In Vitro Studies on the Anti-tick Properties of Selected Plant Species Against Veterinary and Economically Important Ticks in South Africa

by

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ABSTRACT

The study examined the anti-tick properties of the extracts of *Aloe ferox*, *Leonotis leonurus* and *Thymus vulgaris* against *Amblyomma hebraeum* and *Rhipicephalus appendiculatus*. The extensive literature review as captured in chapters 2 to 4 of this study provides a critical outlook on the current understanding of the structural and behavioural features of ticks which enhance their parasitic way of life and their competence as vectors of various disease causing agents. In addition, the current tick control methods were explored with the specific intent to highlight their strengths and shortfalls. The literature review reveals that the current tick control methods are at the most partially effective since tick infestations on livestock and other animals continue to cause enormous economic losses particularly in developing countries.

In this study, the acetone, ethanol and methanol extracts of *A. ferox*, *L. leonurus* and *T. vulgaris* were tested for anti-tick properties using modified versions of the *in vitro* petri dish and tick climbing bioassays to determine their repellency against adults of *Am. hebraeum* and *Rh. appendiculatus* ticks. *In vitro* models offer a viable alternative to the use of animal models. Results from the choice chamber bioassay used in this study show that the most repellent extracts against both tick species included 20% acetone extract of *A. ferox* (percentage repellency: 56.10%), 10% ethanol extract of *L. leonurus* (percentage repellency: 85.58%) and 10% acetone extract of *T. vulgaris* (percentage repellency: 99.59%). Results obtained using the tick climbing bioassay affirmed the observations from the choice chamber bioassay against *Rh. appendiculatus*. In particular data obtained from this study positions acetone extracts (EC_{50} = 2.49%), ethanol (EC_{50} = 21.47%) and methanol (EC_{50} = 0.89%) of *T. vulgaris* and ethanol extract (EC_{50} = 7.33%) and methanol extract (EC_{50} = 4.83%) of *L. leonurus* as potential repellent candidates against *Am. hebraeum* and *Rh. appendiculatus* ticks and should be further explored as repellents during *in vivo* studies. The GC-MS analysis conducted in this study revealed that *A. ferox* extracts had the lowest number of compounds (22) known to have anti-arthropod properties compared to extracts of *L. leonurus* (30)

and *T. vulgaris* (39) suggesting that it is the number of these compounds in an extract that contributes towards the repellent strength compared to the amount of each individual compound.

Key words: *Aloe ferox*, *Leonotis leonurus*, *Thymus vulgaris*, *Amblyomma hebraeum*, *Rhipicephalus appendiculatus*, *in vitro*, petri dish bioassay, tick climbing bioassay, repellency, tick control, gas chromatography, biological active compounds

OPSOMMING

Die studie het die teen-bosluis eienskappe van die ekstrakte van *Aloe ferox*, *Leonotis leonurus* en *Thymus vulgaris* teen *Amblyomma hebraeum* en *Rhipicephalus appendiculatus* ondersoek. Die omvattended literatuuroorsig, soos beskryf in hoofstukke 2 tot 4 van die studie, verskaf ‘n kritiese oorsig oor die huidige begrip van die strukturêle asook die gedrags kenmerke van bosluise wat hul parasitiese leefwyse en hul vermoë om as vektore van verskeie organismes verantwoordelik vir siektes op te tree moontlik maak. Daarbenewens is die huidige bosluis-beheer metodes ondersoek met die spesifieke voorneme om hul sterkpunte en tekortkominge te identfiseer. Die literatuuroorsig het getoon dat die huidige bosluis-beheer metodes slegs gedeeltelik effektief is aangesien bosluis infestasies van vee en ander diere steeds bydra tot enorme ekonomiese verliese veral in ontwikkelende lande.

