paper, creating a sandwich. Finally, 3 mL of the bio-acaricide was added and the whole experiment setup was covered by another petri dish of the same size, and left to stand for 10 minutes at room temperature. After 10 minutes, the sandwich was opened and left to dry after initial absorption. The larvae were then taken from the Whatman filter paper and placed in the apex of a folded Whatman filter paper that was segmented and clipped using 'bulldog' clips of 5 cm long on the sides making a packet. The open end of the filter paper was closed with clips, placed in a rack and incubated at 28°C±1 and RH≥80% in BOD incubator for 24 hrs. Thereafter, dead and alive larvae were counted, and the values were used for calculating percentage mortality (Fig. 4). Larvae without movement were considered dead after 48 hours. These procedures were repeated for each test concentration in triplicate for each of the selected genera

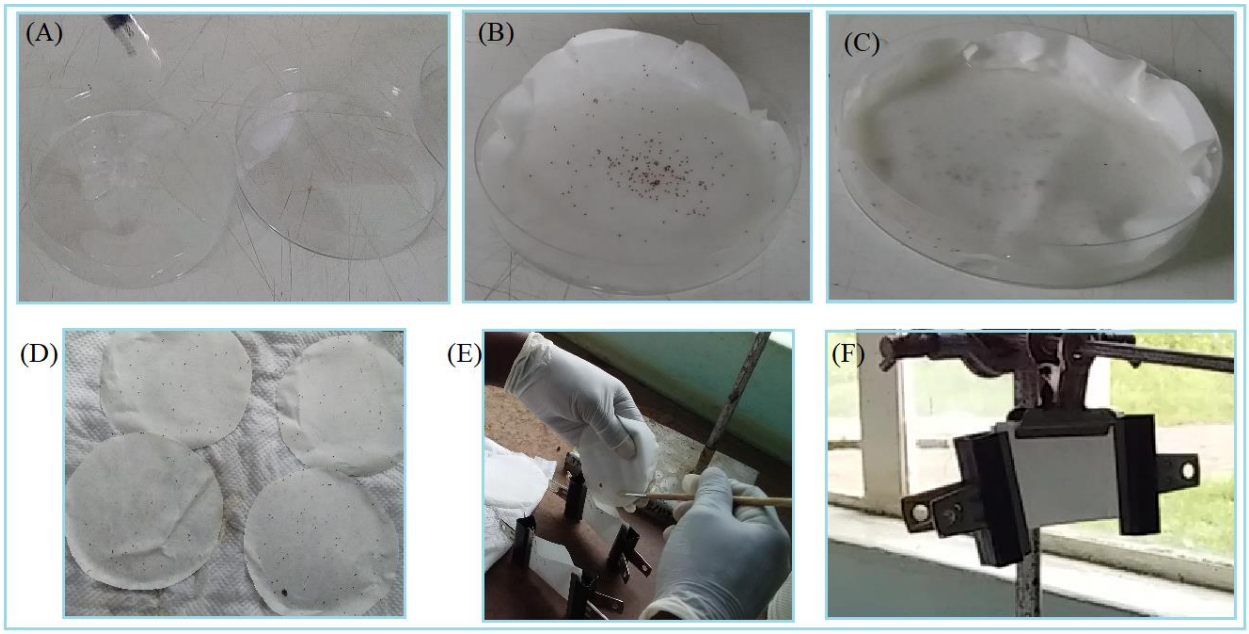

Figure 4: Pictorial presentation of larval sensitivity test. 3 mL of bio-acaricide on petri dish (A); Larvae on soaked filter paper (B); Sandwich incubated for 10 minutes (C); Drying larvae on filter paper after absorption (D); Larvae into filter paper packet (E); Packet on a rack for incubation (F)

3.4.2 Adult Sensitivity Test

The adult sensitivity test was determined by using fully engorged female ticks using the adult immersion test as described by Drummond *et al.* (1973) with some small modifications. The test was done in two replicates for each concentration in each of the selected genera. Sterile distilled water was used as control. Groups of 5 engorged female ticks from each genus were weighed to get a uniform constant sample weight (De Sousa *et al.*, 2020). The fully-engorged females were then immersed in the different concentrations of the bio-acaricide under the same procedure as for the larvae. Then, the ticks were dried using a paper towel, placed in the petri dish with the adhesive material to prevent their movements and incubated at 28°C±1, RH ≥ 80%. Mortality was monitored daily from the time of commencing laying eggs up to finishing (Fig. 5). The laid eggs were weighed and recorded. Failure to lay eggs was interpreted as an indication of death of the tick.. The laid eggs were kept under the same conditions to hatch, and failure to hatch indicated that the egg was not viable and this was observed visually. Then, egg production index, oviposition, product efficacy (De Sousa *et al.*, 2020; Temba *et al.*, 2018) and lethal dose (LC_{50} and LC_{99}) were calculated using prior calculated female's initial weight, egg mass weight and hatching percentage.

Egg production index (EPI) was calculated using the equation:

$$\%EPI = (egg\ weight \div initial\ female\ weight) \times 100$$

Oviposition reduction (OR) was obtained according to the equation:

$$\%OR = ((EPI\ control\ group - EPI\ experimental\ group) \div (EPI\ control\ group)) \times 100$$

Reproductive efficiency (RE) was calculated using the formula:

$$RE = (weight\ of\ egg\ mass \times \%\ hatching\ egg \times 20\ 000) \div weight\ of\ female$$

where 20 000 is the average number of eggs per gram.

Product efficiency (PE) was estimated by the formula:

$$\%PE = ((RE\ control\ group - RE\ experimental\ group) \div (RE\ control\ group)) \times 100$$

(De Sousa *et al.*, 2020; Temba *et al.*, 2018).

