FIELD ASSESSMENT OF THE EFFICACY OF *TEPHROSIA VOGELII (HOOK)* PLANT LEAF EXTRACTS FOR CONTROL OF TICKS ON NATURALLY INFESTED CATTLE IN NJOLA VETERINARY CAMP OF MONZE DISTRICT, ZAMBIA.

By

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A dissertation submitted to the University of Zambia in fulfilment of the requirements for the award of the Degree of Master of Science in Veterinary Parasitology

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DECLARATION

I, Christopher Pakula Siame, declare that the work presented in this dissertation was performed entirely by myself and has not previously been presented to this or any other University for the award of any degree.

Signature of the Candidate……..

Date…….

ABSTRACT

Ticks and tick borne diseases (TBDs) are responsible for substantial direct and indirect economic losses in livestock production in the sub-Saharan countries. Conventional acaricide methods for controlling ticks and TBDs have not been very effective among small scale livestock farmers largely due to the high cost of acaricides and lack of adequate extension and outreach services provided to them. Failure to follow instructions and adherence in preparation of the right dilutions of acaricides has been considered to be responsible for development of tick resistance to most acaricides in use which resulted in increase of TBDs out breaks. This occurrence has prompted livestock farmers and researchers to seek none conventional alternative and sustainable tick control methods. None conventional method should be readily available, simple in processing, cheap, and preferably not harmful to the environment. We identified *T.vogelii* a tropical leguminous herbal plant with natural pesticide and acaricidal properties, as such a bio-acaricide candidate.*Tephrosia* is a short lived, slow growing, herbaceous, frost susceptible perennial plant and has soft hairy velvet leaves which are pleasant to touch. A baseline survey was done using a questionnaire to find out the levels of indigenous knowledge existing for tick control management. The results indicated that 51 percent of the respondents knew that ticks transmit diseases to cattle and 41 percent revealed the use of cow dung as a method to kill ticks. Cattle used were selected for field experiments from small scale farmers' herds with no or, poor record of tick control in Monze district. Six groups of five (5) each were allotted into six (6) treatment groups of5, 10, 20, 40 percent w/v whilea negative control group of five (5) animals were also sprayed with ordinary water. A positive control group of five (5) animals were sprayed with a commercial acaricide (Amitraz®) at recommended dosage of 1:500 dilution by the manufacturer (Ecomed limited company of south Africa). All treatment groups were sprayed with same quantities in volume of the different preparations. Tick counts were conducted on each animal before and after treatments. The results showed that, the efficacy of the botanical extracts was sustained up to six days post treatment.There was reduction in tick counts at all concentration levels used in groups. It was however observed that 0.05 percent w/v concentration level of *T.vogelii* plant leaf extracts had the highest reduction on tick counts of up to 88 percent within 48 hours post treatment. The 0.1percent w/v had 71 percent reduction, 0.2 percent w/v had 74 percent, and lastly 0.4percentw/v had 80 percent reduction The observed tick reductions were found to be statistically significant at all treatment levels (p-value < 0.001). The protection period against reinfection is in excess of six days. The use of *T. vogelii as* bioacaricide proved effective against ticks at very low concentrations of only 5 percent w/v leaf extract. The extract is cheaper to use by small scale livestock farmers where they cannot afford to use chemical acaricides

DEDICATION

This dedication is first given to my lovely and wonderful wife Elizabeth for her genuine love, patience, support and understanding all these years. Secondly, to my children for being good and lastly, my late elder brother (Dr Crispin M Siame) for everything he did to me.

APPROVAL

This Dissertation of CHRISTOPHER PAKULA SIAME is approved as fulfilling the requirements for the award of Degree of Master of Science in Veterinary Parasitology of the University of Zambia.

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Supervisor……..Date……....

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Chairman, Board of Examiners……Date……

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LIST OF ACRONYMS/ABBREVIATIONS

ANOVA	Analysis of variance
CI	Confidence interval
DVLD	Department of Veterinary and Livestock Development
DVO	District Veterinary Office
ECF	East Coast Fever
˚C	Degrees Celsius
FAO	Food Agriculture Organization
G	Gram
hr	Hours
IACUC	Institute of Animal care and Use committee
IRR	Incident rate ratio
kg	Kilogram
KM	KILOMETER
%	Percentage
LIT	Larval Immersion Test
LC_{50}	Lethal Concentration
LD_{50}	Lethal Dose
MVRS	Mazabuka Veterinary Research Station
NISIR	National Institute for Scientific and Industrial Research
OECD	Organization for Economic Co-operation and Development
RDL	Regional Diagnostic laboratory
SANBIO	Southern Africa networking for Biosciences

SHDDP	Southern Highlands Dairy Development project
SPSS	Statistical package for Social Sciences
SD	Standard deviation
TBDs	Tick borne diseases
UNZA	University of Zambia
USA	United States of America
WT	Weight
W/V	Weight per Volume

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

The continuous presence of tick infestations and tick-borne diseases is a worry to the livestock industry. Ticks are nuisance to livestock and have caused economic losses in milk production, quality of hides and productive potential of Zambia's livestock sector. According to Wanzala *et al.* (2005), most animals kept by poor resource farmers are affected by endemic pathogens. Tick are hematogenous vertebrates ectoparasites which transmit viral, bacterial and protozoal diseases (De la Fuente *et al.*,2008). The presence of the ectoparasites cause high economic losses due to effect on the skin and causes anaemia by ingesting the blood of the host (Abbas *et al.,* 2014). The harmful effects of ectoparasites on the productivity of livestock are well documented (Bagavan *et al.,* 2009, Gazim *et al.,* 2011).Commercial and/or synthetic acaricides are toxic to both livestock and can cause cytotoxic and genotoxic effect on man, and can also be destructive to the environment and the ecosystem if incorrectly handled (Ündeğer and Basaran, 2005). Although ticks and tick borne diseases are a major challenge for many small scale livestock farmers in the developing world, farmers don't have the resources to solve the problem, and generally they cannot afford the high cost of these conventional acaricides (Mwale *et al*., 2005). The control of parasitism largely depends on the use of synthetic drugs, however, there is development of resistance in parasites against the drugs (Miller *et al.,* 2007, Saeed *et al.,* 2007).Use of

acaricides has also got disadvantages of harmful effects on environment, ecosystem, as well as to humans and livestock (Garcia-Garcia *et al.,* 2000).In addiction there is the problem of chemical residue in animal by products (Turbin *et al.*, 2006). The application of acaricides in dips and sprays to control cattle ticks has had a profound influence on livestock productivity through the significant reduction in the prevalence of tick infestations and tick borne diseases (Yilima *et al.*, 2001). However, the progressive evolution of resistance of ticks to almost every available class of acaricide continues to frustrate the efforts of cattle farmers (George *et al.*, 2004).

In view of the problem associated with use of chemical acaricides, there is need to search for alternatives control strategies that can overcome the difficulties with synthetic product (Christopher *et al.*, 2009, Hamad *et al.*,2014, Masood *et al*., 2013). This paper is a report on the research of the assessment of the efficacy of *Tephrosia vogelii* (*T.vogelii)* plant extracts in tick control and reduction of ticks on selected animals for experiment at Njola veterinary camp in Monze district of Southern province, Zambia.

1.2 Statement of the problem

Tick-borne diseases (TBDs) are responsible for economic losses in livestock production in the Sub-saharan countries (Makala, 2003).Farmers, lack access to the conventional livestock management skills and financial resources to afford chemical acaricides and curative substances. Njoroge and Bussmann (2006) reported that conventional methods for control of ticks and TBDs are costly for most small scale livestock farmers.Ticks and TBD's are still

the major constraints of cattle production in Zambia. Southern Province lost 995 (28.9%) cattle out of 34437 due to ECF in 2008 alone (Anon, 2009).

It is, therefore, very desirable that alternative methods of tick control that are less costly are sought in order to alleviate the problem of TBD's in resource poor livestock farming sector.

1.3 Study justification

More than 80% of cattle in Zambia is kept under traditional farming system. This sector has often been constrained by non accessibility to diptanks, High cost of synthetic acaricides, and therapeutic drugs for the control of ticks and TBDs. 70% of the small scale poor resource population who live in the rural areas depend on the role of livestock in their daily livelihood. (LID, 1999). The use of *T. vogelii* as a botanical acaricide provides simple, cheap and sustainable alternative. Stoll (2001) reported that *T.vogelii* is useful against most of the sucking and biting insects. It is an evergreen perennial plant, ease to grow with minimal management and can provide required biomass throughout the year, Once established it can last up to 4years (Barnes and Fryer, 1969). It is easy for small scale farmers to prepare *T. vogelii* biomass into acaricide using basic tools and equipment. Control of ticks and TBD's cannot be achieved by use of commercial acaricides alone, But through an integrated effort to achieve maximum impact. According to Belmain and Stevenson (2001) the use of Indigenous plants for pest control by poor resource farmers is cost effective, easy, and environmentally friendly.

.4 Objectives

1.4.1 General objective

To assess the efficacy of *T. vogelii* leaf plant extracts in killing ticks both under laboratory and field conditions.

1.4.2 Specific objectives.

1.4.2.1 To assess and document the existing knowledge of local small scale livestock farmers on traditional methods used to control ticks on cattle in Njola veterinary camp in Monze District.

1.4.2.2To assess the efficacy of *T. vogelii* plant leaf extracts on *Rhipicephalus Boophilus microplus* R. (B.) *microplus*)ticks under laboratory (*in-vitro)* and also on cattle naturally infested by ticks in the field condition.

1.4.2.3 To determine the toxicity and safety levels of *T. vogelii* plant leaf extracts using mice model assays.

1.5 Hypothesis

1.5.1Null hypothesis

1.5.1.1*T. vogelii* leaf extracts cannot reduce the tick burden on infected cattle when used as crudely processed bioacaricide.

1.5.2 Alternative hypotheses

1.5.2.1*T.vogelii* leaf extracts have an effect in reducing tick burden on infected cattle when used as a crudely processed bioacaricide.

CHAPTER TWO

2.0 LITERATURE REVIEW.

2.1 Taxonomy of ticks

Ticks belong to the phylum Arthropoda, Subphylum Chericerate, Class Arachinida and Subclass Acari. (syn. Acaria, Acarina, Acarida) include ticks. There are two well established families of ticks, the Ixodidae (hard ticks) and the Argasidae (soft ticks), (Sonenshine, 1991). According to (Horak *et al.,* 2002) and (Horak, 2009), there are 867 described species of ticks in the World of which 48 are important species to the health of domestic animals in Africa (Walker *et al.,* 2007). The most important Ixodid tick genera include *Amblyomma, Boophilus, Dermacenter, Hyalomma, Haemaphysalis, Ixodes and Rhipicephalus* (Jongejan and Uilenberg, 2004). Studies by (Murrel *et al.,* 2000) and Baker and Murrel (2002) concluded that the genus *Rhipicephalus* is associated to the genus *Boophilus.* Also Horak in 2009 reclassified this genera as Subgenera to the genus *Rhipicephalus* in their world list of the valid tick names. Acari consists mostly of mites and ticks, which can be distinguished by their larger size and their exclusive parasitic mode of feeding (Walker *et al*., 2007). All cattle ticks belong to the family Ixodidae. Thus, the important tick species that infest cattle in Zambia are *Rhipicephalus (Boophilus) decoloratus* (Koch), *Rhipicephalus (Boophilus) microplus* (Canestrini), *Rhipicephalus appendiculatus* Neuman, *Rhipicephalus zambeziensis* Walker, Norval and Corwin, *Amblyomma variegatum* (Fabricius), *Hyalomma trancatum* Koch and *Hyalomma mariginatum rufipes* Koch (Speybroeck *et al*., 2002 and Berkvens *et al*., 1998).

2.2 Geographical distribution of ticks

According to Speybroeck *et al.* (2002), there is significant variability in the species composition and relative abundance of ticks across the country with all major genera being reported in most parts of Zambia. However, Tandon (1991) has provided a detailed account of the geographic distribution of ixodid ticks in each district of Zambia. The specific geographical distribution of tick species is difficult to establish as the type of habitat in which species are found is widely distributed than their current geographical range (Walker *et al*., 2003 and Horak *et al*., 2009) According to Mtshali *et al.* (2004), tick distribution and occurrence differs with vegetation type. In Zambia, it is known that there is significant variability in the species composition and relative abundance of ticks across the country with all major genera being reported in most parts (Speybroeck *et al*., 2002 and Berkvens *et al*., 1998).For any effective control programme of arthropods Knowledge of tick distribution is an essential pre-requisite (De castro, 1997).

2.3 Biology of ticks and life cycle

Mating of adult ixodid ticks occurs on the host except in the genus *Ixodes* where mating can occur while ticks are still on vegetation (Klompen *et al*., 1996 and Walker *et al*., 2003). Male ticks remain on the host and will mate with as many females as possible resulting in the transfer of spermatheca (sperm sac) to females. Females mate only once. Fully engorge detach from their host to lay between 2000 and 20000 eggs, in a single batch (Walker *et al*., 2007). Eggs are never laid on the host. Three distinct life cycles occur among ixodid ticks.

The one-host tick life cycle is less common but occurs in all *Boophilus* subgenera of *Rhipicephalus* genus (Horak *et al*., 2002). Larvae hatch from eggs and crawl onto vegetation to wait for a host. This behaviour of waiting in ambush on vegetation is called questing (Sonenshine, 1991). These ticks are called "one host ticks". They remain on same host during the larval and nymphal stages until they become adults. Adult females drop off the host after feeding to lay their batch of eggs.

The two-host tick life cycle is similar to the one-host tick with larvae and nymphs feeding on the same host while adults attach to another host (Mathysse *et al*., 1987 and Jongejan and Uilenberg, 2004). Ticks that feed on two hosts during their lives are called "two host ticks". These ticks feed and remain on the first host during the larval and nymphal life stages, drop off and attach to a different host as an adult for the final blood meal. The engorged adult female drops off from the host after feeding to lay their batch of eggs (Walker *et al*., 2003).

The commonest life cycle is the three-host tick life cycle (Sonenshine, 1991 and Jongejan and Uilenberg, 2004) in which larvae, nymphs and adults attach to separate hosts. (See figure 3) Upon hatching, larvae will attach and feed on a host, detach from it and hide in the soil or vegetation to moult into a nymph. Nymphs attach to another host, feed and detach to moult into a female or male tick. Females will feed once and lay a huge batch of eggs, after which the depleted females dies. The male will take several small feeds, mate and then die. The three-host life cycle is slow, lasting from six months to several years (Walker *et al*., 2007).

2.4 Biology of important species' of ticks

Ticks are blood-sucking obligate external parasites belonging to the phylum *Arthropoda* and make up the largest collection of creatures in the order *Acarina* (Rajput *et al.*, 2006).But more importantly, they transmit various of pathogenic micro-organisms from infected animals to healthy ones (Jongejan, 2007).