In die studie was die asetoon, etanol en metanol ekstrakte van *A. ferox*, *L. leonurus* en *T. vulgaris* getoets om hul teen-bosluis eienskappe te bepaal deur gebruik te maak van gewysigde weergawes van die *in vitro* petri bakkie en bosluis-uitklim essaiërings deur hul afwering van volwasse *Am. hebraeum* en *Rh. appendiculatus* bosluise te toets. *In vitro* modelle verskaf doeltreffende alternatiewe teenoor die gebruik van diere modelle. Resultate van die keuse-afdeling petri bakkie essaiëring gebruik tydens die studie het getoon dat die mees afwerende ekstrakte teen beide spesies die 20% asetoon ekstrak van *A. ferox* (persentasie afwering: 56.10%), 10% etanol ekstrak van *L. leonurus* (persentasie afwering: 85.58%) en 10% asetoon ekstrak van *T. vulgaris* (persentasie afwering: 99.59%) ingesluit het. Restulte verkry deur die bosluis-uitlim essaiëring het die waarnemings tydents die keuse-afdeling petri bakkie essaiëring teen *Rh. appendiculatus* bevestig. Die data verkry uit die studie dui op die asetoon ekstrakte (EC_{50} = 2.49%), etanol ekstrakte (EC_{50} = 21.47%) en metanol ekstrakte (EC_{50} = 0.89%) van *T. vulgaris* en etanol ektrakte (EC_{50} = 7.33%) en metanol ekstrakte (EC_{50} = 4.83%) van *L. leonurus* as moontlike afwerende kandidate teen *Am. hebraeum* en *Rh. appendiculatus* bosluise en moet verder ondersoek word as afweerders deur *in vivo* studies. Die GC-MS analise gedoen tydens die studie het getoon dat *A. ferox* ekstrakte die laagste aantal

samestellings (22) geassosieër met teen-geleedpottige eienskappe het invergeleke met die ekstrakte van *L. leonurus* (30) en *T. vulgaris* (39) wat voorstel dat die aantal samestellings in ‘n ekstrak bydra tot die afweringsterkte invergelyking met die hoeveelheid van individuele samestellings.

Sleutelwoorde: *Aloe ferox*, *Leonotis leonurus*, *Thymus vulgaris*, *Amblyomma hebraeum*, *Rhipicephalus appendiculatus*, *in vitro*, petri bakkie essaiëring, bosluis-uitklim essaiëring, afwering, bosluit beheer, gas chromatogafie, biologiese aktiewe samestellings

TSHOSOBANYO

Thuto e, e sekasikile boleng ba dithlotlhoa tsa kgopa (*Aloe ferox*), motekwane wa naga (*Leonotis leonurus*) le setlhatshana sa Thime (*Thymus vulgaris*) kgatlhanong le kgofa ya mohuta wa mebala (*Amblyomma hebraeum*) le kgofa e tshetlha ya tsebe (*Rhipicephalus appendiculatus*). Puiso e e tseneletseng ya dikwalo jaaka e supilwe mo kararolong ya bobedi go fitlha ya bone, e neela tshupo e e sekasikilweng mabapi le leago le mekgwa ya dikgofa e e tiisang mokgwa wa go ba setshedi se se tshelang ka tse dingwe (parasite) le bokgoni jwa sona go fitisa ditwatsi. Mo tlaleletsong, tsela tsa ga jaana tsa go laola dikgofa di sekasikilwe ka maikemisetso a go supa bokgoni le makowa a tsona. Tshekatseko ya dikwalo e supa go re tsela tsa ga jaana tsa go laola dikgofa ga di na bokgoni jo be feleletseng ke ka tsela eo dikgofa di tsweletseng go dira go re go nne le ditatlhegelo tsa ikonomi se golobogolo mafatsheng a a ntseng a gola.