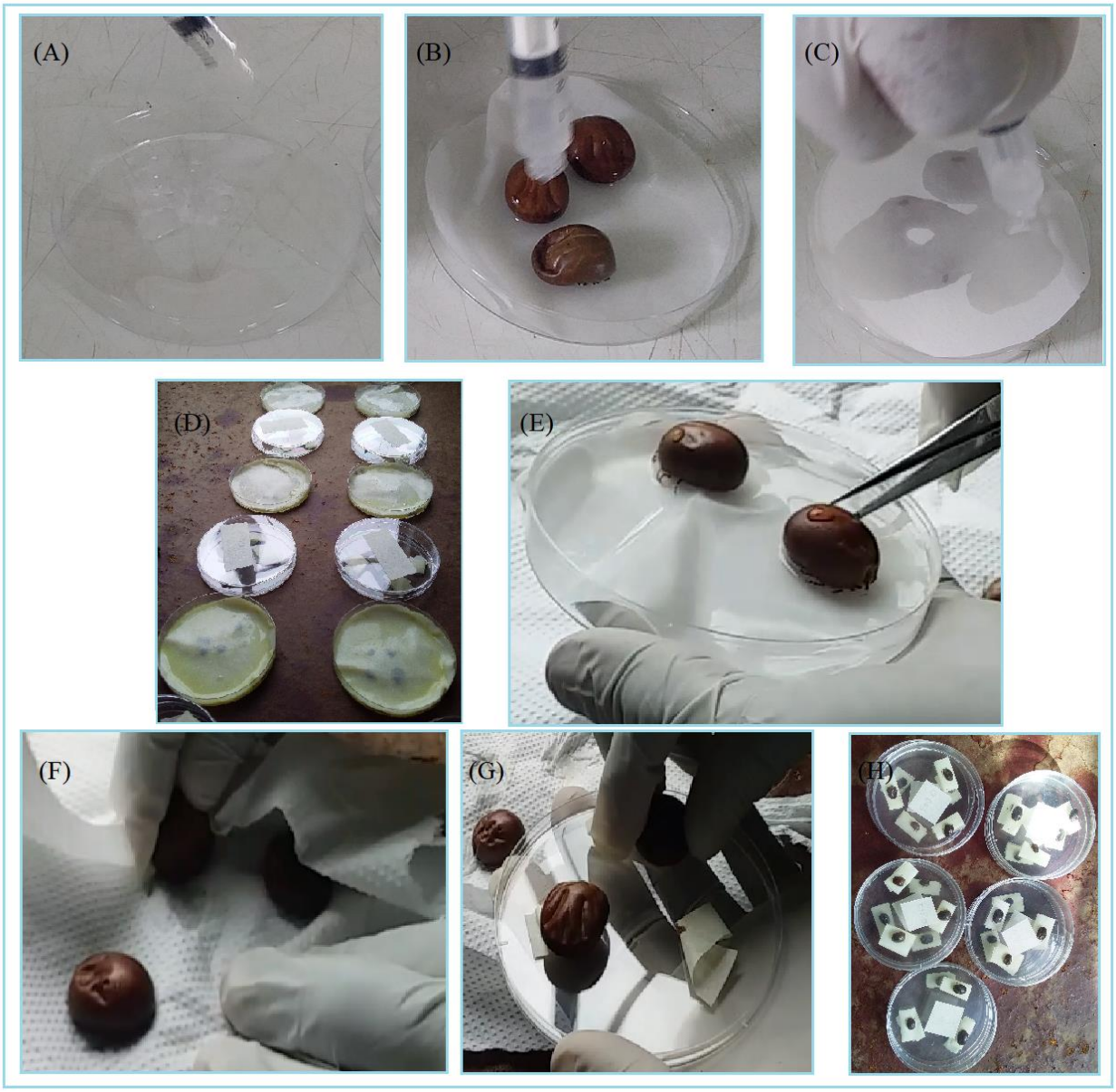

Figure 5: **Adult ticks' sensitivity test procedures. (A) 3 mL of bio-acaricide on petri dish; (B): 4 mL of bio-acaricide over female engorged ticks; (C): 3 mL of bio-acaricide over covered ticks; (D): Sandwich incubated for 10 minutes; (E): Ticks removed from sandwich; (F): Drying ticks with absorbent paper; (G): Ticks on petri dish with adhesive materials; (H): Ticks on petri dishes ready for incubation**

3.5 Application of Bio-acaricide on Laboratory Animals

Rabbits were used for testing the safety of *A. oryzae* product. A total of 20 non-pregnant rabbits of both sexes were purchased for this activity and placed in cages. Four cages, each with five rabbits were supplied with water and food. Cages were named as A, B, C and D for easy identification. Each rabbit was ear-tagged for subsequent identification. Before being subjected to the experiment, rabbits were acclimatized for seven days. Prior to infecting with ticks, a collar was placed around the rabbit's neck to prevent it from scratching and removing the ticks during experimentation (Figure 6). Both ears were shaved to facilitate tick's attachment. A total of 50 ticks were introduced into each ear and allowed to attach themselves to the rabbit's ears for two days. Once the ticks had attached themselves to the rabbits, they were treated with *A. oryzae*. Rabbits in cages A, B and C were sprayed with the bio-acaricide at a concentration of $1x10^4$ conidial/mL, 1×10^5 conidial/mL and 1×10^6, respectively. These concentrations were used to test for any side effects of *A. oryzae* on the rabbits from the minimal, median and higher concentrations. Hand spraying was used and targeted the ears. Rabbits in cage D acted as controls and were sprayed with distilled water. Each rabbit was sprayed with 50 mL of bio-acaricide (Cage A, B & C) and distilled water (Cage D), respectively. The experimental rabbits were monitored every morning for 14 days for any clinical signs and drop-off ticks.

Figure 6: Laboratory animals test procedure. (A): Neck collar in place; (B):Shaving to expose blood vessels; (C): Placing ear tags; (D): Infecting rabbits with ticks; (E): Ticks attached on rabbit's ear

3.6 Application of Bio-acaricide in Cattle under Field Conditions

In the field, two pastoral cattle herds (herd size ≥50 cattle) at Duka Mbili and Shakape sub-villages in Meserani Juu village, Monduli district were purposively selected for this study. Verbal consent was given by the farmers to use their animals.. The tick population on cattle was assessed before treatment and monitored throughout the experiment period and these data were used to determine tick reduction. Seven animals were randomly selected in each herd, where five animals were marked and treated with bio-acaricide while two animals that were used as controls were marked differently for easy identification (Fig. 7). Prior to bio-acaricide application, the number of ticks on each animal was established on the same day. The animals were restrained and sprayed with 500 mL of bio-acaricide at a concentration of 1 x 10^6 conidial/mL of the bio-acaricide on all the areas infested with ticks. A higher concentration was used in the field because ticks are physically and structurally tolerant to fungi infection (Fernandes *et al.*, 2012) and also, to overcome environmental challenges such as UV-light. A concentration of 1 x 10^6 conidial/mL of *A. oryzae* was used in the field because it has shown higher tick mortality in the laboratory. Close monitoring was done to establish drop-off ticks on the treatment day, the first three days consecutively and the seventh day after treatment by counting the number of ticks before releasing the animals and after grazing, a reduction in number of ticks indicates tick drop-off. Similar monitoring was done for the control individuals. Re-infestation was monitored until the next spraying/application by counting the number of ticks on the animals before treatment and after treatment, any additional number of ticks on animal's body indicates reinfection. Subsequent application was repeated on the 14^{th} day to further monitor the performance of the bio-acaricide. Thereafter, a random picking of 30 engorged, female ticks from the animal's body was carried out by bending the tick upward and forward then exerting a steady pull, placed in ventilated tubes with absorbent paper and taken to the laboratory. In the laboratory, the ticks were treated under controlled environmental conditions as described earlier to assess mortality and hatching percentage.

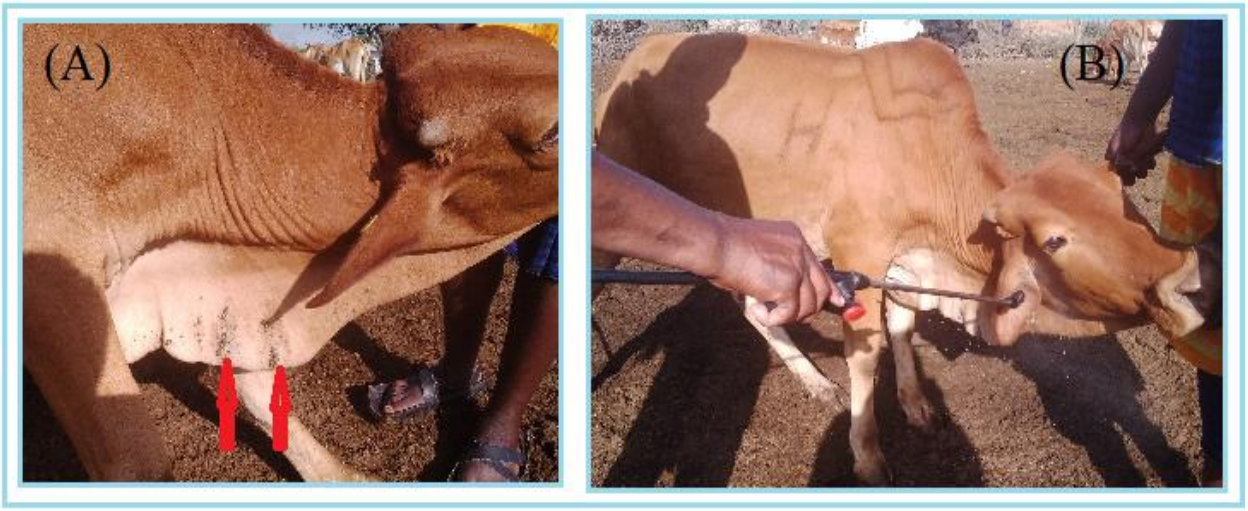

Figure 7: Bio-acaricide application in the field. Arrows show ticks on cattle neck. (A): Cattle infested with ticks; (B): Spraying *A. oryzae* on infested areas of the cattle

3.7 Data analysis

Descriptive statistics were used to summarize the data into means, percentages and standard deviations. A one-way analysis of variance (ANOVA) was used for analyzing tick mortality while the pairwise comparison test (Turkey test) was used to analyse variations between dose concentrations at a 5% significance level. Variables related to the production of eggs were subjected to Kruskal Wallis rank sum test. The probit regression analysis was used to analyze the lethal concentration 50 (LC_{50}) and LC_{99} for the mortality of ticks. Normality test was done by using Shapiro test (log transformation). The critical probability level used was 0.05. All statistical analyses were carried out using R software (4.0.3).

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Results

4.1.1 The Effect of A. oryzae on Different Tick Life Cycle

(i) The Effect on Larvae Mortality

Larvae from the three genera (*Boophilus, Rhipicephalus* and *Amblyomma)* were subjected to different concentrations of *A. oryzae* and mortalities recorded for 24 hours. Although variation on larva mortality was observed across the three species, *Rhipicephalus* showed high mortality only in higher concentrations (Table 1).