2.4.1 *Rhipicephalus (Boophilus) microplus*

Rhipicephalus microplus (formerly *Boophilus microplus*) is considered to be the most important tick parasite of livestock in the world. It is a one-host tick (Sonenshine, 1991. The tick transmits bovine babesiosis (caused by *Babesia bovis* and *B. bigemina*). *Babesia bovis* infection is acquired by the adults of one generation of ticks and transmitted transovarially by the larvae of the next generation. Because this tick transmits both *B. bovis* and *B. bigemina* it poses a greater potential threat to livestock production than *R. (B.) decoloratus*. Bovine anaplasmosis (*Anaplasma marginale*) and spirochaetosis (*Borrelia theileri*) are also transmitted by *R. (B.) microplus*.It has been postulated that *R. (B.) microplus* was introduced into East and South Africa from Madagascar, where it had originally arrived with cattle from southern Asia (Speybroeck *et al*., 2002 and Berkvens *et al*., 1998).There is evidence that where favourable moist and warm climatic conditions exist it competes with and is able to replace the indigenous *R. (B.) decoloratus* (Berkvens *et al*., 1998).

2.4.2 Rhipicephalus (Boophilus) decoloratus

This is the commonest, most widespread and frequently encountered one-host ticks pecies in Africa (Walker *et al*., 2007). It is indigenous to Africa and has a monotrophictype of behaviour with cattle being the main maintenance host (Sonenshine, 1991 and Benitez *et al*., 2012). However, it may also be found on horses, donkeys, sheep, goats and wild ungulates (Sonenshine, 1991). Engorged females lay 1000 to 2500 eggs which hatch about one week after detachment from the host (Sonenshine, 1991). Eggs hatch into larvae in 3 to6 weeks after which they climb vegetation and attach to a host. Larval, nymph and adult stages spend a total of about 3 weeks on the same host. The entire life cycle, including the non-parasitic phase, can be completed in approximately two months, with more than one life cycle being completed annually (Walker *et al*., 2000 and Speybroeck *et al*.,2002). *R. (B.) decoloratus* has no strict seasonal occurrence in southern Africa, with adults occurring on host throughout the year (Walker *et al*., 2007).

2.4.3 ***Rhipicephalus appendiculatus***

This is a three-host tick, known as the brown ear tick because of its colour and preference of feeding on the ears of cattle. All the developmental stages engorge within four to seven days (Mtambo, 2008). Its entire life cycle can be completed in 3 months but in the southern regions of the tick's distribution it probably takes a year to complete. *Rhipicephalus appendiculatus* has a strictly seasonal, single annual life cycle in southern Africa (Muyobela, 2015) It is the chief vector of the pathogen Theileria parva which causes East Coast fever (Walker *et al.,* 2003). Adults occur during the rainy period (December to March), larvae in

the cooler late summer to winter period after the rains (March to July) and nymphs in the winter and early spring July to October (Okello-Onen *et al*., 1999a and Walker *et al*., 2007). The pattern of seasonal occurrence is regulated by the unfed adults, which enter diapause and do not engage in host seeking until the rains start (Sonenshine, 1991 and Madder *et al*., 1999). In regions close to the equator more than one life cycle can be completed annually and no clear pattern of seasonal abundance may be evident.

2.4.4 *Amblyomma variegatum*

This tick is a three-host tick that also has a clearly defined seasonal pattern of occurrence (Stachurski, 2006 and Walker *et al*., 2007). In Zambia, adults are most abundant in the wet season (October to February), larvae from March to May and Nymphs from May to September. Delayed development of females (Morphogenetic diapause) resulting in delayed oviposition is responsible for this pattern of seasonal abundance (Sonenshine, 1991 and Walker *et al*., 2007). All feeding stages of this tick infest cattle, sheep and goats (Jongejan and Uilenberg, 2004 and Nyangiwe and Horak, 2007).

2.5 Tick control methods

2.5.1 Chemical control of ticks (Conventional methods)

Ticks and Tick borne diseases control is mainly by the use of commercial acaricides such as organophosphates, Carbamates, pyrethroids, etc (Ghosh *et al.,* 2007).Acaricides can be applied to cattle using hand sprayers, spray races and in dipping vats (George *et al*., 2004), in order to control ticks and tick borne diseases.They are often accompanied by serious

drawbacks, including the selection of acaricide-resistant ticks, environmental contamination and contamination of milk and meat products with drug residues(Graf *et al.,* 2004).Some reports show that ticks are developing some acaricidal resistance in many African countries where cattle have been treated with conventional acaricides to control tick infestations (Martins *et al*., 1995; Latif and Jogejan, 2002).Tick resistance to chemical acaricides has been on the rise (Lane and Crosskey 1996).Chemical acaricides made from arsenic solutions were the first to be used for tick control (Angus, 1996) and in the eradication of *R.(B)microplus* in the United states were dependable products (George *et al*., 2004). The problem of resistance to arsenic, between effective concentration for tick control and the toxic concentration to cattle, led to its replacement by synthetic organic compounds (George *et al*., 2004).Chlorinated hydrocarbon acaricides have been withdrawn from the market (Grahman and Hourrigan, 1977; Spickett, 1998) because of their high toxicity and long residual effect (lifespan) on cattle products. Carbamates are a little more toxic than the organophosphates for mammals and are much more expensive (Spickett, 1998).There are reports of amitraz resistance to be on the increase with confirmed cases of resistance being reported in Brazil (Furlong, 1999, Miller *et al*., 2002 and Mendes *et al*., 2013) and South Africa (Mekonnen, 2002; Ntondini *et al*., 2008).). Organophosphates are generally the most toxic of all pesticides to vertebrates (Ware, 2000)

2.5.2 Non-conventional methods of tick control.

Management of traditionally reared animals by small scale livestock farmers depends on the acquisition of indigenous knowledge, skills, methods, practices and beliefs in animal

husbandry (McCorkle *et al.,* 1996).Many small scale livestock farmers either do not implement tick control programmes at all and/or complement conventional methods with indigenous tick control methods which may include the use of used motor oil, household disinfectants, paraffin, or manually plucking off ticks from the animals (Masika, 1997; Hlatshwayo, 2005).

Pasture spelling, pasture burning and use of certain grasses and legumes are also practiced for inhibition or killing of ticks (Branagan, 1973,Suthrest *et al*., 1982,Chiera *et al.*, 1984).Bush clearing through burning of land annually in Zambia reduces the number of ticks (Baars, 1999). Mbati *et al.*(2002) reported that farmers also used oil (12%), Jeyes fluids (24%) and De-ticking(2%). Kaaya and Hassan (2000) reported that the use of entomological fungi to control ticks may reduce the frequency of chemical acaricides applications.

Ethno veterinary medicine and medical knowledge offers a range of herbal plants with insecticidal and acaricidal properties which needs to be fully investigated and evaluated in their preparations and efficacies in ticks and TBDs control (Njoroge and Bussmann 2006; Ghosh *et al*., 2007). In Eastern Cape Province of South Africa, farmers complemented government dipping services with their own indigenous knowledge to control ticks (Moyo and Masika(2009). However, the use of tick repellents as a method for tick control on livestock are limited (Mwase *et al*., 1990). Oil extract from the leaves of a tropical shrub *Ocimum suave* was found to repel insects as well as all stages of the tick *R. appendiculatus* (Mwangi *et al.,* 2004). Significant numbers of ticks were attracted by odours emanating from the leaves of a plant called *Acalypha fruticosa* (Hassan *et al.,* 1994). Certain pasture legumes plants produce sticky secretions which have been reported to immobilize and to kill

tick (Elliot *et al.*, 1978; Sutherst, 1982). Some plant products have also been shown to kill ticks and inhibit tick oviposition (Chabra and Saxena 1998). Different reports showed that certain plants and herbs have anti-tick insecticidal properties (Ghosh *et al*., 2007).

The biological control of ectoparasites of veterinary importance is triggering and assuming widespread interest in the developing countries (Chabra and Saxena, 1998; Robert *et al.*, 2010). Chickens and certain birds provide natural biological control of ticks (Dreyer *et al.*, 1997). *T. vogelii* plant leaf extracts have been recommended as a biological candidate for controlling external parasites on cattle (Mwale *et al.*, 2006). In Zambia, the search for indigenous plants in tick control started in 1986 by Kaposhi *et al*. (1992).*Tephrosia vogelii*, a leguminous plant, has been identified as the most readily available potential plant with insecticidal properties to reduce tick infestation in cattle (Gaskins *et al.,* 1972).According to Muyobela *et al.* (2016) *T.vogelii* plant extracts have excellent acaricidal activity against ticks and persisted for 8days with 100% mortality of *A. variegatum* ticks in 24hrs.

2.5.3Challenges of chemical tick control

Management of ticks and TBDs by small scale farmers has been facing a number of challenges. The cost of conventional acaricides is generally expensive and unsustainable, as such it has resulted in the increase of some of the TBDs which includes Theileriosis, Anaplasmosis, Heart Water and Babesiosis.They are also expensive and unaffordable to resource-limited farmers; as a result the farmers have resorted to ethno-veterinary practices and remedies (Laffont *et al*., 2001).The conventional control methods include the use of chemical acaricides with partially successful results but this treatment has certain implicit drawbacks,

such as the presence of residues in the milk and meat and the development of chemical resistant tick strains (Willadsen and Kemp, 1988; Nolan, 1990).However there is development of resistance in parasite drugs.Tick resistance to acaricide is an increasing problem and real economic threat to the livestock and allied industry (Rajput *et al.,* 2006).The use of acaricides has disadvantages, such as the selection of resistant tick populations and harmful effects on the animals, human beings and the environment (García-García *et al.,*2000). Increased resistance in target species of ticks to chemical acaricide has been reported (Currie *et al.,* 2004). Dip tanks are located far away from where cattle are kept and in some cases the dip tanks are non-functional due to the non-availability of water pumps, water and acaricides (Moyo and Masika, 2009).

2.5.4 Economic importance of ticks and Tick borne diseases

According to Simuunza *et al.* (2011) tick borne diseases are the constraints to livestock production in many developing countries, which has caused high morbidity and mortality, resulting in decreased production of meat, milk and other livestock by-products. Ticks and TBDs continue to be the major constraint to livestock production not only in Zambia, but in many parts of Eastern Southern and Central Africa (Makala *et al.,* 2003. Milk production and weight gain are indirectly affected by tick bites (Hostis and Seeger, 2002; Peter *et al.,* 2005).Increased tick numbers on animals were found to cause proportional bigger live weight losses in tick susceptible Boran cattle than in tick resistant animals of the same breed (De Castro, 1987). Ticks also transmit viral, rickettsial, bacterial and protozoal diseases that affect wild animals, domestic animal, both domesticated and wild animals and indeed human

beings (Lane and Crosskey, 1996).Apart from acting as vectors for diseases (TBDs), ticks have been recognized as important ectoparasites of livestock. They are bloodsuckers, causing local necrosis which results to low-quality hides (Jongejan and Uilenberg, 2004). It is also known that ticks cause pain, irritation, discomfort leading to loss of production of meat, milk and other animal by products (Moyo *et al*., 2009). The effects of ticks limit the livestock production and improvement (Latif and Jongejan, 2002). Ticks and tick-borne diseases have been and continue to be one of the major constraint in livestock production in many African countries especially in East and Central African countries including Zambia inclusive (Chizyuka and Mangani 1987; Masiga, 1996).The fact is that world-wide, tick bites and tick-borne diseases are considered to be a very serious public health problem (Carroll*et al.*, 2004). East Cost Fever (ECF) control was reported to have costed a total ofUS$168million in the affected African countries (Eisler *et al*., 2003). The problems that are related to ticks and TBD's of cattle, created a demand for other more appropriate methods on how to control ticks and TBDs in order succeed in reducing productivity losses in livestock, especially cattle (George *et al*., 2004).According (Moyo and Musika 2009, Moyo *et al*., 2009) farmers have resorted to look for alternatives such as: herbal extracts, used oil, jeyes fluid, Aloe ferox Mill and Ptaeroxylon obliquum for tick control. According to Mwale *et al.* (2005) the demand for herbal use has increased more than chemical substances due to effectiveness, easy access and low costs

2.5.5 Vaccines against ticks

Studies have shown that, targeting tick protein by vaccination can not only reduce tick feeding and reproduction but also the infection and transmission of pathogens from the tick to the vertebrate host (Merino *et al.*, 2013). One example of a vaccine in Australia and Cuba was developed from *R.microplus* Bm86 gut antigen (TickGARD®)and proved to be effective (Lightowlers,2013).These vaccines proved to be a cost-effective alternative for cattle tick capacity and the prevalence of some tick-borne pathogens(de la Fuente *et al.*, 2007).

Vaccination is an attractive alternative for the control of tick infestations and pathogen infections as it is a more environmentally friendly method (Merino *et al.*,2013).Several tick borne diseases can be controlled if a common vector is targeted (de la fuente *et al.*, 2011).Globally, most of the vaccines available to overcome TBDs are attenuated or live blood-derived (Domingos *et al.*,2013).Theileriosis can be controlled by immunization using the infection and treatment procedures (Mbao *et al*., 2006).

2.6 Botany of *Tephrosia. vogelii*

Tephrosia vogelii (T.vogelii) is native to tropical Africa and it is found growing naturally in wide and varying habitats which include savannah vegetation, grasslands, forest margins and shrub land, wasteland and fallow fields (www.worldagroforestry.org). In English *Tephrosia vogelii* is called *Vogel's Tephrosia*, which belongs to the Kingdom: Plantae, Genus: *Tephrosia*, Family*: fabaceae* and Epithet: *vogelii* Hook.