Mo thutong e, ditlhothloa tsa asetone, ethanole le methanole tsa kgopa (*A. ferox*), motekwana wa naga (*L. leonurus*) le setlhatsana sa Thime (*T. vulgaris*) di sekasekilwe go batlisisa fa di nale boleng kgatlhanong le dikgofa. Mo tshekatshekong e, go dirisitswe mananeo a a fetotsweng a a akaretsang tiriso ya sekotlojana le mokgwa wa go pagama go netefatsa ga ditlhotlhoa tse di na le boleng ba go koba dikgofa (*A. hebraeum* le *R. appendiculatus*). Mekgwa ya dipatlisiso e e sa diriseng diphologolo, ke tsela e e farologaneng ya go dira dipatlisiso. Dipoelo tsa tiriso ya mokgwa wa sekotlojana e e dirisitsweng mo thutong e, e supa go re dithlotlhoa tse di neng di le matla kgatlhanong le dikgofa di akaretsa tsa 20% asetone ya kgopa (*A. ferox*) (di thibela ka diperesente tse 56.10), 10% ethanole ya motekwane wa naga (*L leonurus*) (e thibela ka diperesente tse 85.58) le 10% acetone ya setlhatshana sa Thime (*T. vulgaris*) (e thibela ka diperesente di le 99.59). Dipoelo tse di fitlheletsweng fa go dirisiwa lenaneo la go pagama, di tiisa tse di bonweng ka mokgwa wa se kotlojana mabapi le *Rh. appendiculatus*. Ka go nepagala, dipoelo tse di fitlhetsweng, di supa ditlhotlhoa tsa asetone (EC_{50} = 2.49%), enthanole (EC_{50} = 21.47%) le methanole (EC_{50} =0.89%) tsa *T. vulgaris* le tsa ethanole (EC_{50} = 7.33%) le methanole (EC_{50} = 4.83%) tsa *L. leonurus* e le tse di na leng boleng ba go thibela dikgofa tsa *Am hebraeum* le *Rh appendiculatus*, ka jalo di ka sekasekiwa go diriswa mo diphologolong. Dipatlisiso ka GC-MS mo thuton

e, di supa go re ditlhotlhoa tsa kgopa (*A. ferox*) di ne di na le palo e e ko tlase (22) go farologana le 30 ya motekwane wa naga (*L. leonurus*) le 39 ya setlhatshana sa Thime (*T. vulguris*). Se, se tshitshinya go re ke nomore ya dikompone e e natlafatsang ditlhothloa kgatlhanong le dikgofa go na le bontsi ba dikompone.

Mantswe a senotlolo: Kgopa (*Aloe ferox*), motekwane wa naga (*Leonotis leonurus*), setlhatshana sa Thyme (*Thymus vulgaris*), kgofa ya mebala (*Amblyomma hebraeum*), kgofa e tshetlha ya ditsebe (*Rhipicephalus appendiculatus*), Go ntle le tiriso ya diphologolo (*in vitro*), lenaneo la sekotlojana (petri dish bioassay), lenaneo la go pagama ga kgofa (tick climbing bioassay), go thibela (repellency), taolo ya dikgofa (tick control), pharologanyo ya dikomone ka mowa (gas chromatography), dikompone tse di dirang (biological active compounds)

Table of Contents

LIST OF FIGURES

LIST OF TABLES

ABBREVIATIONS AND ACRONYMS

AIT	Adult immersion test
A. ferox	*Aloe ferox*
Am. cajennense	*Amblyomma cajennense*
Am. hebraeum	*Amblyomma hebraeum*
ANOVA	Analysis of Variance
ATP	Adenosine triphosphate
B. bovis	*Babesia bovis*
B. bigemina	*Babesia bigemina*
BHC	Benzenehexachloride
C. aurea	*Calpurnia aurea*
CO_2	Carbon dioxide
CPD	Citrate-phosphate-dextrose
DCM	Dichloromethane
DDT	Dichlorodiphenyl trichloroethane
DEET	N,N-diethyl-m-toluamide
DEM	Mandelic acid diethyl amide
DEPA	*N,N*-diethyl-2-phenyl-acetamide
DMP	Dimethyl phthalate
EDTA	Ethylenediaminetetraacetic acid
E. ruminantium	*Ehrlichia ruminantium*
FAO	Food and Agriculture Organization of the United Nations
GC	Gas chromatography
GCxGC-TOF-MS	Gas Chromatography x Gas Chromatography Time of Flight Mass Spectrometer
G. gynandra	*Gynandropsis gynandra*
Hy. rufipes	*Hyalomma rufipes*
Hy. truncatum	*Hyalomma truncatum*
Ix. ricinus	*Ixodes ricinus*
L. leonurus	*Leonotis leonurus*
LIT	Larval immersion test
LPT	Larval packet test
Neem	*Azadirachta indica*