Table 1: The effect of *A. oryzae* on the larvae mortality of the three genera of ticks after exposure to treatment conditions after 24hours

Concentration (conidial/mL)	No. of Exposed larva	*Rhipicephalus*			*Boophilus*			*Amblyomma*		
		Live	Dead	Mortality %	Live	Dead	Mortality %	Live	Dead	Mortality %
Control	300	300	0	0	300	0	0	300	0	0
$1.5625*10^4$	300	90	210	70	23	277	92.3	64	236	78.7
$3.125*10^4$	300	65	235	78.3	16	284	94.7	30	270	90
$6.25*10^4$	300	66	234	78	14	286	95.3	17	283	94.3
$1.25*10^5$	300	47	253	84.3	13	287	95.7	13	289	96.3
$2.5*10^5$	300	38	262	87.3	5	295	98.3	10	290	96.7
$5.0*10^5$	300	5	295	98.3	0	300	100	0	300	100
$1.0*10^6$	300	0	300	100	0	300	100	0	300	100

The mean larvae mortality for all genera was significant at $p<0.05$, with *Boophilus* having the highest mean mortality (Table 2). However, the difference in larvae mortality between *Rhipicephalus* and *Amblyomma* was not significant ($p = 0.512$).

Table 2: The effect of *A. oryzae* on larva of three genera of ticks after exposure to treatment conditions after 24hours

Genus of Tick	Mortality (Mean ± SD)%
Boophilus	95.57^{b}±3.25
Rhipicephalus	84.95^{a}±10.71
Amblyomma	87.90^{a}±9.89

$F_{(2, 60)} = 8.484$, $p = 0.0006$. Different letters show significant difference; same letters show no significant difference

The effect of *A. oryzae* on larvae was so high that led to the lethal concentrations in larvae mortality to be at least 50% and 99% for the three genera as shown in Table 3. A concentration increase in mortality of larvae was observed in all three genera as shown in Fig.8.

Table 3: The lethal concentration (LC_{50} and LC_{99}) of *A. oryzae* against *Boophilus, Rhipicephalus* and *Amblyomma larvae*

Genus of Tick	LC_{50} (conidial/mL)	95% CL	LC_{99} (conidial/mL)	95% CL	R^2
Boophilus	99.610	5.431-473.55	9.8×10^5	4.7×10^4 -3.5×10^6	0.36
Rhipicephalus	5.0×10^3	2.1×10^3- 8.8×10^3	3.0×10^6	1.4×10^6 -1.0×10^7	0.55
Amblyomma	3.9×10^3	2.2×10^3 -6.0×10^3	1.6×10^6	1.0×10^6-3.0×10^6	0.50

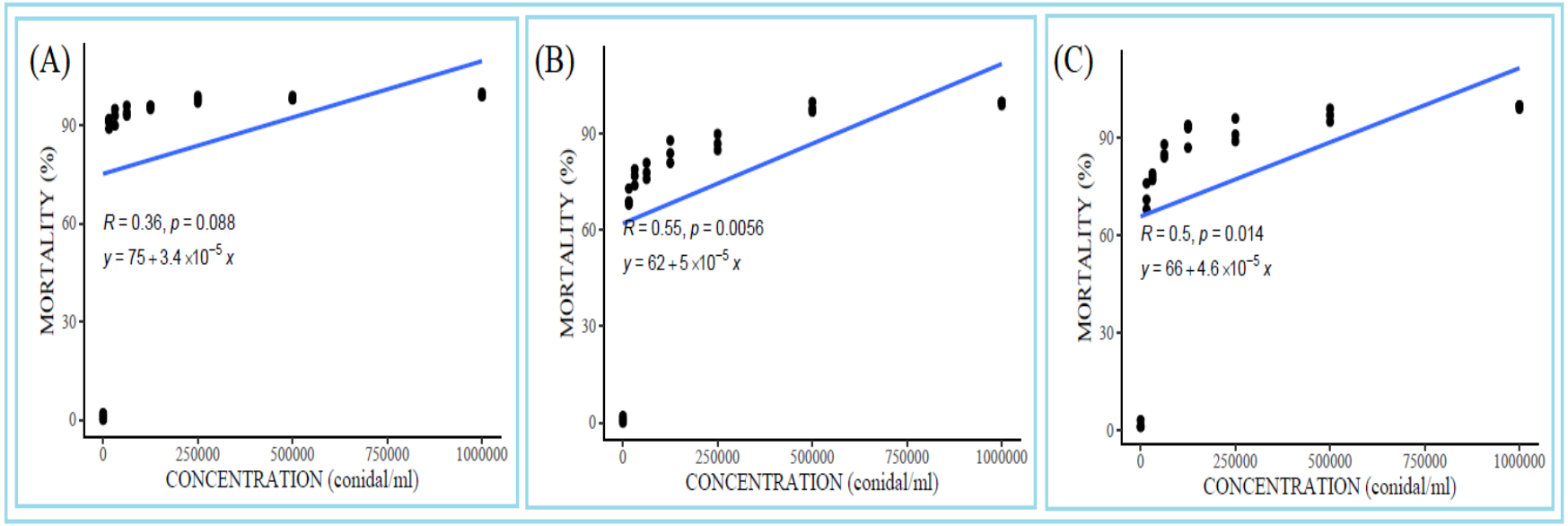

Figure 8: Effectiveness of A. oryzae on larva mortality at different concentrations (Boophilus (A), Rhipicephalus (B) and Amblyomma (C))

(ii) The Effect of *A. oryzae* Product on Female Engorged Ticks

The results showed that female engorged ticks respond differently when exposed to different concentrations of *A. oryzae*. Few female engorged ticks died before they began to lay eggs (Table 4).

Table 4: The effect of *A. oryzae* on female engorged ticks after exposure to different treatment conditions

Concentration (conidial/mL)	No. of ticks exposed	*Rhipicephalus*				*Boophilus*				*Amblyomma*			
		Average Weight of Female(g)	Weight of Eggs (g)	Mortality %	Hatching %	Average Weight of Female (g)	Average Weight of Eggs (g)	Mortality %	Hatching %	Average Weight of Female (g)	Average Weight of Eggs (g)	Mortality %	Hatching %
Control	10	1.65	1	0	100	1.5	0.949	0	100	3.1	1.995	0	100
$1.5625*10^4$	10	1.65	0.825	0	20	1.5	0.021	30	10	3.1	1.53	10	35
$3.125*10^4$	10	1.65	0.457	0	10	1.5	0.0156	40	5	3.1	1.493	0	21
$6.25*10^4$	10	1.65	0.383	0	5	1.5	0.0146	50	3	3.1	1.393	0	5
$1.25*10^5$	10	1.65	0.287	10	2	1.5	0.0138	50	2	3.1	1.269	10	2
$2.5*10^5$	10	1.65	0.082	20	1	1.5	0.0068	50	1	3.1	1.229	20	1
$5.0*10^5$	10	1.65	0.058	20	1	1.5	0.0046	60	1	3.1	0.9175	30	1
$1.0*10^6$	10	1.65	0.016	40	1	1.5	0.003	70	1	3.1	0.658	30	1

Variation in the mean female engorged tick mortality rate among all genera was significant at ($p < 0.001$) as shown in Table 5 although by using post hoc test of *Boophilus*, the mean mortality rate was found to be significantly higher than that of *Rhipicephalus* and *Amblyomma* ($p < 0.001$).

Table 5: Mean mortality of female engorged ticks of three genera after exposure to different treatment

Genus of Tick	Mortality (Mean ± SD) %
Boophilus	62.86^{b}±23.90
Rhipicephalus	21.90^{a}±24.42
Amblyomma	23.81^{a}±24.99

$F_{(2, 60)} = 18.78$, $p < 0.001$. Different letters show significant difference; same letters show no significant difference

The lethal concentration of *A. oryzae* for female engorged tick mortality rate in all genera was established as shown in Table 6.

Table 6: The lethal concentration (LC_{50} and LC_{99}) of *A. oryzae* against *Boophilus, Rhipicephalus* and *Amblyomma* engorged ticks

Tick Genera	LC 50 (conidial/mL)	95% CL	LC 99 (conidial/mL)	95% CL	R^2
Boophilus	5.3×10^4	2.2×10^4-9.3×10^4	7.9×10^6	1.9×10^6 -2.6×10^8	0.79
Rhipicephalus	5.7×10^5	4.5×10^5-8.0×10^5	1.0×10^7	5.1×10^6 -3.2×10^7	0.98
Amblyomma	5.3×10^5	3.4×10^5-1.0×10^6	1.2×10^7	3.8×10^6 -1.6×10^8	0.97

A concentration dependent increase in the mortality was observed in all the genera although a 100% mortality was not observed even at the higher tested concentration (Fig. 9).