Figure 1: *Tephrosia vogelii* leaves (left), flowers (middle) and pods (right)

The name *vogelii* was given in honour of Dr. Theodore Vogel a botanist who was sent on an expedition by Her Britannic Majesty to the river Niger in 1841. (http://www.virboga.de/tephrosia-vogelii.htm). *Tephrosia* is a Genus of legumes which belong to the family Fabaceae with about 300 *vogelii* species (Zambia-ICRAF Agroforestry project, 2009). It was initially used by indigenous people as a mild fish poison. (en.wikipedia.org/wik/*Tephrosia*). It is a shrubby plant mainly found in the tropical and subtropical regions of the world (Barnes, 1967, Gaskins, 1972). *T. vogelii* is easy crop to grow from the seeds and manage, it remains ever green for more than four (4 yrs.) when it is established (Barnes and Fryer, 1969). It can be used as a cover crop, a hedge and/or for shelter while fixing nitrogen in the soils where it is planted. Certain species of *Tephrosia* shrub can grow 2 to 3m in 7 months (Hutchinson, 1958). *T. vogelii* and *T. diversifolia* can accumulate substantial amounts of biomass and nutrients in soils that are not fertile (Rutunga *et al.,* 1999). The common names of *Tephrosia vogelii* are: fish bean, fish poison bean,

Vogel's Tephrosia (*http://www.worldagroforestry.org)*. Physically, this plant has branches and stems with long and/or short white or rusty brown hair coat. (Fig 1)

2.7 Uses of *Tephrosia. vogelii*

Although *T. vogelii* has been known to have many uses in Agriculture and human health, the effectiveness of *Tephrosia vogelii* in the control of Acarina has not been fully exploited in developing countries where its use is required most (Matovu and Olila, 2007). *T. vogelii* is also used as an abortifacient emetic and purgative therapy for the skin diseases (Ethno medical and veterinary/Free library, 2008). Helminthes have been also treated with *T. vogelii* leaf extracts. *T. vogelii* has been found to possess antimicrobial activity. The dichloromethane extract from the roots and leaves was tested against *staphylococcus aureus, Escherilishia coli and staphylococcus paratyphi* (Wanga *et al.,* 2007) and the roots decoctions are used to treat constipation. It is also used in plant protection and storage, *Lantana camara* and *T. vogelii* powders have been found to be very potent natural pesticides in maize or beans storage. There was a significant reduction in grain damage with no adverse effects on seed germination (Ogendo *et al*., 2004). *T. vogelii is* very useful in soil enrichment through nitrogen fixation. It has a high potential of improving soil fertility when used in improved fallow situations (Balasubramanian and Sekayange 1992). *T.vogelii* plantations has been reported to increase subsquent maize yields in Tanzania (Mgangamundo, 2000). *Tephrosia vogelii* and other species have been grown for crop protection in Eastern and Southern Africa. Preliminary studies in Zambia, using crude *T. vogelii* water soluble extracts

in the field experiments, showed that at a concentration of 10 percent w/v it was possible to protect cattle from tick infestation (Kaposhi, 1992).

Results from another experiment done in Zimbabwe indicated a decline in the number of engorged ticks from different dilutions levels after treatment with *T. vogelii* on dairy animals (Gadzirayi *et al.,* 2009). About 90 % of farmers interviewed in Iringa and Mbeya regions in Tanzania reported that *T. vogelii* was quite effective against many external parasites (SHDDP, 2000). It is probably thought that farmers don't adopt the use of *T. vogelii* and other plants due to labour involved in their production and processing (Barnes, 1967 and Gaskin 1972). Gadzirayi *et al.* (2009) showed that there was no significant difference in performance by *using T. vogelii* and Triatix dip for tick control.

2.8 Pharmacokinetics of *T.vogelii* extracts

T. vogelii has been found to be a potential source of non-residual insecticide. The principle active ingredient of *T. vogelii* is rotenone (Zambia-ICRAF Agroforestry project, 2009; Blommaert, 1950).*Tephrosia* species contain complex mixtures of rotenoids and other flavonoids (Go´mez-Garibay *et al.*,2002),known to be mitochondrial chain inhibitors, inhibiting cellular respiration in almost every living organism including insect and mammals. These compounds block the enzymes glutamate and succino dehydrogenase and thus H+transport (Neuwinger, 2004).*T. vogelii* has wide range of rotenoid compounds: rotenoid, tephrosin, deguelin and 6a 12-dehydrodeguelin (Lambert *et al.,* 1993). Rotenone is a compound which has no color or smell, it has an empirical formula of C23H22O6 and Molecular weight of 394.41

The melting point for this substance is between 165 -166ºC (Watt, 1962) and soluble in acetone and ethanol. The pharmacokinetics of rotenone is attributed to the mitochondrial electron transport destruction in the cells which hinders the utilization of oxygen in the respiration process of the organism leading into cell death (Islam, 2006).Despite the toxic properties of rotenone to fish and arthropods, it is relatively safe to humans and animals when ingested, as the changes in the gut transform it into less toxic substances before it enters the blood stream where its toxicity matters (McClay, 2000).

2.9 Other Species of *Tephrosia* and their uses

Tephrosia purpurea, an important plant of the genus is used as tonic, laxative, antivenom, antiulcer, antidiarhheal, and in leprosy (Virupanagouda *et al.,* 2011). The insecticidal effects of rotenone has been shown on several arthropod species using different species of *Tephrosia* which include *T. bracteolata Guill and Perr,*which is wide spread in tropical Africa and provides grazing material for horses and other livestock (Dalziel, 1937; Grainge and Ahmed, 1988). In Tanzania roots are taken as a therapeutic agent for pregnant Sukuma women who are infected with syphilis (Burkill, 1995).*T.nana Kotschy exschweinf*is grazed by livestock in eastern Cameroon while in Ivory Coast and Congo it is used as fish poison (Kerharo, 1950*).* Many plants from this genus have been used traditionally for the treatment of diseases like rheumatic pains, syphilis, dropsy, stomach ache, diarrhea, asthma, abortifacient, respiratory disorderrs, laxative, diuretic, and inflammation etc (Qureshi *et al.*, 2010; Dzenda *et al.*, 2007).

2.10 Propagation of *T. vogelii* Hook. f

Tephrosia plant is propagated by seeds. Viable seeds of *T. vogelii* are selected by soaking in water stirred and left overnight. Bad seed would float and should be discarded. The seeds do very well in loamy soil with compost manure and/or planted in portable poly packs. It is advisable to ensure that the soil is watered prior to planting. When seedlings are out they should be watered until they become strong enough for transplanting. The germination percentage is about 65percent and survival of the seedling is about 60 percent. Germination rate can be enhanced by putting the seeds in warm water of about 45°C for 5 minutes and can be soaked in cold water for 24 hours (http://www.worldagroforestry.org/). Seeds are planted along 90 cm rows at 45cm spacing in row. It is recommended to plant three (3) in each hole at the depth of about 3 cm (http://www.fao/ag/ags).

Barnes and Freire (1967) suggested that commercial production of rotenone which is derived largely from leaves, plants should be grown at the rate of 30,000 to37,000 per hectare. They further reported that when plants were spaced 1.0 to 8.9m apart, the seed yield per plant ranged from 1.1 to 8.9g with the highest yielding line producing at the rate of 70kg seed per ha. It grows well at attitudes of up to 2,100m, with average temperature of 12 to 27ºC, rainfall of about 850 to 2650mm and at pH of 5 to 6.5. It is however sensitive to soils prone to flooding which are poorly drained loamy soils. It is however also tolerant to poor soils acid soils.

2.11 Toxicity tests of *T. vogelii*

Many toxicity tests examine specific types of adverse effects known as end point, such as eye irritation or cancer development. Other tests are more general in nature, ranging from acute (single to exposure in which animals are administered daily dose of test substance (Alttox.org/map/toxicity testing overview).

The fixed dose method or test is a procedure that reduces the number of animals required to estimate the acute oral toxicity of a chemical under study (Stallard and Whitehead, 1995).The focus of the tests is to determine the range between the dose that causes no adverse effect and the dose that is life-threatening. The test consists in dosing groups of animals (single sex, normally females) in a stepwise procedure using the fixed doses of 5, 50, 300, and 2000 mg/kg (exceptionally an additional dose of 5000 mg/kg may be considered), (OECD, 2000). Depending on the signs of toxicity, further groups of mice may be dosed at higher or lower fixed doses. The test is observed for a period of 14 days. This procedure continues until the dose causing evident toxicity or death is identified, or when no effects are seen at the highest dose or when deaths occur at the lowest dose. The test substance is administered in a single dose by gavage using a stomach tube or a suitable intubation cannula. Animals should be fasted prior to dosing. A total of five animals of one sex are normally used for each dose level investigated. The results of this study include: measurements of weight on a weekly interval, detailed overall observations on a daily interval, as well as gross necropsy. This method provides information on the hazardous

properties and allows the substance to be classified for acute toxicity according to the Globally Harmonized System of classification and labelling of chemicals (OECD, 2001).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

Both a baseline study using a questionnaires and field experiments were conducted at Njola Veterinary camp (See Fig 5) which is (16° 15.838S and 27° 41. 24 13E and elevation 1,138m), located 27 km East of Monze District, Southern province of Zambia. Monze town is located 185km south of the capital Lusaka, enroute to Livingstone. Geographically, Monze shares its border with Mazabuka, Namwala, Pemba and Gwembe districts, with estimated human population of about 200,000 people (DVO/Monze Annual report, 2009).The estimated cattle population in this area is about 152 (DVO Annual report, 2016). Monze has three seasons, namely; hot-wet season which runs from late November through to April, cool - dry from May to August and lastly hot-dry season which is from September to October. The Mean annual temperatures range from 21°C to 29°C. It has a typical savanna vegetation environment, balanced natural setting for livestock, tick and human population of diverse personalities and characteristics which provides an enabling environment for interactions and indeed livestock diseases.

Njola is a traditional habitat well known for its livestock activities by the small scale farmers. Njola has repeatedly been prone to a number of livestock diseases especially TBDs. Sadly this area acts as a filter point for most of the livestock leaving the province for sale on the line of rail. Major TBDs in Monze district are East Coast fever, Anaplasmosis and

Heartwater (DVO/Monze Annual report, 2009). The Laboratory (Invitro) experiments were done at the Regional Diagnostic Laboratory in Mazabuka.

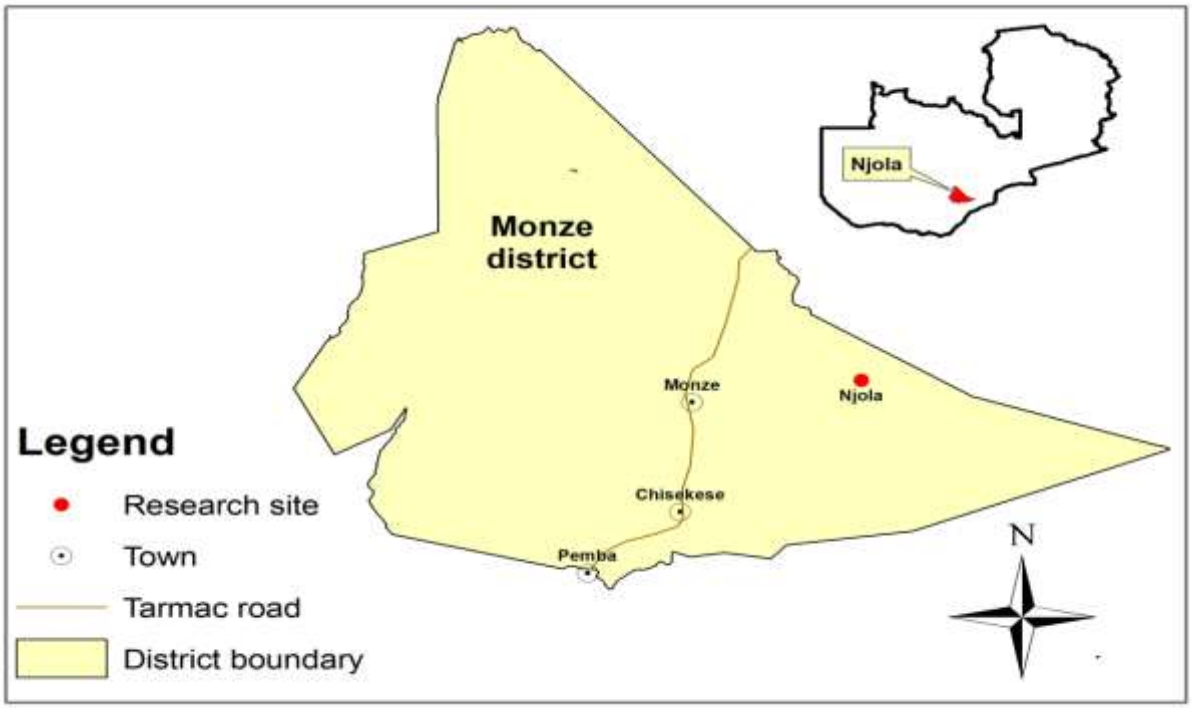

Figure 2: Njola veterinary camp in Monze District

3.2 Traditional tick control questionnaire survey

The research design was non-experimental hence no control groups were used for comparison. The survey was mainly aimed at collecting descriptive information. The threats to validity of this evaluation study were both internal and external. The internal threats included the selection bias in that though the underlining assumption in selecting respondents

there could be some bias in the identification of background characteristics of the respondents. Instrumentation bias is another internal validity threat, this applies to the way each respondent understood and truthfully answered the questions posed to them in the questionnaire. The external validity threats included the sample size from Njola which was too small to be a true representative of the total population of Monze in particular and Zambia at large. It suffices therefore, that our findings from the Njola survey cannot be generalized to be representative for Monze or the entire country of Zambia.

3.2.1 Sample size

There are about 400 small scale livestock farmers in Njola Veterinary Camp. We randomly selected total of 100 farmers asour respondents who were interviewed on the indigenous traditional methods used to control ticks and tick-borne diseases in the area. Sample size was calculated using the formula (Naing *et. al*., 2006).n =Z^2 P (1- P)/d^2

Where: n= Sample size, Z = Critical value for 95% level of significance of a normal distribution (1.96; 95% CI), P= expected proportion (10% or 0.10)d^2 = Standard error (5% or 0.05).The calculated sample size was therefore =139small scale farmers

One hundred and thirty nine (139) farmers were targeted to be interviewed but due to circumstances beyond our control, only one hundred (100) representative farmers were available to be interviewed.

3.2.2 Questionnaire data

Data was collected using a structured questionnaire (Appendix1) included, household demography and their knowledge of cattle diseases in the area, ticks common in the area, the

effect of ticks on cattle, the tick control methods that traditional livestock farmers were practicing, types of conventional acaricides they knew, herd dynamics, herd size and availability of livestock extension services in the area.

3.3. Rearing and maintenance of a tick colony.

A tick colony of *Rhipicephalus appendiculatus* was raised and maintained at the Regional Diagnostic Laboratory in Mazabuka under the following conditions:

(a) Engorged ticks from animals of Njola veterinary camp of Monze District were collected and kept in test tubes at 24ºC +/- 1 and relative humidity of 85 percent for their further development.

(b) Under favourable conditions, females laid several thousand eggs each.

(c) Potassium chloride was used in desiccators to maintain the humidity of 85 percent.

(d) Oviposition lasted up to three weeks, which was dependent on the temperatures.

(e) Eggs laid hatched and the larvae waited for or actively sought a host (questing behaviour).

(f) The larvae were fed on the ears of rabbits, those that detached, were collected and taken to the laboratory. They molted into a nymphs. The nymphs were fed and repeated the same process as the larva, but emerged with developed anatomy of either an adult female or male. The different stages were used in the bioassays.