Rh. appendiculatus	*Rhipicephalus appendiculatus*
Rh. (Bo.) decolaratus	*Rhipicephalus (Boophilus) decolaratus*
Rh. (Bo.) microplus	*Rhipicephalus (Boophilus) microplus*
Rh. evertsi evertsi	*Rhipicephalus evertsi evertsi*
Rh. sanguineus	*Rhipicephalus sanguineus*
Rh. simus	Rhipicephalus simus
TIC	Total Ion Chromatograph
TLC	Thin Layer Chromatography
T. vulgaris	*Thymus vulgaris*
w/v	Weight per volume

CHAPTER 1
THE RESEARCH PROBLEM

1.1 Background

Ticks are blood feeding ecto-parasites of amphibian, reptilian, avian and mammalian hosts throughout the world (Walker *et al.,* 2003; Jongejan and Uilenberg, 2004). They belong to the phylum Arthropoda, class Arachnida, order Acari and sub-order Ixodida (Walker *et al.,* 2003). To date 907 valid Ixodida species have been identified and are divided into three families namely, the Argasidae (soft ticks) consisting of approximately 191 species, Ixodidae (hard ticks) consisting of approximately 720 species and Nuttalliellidae which includes only one species (Barker and Murrell, 2004; Barker and Murrell, 2008; Pfäffle *et al.,* 2013). Olivier (1989) estimated that approximately 10% of all ticks are of concern to humans and livestock due to their parasitic nature (direct impact) and their potential to transmit diseases (indirect impact). Waladde *et al.* (1996) estimated 650 species of Ixodidae which are important vectors of diseases, with genera *Amblyomma, Hyalomma*, and *Rhipicephalus* (which include the genus previously classified as *Boophilus*), considered to be amongst the most economically important (Minjauw and McLeod, 2003; Rajput *et al.,* 2006; Mapholi *et al.,* 2014). Of the approximately 80 ixodid tick species found in South Africa, four namely *Amblyomma hebraeum*, *Rhipicephalus appendiculatus*, *Rhipicephalus (Boophilus) decoloratus* and *Rhipicephalus (Boophilus) microplus* are considered to be the most important vectors of cattle disease (Nyangiwe *et al.,* 2011).

Ticks can affect their hosts, directly by rendering them anaemic, transmitting salivary toxins which can cause paralysis, and damaging their hide (Sonenshine, 1991; de Castro, 1997; Walker *et al.,* 2003; Jongejan and Uilenberg, 2004). Indirect effects are associated with transmitting disease-causing agents such as bacteria, fungi, viruses and protozoans (Sonenshine, 1991; Jongejan and Uilenberg, 2004, Mapholi *et al.,* 2014); and / or by causing wounds which permit secondary infection by opportunistic pathogens (Sonenshine, 1991; Walker *et al.,* 2003; Jongejan and Uilenberg, 2004). According to Dantas-Torres *et al.* (2012) various domestic animals may also be hosts to pathogen infected ticks

which could in effect also represent a risk to humans, making this an important aspect to consider when planning tick control and strategies against tick-borne diseases. Consequently, effective tick control methods are needed in order to reduce the impact of ticks on animals and man.

1.2 Hypothesis

The hypothesis for this study was:

Plant compounds with anti-tick properties can alter the questing behaviour of ticks and may serve as agents for effective tick control.