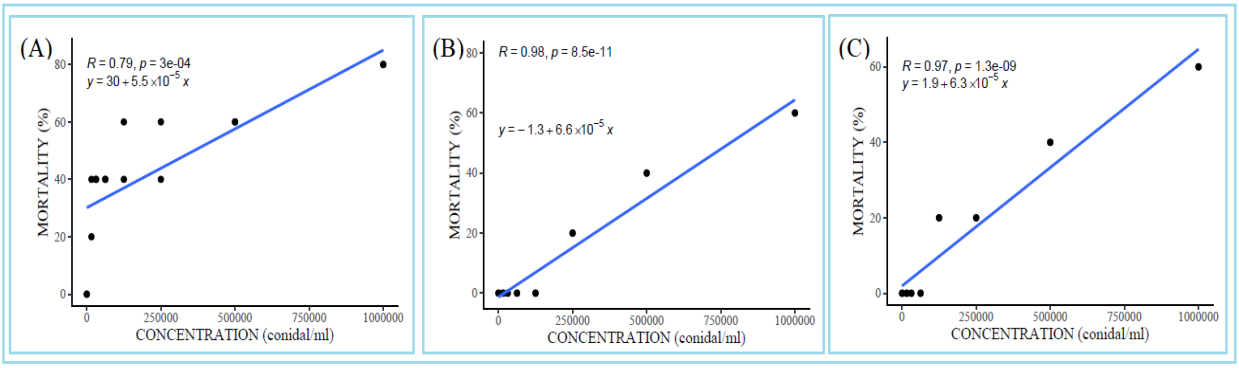

Figure 9: Effectiveness of *A. oryzae* on ticks mortality at different tested concentrations *Boophilus* (A), *Rhipicephalus* (B) and *Amblyomma* (C)

By visual observation, it was found that the hatching percentage lowered to 1% on $2.5^{x}\ 10^{5}$ conidial/mL concentration (Fig. 10) although the variation across all genera was not significant (P=0.89) as shown in Table 8. Similarly, there was a high reduction in oviposition with the increase in concentration of the bio-acaricide, which was significantly different (P=0.009) across the genera.

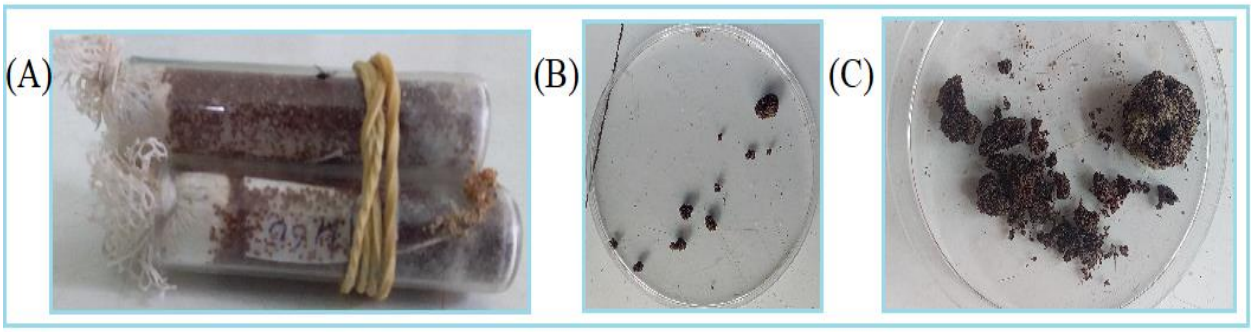

Figure 10*:* **Effect of *A. oryzae* on eggs. Control 100% hatched (A); Unhatched eggs hence not viable (B & C)**

The egg production index was found to decrease as product concentration increased with *Boophilus* having the lowest egg production index followed by *Rhipicephalus* and *Amblyomma*, respectively (Table 7). Although variation in egg production index and oviposition reduction was significant at p=*0.009*, variation in egg hatching and product effectiveness across the general was not significant (*p=0.89* and *p=0.004*, respectively) as shown in Table 8.

Table 7: Mean percentages for egg production index, oviposition reduction, hatchability and efficacy against female engorged ticks exposed to different *A. oryzae* concentrations

	Concentration conidial/mL	Egg production index (%)	Oviposition reduction %	Egg hatching %	Product effectiveness
Rhipicephalus	0.0000	60.6		99.9	
	$1.5625*10^4$	50	17.49	20	91.74
	$3.125*10^4$	27.7	54.29	10	97.74
	$6.25*10^4$	23.2	61.71	5	98.84
	$1.25*10^5$	17.39	71.3	2	99.7
	$2.5*10^5$	0.05	99.9	1	99.9
	$5.0*10^5$	0.035	99.9	1	99.9
	$1.0*10^6$	0.009	99.99	1	99.9
Boophilus	0.0000	63.26		99.9	
	$1.5625*10^4$	1.4	97.78	10	99.7
	$3.125*10^4$	1.04	98.35	5	99.9
	$6.25*10^4$	0.97	98.46	3	99.9
	$1.25*10^5$	0.92	98.54	2	99.9
	$2.5*10^5$	0.45	99.28	1	99.9
	$5.0*10^5$	0.31	99.5	1	99.9
	$1.0*10^6$	0.2	99.68	1	99.9
Amblyomma	0.0000	64.3		99.9	
	$1.5625*10^4$	49.35	23.25	35	73
	$3.125*10^4$	48.16	25.1	21	84.2
	$6.25*10^4$	44.9	30.1	5	96.5
	$1.25*10^5$	40.93	36.35	2	98.72
	$2.5*10^5$	39.69	38.27	1	99.38
	$5.0*10^5$	29.59	53.98	1	99.53
	$1.0*10^6$	21.23	66.98	1	99.67

Table 8: Effect of *A. oryzae* on egg production index, oviposition reduction and egg hatching

Variable	Tick Genera			Kruskal-Wallis Rank Sum Test	
	Amblyomma	***Boophilus***	***Rhipicephalus***	**χ^2-value**	**P-value**
Egg production index	$39.0^{a} \pm 10.0$	$0.76^{b} \pm 0.44$	$17.0^{ab} \pm 19.0$	9.3803	0.009185
Oviposition reduction	39.0^{a}±16.0	99.0^{b}±0.7	72.0^{ba}±31.0	9.3864	0.009157
Egg hatching	9.4^{a}±13.0	3.3^{a}±3.3	5.7^{a}±7.1	0.22476	0.8937
Product effectiveness	93.0^{a}±10.0	100.0^{a}±0.076	98.0^{a}±3.0	10.879	0.004342

Descriptive statistics (mean±SD); Kruskal-Wallis Rank Sum Test followed by post hoc analysis using Bonferroni test at 5% confidence level, Different letters show significant difference otherwise none significance

4.1.2 Response of *A. oryzae* Application to Animal Behavior

Neither mortality nor signs of toxicity were observed on the rabbits subjected to all the tested concentrations. Their behaviors as well as physiological performance were generally normal throughout the study period.

A delay in the feeding habits of the ticks was observed as the concentration increased. The ticks on the control group were fully engorged after three days of incubation and dropped from the rabbits' bodies whereas, it took 7, 10 and 14 days for the treated groups, respectively to be fully engorged. There was a decrease in the hatching of the laid eggs in the treated groups (1×10^4 conidial/mL and 1×10^5 conidial/mL) and no hatching for eggs laid by engorged ticks exposed to 1×10^6 conidial/mL (Fig.11).