3.4 Preparation and extraction of the aqueous *T.vogelii* fresh leaf extracts

The procedure for preparation and extraction of the aqueous of *T.vogelii* leaf extracts were as follows:

(a) Fifty (50) grams of poultice paste of freshly harvested leaves of *T. vogelii* was weighed on an electric beam balance.

(b) The poultice paste of leaves was then transferred to a 1000ml conical flask.

(c) 500mls tap water was added to the paste in the flask.

(d) The flask was sealed with parafilm and then put on a shaker for 1hour while continuously mixing.

(e) The mixture in the flask was allowed to stand for 2hours for sedimentation to take place. The solution (crude extracts) was then filtered immediately through a cotton wool and thereafter through a filter paper.

(f) From this preparation a stock solution of 10 percent w/v stock crude extract was obtained.

3.5 *In vitro* assessment of *T. vogelii* leaf extract

3.5.1 Larval immersion bioassay

Tick Larval immersion bioassay for efficacy of *T vogelii* extract in larval tick was conducted according to Shaw (1966), as follows:

a) From the 10% w/v stock solution, serial dilutions of 5%, 2.5%, 1.25% and 0.625% w/v were prepared using ordinary water from the tap.

b) Distilled water served as negative control, while Amitraz12.5%M/w at the dilution of 1: 500 served as positive control.

c) Each treatment level was run in three (3) replicates. Petri dishes were prepared and appropriately labelled.

d) A filter paper (whatman No.1, 11cm in diameter) was cut to size to fit tightly in the petri dishes and placed in each petri dish.

e) 5ml of each treatment levels was added onto the petri dishes using a 5ml syringe beginning with the negative control, then from the lowest concentration to the highest concentration.

f) The filter paper was left to soak for 10minutes a room temperature.

g) A large number of *R.appendiculatus* larvae were scooped using soft brush from the test tubes where they were kept and a total of 150 larvae required for each petri dish.

h) Another filter paper was cut to fit tightly as a cover after the larvae were exposed to the surface of the treated filter paper surfaces and left for 10minutes.

i) A pair of forceps was used to remove the filter papers with treated larvae and placed them on blotting paper for drying for 10-15minutes.

j) New filter papers were folded into two halves to get a triangular shape and these were well labelled with each particular concentration using a pencil.

k) After 15 minutes of drying, the dry filter papers were opened and tick larvae were gently brushed into the labelled filter papers per concentration starting from the control to the highest 10%w/v concentration. The labelled filter papers were clipped

on the open ends and placed in a desiccator and later on put in an incubator at a regulated temperature and relative humidity of 25ºC+/-1ºC and 80+/-5 percent respectively.

l) The filter papers were opened after the exposure time of 24 hours and placed under a light source on a stereo microscope.

m) The mortality rates were recorded.

n) Any larvae found moving were considered alive.

o) Two replicates were prepared for each concentration.

3.5.2 The dipmethod bioassays.

The dip bioassay was done according to Pirali-Kheirabadi *et al*.(2007) to assess efficacy of *T. vogelii* extracts concentration levels on nymphs and adult ticks of *R appendiculatus* species as follows:

(a) Batches of nymphs and adult ticks were allotted into groups of 10 and each group were subjected to different *T. vogelii* extract stock solution. and replicated three times.

(b) Nymphs were immersed in a different concentrations of 0.625, 1.25, 2.5, 5, 10 and 20 percent of the stock extract. (Weight per volume preparation)

(c) Adult's ticks were subjected to the following concentrations 5, 10, 15, 20, 30 and 40 percent for one minute (Weight per volume preparation).

(d) Distilled water and a conventional acaricides (12.5% amitraz) were used as negative and positive control, respectively.

(e) After dipping in the respective materials, each group was placed into separate petri dishes containing moist Whatman No. 1 filter papers measuring 11cm in diameter, with pieces of green grass to provide an environment conducive for tick survival (Thorsell *et al*., 2005).

(f) The tick samples were incubated for 7 days at 25°C and 85% relative humidity in the dark as described by Pirali-Kheirabadi *et al*.(2007).

(g) The petri dishes were examined hourly for the first 6 hours post-treatment, thereafter 24 hourly every morning to count and remove the dead ticks as described by Pamo *et al*.(2005).

(h) Ticks that did not move their legs after treatment with the crude extracts were considered dead.

(i) Tick mortality rates were calculated as described by Abbott (1925) and Chungsamarnyart *et al*. (2003).

(j) Corrected Tick Mortality (%) = (1 - T/C) x 100.Where; T is the number of ticks that walked around after treatment & C is the number of ticks that walked around in the control group.

(k) Water was used as a negative control on larvae in this experiment, while on adult ticks Amitraz was used as positive control and water as a negative control.

3.6 The efficacy of *T. vogelii* leaf extracts in the field condition.

3.6.1 Preparation of Plant leaf extracts

Fresh leaves from *T.vogelii* plants were harvested, weighed into four groups of different kilograms (kg), (ie 1kg, 2kgs, 4kgs and 8kgs). Each of these groups of kilograms of leaves were pounded in a mortar and soaked each in separate buckets of 20litres of water overnight. The juice was filtered through a muslin cloth to prepare different leaf extract concentrations of (5, 10, 20 and 40 percent w/v).

In this study, 4litres of the extracts was used to spray each animal in the study group.

Amitraz 12.5% by volume was prepared and used as a positive control at the same volume and concentration at recommended dilution of 1:500 by the manufacturer (Ecomed Limited Company of South Africa) while ordinary water was used as a negative control.

3.6.2 Experiment design –Randomisation

a) Herds of cattle were selected after consultations with the District Veterinary Office in Monze.

b) As far as possible, the study was done in the herds with poor tick control programmes and also where high incidences of ECF and other TBDs were reported in previous years.

c) Both males and females cattle aged one year and above and had visible ticks on the skin were randomly selected from volunteer small scale livestock farmers in Njola Veterinary Camp.

d) The animals were examined for the presence of ticks.

e) The animals were divided into five (5) groups comprising six (6) animals in each group.

f) Tick counts *in situ* were carried on daily for a week after spraying. This was repeated three (3) times, during the period December-March which is a peak season for ticks.

g) The animals were sprayed with different concentrations of crude extracts of *T.vogelii* leaves per different groups and ordinary water was used as a control.

3.6.3 Experimental Animals

Experimental animals were selected based on the following criteria: (i) had many visible ticks on the skin (positive cases of tick infestation), (ii) the age was one year and above, (iii) ECF cases and TBD's in the area and (iv) no history of tick control was practiced before commencement of the experiment.

A total of thirty (30) cattle of both sex, aged one year and above with visible tick infestation on their skin were randomly selected from volunteer small scale livestock farmers in Njola Veterinary Camp. All the selected animals had their identity recorded at the beginning of the experiment. The animals were allotted into five (5) groups of six (6) animals. The selected animals were sourced from different herds, but same communal grazing area with other animals not in the experiment.

3.6.4 The Ethical Clearance

The ethical clearance was sourced and granted by the ethics committee of the Ministry of Fisheries and Livestock in the Department of the Veterinary Services,in Lusaka (Ref No.2015/002) to allow the use of mice in the evaluation of the safety and toxicity of the leaf

extracts of *T.vogelii* using the fixed Dose Method. The committee is based at Mulungushi House.

3.7 Toxicity tests in mice

3.7.1 Preparation of *T. vogelii* extracts for safety test

a) Fresh leaves of *T.vogelii* were pounded and weighed into5, 10, 20, 30 and 40grams

b) Each of these grams were soaked in 100mls of tap water for 2hours.

c) The crude extract was filtered through clean cotton wool or muslin cloth to remove large particles.

3.7.2. Determination of the concentrations of crude extract

From the preparations, 50mls of 10% of the crude extract of *T.vogelii* was used to calculate the concentration of the extract.

a) An empty petri dish was weighed and then 50mls of the extract solution was poured on it.

b) The extract was then evaporated to dry up water using the room temperature on the tray.

c) The difference between the weight of petri dish and the extract was calculated.

d) The concentration of the extract was then calculated using the following formula below:

$C_1V_{1=}C_2V_2$. (www.emsb.qc.ca*/laurenhill/science/cv.html*) Where:

$C_{1=}$ is the first known concentration.

$V_{1=}$ is the first known volume.

$C_{2=}$ is the unknown concentration and lastly

$V_{2=}$ is the known volume

From the above formula, the concentration of the extract in 50 mls leaf extract worked out to be 0.00131g/ml or 13.1mg/ml.

a) The maximum volume of liquid for oral gavage recommended is 10ml/mouse. (www.iacuc.ucsf.edu). 0.25ml of the leaf extracts was given at the average weight of 25g per mouse in the group).
b) A dose of 0.25ml x 13.1mg/ml was determined to 3.275mg per mouse therefore the dose rate is 3.275mg per mouse was divided by 0.025kg mouse or 131mg/kg which was between 50mg and 500mg per kg.

3.7.3. Experimental mice

The BALB/c mice specie was used to evaluate the toxicity of the *T. vogelii* plants leaf extracts. Adult mature and health mice of between 8 and 12 weeks were employed in the experiment. The females were those that were nulliparous and not pregnant. The average weight of mice was between 25 grams and 30 grams. The cages were labelled with a marker for a particular concentration and controls.

3.7.4 Oral administration into the mice

After the completion of extraction, the mice were orally administered with these leaf extract preparation representing 5, 10, 20, 30 and 40 percent w/v.

a) Weighed the animal and determined the appropriate dosing volume.

b) Checked the length of the gavage tube by measuring from the tip of the animal's head to the last rib to mark the tube at the nose.

c) The mice were restrained by grasping the skin over the shoulders with the thumb and middle fingers extended out to the side and kept the front feet from pushing the gavage tube away .The heads were placed gently to create a straight line through the neck and esophagus.

d) The gavage tube was placed in the diastema of the mouth. The tube was then gently advanced along the upper palate until the esophagus is reached. The tube passed easily into the esophagus.

e) Once proper placement was verified, the extract was administered by a syringe attached to the end of the tube. After dosing, the tube was gently removed following the same angle of insertion. The mice were returned to the cage and monitor for 5-10 minutes, looking for signs of laboured breathing

3.7.5. Fixed dose method

3.7.5.1. Test Procedures in groups

a) The first group had used six (6) mice randomly selected, of which five (5) were administered with five (5) percent of the extract while one (1) with water as a negative

control. They were administered at dose of 10ml/kg (1ml per 0.100kg mouse).The mice in groups were marked and kept for overnight fasting in separate labelled cages. After 24 hours, they were sacrificed by cervical dislocation. Heart, liver and kidneys were removed and subjected to gross pathological examination.

b) The second experiment had ten (10) mice of either sex weighing between 25 and 30 g were selected for the experiment. They were divided into five(5) groups of two (2)each, of which the first four (4) groups were randomly allotted and administered with 10, 20, 30 and 40 percent w/v concentration respectively and two (2) other with water as negative control. All the mice had free access to food and water after extract administration. They were observed for a period of 14 days, thereafter, the test mice were subjected to gross necropsy. Any pathological changes were recorded for each mouse. All observations were systematically recorded.

c) So further test was conducted at 2000mg/kg, where a 76% w/v solution of the plant extract materials was prepared of 0.25ml per mouse twice at an interval of 1 hour was administered to two groups of 5 males and 5 females.

d) The dose rate of 10ml/kg (1ml per 0.100kg mouse) is the maximum recommended mls for mice by the Institute of Animal Care and Use Committee (IACUC). BALB/c mice (www.thebts.org/) were used to evaluate the toxicity and safety of the plant extracts of *T.vogelii* in laboratory using fixed dose method. This test was done in line with the Organization for Economic Cooperation and Development (OECD 420) guidelines. All the mice were observed for any abnormal signs including changes in the skin, eyes, fur, mucous membrane, salivation, diarrhoea, lethargy, central nervous system, coma, ataxia,

convulsions, tremors, sleep and breathing patterns etc.etc.). The experimental mice were observed closely for the first 30minutes after administration of the crude extracts of *T. vogelii,* then 1hour and thereafter daily for a period of 14 days for any clinical signs or any reactions.

3.8Statistical analysis

Questionnaire data generated was recorded in excel spread sheet and analysed using Epi Info™ 7 statistical package to described the statistics (mean, percentage and Confidence intervals). T-test was used to determine whether there was a significant different in the mean tick counts between the lower concentrations of 5%w/v and the higher concentration of 40%w/v. Also Analysis of variance (ANOVA) was used to evaluate whether there was any significant difference in the treatment outcome among groups from day 1 to 5 of treatment. STATA® statistical package version 12 was used to analyze the experimental data and significance level was set at 95% (p= 0.05). The incidence rate ratio (IRR) was evaluated by comparing the negative control and the different efficacies of the concentrations. Log rank test result on treatment groups' analyses was used to determine whether there was any difference in tick survival times at given *T. vogelii* leaf extract concentrations used in this study.

CHAPTER FOUR

4.0 RESULTS

4.1 Distributions of respondents by age group

A total of 100 survey questionnaires were administered. This study was both qualitative and quantitative. The majority of respondents were between the age of 40 and 49 years olds (36 percent) of the total respondents. While the smallest age group was 70 to 79 (representing 5 percent of the respondents). Overall age groups of respondents, their frequencies and percentages are shown in Table I.

Table1: Frequency distribution of age group of respondents

Age group	Frequency	Percent (%)
30-39	23	22.8
40-49	36	35.6
50-59	28	27.7
60-69	9	8.9
70-79	4	5
Total	100	100

4.2 Livestock farming experience

Results revealed that 53 (52.5 percent) of the respondents had more than 20 years experience in livestock farming,while31 respondents said they had been keeping livestock for 15 years (Table2).

Table 2: Livestock farming experience

Experience of keeping Cattle	Frequency	Percent (%)	Valid (%)	Cumulative (%)
5yrs	2	2	2	3.0
10yrs	13	12.9	12.9	15.8
15yrs	31	30.7	30.7	46.5
more than 20yrs	53	52.5	52.5	99.0
Total	100	100	100	

4.3 Number of Cattle Owned by respondents

The results revealed that (12%) at 95% CI (6.36, 20.02) of the respondents had small herd sizes of 10, while (1%) at 95% CI (0.03, 5.45) had 71 herd size (See Table 3 below).