1.3 Justification

As indicated in the foregoing paragraphs ticks are considered to be a problem with regard to the direct and indirect impacts they cause to animals and humans, resulting in high losses of revenue occurring from the loss of livestock (Bishop *et al.,* 2004; Jongejan and Uilenberg, 2004; Kiss *et al,* 2012; Mapholi *et al.,* 2014). Current control methods rely largely on the use of synthetic acaricides and are not entirely effective since tick infestations on livestock and the diseases they transmit continue to be a problem globally (Minjauw and McLeod, 2003; Adenubi *et al.,* 2012; Spickett 2013). Furthermore, the reliance on synthetic acaricides is fraught with problems including high costs of acaricides which cannot be afforded by small-scale farmers, environmental pollution and contamination of animal products by chemical residues and the emergence of tick strains resistant to acaricides (Kaaya *et al.,* 1995; de Castro, 1997; Tisdell *et al.,* 1999; Minjauw and McLeod, 2003; FAO, 2004; Zorloni *et al.,* 2010; Mapholi *et al.,* 2014). Therefore, there is a need to explore alternative means of tick control which are effective and exclude pollution and contamination of the environment as well as animal products destined for human consumption.

Two of the most important avenues in tick research include the testing and development of tick control methods and / products (Willadsen and Kemp, 1988; de Castro, 1997; Minjauw and McLeod, 2003; FAO, 2004; Ghosh *et al.,* 2007; Mapholi *et al.,* 2014), as well as investigation of tick-borne diseases and their transmission (Kröber and Guerin, 2007a and 2007b).

According to Costa-da-Silva *et al.* (2014) bioethical evaluation of research involving animals should consider the '3Rs'-principle of replacement, reduction and refinement focused on animal welfare. In research involving haematophagus arthropods, sufficient quantities of blood are required to allow for the completion of their life cycle. As a result, laboratory animals including mice, guinea pigs, or rabbits are generally used, and the protocols should therefore include information on anesthetization or immobilization (Costa-da-Silva *et al.,* 2014). While performing tests on live animals provides a constant availability of blood at the correct temperatures and required stimuli (Kasap *et al.,* 2003), *in vivo* studies are influenced by a number of limiting factors. For example, *in vivo* studies aimed at testing animal health products against ticks require a large quantity of animals and test material. Therefore, in addition to ethical considerations, there are high costs involved with the maintenance of the host animals (Kuhnert, 1996; Eckert, 1997; Kasap *et al.,* 2003; Kröber and Guerin, 2007a), and the requirement of specialised facilities and staff are a problem which contribute to the cost during development (Eckert, 1997; Kröber and Guerin, 2007a). Also, strict legal regulations put a constraint on many investigations that are reliant on animals (Eckert, 1997). Bioassays using live hosts, for example, might also place the host animals and / or humans, at risk of tick-borne diseases (Kasap *et al.,* 2003; Bissinger and Roe, 2010) or cause hypersensitivity (Kasap *et al.,* 2003). Nonetheless, it is acknowledged that laboratory assays using live hosts might have a reduced chance of disease transmission (Bissinger and Roe, 2010), due to a more controlled environment in the laboratory compared to field assays. Field trials with ticks are also associated with several other disadvantages, including, difficulty in separating effects of different ticks, general conditions of production and herd structure (de Castro *et al.,* 2007). These considerations influenced and guided the choice of bioassays used in this study.