Table 9: Response of *A. oryzae* application to animal's behavior

Animal response	Concentration (conidial/mL)			
	Control	**1×10^4**	**1×10^5**	**1×10^6**
Eye lid closure	-	-	-	-
Difficulty in breathing	-	-	-	-
Change in skin fur	-	-	-	-
General body weakness	-	-	-	-
Loss of appetite	-	-	-	-
Excitement	-	-	-	-
Mortality	Nm	Nm	Nm	Nm

Keys: – = no physiological/behavioral changes, + = observed physiological/behavioral changes, Nm = no mortality

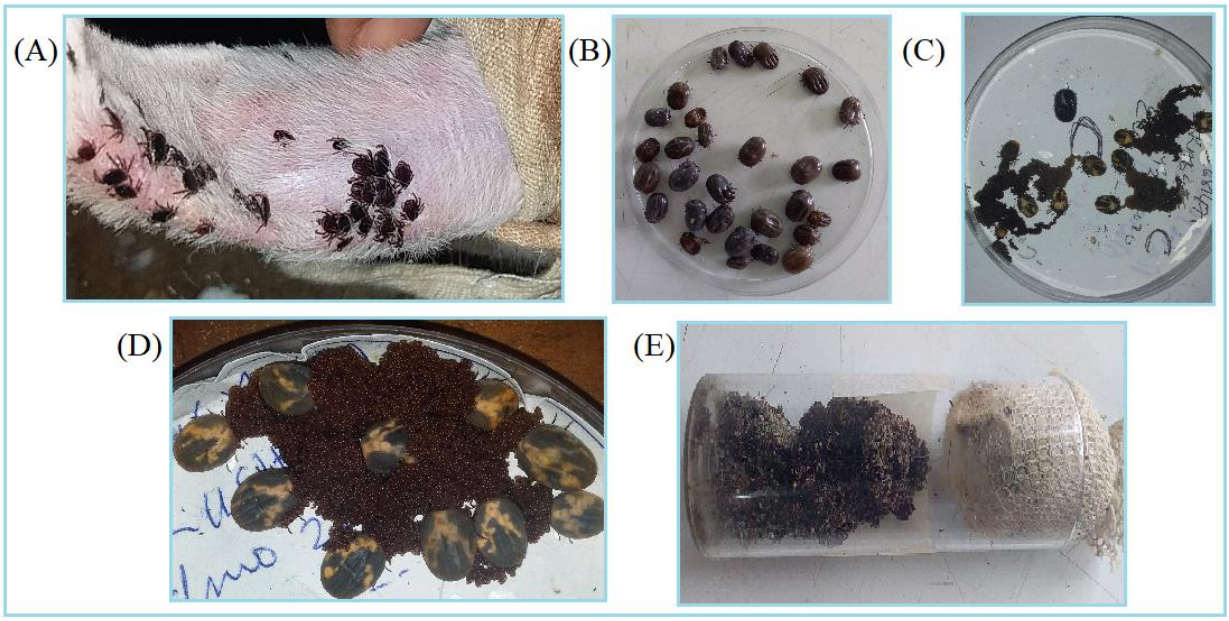

Figure 11*:* **Effect of *A. oryzae* on laboratory Animals: Ticks attached on rabbit's ear (A); Dropped engorged ticks (B); Treated ticks eggs after incubation (C); Control ticks eggs after incubation (D); Unhatched eggs i.e. non-viable(E)**

4.1.3 Effect of *A. oryzae* on Ticks under Field Conditions

It was observed that the number of ticks gradually decreased from day one to day seven on animals treated with *A. oryzae* as compared to control groups. Variation in ticks mortality across different herds after 14 days was significant at (P<0.001) as shown in Table 10 with day seven being observed to have the least number of ticks.

After 14 days, new ticks were observed to re-infest the cattle although the number was less when compared to the control group.

Table 10: Effect of *A. oryzae* product on ticks under field conditions

Treatment	Ticks (Mean ± SD)
Before	107.0^{c}±35.39
After	
Day 1	103.2^{c}±35.15
Day 3	42.4^{ab}±19.39
Day 7	1.6^{a}±2.07
Day 14	55.4^{b}±17.81

$F_{(4, 20)}$ =15.3, p < 0.001 Different letters show significant difference; same letters show no significant difference

Ten of the 30 engorged female ticks that were taken to the laboratory died after two days. The remaining 20 engorged female ticks under controlled laboratory conditions laid eggs but none of the eggs hatched.

4.2 Discussion

Ticks are important ectoparasites of livestock because they are responsible for great economic losses due to their ability to transmit tick-borne diseases (Jongejan, 1999; Jongejan & Uilenberg, 1994; Jongejan & Uilenberg, 2004; McLeod & Kristjanson, 1999; Rodriguez *et al.*, 2018; Samish *et al.*, 2004; Wharton, 1983). The use of chemical acaricides is the most common method used to control ticks in livestock. However, over time ticks have become resistant to acaricides due to their indiscriminate and long-term application. Additionally, chemical acaricides have effects on non-target organisms, cause environmental pollution and pose a threat to human health. Moreover, chemical acaricides leave residues on animal products such as meat and milk, thus becoming a great public health concern (Alonso-Díaz & Fernández-Salas, 2021; Bittencourt, 2008; Fernandes *et al.*, 2012; Fernandes & Bittencourt, 2008). To address the above challenges, the adoption of environmentally-friendly methods for ticks control is considered to be a worthwhile alternative such as entomopathogens (Adrian, 2012; Jongejan, 1999; Kerario *et al.*, 2018; Laisser *et al.*, 2017; Nagagi *et al.*, 2020; Nejash, 2016). Entomopathogenic fungi are promising alternatives for controlling ticks given the lack of fungal-resistance and environmental safety (Fernandes *et al.*, 2012; Fernandes & Bittencourt, 2008; Ghany, 2015; Jiang *et al.*, 2020).

This study aimed at evaluating the acaricidal activity of *A. oryzae* against different stages of the tick life cycle for the three different tick genera; *Rhipicephalus, Boophilus* and *Amblyomma*. Findings from this study have revealed that *A. oryzae* induces mortality of tick larvae and adults. Additionally, *A. oryzae* was found to lower egg production capability and viability of eggs. The tick mortality rate was found to increase with an increase in the concentration of *A. oryzae* in all the three genera, and a 100% mortality of larvae was observed at 1×10^6 conidial/mL concentration of *A. oryzae*. The potential acaricidal activity of entomopathogenic fungi such as *B. bassiana* and *M. anisopliae* has also been reported by other workers on genus *Rhipicephalus* (*R. microplus)* (Fernandes *et al.*, 2012; Perinotto *et al.*, 2012; Pirali-Kheirabadi *et al.*, 2007). The mortality of ticks in this study was attributed to the virulence effect of *A. oryzae* as also reported by Zekeya *et al*. (2020).

This study further assessed the ability of *A. oryzae* to cause mortality of tick larvae. It was revealed that the LC_{50} and LC_{99} values resulted in a higher activity of *A. oryzae* on larvae than in engorged female ticks. This finding agrees with observations in other studies on entomopathogenic fungi on their effect on tick larva (Fernandes *et al.*, 2012; Fernandes & Bittencourt, 2008). This observation

indicates that *A. oryzae* is a promising alternative bio-acaricide for the control of ticks since it has shown acaricidal effects on the entire tick life cycle.

Both mean larva mortality and mortality of engorged ticks were significantly higher in *Boophilus* compared to other genera. In both cases, *Rhipicephalus* responded the least. This could be due to a low chitin composition of *Boophilus* cuticle thereby influencing the penetration of the fungi (Flynn & Kaufman, 2015; Hackman, 1975; Hackman & Goldberg, 1987).

The present study also observed that the egg production index decreased as *A. oryzae* concentration increased, with *Boophilus* having the least index followed by *Rhipicephalus* and *Amblyomma* respectively. Furthermore, the hatching percentage was found to relate inversely with product concentration. This may be attributed to infertility effects caused by entomopathogenic fungi. This finding is comparable to Ghany, (2015) who reported that *A. oryzae* causes sterility in female ticks due to adverse effects in ovarian development. It has also been reported that fungus suppresses and reduces the rate of ovarian development hence, reducing the number of laid eggs (Ghany, 2015; Wasinpiyamongkol & Kanchanaphum, 2019). These findings suggest that the use of *A. oryzae* may reduce the tick population with subsequent reduction of tick-borne diseases.

The response to changes in animal behavior was assessed by using experimental animals. There was no behavioral and physiological difference between treated animals and the controls. Generally, the physiological activities of the animal were normal, which suggests that *A. oryzae* may not have toxic effects on the rabbits. This agrees with other studies which indicated that *A. oryzae* and other entomopathogenic fungi have no effect on non-target organisms and are environmentally friendly (Fernandes & Bittencourt, 2008; Ghany, 2015; Kaaya & Hassan, 2000; Kalsbeek *et al.*, 1995; Stafford & Allan, 2014; Zekeya *et al.*, 2020; Zekeya *et al.*, 2019; Zhang *et al.*, 2015).