Table 3: Number of Animals owned by farmers

No of Cattle	Frequency	Percent (%)	95% CI
0	2	2.0	0.24 -7.04
2	2	2.0	0.24 - 7.04
3	2	2.0	0.24 - 7.04
4	8	8.0	3.52 - 15.16
5	5	5.0	1.64 - 11.28
6	4	4.0	1.10 - 9.93
7	4	4.0	1.10 - 9.93
8	6	6.0	2.23 - 12.60
9	8	8.0	3.52 - 15.16
10	12	12.0	6.36 - 20.02
11	2	2.0	0.24 - 7.04
12	8	8.0	3.52 -15.16
13	6	6.0	2.23 -12.60
14	3	3.0	0.62 - 8.52
15	4	4.0	1.10 - 9.93
16	2	2.0	0.24 -7.04
18	3	3.0	0.62 - 8.52
19	1	1.0	0.03 - 5.45
21	1	1.0	0.03 - 5.45
22	2	2.0	0.24 - 7.04
23	1	1.0	0.03 - 5.45
24	2	2.0	0.24 - 7.04
26	1	1.0	0.03 - 5.45
37	4	4.0	1.10 - 9.93
42	1	1.0	0.03 -5.45
46	2	2.0	0.24 - 7.04
59	1	1.0	0.03 - 5.45
60	2	2.0	0.24 - 7.04
71	1	1.0	0.03 -5.45
Total	100	100.0	

4.4 Use of commercial acaricide by respondents

Results indicated that 34 percent with confidence interval (24.82- 44.15) of respondents used at least5litres of commercial acaricide per week. Table 5 shows overall responses of respondents on the usage of commercial acaricide.

Table 4: Usage of acaricide per week

Quantity (Litres)	Frequency	Percent (%)	95% CI
5 litres	34	34.0	24.82 – 44.15
10 litres	28	28.0	19.48 – 37.87
20litres	7	7.0	2.86 – 13.89
> 20litres	31	31.0	22.13 -41.03
Total	100	100.0	

4.5 Amount of money spent by respondents on tick control

Results on the money spent on commercial acaricide use for tick control is given in Table 5.Most farmers spent their money ranging from K100 to K2400, of which18 percent of the farmers interviewed said that they spend K300 on dipping.

Table 5: Amount of money spent on dipping in kwacha per week

Amount (K) per week	ency	Percent (%)	95% CI
0	4	4.0	1.10 - 9.93
100	2	2.0	0.24 - 7.04
104	2	2.0	0.24 - 7.04
120	2	2.0	0.24 - 7.04
150	2	2.0	0.24 - 7.04
180	2	2.0	0.24 - 7.04
200	2	2.0	0.24 - 7.04
225	1	1.0	0.03 - 5.45
250	2	2.0	0.24 - 7.04
300	18	18.0	11.03 - 26.95
340	2	2.0	0.24 - 7.04
350	10	10.0	4.90 - 17.62
380	1	1.0	0.03 - 5.45
384	2	2.0	0.24 -7.04
396	1	1.0	0.03 - 5.45
400	6	6.0	2.23 - 12.60
440	2	2.0	0.24 - 7.04
450	2	2.0	0.24 - 7.04
468	2	2.0	0.24 - 7.04
480	4	4.0	1.10 - 9.93
500	14	14.0	7.87 - 22.37
520	2	2.0	0.24 - 7.04
550	1	1.0	0.03 - 5.45
600	2	2.0	0.24 - 7.04
624	2	2.0	0.24 - 7.04
764	1	1.0	0.03 - 5.45
1200	3	3.0	0.62 - 8.52
1800	2	2.0	0.24 - 7.04
2208	2	2.0	0.24 - 7.04
2400	2	2.0	0.24 - 7.04
Total	100	100	

4.6 Knowledge levels on Tick borne diseases by respondents

Forty one (41) percent of the participants knew that corridor disease was a TBDs. While two (2) percent mentioned Corridor disease Babesiosis and Anaplasmosis as TBDs. Fifty-six (56) percent mentioned Corridor disease and Heartwater as the TBDs they knew (See Table 6).

Table 6: Type of tick borne diseases recognised by respondents

Diseases	Frequency	Percent (%)	95% CI
Corridor	41	41.0	31.26 -51.29
Corridor/Anaplasmosis/Babesiosis	2	2.0	0.24 -7.04
Corridor/Heartwater	56	56.0	45.72 -65.92
Heartwater/	1	1.0	0.03 - 5.45
Total	100	100.0	

4.7 Knowledge on traditional methods of TBDs Control

Table 7 indicates that out of 100 respondents, thirty three (33) respondents were able to mention the indigenous knowledge which could be used to control tick infestations on livestock, while sixty seven (67) respondents did not mention any method.

Table 7: Indigenous knowledge indicated for use in traditional tick control

Traditional methods	Frequency	Percent (%)	95% CI
Dung	9	9.0	4.20 – 16.04
Dung, birds, scissor	2	2.0	0.24 – 7.04
Dung, scissors, birds	2	2.0	0.24 – 7.04
Dung, birds, thorns	5	5.0	1.64 – 11.28
Mupulanga	2	2.0	0.24 – 7.04
Nil	67	67.0	56.88 – 76.08
Thorns	5	5.0	1.64 -11.28
Thorns/scissors	8	8.0	3.52 – 15.16
Total	100	100.0	

Thirty six (36) respondents acknowledged that they had used traditional tick control methods in their farming practice, while 64 respondents did not use any traditional tick control method (Table 8).

Table 8: Number of respondents who had used traditional tick control method

Response	Frequency	Percent (%)	95% CI
Yes	36	36.0	26.64 – 46.21
No	64	64.0	53.79 – 73.36
Total	100	100.0	

4.8 Bioassay test on larvae survival

The efficacy of the crude extracts bioassay tests of *T. vogelii* on larva *in vitro* at varying concentrations was measured as live larva or dead larva post treatment. Table 9 shows the mortalities of different concentrations of *T. vogelii* on eliminating *R. appendiculatus* larvae *in vitro*. The mortality rate of various treatment levels of larvae with T. vogelii extracts was proportional to the concentrations used.

Table 9: Mortalities and Survival larvae to various concentrations of*T.vogelii*extracts after 24 hours.

Concentration of Extract (%)	Log 10Conc	Number Dead	Number Alive	Total	Mortality (%)
0.63%	-0.20	25	125	150	17.0
1.25%	0.10	39	111	150	26.0
2.50%	0.40	88	62	150	59.0
5%	0.70	148	2	150	99.0
10%	1.00	149	1	150	99.0
Negative control (water)		2	148	150	1.0
Positive control (Amitraz)	1.10	150	0	150	100.0

The efficacies of the crude plant leaf extracts were observed within 24hourspost-treatment. Interesting observation was that at 5 and 10 w/v concentrations, gave the same mortality rates of 99 percent, as compared to negative control which gave a mortality rate of by only 1 percent. In both cases the results were found to have a very significant effect in killing the larva, see Fig 3.

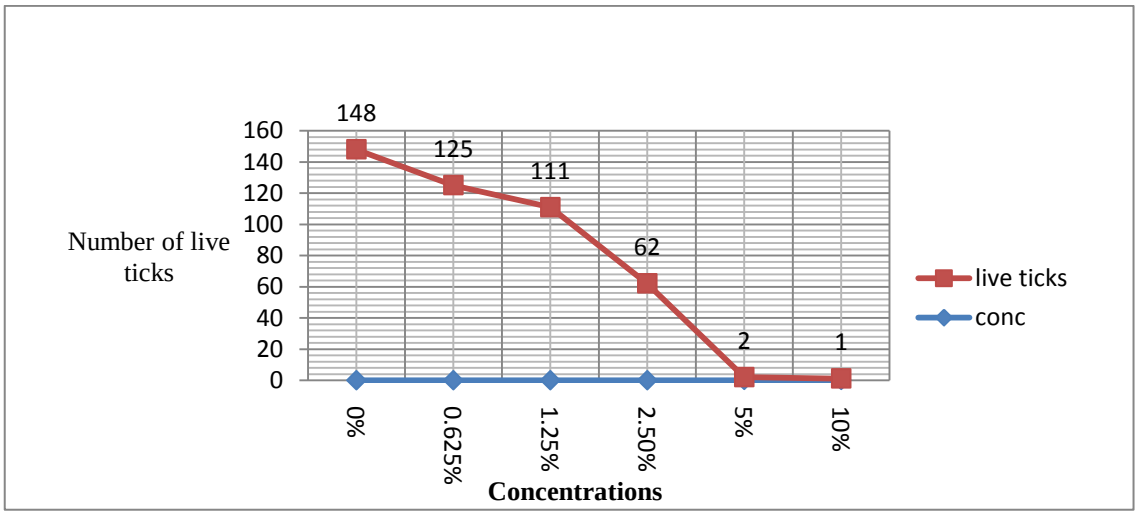

Figure 3:Live larva of *R. appendiculatus* after treatment at different concentrations of *T. vogelii*.after 24 hours.

4.9 Analysis of nymphal tick data

The mean numbers of live nymphs per day were observed after treatment with *T. vogelii* leaf extracts. There was a significant (p-=0.0001, reduction in numbers of nymphs from day 1 to day 7 post treatment (see Table 10).

Table 10: Summary statistics of number of nymphal ticks surviving over time

Time(days)	Mean of Live ticks	SD	Min	Max
0	10	0	10	10
1	9	0.68	5	10
2	7	1.46	5	10
3	6	1.93	0	10
4	4	2.45	0	9
5	2	2.15	0	9
6	1	2.08	0	9
7	1	2.08	0	9

The calculated mean reductions, standard deviations, the minimum and maximum counts are given in Table 11 below. There was a variation in mean tick counts according to the concentrations used, while negative control had higher mean tick counts.

Table 11: Showing reduction of nymphal ticks following exposure to *T. vogelii* leaf extract

Concentration (%)	Mean of live ticks	SD	Min	Max
0.625	5.0	3.7	0	10
1.25	5.0	3.6	0	10
2.5	6.0	3.5	0	10
5.0	5.0	3.7	0	10
10.0	5.0	4.1	0	10
20.0	3.0	4.1	0	10
-ve control	9.0	0.5	9	10

The mean number of nymphs per concentration of *T. vogelii* extract showed that, there was a significant reduction (P=0.011) after treatment. The negative control remained with the initial average numbers of nymphs (See table 11).

Table 12: Showing the effects of concentrations on incident rate ratio of nymphs**.**

Concentration (%)	Incident Rate ratio(IRR)	P-value	95% CI
0.625	0.542	<0.001	0.407, 0.723
1.25	0.498	<0.001	0.372, 0.667
2.5	0.627	<0.001	0.379, 0.678
5.0	0.551	<0.001	0.474,0.829
10.0	0.507	<0.001	0.259, 0.487
20.0	0.356	<0.001	0.414,0.734
-ve Control	1.000	-	-

The incidence rate ratios (IRR) were calculated using the Poisson regression model to compare the efficacies of the *T. vogelii* leaf extract concentrations and the negative control groups (See table 12). The numbers of tick counts were observed from each concentration treatment groups of 0.625, 1.25, 2.5, 5.0, 10 and 20.0 w/v percent. Each group had the same of ticks on day zero (0). The average tick counts were converted to percentage of number of ticks counted on each group on day zero (0). Results in Table 12 shows that there was a reduction in tick counts at all concentrations levels used. 0.625,1.25, 2.5, 5.0, 10 and 20.0 percent w/v which gave 46, 50, 37, 45, 50 and 34 percent reduction of tick counts, respectively.All the treatment groups were statistically significant with the p-value <0.001.

Results in Fig 4 shows the number of surviving nymphal ticks post exposure up to 144 hours to different concentrations of *T. vogelii* extracts. The results show that the negative control group had one (1) mortality of out of ten (10) ticks exposed, while all other concentration levels had 100 percent mortality rates by the end of 144 hours. Twenty percent treatment group had 100 percent mortality rate in 96 hours post treatment.

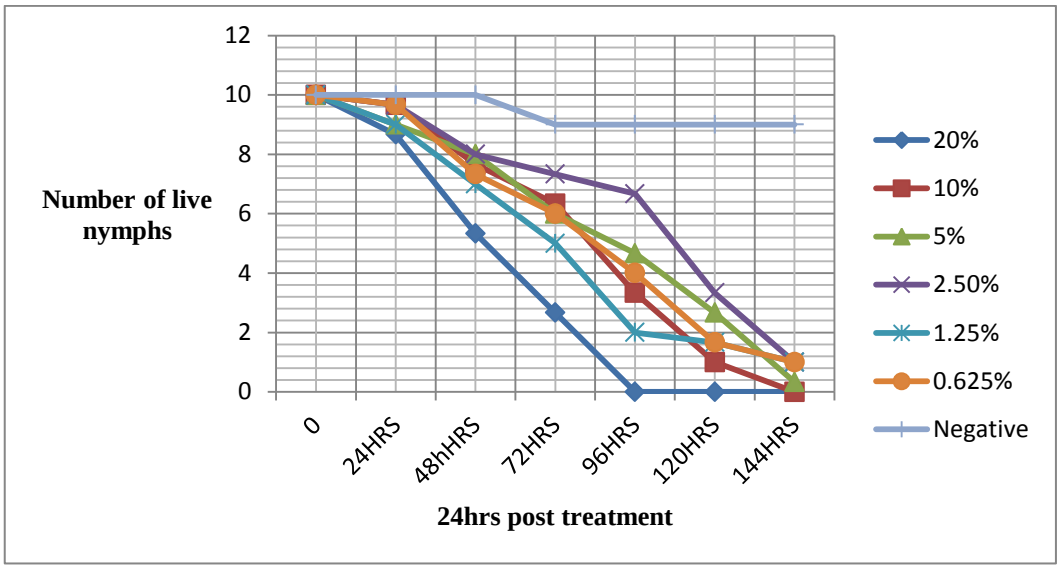

Figure 4: Reduction in total nymphal tick counts at different concentrations and time

The mean efficacy was calculated from all the concentrations used from day one (1) to day six (6).The results in Fig 5 shows the increase in mortality risk on a daily basis from day one (1) to day six (6) post treatment, by steady increase in the number of nymphs dying.