To the best of our knowledge, this is the first field trial to study the effect of *A. oryzae* in Tanzania. The bio-acaricide (*A. oryzae*) has shown to be effective in the field at a concentration of 1×10^6 conidial/mL which is lower than the recommended concentration by Fernandes and Bittencourt (2008); Alonso-Díaz and Fernández-Salas (2021) and Perinotto *et al*. (2012) that was $1X10^9$ conidial/mL. These findings indicate that the bio-acaricide is effective in reducing tick population on cattle under field conditions. Tick reduction on the observed herds was noticed up to day seven which had the least number of the ticks on study animals. Ticks re-infestation started to be noticed thereafter. This suggests that *A. oryzae* has a residual effect of up to seven days after application. This finding implies that application of the *A. oryzae* in the field

can be carried out once a week for the effective control of ticks. Since *A. oryzae* has shown high efficacy against tick infestation, it may be considered as one of the bio-acaricides for integrated management of ticks in the livestock sector.

This study has also revealed that *A. oryzae* has a delaying acaricidal effect like other entomopathogenic fungi as it took up to three days before its effect was observed when compared to chemical acaricides, which are rapid, and takes few hours to show effect. This delay may cause some farmers not having confidence in its efficacy. Further studies on the efficacy of *A. oryzae* need to be undertaken to explore the potential for improving the same by using molecular techniques, nanotechnology or other technologies to enhance its uptake. Improvement of other entomopathogenic fungi has been pointed out in other studies (Fernandes *et al.*, 2012; Leger & Wang, 2010; Maina *et al.*, 2018; St Leger *et al.*, 1996; Sun *et al.*, 2011; Winder *et al.*, 2003).

CHAPTER FIVE

CONCLUSION AND RECCOMENDATIONS

51 Conclusion

Findings from this study indicate that, *A. oryzae* both in the laboratory and under field conditions had acaricidal effects and hence, it can be considered as a promising bio-acaricide. The fungal product has shown significant effects on eggs and larvae, thus breaking the tick's life cycle. The use of this product may reduce chemical acaricide application and thus, protect the environment from chemical residues accumulation, overcome tick resistance and reduce costs associated with the use of chemical acaricides, hence it has to be considered as an alternative in the integrated management of ticks..

52 Recommendations

Further field studies in different geographical locations should be carried out to further validate the bio-acaricidal of the *A.oryzae* as environmental conditions may affect the susceptibility of ticks to fungi. Moreover, more studies should be done on the virulence, safety, shelf life and efficacy of *A. oryzae* for the potential management of ticks. Targeting eggs and larvae of the three cattle tick genera may pave the way to managing tick-borne diseases and therefore more systematic field studies to further validate these findings are required.

REFERENCE

Abbas, R. Z., Zaman, M. A., Colwell, D. D., Gilleard, J., & Iqbal, Z. (2014). Acaricide resistance in cattle ticks and approaches to its management: The state of play. *Veterinary Parasitology*, *203*(1-2), 6-20.

Adehan, S. B., Adakal, H., Gbinwoua, D., Zoungrana, S., Toé, P., Ouedraogo, M., Gbaguidi, A. M., Adoligbé, C., Fandohan, A. B., & Hounmanou, G. (2018). West African Cattle Farmers' Perception of Tick-Borne Diseases. *EcoHealth*, *15*(2), 437-449.

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), 224.

Almazan, C., Tipacamu, G. A., Rodriguez, S., Mosqueda, J., & Perez de Leon, A. (2018). Immunological control of ticks and tick-borne diseases that impact cattle health and production. *Frontiers in Bioscience*, *23*, 1535-1551.

Alonso-Díaz, M. Á., & Fernández-Salas, A. (2021). Entomopathogenic fungi for tick control in cattle livestock from Mexico. *Frontiers in Fungal Biology*, *2*, 18.

Beguin, H., & Nolard, N. (1994). Mould biodiversity in homes I. Air and surface analysis of 130 dwellings. *Aerobiologia*, *10*(2-3), 157.

Benelli, G., Pavela, R., Canale, A., & Mehlhorn, H. (2016). Tick repellents and acaricides of botanical origin: a green roadmap to control tick-borne diseases? *Parasitology Research*, *115*(7), 2545-2560.

Bennett, J. W. (2010). An overview of the genus Aspergillus. *Aspergillus: Molecular Biology and Genomics*, 1-17.

Beys-da-Silva, W. O., Rosa, R. L., Berger, M., Coutinho-Rodrigues, C. J., Vainstein, M. H., Schrank, A., Bittencourt, V. R., & Santi, L. (2020). Updating the application of *Metarhizium anisopliae* to control cattle tick *Rhipicephalus Microplus* (Acari: Ixodidae). *Experimental Parasitology*, *208*, 107812.

Bittencourt, V. R. E. P. (2008). Entomopathogenic fungi against South American tick species. In *Diseases of Mites and Ticks* (p. 71-93). Springer.

Bram, R. A. (1983). Tick-borne livestock diseases and their vectors. *FAO Animal Production and Health Paper*, *36*.

Brunner-Mendoza, C., Reyes-Montes, M. D. R., Moonjely, S., Bidochka, M. J., & Toriello, C. (2019). A review on the genus *Metarhizium* as an entomopathogenic microbial biocontrol agent with emphasis on its use and utility in Mexico. *Biocontrol Science and Technology*, *29*(1), 83-102.

Byaruhanga, C., Oosthuizen, M. C., Collins, N. E., & Knobel, D. (2015). Using participatory epidemiology to investigate management options and relative importance of tick-borne diseases amongst transhumant zebu cattle in Karamoja Region, Uganda. *Preventive Veterinary Medicine*, *122*(3), 287-297.

Dahliz, A., Lakhdari, W., Soud, A., Hammi, H., Bouchekima, H., & Belaidi, M. (2013). Complex of natural enemies and control methods of the exotic invasive pest *Tuta absoluta* (Lepidoptera: Gelechiidae) in Southern Algeria. Crop Protection Management in Mediterranean Organic Agriculture,Symposium 14-16 May 2013 in Sousse, Tunisia (M. Ben Kheder, D. Neuhoff, eds.)(Book of Abstracts), 48 .

de la Fuente, J. (2018). Controlling ticks and tick-borne diseases… looking forward. *Ticks and Tick-Borne Diseases*, *9*(5), 1354-1357.

de la Fuente, J., Kopáček, P., Lew-Tabor, A., & Maritz-Olivier, C. (2016). Strategies for new and improved vaccines against ticks and tick-borne diseases. *Parasite Immunology*, *38*(12), 754-769.

De Sousa, E. O., Lima, A. D. S., Lopes, S. G., Costa-Junior, L. M., & Da Costa, J. G. M. (2020). Chemical composition and acaricidal activity of *Lantana camara L.* and *Lantana montevidensis Briq*. essential oils on the tick *Rhipicephalus microplus*. *Journal of Essential Oil Research*, 1-7.

Di Giulio, G., Lynen, G., Morzaria, S., Oura, C., & Bishop, R. (2009). Live immunization against East Coast fever–current status. *Trends in Parasitology*, *25*(2), 85-92.

Drummond, R. (1976). Tick-borne livestock diseases and their vectors. 4. Chemical control of ticks. *World Animal Review (Italia) no. 19,* 28-33.

Ekesi, S., & Maniania, N. K. (2007). Use of entomopathogenic fungi in biological pest management. *Research Signpost*, 274-294.

Fernandes, É. K., Bittencourt, V. R., & Roberts, D. W. (2012). Perspectives on the potential of entomopathogenic fungi in biological control of ticks. *Experimental Parasitology*, *130*(3), 300-305.

Fernandes, E. K. K., & Bittencourt, V. R. E. P. (2008). Entomopathogenic fungi against South American tick species. *Experimental and Applied Acarology*, *46*(1), 71-93.

Flynn, P. C., & Kaufman, W. R. (2015). Mechanical properties of the cuticle of the tick *Amblyomma hebraeum* (Acari: Ixodidae). *The Journal of Experimental Biology*, *218*(17), 2806-2814.

Ganga, N., Ananda, K., & Rani, B. (2010). Theileriosis in calves and its successful treatment. *Veterinary World*, *3*(4).

George, J., Pound, J., & Davey, R. (2004). Chemical control of ticks on cattle and the resistance of these parasites to acaricides. *Parasitology*, *129*(S1), 353-366.

Ghany, T. (2015). Entomopathogenic fungi and their role in biological control. *OMICS International. http://doi. org/10.4172/978-1-63278-065-2-66*.

Gilioli, G., Groppi, M., Vesperoni, M., Baumgärtner, J., & Gutierrez, A. (2009). An epidemiological model of East Coast Fever in African livestock. *Ecological Modelling*, *220*(13-14), 1652-1662.

Gonzo, L., Mukwembi, P., Mashiri, C., Pfupajena, M. H., Chandiwana, E., Chipato, S., Maposa, S., & Mpondo, C. (2014). Comparison of the Performance of Garlic, Tobacco and Tephrosia as Natural Tick Control Biocides on Mashona Cows. *International Journal of Mathematical Archive*, *5*(2).

Hackman, R. (1975). Expanding abdominal cuticle in the bug *Rhodnius* and the tick *Boophilus*. *Journal of Insect Physiology*, *21*(9), 1613-1623.

Hackman, R., & Goldberg, M. (1987). Comparative study of some expanding arthropod cuticles: the relation between composition, structure and function. *Journal of Insect Physiology*, *33*(1), 39-50.

Hubka, V., Kolařík, M., Kubátová, A., & Peterson, S. W. (2013). Taxonomic revision of *Eurotium* and transfer of species to *Aspergillus*. *Mycologia*, *105*(4), 912-937.

Jabbar, A., Abbas, T., Saddiqi, H. A., Qamar, M. F., & Gasser, R. B. (2015). Tick-borne diseases of bovines in Pakistan: major scope for future research and improved control. *Parasites & Vectors*, *8*(1), 283.

Jiang, W., Peng, Y., Ye, J., Wen, Y., Liu, G., & Xie, J. (2020). Effects of the entomopathogenic fungus *Metarhizium anisopliae* on the mortality and immune response of *Locusta migratoria*. *Insects*, *11*(1), 36.

Jongejan, F. (1999). Integrated control of ticks and tick-borne diseases. *Parassitologia*, *41*, 57-58.

Jongejan, F., & Uilenberg, G. (1994). Ticks and control methods. *Revue Scientifique et Technique-Office International des epizooties*, *13*(4), 1201-1242.

Jongejan, F., & Uilenberg, G. (2004). The global importance of ticks. *Parasitology*, *129*(S1), 3-14.

Kaaya, G. P., & Hassan, S. (2000). Entomogenous fungi as promising biopesticides for tick control. *Experimental & Applied Acarology*, *24*(12), 913-926.

Kalsbeek, V., Frandsen, F., & Steenberg, T. (1995). Entomopathogenic fungi associated with *Ixodes ricinus* ticks. *Experimental & Applied Acarology*, *19*(1), 45-51.

Kerario, I. I., Muleya, W., Chenyambuga, S., Koski, M., Hwang, S. G. & Simuunza, M. (2017). Abundance and distribution of Ixodid tick species infesting cattle reared under traditional farming systems in Tanzania. *African Journal of Agricultural Research*, *12*(4), 286-299.

Kerario, I. I., Simuunza, M., Laisser, E. L., & Chenyambuga, S. (2018). Exploring knowledge and management practices on ticks and tick-borne diseases among agro-pastoral communities in Southern Highlands, Tanzania. *Veterinary World*, *11*(1), 48.

Kioko, J., Baker, J., Shannon, A., & Kiffner, C. (2015). Ethnoecological knowledge of ticks and treatment of tick-borne diseases among Maasai people in Northern Tanzania. *Veterinary World*, *8*(6), 755.

Kivaria, F. (2006). Estimated direct economic costs associated with tick-borne diseases on cattle in Tanzania. *Tropical Animal Health and Production*, *38*(4), 291-299.

Laisser, E., Chenyambuga, S., Karimuribo, E., Msalya, G., Kipanyula, M., Mwilawa, A., Mdegela, R., & Kusiluka, L. (2017). A review on prevalence, control measure, and tolerance of Tanzania Shorthorn Zebu cattle to East Coast fever in Tanzania. *Tropical Animal Health and Production*, *49*(4), 813-822.

Lakhdari, W., Dehliz, A., Acheuk, F., Mlik, R., Hammi, H., Matallah, S., & Doumandji-Mitiche, B. (2016). Biocontrol test against the leaf minerof tomato *Tuta absoluta* (Meyrick)(Lepidoptera: Gelechiidae) by using entomopathogenic fungi in the Algerian Sahara. Abstract in Algeria Conference,

Leger, R. J. S., Staples, R. C., & Roberts, D. W. (1992). Cloning and regulatory analysis of starvation-stress gene, ssgA, encoding a hydrophobin-like protein from the entomopathogenic fungus, *Metarhizium anisopliae*. *Gene*, *120*(1), 119-124.

Leger, R. J. S., & Wang, C. (2010). Genetic engineering of fungal biocontrol agents to achieve greater efficacy against insect pests. *Applied Microbiology and Biotechnology*, *85*(4), 901-907.

Lew-Tabor, A., & Valle, M. R. (2016). A review of reverse vaccinology approaches for the development of vaccines against ticks and tick borne diseases. *Ticks and Tick-Borne Diseases*, *7*(4), 573-585.

Lynen, G., Zeman, P., Bakuname, C., Di Giulio, G., Mtui, P., Sanka, P., & Jongejan, F. (2007). Cattle ticks of the genera *Rhipicephalus* and *Amblyomma* of economic importance in Tanzania: Distribution assessed with GIS based on an extensive field survey. *Experimental and Applied Acarology*, *43*(4), 303-319.

Lynen, G., Zeman, P., Bakuname, C., Di Giulio, G., Mtui, P., Sanka, P. & Jongejan, F. (2008). Shifts in the distributional ranges of *Boophilus* ticks in Tanzania: Evidence that a parapatric

boundary between *Boophilus Microplus* and *B. Decoloratus* follows climate gradients. *Experimental and Applied Acarology*, *44*(2), 147-164.

Magulu, E., Kindoro, F., Mwega, E., Kimera, S., Shirima, G. & Gwakisa, P. (2019). Detection of carrier state and genetic diversity of *Theileria parva* in ECF-vaccinated and naturally exposed cattle in Tanzania. *Veterinary Parasitology: Regional Studies and Reports*, *17*, 100312.

Maharana, B. R., Tewari, A. K., Saravanan, B. C., & Sudhakar, N. R. (2016). Important hemoprotozoan diseases of livestock: Challenges in current diagnostics and therapeutics: An update. *Veterinary World*, *9*(5), 487.

Maina, U., Galadima, I., Gambo, F., & Zakaria, D. (2018). A review on the use of entomopathogenic fungi in the management of insect pests of field crops. *Journal of Entomology and Zoology Studies*, *6*(1), 27-32.

Mamiro, K. A., Magwisha, H. B., Rukambile, E. J., Ruheta, M. R., Kimboka, E. J., Malulu, D. J., & Malele, I. I. (2016). Occurrence of Ticks in Cattle in the New Pastoral Farming Areas in Rufiji District, Tanzania. *Journal of Veterinary Medicine*, *2016*.

Maniania, N. K., Nchu, F., & Ekesi, S. (2007). 10 Fungal pathogen for biocontrol of ticks. *Research Signpost*, *2*(37/661).

Mbassa, G., Mgongo, F., Melau, L., Mlangwa, J., Silayo, R., Bimbita, E., Hayghaimo, A., & Mbiha, E. (2009). A financing system for the control of tick-borne diseases in pastoral herds: The Kambala (Tanzania) model. *Livest. Res. Rural Dev*, *21*(3), 44.

McKeever, D. J. (2007). Live immunisation against *Theileria parva*: containing or spreading the disease? *Trends in Parasitology*, *23*(12), 565-568.

McLeod, R., & Kristjanson, P. (1999). Economic impact of ticks and tick-borne diseases to livestock in Africa, Asia and Australia. *Report to the International Livestock Research Institute, Nairobi, Kenya*.

Mora, M. A. E., Castilho, A. M. C., & Fraga, M. E. (2018). Classification and infection mechanism of entomopathogenic fungi. *Arquivos do Instituto Biológico*, *84*.

Mtei, B. J., & Msami, H. M. (1996). Reflections on current animal health status in Tanzania. *Tanzania Veterinary Journal*, *16*(suppl. 01), 45-64.

Murigu, M. M., Nana, P., Waruiru, R. M., Nga'nga, C. J., Ekesi, S., & Maniania, N. K. (2016). Laboratory and field evaluation of entomopathogenic fungi for the control of amitraz-resistant and susceptible strains of *Rhipicephalus decoloratus*. *Veterinary Parasitology*, *225*, 12-18.

Nagagi, Y. P., Kimaro, E. G., & Temba, V. (2020). Practical application and the possible emergence of tick resistance to commonly used acaricides in various districts of Tanzania. *Livestock Research for Rural Development*, *32*(127), 2020.

Narladkar, B. (2018). Role of entomopathogenic fungi in tick control: A Review. *Journal of Entomology and Zoology studies*, *6*(1), 1265-1269.