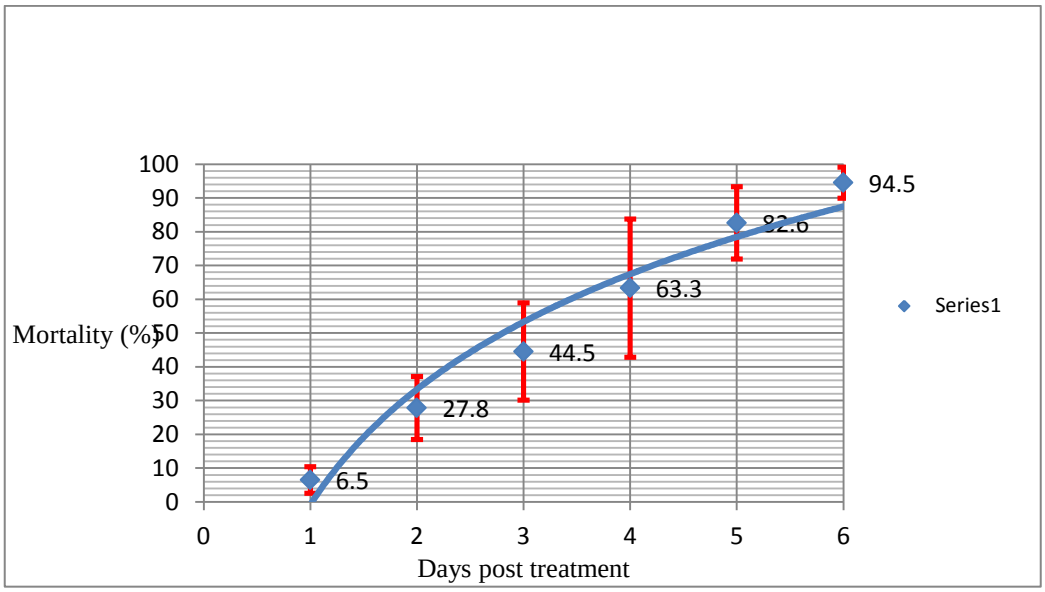

Fig 5: Mean mortalities on nymph ticks days post treatment

Mean Mortalities of on nymphal ticks of the concentrations 2.5, 5 and 10 percent w/v of day four(4) was used to calculate the EC50 value. The mortalities were conversted to LogConc to express the range of values so that EC50 is interpreted and determined within the values. The linear formula was influenced by the data used (Below is fig 6).

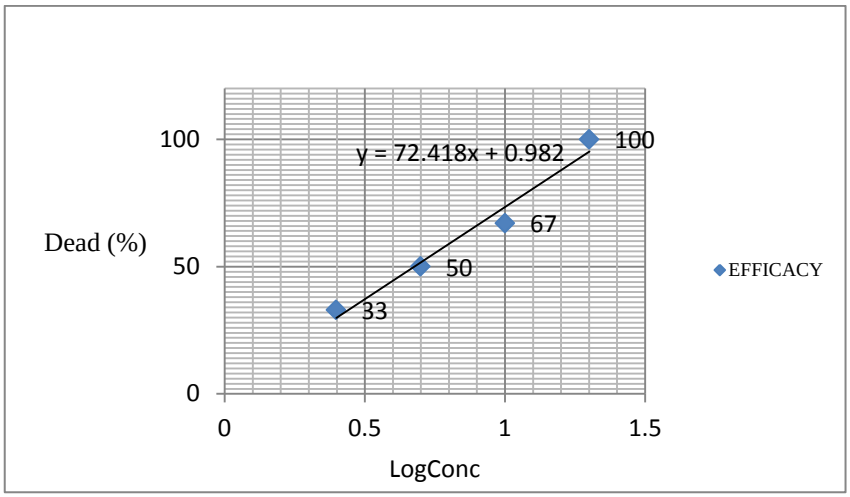

Figure 6: The efficacy of *T. vogelii* in killing and the calculated EC50 at day 4 post treatment.

Linear formula which was used to determine the EC50 was formulated after entry of values.

Below is the formula:

Y=72.41x + 0. 982

50 = 72.41x + 0.982

50 – 0.982 = 72.41x

49.018 = 72.41x

x = 49.018/72.41

x = 0.67

Antilog = 4.752

The Log concentration that is expected to kill 50 percent of ticks exposed to the extracts was 0.67. This is the amount of *T. vogelii* leaf extracts concentration needed to achieve 50

percent mortality rates of the nymph ticks post exposure. The EC50 for *T vogelii* extract was apparently very low.

4.10 Comparison of two means from low and high concentrations of *T. vogelii* extracts.

A T-test was performed using the Excel Microsoft 2007 programme to calculate the p-value of the test and determine whether there any significant difference between the lowest 0.625 percent w/v and highest 10 percent w/v *T. vogelii* leaf extract concentrations on day 1.It was found to be 0.3. The calculated T-test value was one (1) which was above the standard p–value of 0.05 indicating no significant differences between the two means.

4.11 Analysis of Adult ticks treatment with *T.vogelii* extracts

Exposure of adult ticks to *T .vogelii* leaf extract presented different results to each concentration level used (See table 13). There was 100 percent mortality rate of adult ticks from day four (4) to day six (6) at 30 and 40 percent concentrations. Positive controls showed 100 percent mortality rate after three (3) days post treatment while, negative control presented no mortalities. At 5 percent concentration, ticks reduced to zero by day 6 post treatment.

Table 13: Number of adult *R.appendiculatus* ticks remaining on host days post treatment.

CONC (%)	DAY 0	DAY 1	DAY2	DAY 3	DAY 4	DAY5	DAY 6
40	10	5	3	1	0	0	0
30	10	7	3	1	0	0	0
20	10	8	7	4	2	0	0
15	10	7	7	5	3	0	0
10	10	8	7	3	2	0	0
5	10	9	9	7	6	3	0
+ve Control	10	5	3	0	0	0	0
-ve Control	10	10	10	10	10	10	10

4.12 Poisson regression analysis on adults ticks

Poisson regression model analysis on the adult tick reduction rates from day 1 to day 6 at different *T. vogelii* concentrations levels used in comparison to the negative control, 0.05 percent w/v was 37 percent reduction rate while 0.4 percent w/v was 73 percent. The P-value at all the concentrations levels were significant (See table 14 below).

Table14: Modeling the incident rate ratios, using negative control as the reference group.

Concentration of extract	Incidence Rate ratio (IRR)	P-value	95% CI
0.05	0.63	<0.016	0.431, 0.917
0.10	0.43	<0.000	0.279, 0.657
0.15	0.46	<0.000	0.301, 0.694
0.2	0.44	<0.000	0.290,0.676
0.3	0.30	<0.000	0.184, 0.488
0.4	0.27	<0.000	0.163,0.451
-ve control	1.00	-	-

The results from Table 15 below, shows the reduction of mean adult tick counts (10 to 1.2) from day zero (0) to day six (6). The average mortality rate decreased steadily with time after treatment.

Table15: Showing the summary number of adult ticks by day

Day	Mean	SD
0	10	0
1	7.4	1.77
2	6.1	2.79
3	3.9	3.39
4	2.9	3.52
5	1.6	3.54
6	1.2	3.53

Table 16 illustrates the summary results on the steady adult tick reduction at concentrations ranging from 0.5 to 0.4 *T. vogelii* leaf extract concentrations. The number of ticks where reducing from day one (1) to Day six (6) of observations.

Table16: Showing the summary values for number of adult ticks by concentration

Concentration	Mean	SD
0.5	6.28	3.638
0.1	4.28	4.09
0.15	4.57	3.77
0.2	4.42	3.99
0.3	3.0	4.0
0.4	2.71	3.72
-ve control	10.0	0.0
+ve control	2.57	3.82

Fig 7 is shows results of the Log Concentration against the mortalities that was used to calculate the lethal dose (LD_{50}). The equation (Y=132.8x – 106.2) was derived from the two(2) points of mortalities (50 and 90) stretched in the same line to determine the LD_{50}.Where Y stands for the 50 percent mortality while x is the LogConentration that is needed to kill 50percentage of ticks. The lethal dose at which 50 percent of the adult tick population were killed was at Log Concentration1.17.

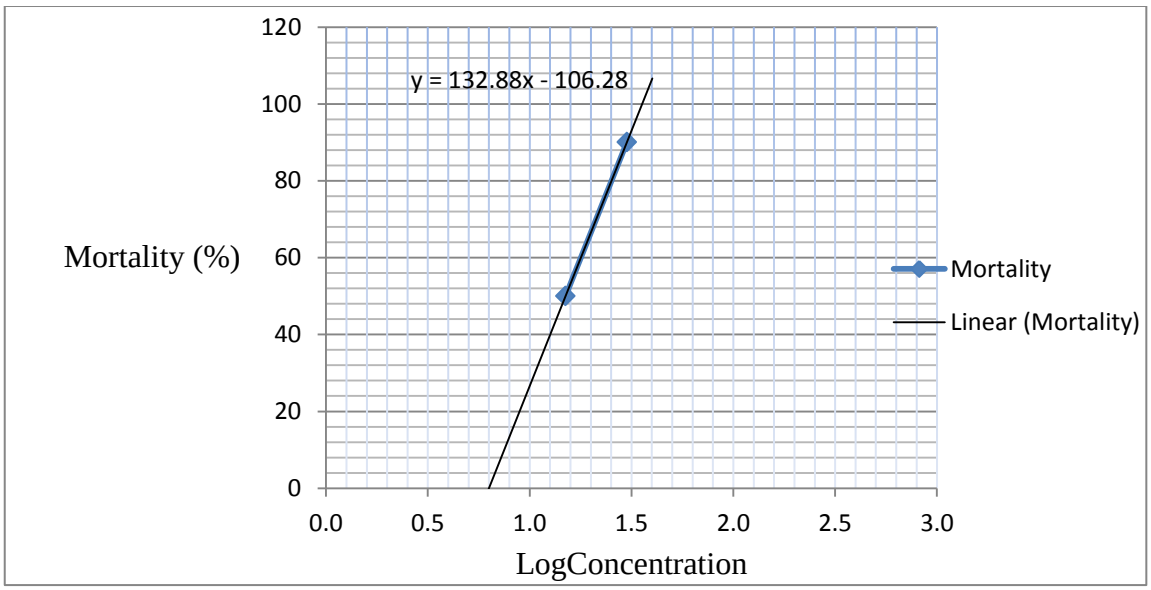

Figure 7: Mortality of *R. appendiculatus* adult ticks against log concentration

The effective concentration levels ranged from 5 to 40 percent which represented mortalities of 35 to 100 percent respectively. Figure 8, further shows the calculated antilog of 15 percent w/v which can kill 50% of the ticks.

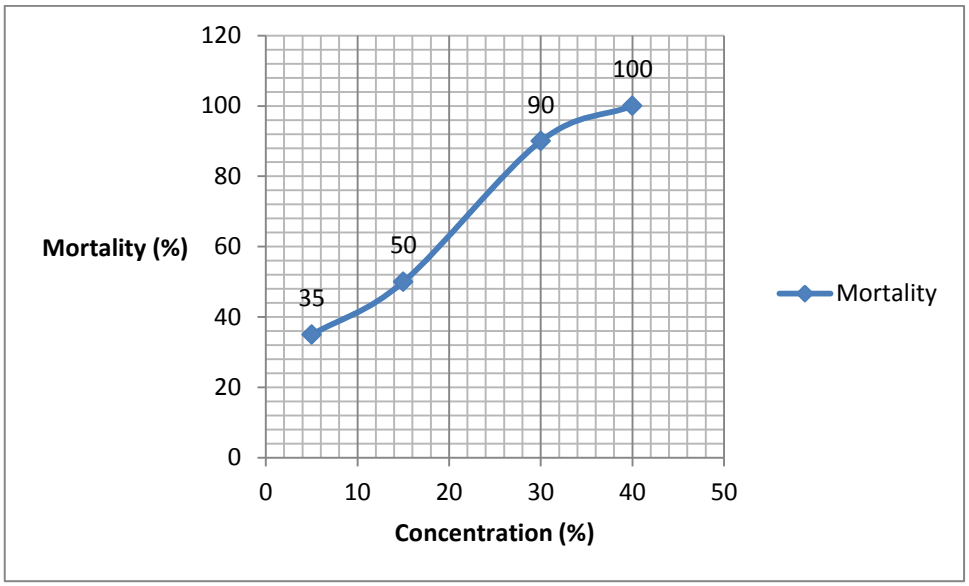

Figure 8: Results showing percentage efficacies at varying concentrationrates

Y = 132.8x – 106.2

50 = 132.8x – 106.2

50=132.8x – 106.2

50 + 106.2 = 132.8x

156.2 = 132.8x

X = 156.2/138

X = 1.176the Antilog = 15%

4.13 Efficacy of *T. vogelii* leaf extracts on cattle under field condition

Table 17 below shows the results of *T. vogelii* leaf extract on reducing tick load infestation on cattle under field condition. The mean tick's counts reduced from 32.25 on day 0 to 5.25 in five (5) days. This reduction was statistically significant.

Table 17: Mean number of ticks in the field for a period of Six (6)days**.**

Days	Mean	SD
0	32	14
1	12	8
2	8	10
3	6	11
4	5	13
5	5	14
6	0	16

Table 18 below shows the comparisons of number of the tick counts post treatment in the negative control (water) group, positive control groups and in the *T. vogelii* leaf extract treatment groups at 0.05 to 0.4 concentration levels.

Table 18: Showing the summary values for number of ticks by concentration

Concentration of extract (%)	Mean	SD
0.05	4.25	6.023
0.1	10.29	14.78
0.2	9.06	14.57
0.4	7.26	10.70
Positive (Amitraz)	10.05	17.90
Negative (Water)	34.8	5.109

Interestingly, lowest treatment concentration level of 0.05 had significantly lower mean count of ticks than other treatment concentration levels of 0.1 to 0.4.The negative control group had no effect on the ticks instead there were more ticks. This variation in responses is clearly illustrated in Table 18.

The incidence rate ratios (IRR) calculated using the Poisson regression model to compare the mortality rate of ticks in different concentrations and the negative control groups are indicated in Table 19. The numbers of tick counts were observed from each concentration treatment groups of 0.05, 0.10, 0.20 and 0.40. Each group had different number of ticks on animals on day zero (0). The average tick counts were converted to percentage of number of ticks counted on each group on day zero (0). Results in Table19 shows reductions in tick counts at all concentrations levels used. Five (5) percent w/v concentration of *T.vogelii* leaf extracts had the highest reduction rate of ticks of 88 percent, while 0.10, 0.20 and 0.40 percent w/v gave 71, 74, and 80 percent reduction of tick counts, respectively. The

associations between leaf extract concentrations used and tick mean count reduction, was significant with p-value of 0.001.

Table: 19 Poisson regressions Model

Concentration of extract (%)	Incident rate ratio (IRR)	P-value	95% CI
0.05	0.122	0.000	0.073 - 0.204
0.1	0.295	0.000	0.180 - 0.482
0.2	0.260	0.000	0.162 - 0.416
0.4	0.208	0.000	0.130 - 0.335
+ve Control	0.288	0.000	0.170 - 0.489
-ve Control	34.888	0.000	24.261 - 0 50.17

Figure 9 below, shows the general trend of tick reduction after treatment with different concentrations of *T. vogelii* extracts from day (one) 1 to day six (6). Although the initial tick challenge was lower on animals, the study commenced at a peak period for tick infestation in the area, there was notable reduction in the teak loads after treatment with all levels of *T. vogelii* leaf extracts concentrations.

On day zero (o), the numbers of ticks were lower than 40 on each animal. Notable reduction was observed on day one (1)) post treatment. The gradient of tick reduction rate was directly proportional to the increase in *T. vogelii* leaf extract concentrations (See figure 9).This is even evident when the slops for all the concentrations are compared to the control with lowest reduction seen on day six (6) post treatment The knock out effect of *T. vogelii* leaf extract was evident and high at all concentration levels as seen with steep slope.

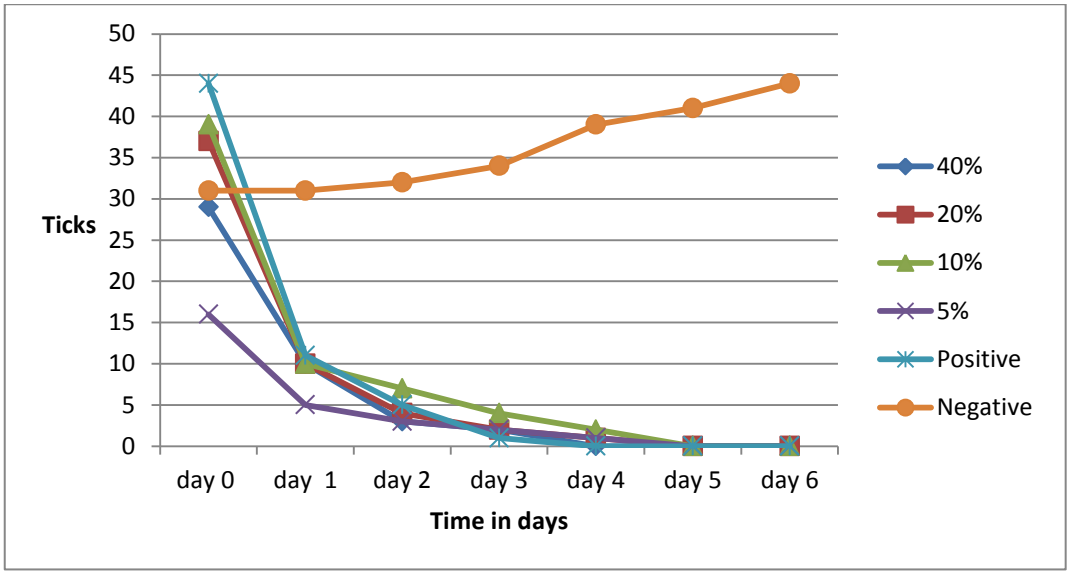

Figure 9: Rate of decrease in the tick counts on cattle days post treatment at different concentrations.

Figure 9 shows the overall reduction pattern in tick counts after treatment. There was a significant reduction in tick loads on animals within 24 hours after treatment and remained steadily low for close to a week. The general impression shows that, the acaricidal effects of the leaf extracts on ticks was significant.

The overall outcome in the four different concentrations levels used in box plot shows (Fig 10) similar trends in the killing effect or reduction of ticks on animals. The median line in concentrations of 5% and 10% were symmetrical, while the concentration of 20% and 40% had different symmetrical line appearance.

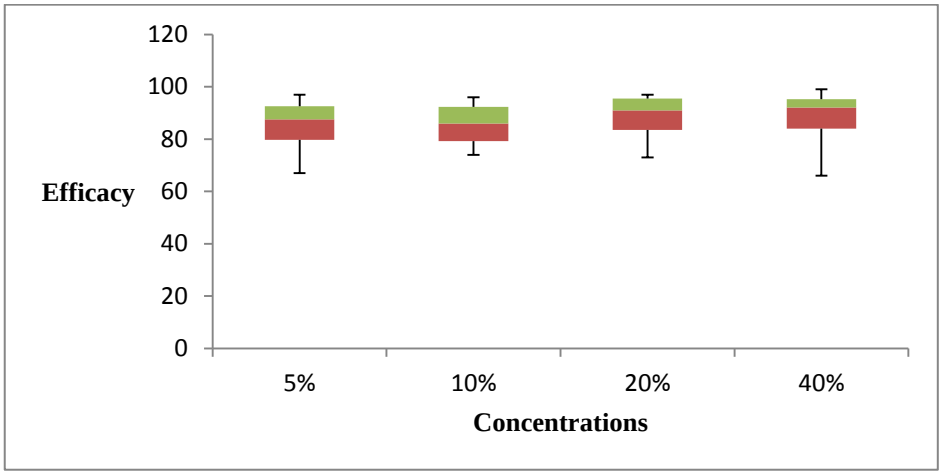

Figure 10: Overall pattern of response for each group per concentration

4.14 Survival analysis

The data from the field experiment were analysed using the survival analysis. The estimated mean time until death was found to be 1.451 days for the ticks exposed to 40% concentration, estimated mean time until death is 1.433 for the ticks exposed to 20%, estimated mean time until death is 1.549days for the ticks exposed to 10% and estimated mean time until death 1.729 is for ticks in 5% concentration. The time at which 50% of the ticks had reached the event in all the groups is 1.000.

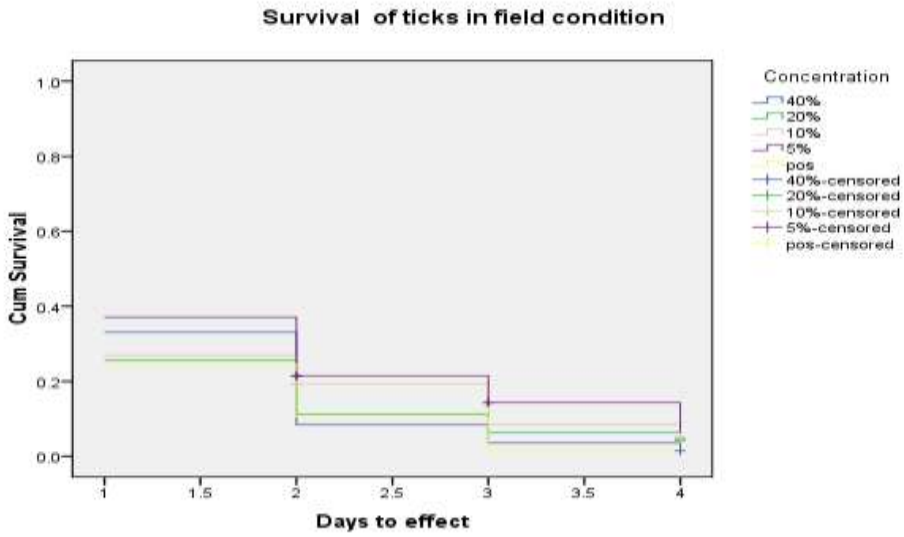

Figure 11: The Kaplan Meier plot of ticks in the field experiment at different concentrations

Although the survival probability seemed to be lower at 20 and 40 percent and higher at lower at concentration 5 percent, treatment with *T. vogelii* extracts *in vivo*, (See figure 11), there was no significant differences in the ultimate killing effects between the lower and higher concentrations after the end of the observation period (P-value= 0.001).

4.15 Toxicity of *T. vogelii* leaf extracts in mice

4.15.1 Acute toxicity test

Table 20: Group treated with 5 percent w/v extract and water, single dose, oral

Serial No.	Mouse body weight (grams)	Sex	Concentration of extracts(5%) dose	Mortality
1	25g	Female	0.25ml	No mortality
2	22g	Female	0.22ml	No mortality
3	24g	Female	0.24ml	No mortality
4	23g	Male	0.23ml	No mortality
5	21g	Male	0.21ml	No mortality
6	23g	Male	Water (0.23ml)	No mortality

Results were interpreted in relation to mice survival and evident toxicity signs in comparison to the LD_{50} test developed in 1927. This method produces similar results while using fewer animals and causing less pain and suffering (Trevan, 1927).The results in both Table 20 and 21showed no mortality or any signs of toxicity in the tested mice.

Table 21: Group treated at 10, 20, 30 and 40percent w/v of the extract single dose, oral.

Serial No.	Mouse body weight(grams)	Sex	Concentration of extract (%)	Dose in mls	Histopathology		
1	25g	Female	10% w/v	0.25ml	Liver	Kidney	Heart
2	28g	Male	10% w/v	0.28ml	Normal	Normal	Normal
3	26g	Female	20%w/v	0.26ml	Normal	Normal	Normal
4	29g	Male	20%w/v	0.29ml	Normal	Normal	Normal
5	30g	Female	30%w/v	0.30ml	Normal	Normal	Normal
6	25g	Female	30% w/v	0.25ml	Normal	Normal	Normal
7	27g	Female	40% w/v	0.27ml	Normal	Normal	Normal
8	25g	Male	40% w/v	0.25ml	Normal	Normal	Normal
9	30g	Male	water	0.30ml	Normal	Normal	Normal
10	26g	Male	water	0.26ml	Normal	Normal	Normal

Similarly, results from the experiment group of mice given 76 percent w/v dosage had no evidence of toxic signs or histopathological lesions at the highest dose of 2000mg/kg. The *T. vogelii* extract can be considered safe and none toxic in mice under the prevailing conditions.

CHAPTER FIVE

5 0 DISCUSSION

5.1 Questionnaires survey

Most small scale livestock farmers in Monze East, keep cattle traditionally and this skill has been passed on from one generation to other within the families. 70% of the small scale poor resource population who live in the rural areas depend on the role of livestock in their daily livelihood. (LID, 1999). Tick-borne diseases in their herds have continued to be the major economic problem (Makala *et al.,* 2003). All age groups of famers were involved in livestock keeping (See table 1), although the majority were between the age 40 to 49 years. About 53% had the experience of keeping animals for over 20 years, suggesting that these livestock farmers had wide experience with animals (See table 2). This shows that the art of keeping animals involved a wide range of different age groups and animals were for home sustainance, though they have different herd sizes kept by famers. Of all the farmers interviewed 12% of had 10 animals while 1% had 71 herd size (See table 3). Those that used the acaricide showed that 34percent used 5litres per week, which is equally costly. Of the farmers interviewed different ranges from K100 to 2400) of expenditures were recorded, of which 18percent spent K300.per week to dip their cattle (See table 5), which was generally on the higher side for the majority of famers and thus posing a great challenge for sustainability, The cost of these acaricides could have influenced the low demand in tick control(Noroge and Bussmann 2006). The Amount of acricides used to dip animals has

enormous influence on how long a farmer can manage their tick control programme. This study also revealed that the amount of dip acaricide used by farmers per week was largely dependent on availability of resources. It was also observed that tick control management was major concern for farmers depended on their livestock for survival and who may had no any other source of income because of the devastating effect of TBDs. Having viewed the problems and challenges faced by traditional livestock farmers and also the effects of these synthetic acaricides a cheap and cost effective way is needed for resource- poor farmers to control ticks (Christopher *et al.,* 2009) The number of animals owned by farmers is an important variable in the study of tick control as it may influence choice of tick control method used. The farmers enrolled in this study were small scale farmers with average herd of ten cattle. Out of Hundred (100) respondents only 36 percent farmers confirmed that they had used traditional tick control methods as an alternative tick control intervention. Farmers also mentioned that, Dung, thorns, scissors and Mupulanga were used. The use plants to control ticks is common according to Moyo and Musika (2008) and Hlatshwago and Mbati (2005), who found 6.8 percent and zero percent of smallholder livestock farmers using plant to control ticks in Eastern cape province of South Africa. Farmers had the knowledge on tick-borne diseases, forty one (41) percent revealed that Corridor disease locally known as (Denkete) was common and other diseases like Babesiosis, Anaplasmosis and Heart water. *T. vogelii* was known for soil fertility and not for tick control as such adoption levels to use it was a challenge, however, after noticing the positive results from the experiments the farmers had confidence and hence offered their animals for further testing.

This knowledge about traditional control of ticks was in line with Management of traditionally reared animals by small scale livestock farmers which depends on the acquisition of indigenous knowledge, skills, methods, practices and beliefs in animal husbandry (McCorkle *et at.,* 1996).

5.2 *In-vitro* assessment of plant extract

Laboratory bioassays on the effects of *T. vogelii* leaf extracts on *Rhipicephalus appendiculatus* species of ticks at different concentrations levels where done at the Regional Diagnostic Laboratory in Mazabuka, Southern province. The use of bioassay on tick larvae with plant extracts of *T. vogelii* has shown toxic effects (Kaposhi *et al.,* 1994). In Benin similar studies have shown that extraction of leaf materials using ethanol from *T. vogelii* caused 98.51% mortality in *A. variegatum* (Dougnon *et al.,* 2012).Similar studies by Matovu and Olila (2007) were observed on the activity of *T. vogelii* plant leaf extracts on nymphs and adults ticks. The *in vitro* assessment studies (Shaw, 1966) indicated that both 5 and 10 w/v concentrations levels gave the same mortalities of 99 percent as compared to negative control (See table 9).This was not expected but gives a new processing strategy of the biomass which will help farmers conserve by two fold their plant material for more dipping in the three years *T. vogelii* plant remains in their field.

The dip and immersion method bioassay (Pirali-Kheirabadi *et al.,* 2007), on nymphs and adult ticks (respectively) showed that plants extracts of *T. vogelii* were effective in killing

the larvae, nymphs and adult tick stages at all concentrations levels used (p-value <0.001) as shown in Table 12.

5.3 Field assessment of the extract

This study aimed to evaluate the efficacy of different concentrations of *Tephrosia vogelii,* a tropical leguminous herb, crude leaf extract as a natural acaricide to control ticks on naturally infested traditionally reared cattle in selected areas of Monze district of Zambia. Control of ticks and tick borne diseases is mainly known to be done using a wide range of approaches which include commercial acaricides like organophosphates, carbamates and pyrethroids, which unfortunately have led to various problems such as; development of tick resistance strains, residual environment pollution, indiscriminate killing of useful arthropods and the high cost of purchase (Ghosh *et al.*, 2007). In many places in Zambia, different preparations have been used by local farmers for the purpose of controlling ticks on the livestock, without enough proof of which concentration to depend on for daily use within economical usage. In this study, all the preparations gave good results. The results obtained in this study (Table 17) demonstrated the reduction of ticks after application of the leaf extract of *T.vogelii*. The mean tick's counts reduced from 32 on day Zero (0) to 6 on day six (6). This showed that the extracts effectiveness was high. Under field condition, all the four (4) treatment groups allotted to different *T. vogelii* extract concentrations spray gave similar tick count reduction responses compared to controls. In Zambia, preliminary studies using crude *T. vogelii* water soluble extracts in the field experiments showed that at a

concentration of 10 percent w/v it was possible to protect cattle from tick infestation (Kaposhi, 1992). Results from this study shows that tick reductions were found to be statistically significant at all treatment levels (p-value < 0.001). Despite a slight difference in efficacy in the treatment groups, statistically, the different levels of concentration had similar effects. In this study, even at a low concentration of 5%, there was significance reduction in tick numbers compared to what has been observed in previous studies. An experiment done in Zimbabwe on the tick control indicated that there was a decline in the number of engorged ticks from different dilutions levels after treatment with *T. vogelii* on dairy animals (Gadzirayi *et al*., 2009). This study provided further evidence to show that the herbal extract is effective against ticks. According to the PACE project in central Kenya, the use of *T. vogelii* as an acaricide has shown encouraging results (PACE 2013). Studies from Congo Democratic Republic have also shown that mortality of 95% and 100% using 10 and 20mg/ml of leaves of two varieties of *T. vogelii* against *R. appendiculatus* ticks has been observed by (Kalume *et al.*, 2012).