Nejash, A. (2016). Review of Important Cattle Tick and Its Control in Ethiopia. *Open Access Library Journal*, *3*(03), 1.

Ortiz-Urquiza, A., & Keyhani, N. O. (2013). Action on the surface: entomopathogenic fungi versus the insect cuticle. *Insects*, *4*(3), 357-374.

Perinotto, W., Angelo, I., Golo, P., Quinelato, S., Camargo, M., Sá, F., & Bittencourt, V. (2012). Susceptibility of different populations of ticks to entomopathogenic fungi. *Experimental Parasitology*, *130*(3), 257-260.

Pirali-Kheirabadi, K., Haddadzadeh, H., Razzaghi-Abyaneh, M., Bokaie, S., Zare, R., Ghazavi, M. & Shams-Ghahfarokhi, M. (2007). Biological control of *Rhipicephalus (Boophilus) annulatus* by different strains of *Metarhizium anisopliae*, *Beauveria bassiana* and *Lecanicillium psalliotae* fungi. *Parasitology Research*, *100*(6), 1297-1302.

Rajput, Z. I., Hu, S. H., Chen, W. J., Arijo, A. G. & Xiao, C. W. (2006). Importance of ticks and their chemical and immunological control in livestock. *Journal of Zhejiang University Science B*, *7*(11), 912-921.

Ravindran, R., Juliet, S., Gopalan, A. K. K., Kavalimakkil, A. K., Ramankutty, S. A., Nair, S. N., Narayanan, P. M., & Ghosh, S. (2011). Toxicity of DMSO, Triton X 100 and tween 20

against *Rhipicephalus (Boophilus) annulatus*. *Journal of Parasitic Diseases*, *35*(2), 237-239.

Raynal, J. T., Silva, A. A. B. d., Sousa, T. D. J., Bahiense, T. C., Meyer, R., & Portela, R. W. (2013). Acaricides efficiency on *Rhipicephalus (Boophilus) microplus* from Bahia state North-Central region. *Revista Brasileira de Parasitologia Veterinária*, *22*(1), 71-77.

Rodriguez, Y., Rojas, M., Gershwin, M. E., & Anaya, J.-M. (2018). Tick-borne diseases and autoimmunity: A comprehensive review. *Journal of Autoimmunity*, *88*, 21-42.

Samish, M., Ginsberg, H., & Glazer, I. (2004). Biological control of ticks. *Parasitology*, *129*(S1), 389-403.

Skinner, M., Parker, B. L., & Kim, J. S. (2014). Role of entomopathogenic fungi in integrated pest management. *Integrated Pest Management*, 169-191. doi:10.1016/B978-0-12-398529-3.00011-7

St Leger, R., Joshi, L., Bidochka, M. J., & Roberts, D. W. (1996). Construction of an improved mycoinsecticide overexpressing a toxic protease. *Proceedings of the National Academy of Sciences*, *93*(13), 6349-6354.

Stafford, K. C., & Allan, S. A. (2014). Field applications of entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* F52 (Hypocreales: Clavicipitaceae) for the control of *Ixodes scapularis* (Acari: Ixodidae). *Journal of Medical Entomology*, *47*(6), 1107-1115.

Sun, M., Ren, Q., Liu, Z., Guan, G., Gou, H., Ma, M., Li, Y., Liu, A., Yang, J., & Yin, H. (2011). *Beauveria bassiana*: Synergistic effect with acaricides against the tick *Hyalomma anatolicum anatolicum* (Acari: Ixodidae). *Experimental Parasitology*, *128*(3), 192-195.

Tatchell, R., & Easton, E. (1986). Tick (Acari: Ixodidae) ecological studies in Tanzania. *Bulletin of Entomological Research*, *76*(2), 229-246.

Temba, S. G., Erasto, P., Swai, D. E., & Chacha, M. (2018). Acaricidal Effect of *Commiphora swynnertonii* Stem bark Exudate Against *Rhipicephalus appendiculatus*. *Ambient Science*, *5*(1), 28-32.

Tomley, F. M. & Shirley, M. W. (2009). Livestock infectious diseases and zoonoses. *Philosophical Transactions of Royal Society B: Biological Sciences, 364* (1530), 2637-2642. https://doi.org/http://doi.org/10.1098.

Tuppurainen, E. (2015). Evaluation of vector potential of *Rhipicephalus appendiculatus, Amblyomma hebraeum* and *Rhipicephalus decoloratus* ticks for lumpy skin disease virus. Helsinki: University of Helsinki. https://doi.org/http://hdl.handle.net/10138/153942

Valero-Jiménez, C. A., Wiegers, H., Zwaan, B. J., Koenraadt, C. J., & van Kan, J. A. (2016). Genes involved in virulence of the entomopathogenic fungus *Beauveria bassiana*. *Journal of Invertebrate Pathology*, *133*, 41-49.

Wang, C., Huang, Y., Zhao, J., Ma, Y., Xu, X., Wan, Q., Li, H., Yu, H., & Pan, B. (2019). First record of *Aspergillus oryzae* as an entomopathogenic fungus against the poultry red mite *Dermanyssus gallinae*. *Veterinary Parasitology*, *271*, 57-63.

Wang, C. & St Leger, R. J. (2007). The MAD1 adhesin of *Metarhizium anisopliae* links adhesion with blastospore production and virulence to insects, and the MAD2 adhesin enables attachment to plants. *Eukaryotic Cell*, *6*(5), 808-816.

Wasinpiyamongkol, L., & Kanchanaphum, P. (2019). Isolating and identifying fungi to determine whether their biological properties have the potential to control the population density of mosquitoes. *Heliyon*, *5*(8), 1-5.https://doi.org/10.1016/j.heliyon.

Wharton, R. (1983). Tick-borne livestock diseases and their vectors. Acaricide resistance and alternative methods of tick control. *World Animal Review (FAO)*, *36*, 34-41.

Winder, R., Wheeler, J., Conder, N., Otvos, I., Nevill, R., & Duan, L. (2003). Microencapsulation: a strategy for formulation of inoculum. *Biocontrol Science and Technology*, *13*(2), 155-169.

Yeomen, G., & Walker, J. (1967). The Ixodid Ticks of Tanzania: A Study of the Ixodidae of an East African Country. London: Commonwealth Institute of Entomology. https://www.worldcat.org.

Young, A., Groocock, C., & Kariuki, D. (1988). Integrated control of ticks and tick-borne diseases of cattle in Africa. *Parasitology*, *96*(2), 403-432.

Zekeya, N. (2019). *Occurrence, seasonal variation and management of tomato leafminer (tuta absoluta meyrick.)* in Tanzania. Arusha: NM-AIST.

Zekeya, N., Mbega, E., & Ndossi, H. (2020). Susceptibility of Different Species of Ticks (Acari: Ixodidae) to an Entomopathogenic Fungus in Tanzania. *Journal of Animal Science and Research*, *4*(2), 1-6. https://doi.org/dx.dio.org/10.16966/2576-6457.139

Zekeya, N., Mtambo, M., Ramasamy, S., Chacha, M., Ndakidemi, P. A., & Mbega, E. R. (2019). First record of an entomopathogenic fungus of tomato leafminer, *Tuta absoluta* (Meyrick) in Tanzania. *Biocontrol Science and Technology*, *29*(7), 626-637. https://www.tandfonline.com/doi/abs/10.1080/09583157.

Zhang, P., You, Y., Song, Y., Wang, Y., & Zhang, L. (2015). First record of *Aspergillus oryzae* (Eurotiales: Trichocomaceae) as an entomopathogenic fungus of the locust, *Locusta migratoria* (Orthoptera: Acrididae). *Biocontrol Science and Technology*, *25*(11), 1285-1298.

RESEARCH OUTPUTS

(i) Publication

Msangi, S. S., Zekeya, N., Kimaro, E. G., Kusiluka, L., & Shirima, G. (2022). Entomopathogenic fungi (*Aspergillus oryzae*) as biological control agent of cattle ticks in Tanzania. *Journal of Veterinary Medicine and Animal Health*, *14*(3), 52-61.

(ii) Poster Presentation

Msangi, S. S., Zekeya, N., Kimaro, E. G., Kusiluka, L., & Shirima, G. (2022). *Efficacy and Effect of Entomopathogenic Fungi (Aspergillus Oryzae) for Control of Ticks of Major Economic Importance of Cattle in Tanzania [Poster Presentation]. Nelson Mandela African Institution of Science and Technology, Arusha*.