Generally, the results obtained in this study regarding the effectiveness of the extracts suggests that the plant has acaricidal effects on ticks. The overall reduction pattern in tick counts after treatment, suggests that there was significant reduction in tick loads on cattle within 24 hours after treatment and remained steadily low for close to a week (See Fig 12). Tick numbers reduced from day one (1) to day six (6). The general impression showed that, the acaricidal effects of the leaf extracts on ticks was significant, and should be recommended for use by small scale farmers and those venturing in organic farming. From

these results, the protection period was 5 to 6 days post treatment which suggests that under high tick challenge in the field, dipping should be repeated weekly. Interestingly weekly dipping is also recommended when using commercial acaricide during periods of high risk. Studies by Barnes and Freyre (1966), Gaskin *et al*., 1972 have shown that the rotenoids present in leaves of *T. vogelii* are effective in killing numerous pests yet all toxicity is lost in five (5) to six (6) days. According to Koigi (2011) *T. vogelii* crude extracts usually remains for a week after which results appear. In another study, Gadzirayi *et al.* (2009) showed that there was no significant difference in performance by using *T. vogelii* and Triatix dip (a conventional dip chemical) for tick control. These findings show that it is easier to use *T. vogelii* leaf extracts by small scale farmers which is cheaper to propagate for tick control. Similar observations by (Dougnon *et al*., 2014) have supported the findings that the effects of *T. vogelii* and a (Alfapor – cypermethrin) synthetic acaricide are similar. The drastic drop in tick mortalities suggests that the effectiveness of the leaf extract is high and can be observed within 24 hrs after its application (See fig 12). The negative control showed an increase in the number of ticks after treatment with water which had no effect on the ticks on the cattle. The difference in tick counts before treatment to different concentrations were all lowered to zero on the sixth day (6) of observation. The gradient of tick reduction rate was directly proportional to the increase in *T. vogelii* leaf extract concentrations. This is even evident when the slops for all the concentrations are compared to the control with lowest reduction seen on day 6 post treatment. This observation is in agreement with that reported by Muyobela *et al.*(2016),who found that *T. vogelii* plant extracts has excellent

acaricidal activity against ticks and persisted for 8 days with 100% mortality of *A. variegatum* ticks in 24hours. The knock out effect of *T. vogelii* leaf extract was evident and high at all concentration levels as seen with steep slope. The results also showed that the lower concentration treatment group had the lower mean counts ticks than the positive control while in the negative group, the tick counts were higher. This may be related to few number of ticks prior the application in the 0.05 percent treatment group. There was also a variation in the mean count between 0.05 percent and 0.40 percent. The incidence rate ratios (IRR) calculated in (Table 19) indicates the relationship between negative control and treatment groups in action. Five (5) percent w/v concentration of *T. vogelii* leaf extracts had the highest reduction rate of ticks of 88 percent, while 0.10, 0.20 and 0.40 percent w/v gave 71, 74, and 80 percent reduction of tick counts respectively. This observation was not expected, where by the low concentration treatment group had higher mortalities effects on ticks than the higher concentration treatment group. This could probably be attributed to several factors including: the low number of ticks in this treatment group, inadequate hand spraying of the crude extract to the body sites, especially where ticks were observed on each animal in the group. The study has shown the incident rate ratio in other different groups compared with the negative control with significant reduction of $P<0001$. The associations between leaf extract concentrations used on tick mean count reduction, was significant. It was further noted that, despite the different level of concentrations the effectiveness was statistically similar at all concentration. This observation is useful in showing us that, the effect of the lower and the higher concentrations were similar, and the usage of biomass in

the preparation of the crude extract can be minimized in order to preserve the leaves for the next spray than using higher concentrations which may demand a lot of biomass of leaves. There was progressive increase in the efficacy of *T. vogelii* leaf extracts, resulting in reducing the number of ticks on sprayed animals. From day one (1) the efficacy was above 50 percent of tick reduction from different concentrations. It was possible to achieve significant efficacy of *T. vogelii* leaf extract use as bio-acaricide at 5 percent concentration w/v instead of the earlier prescribed 10 percent w/v (Kaposhi, 1994). The implication of this finding will significantly reduce the required biomass needed in the preparation of the *T. vogelii* bio-acaricide by farmers and thus conserving their fields and reducing the cost of treatment using this method. There was no significant difference in the protection period against re-infection between high and low concentration. .The effects are due to the presence of rotenone, the active ingredient, which *T.vogelii* contains and has been reported to be useful against sucking and biting insects. (McDavide and Lesseps, 1995 and Stoll, 2001). According to PACE (2013), the leaf extracts of *T. vogelii* are used as cheap acaricide materials to control ticks in Kenya.

5.4 Safety of extracts in mice

Our findings have also shown that *T .vogelii* leaf extracts are not toxic in mice. There was no visible external signs of abnormality observed. No mortality in the number of mice during and after the orally administration with the leaf extracts was observed. The mice

were sacrificed after a period of study (see table 20 and 21). No visible lesions were observed. All the organs looked normal from outside. However the liver looked congested with the blood. These extracts can be considered safe in mice under the prevailed conditions. The use of these extracts to control ectoparasites of domestic animals which has been proven to be safe and that it does not put them at risk in case it finds its way in the oral cavity. In fact, *Tephrosia* leaves are eaten by domestic animals such as goats and cattle and in some cases the same extracts have been used by small scale livestock farmers to deworm the animals (Mulenga, 2013). This study provided the platform to have the proof of safety of the extract. This finding indicates that typical use of *T. vogelii* is unlikely to expose farmers and their animals to toxic levels of these plant extracts. However, advice on the safe use of these plants should still accompany their promotion extension packages.

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

The reduction of tick infestations on cattle after the application with *T. vogelii* leaf extracts provides the evidence that this bioacaricides can be used to control ticks on cattle within the village environment of poor resource livestock farmers. The (results in figure 19) indicates good performance of the extracts in killing the ticks 24hours after treatment. The findings revealed that *T. vogelii* leaf extract is therefore highly recommended for use as bioacaricide at a lower concentration of 5 percent instead of 10 percent which is currently recommended. The utilization of the biomass can be reduced in order to allow more leaves to grow on the plant. The observations on the safety of extracts showed that the extract is not likely to put the animals at risk when sprayed. The plant is cheap and easy to propagate and it is a perennial plant that can last for 3 to 4years. The fields of the *Tephrosia* plants requires protection from domestic animals since they eat the leaves.

6.2 Recommendations

1**.** From this study, it can be recommended that *T. vogelii* plant leaf extracts be considered for use as a bioacaricide even at the lower concentration of 5% w/v to control of ecto-parasites on cattle in the field condition.

2. Further checking of the leaf performance at every growth stage must be evaluated for its effectiveness.

3. The absorption of the ingredient may differ between different tick species and the bioacaricide thus, presenting varying results. It is therefore recommended that more work be done to asses such variations if any. It is also important to carryout studies on possible development of resistance to potential bioacaricide.

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APPENDIX

7.1 Apendice 1: Questionnaire

KNOWLEDGE ON TRADITIONAL CONTROL OF TICKS AND TICK BORNE DISEASES IN MONZE DISTRICT OF SOUTHERN PROVINCE OF ZAMBIA.

School of Veterinary Medicine, University of Zambia

Preamble

The purpose of this survey is to assess the importance of ticks to the livestock sector in this region. The results of the survey will complement the findings of a larger project on the use of *Tephrosia vogelii* (Ububa) plant in the control of ticks in Monze District.

Your participation in this survey is extremely important because your infomation will provide a basis, if any, to look for alternative and sustainable strategies for tick and tick borne disease control in Monze District.

Thank you for taking the time to participate in this survey.

SECTION 1.

A. IDENTIFICATION INFORMATION

Questionnaire Number:…….

Date of interview:……..

Numerator…….

Farm location:

District	Veterinary camp	Village/Crush pen

Name of Farmer……..…….

Address……..……..

Year of establishment……..

Interviewee's name of if different from owner and relationship……

B.HERD STRUCTURE AND SIZE

1. What type of animals do you have on this farm?

Cattle	Sheep	Goats	Pigs	Dogs	Chickens

2. How many cattle do you have presently on this farm?

Cattle Category	Number	Breeds
Oxen		
Bulls		
Cows		
Heifers		
Steers		
Calves		
Total		

C. EXTENSION SERVICES

Q1.Do you receive any veterinary services (Animal Health extension services)?

0). Yes ☐ 1). No ☐

Q2. Who are the provider(s) of animal health extension veterinary services in your area?

0)Veterinary Department…… ☐

1) Community livestock Auxiliary…… ☐

2) NGO (Provide name).....…… ☐

3) Others specify……..… ☐

Q3. What is your comment on the quality of service you receive?

0) Excellent……..…..…….……. ☐

1) Very good……..…… ☐

2) Good…….. ☐

3) Poor …… ☐

4) Extremely Poor……. ☐

Q 4. If you are currently not accessing animal health extension give reasons why?

.....

……

Q5. Is there any training in livestock management that a member of your family has been involved in?

0)Yes …… ☐

1)No…… ☐

D. HERD DYNAMICS

Q6.Do you take animals to somewhere for grazing?

0) Yes……... ☐

1)No…….. ☐

Q7.If yes specify where you take the animals?**.....**

……

Q8. Do your animals come into contact with wild animals?

0) Yes……..…. ☐

1) No…….. ☐

If yes, specify the type of wild animals……

Q9. For how long do your animals come into contact with wild animals?

0) Less than a month…… ☐

1) 3-6 months…… ☐

2) All year round…… ☐

Q10. Did you experience any cattle death on this farm due to tick-borne diseases?

0) Yes…… ☐

1) No…… ☐

Specify?.....

E. TICK CONTROL

Q11. Do you see ticks on your Animals?

0) Yes…… ☐

1) No…… ☐

Q12. What are the different types of ticks you find on your cattle and goats?

0) Bont tick…….. ☐

1) Blue tick…… ☐

2) Bont Legged…… ☐

3) Red legged……. ☐

4) Brown ear……. ☐

Q13. When do you mostly see ticks on your animals?

0) Jan –March…….……

1) April-June…… ☐

2) July-September……. ☐

3) October-December…….…….. ☐

Q14. Where do you see ticks on the animal?

0) Head…….……. ☐

1) Ears…….……. ☐

2) Udder…….…….. ☐

3) Legs…….…… ☐

4) Whole body……. ☐

Q15. What are the different types of ticks you find on your cattle and goats?

0) Bont tick…….. ☐

1)Blue tick…… ☐

2) Bont Legged……..…… ☐

3) Red legged……. ☐

4) Brown ear……. ☐

Q16. How far is the cattle dip from your village (how long does it take to walk to the dip?)

0) 1 km ……. ☐

1) 2-3 km…… ☐

2) 4 km…… … ☐

3) 5km……. ☐

4) 10km…… ☐

Q17. What problems do ticks bring to your animals…….

……..

Q18. Do you know any Tick borne diseases that are brought by tick bites?

0) Yes…… ☐

1) No…… ☐

Q19. Which are these diseases?

……

……

Q20. Do you practice any tick control on your animals?

0) Yes…… ☐

1) No…… ☐

If answer to Q 20 is Yes, proceed to Q22-77

Q21. If your answer to Q20 is 'No' what are your reasons for not practising tick control?

……

…….

Q22. What are the methods you use to control ticks?

0) Dipping…… ☐

1) Spraying……. ☐

2) Spot on……. ☐

3) Others, specify…… ☐

Q23. What are the common acaricide (dip) that you use in tick control of your herd?

……

……

……

Q24How much of dip (commercial acaricides) do you need to dip your animals?

0) 5 litres…… ☐

1) 10 litres……. ☐

2) 20 litres…….. ☐

3) Other, specify……. ☐

Q25. How often do you send dip wash samples for checking at the veterinary laboratory in?

0) Once in a week……. ☐

1) Once in 2 weeks…….…….. ☐

2) Once in 3 weeks…… ☐

3) Once in 4 weeks…….. ☐

4) None…… ☐

Q26. How much does dipping cost you in Kwacha per year?

……

……

Q27. Of the method(s) of tick control you have stated, what is your frequency like?

0) Once in a week…… ☐

1) Once in 2 weeks…….. ☐

2) Once in 3 weeks…… ☐

3) Once in 4 weeks…… ☐

4) Irregular…… ☐

Q28. Is your tick control method

0) All year round?..... ☐

1) Restricted to certain periods of the year?……. ☐

Q29. If your tick control is restricted to a period or periods which is this?

0) Jan –March……. ☐

1) April –Jun…… ☐

2) Jul-Sep…… ☐

3) Oct- Dec…….. ☐

F. TRADITIONAL METHODS OF TICK CONTROL

Q30. Are there ways of tick control that our ancestors practiced?

0) Yes…… ☐

1) No…… ☐

Q31. If answer to Q1 is YES, which……

……

Q32. Where did you hear from?

0) Parents/Elders……..……. ☐

1) Books……. ☐

2) Radio……..…… ☐

3) Friends……..…… ☐

4) Others, specify…… ☐

Q34. When did you first hear of these methods?

0) 20 years ago……. ☐

1) 10 years ago…….. ☐

2) 5 years ago…… ☐

3) 2 years ago…… ☐

4) Last year 2009…….. ☐

Q35. Which ones are these methods?

……

……

……

Q36. Of these methods mentioned, which one do you practise in your herd to control ticks?

0) Using plants……

1) Dung…… ☐

2) Tobacco…….. ☐

3) Used engine Oil…….. ☐

4) Paraffin…… ☐

5) Other specify…… ☐

Q37. If they are plants, do you know the name of plants?

0) Yes……...

1) No…….. ☐

Q38. What are names of these plants in local language used to control ticks?

……

……

Q39. Did you have good results for using these plants to control ticks?

0) Yes……

1) No……..

G. DISEASES

Q40. Do you have problems of Tick-borne diseases in your area?

0) Yes……..

1) No……

Q41. Which Tick borne diseases are common in your area?

0) East Coast fever (Denkete)…….

1) Heartwater……..…….

2) Anaplasmosis…….

3) Babesiosis……

Q42. What signs do you see on the sick animals?

0) Loss of appetite……..……..

1) Loss of weight……..

2) Diarrhoea…….

3) Swollen lymphnodes……

4) Others, specify……

End of Interview