In vitro models offer a viable alternative (Kröber and Guerin, 2007b; McGaw and Eloff, 2008) to the use of animal models. The most commonly used *in vitro* approaches in tick repellency and / or acaricidal studies include the larval packet test (LPT), adult immersion test (AIT), larval immersion test (LIT) (FAO, 2004), the petri dish assay (Dautel, 2004; Anisuzzaman *et al.,* 2005; Nchu *et al.,* 2005; Del Fabbro and Nazzi, 2008; Mkolo *et al.,* 2011a; Mkolo *et al.,* 2011b), and climbing bioassays (Mkolo and Magano, 2007; Zorloni *et al.,* 2010; Magano *et*

al., 2011b; Nchu *et al.,* 2012). Additional *in vitro* bioassays include the use of *in vitro* (artificial) feeding devices (Kröber and Guerin, 2007a and 2007b). However, these are generally not used for the exploration of repellent and / or acaricidal products. *In vitro* studies, are important avenues, which should form the first option used to explore if compounds or products tested will have an effect prior to introduction to live hosts as well as playing a role in determining costs and thereby provide an opportunity to evaluate the overall effectiveness of a product prior to additional testing.

In an effort to expand the scientific database of effective botanical repellent sources, the repellent effect of extracts from three selected plant species, namely *A. ferox*, *L. leonurus*, and *T. vulgaris,* were tested against *Am. hebraeum* and *Rh. appendiculatus*, which are two of the important tick species in South Africa. In addition, the study also explored modifications to commonly used *in vitro* repellent bioassays in order to improve the sensitivity of the bioassays for future use.

1.4 Study Significance

Data collected from this study may extent the options for *in vitro* bioassays, to include the modified versions to the conventional petri dish and tick climbing bioassays. In addition, data collected may also extent the current understanding of how plant based compounds affect the behaviour of ticks during *in vitro* repelling studies. Furthermore, this study may be used as a precursor of an *in vivo* study, to further test botanical extracts as repellents, by ensuring that only effective and successful botanical extracts are included during the planning of *in vivo* bioassays. Following this as an initial approach has a dual benefit as firstly it will eliminate the possibility of unnecessarily exposing animals to products that are not effective against ticks and secondly it will reduce the number of animals required for an *in vivo* study or bioassay.

1.5 Study Goal

This study proposed to examine the repellent properties of three selected plant species namely, *Aloe ferox*, *Leonotis leonurus* and *Thymus vulgaris* against *Amblyomma hebraeum* and *Rhiphicephalus appendiculatus* and to evaluate the use of the modified petri dish and tick climbing bioassays for tick repellency. To attain this goal, the following objectives were formulated:

1.5.1 Study Objective One

The first study objective focused on an in-depth literature study to reveal the current understanding and limitations of knowledge on ticks, their impact and control.

1.5.2 Study Objective Two

The second study objective focused on testing and comparing the extracts of *A. ferox*, *L. leonurus* and *T. vulgaris* for their tick repellent strengths, and to determine their potential as anti-tick agents. To achieve this objective *Am. hebraeum* and *Rh. appendiculatus* adults were exposed to various concentrations of the extracts of *A. ferox*, *L. leonurus* and *T. vulgaris*, using modified *in vitro* petri dish (for both species) and tick climbing (for only *Rh. appendiculatus*) repellency bioassays.

1.5.3 Study Objective Three

The third study objective focused on the separation and characterisation of the biologically active compounds from plant extracts and to determine the associated repellent properties of these plant extracts. To achieve this objective, all plant extracts used during the study were analysed using Thin Layer Chromatography (TLC) as well as Gas Chromatography (GC).

1.6 Document outline

The structure of the thesis is outlined as follows:

I. **Chapter One** provides a case for the study by justifying and highlighting the need for investigating alternative tick control methods that are effective yet friendly to the environment and accessible even by small-scale livestock farmers.

II. **Chapter Two** provides an in-depth literature review on the structural and behavioural adaptations of ticks which enhance their parasitic way of life. In this chapter it is argued that knowledge on these biological aspects of ticks is foundational in any effort aimed at designing and developing effective tick control methods.

III. **Chapter Three** provides and in-depth literature review on the veterinary, medically and economic importance of ticks as reinforcing the need to find alternative tick control methods that are effective and safer to the environment.

IV. **Chapter Four** provides a critical review of the current tick control practices and explores the potential impact of botanicals as alternative sources of anti-tick agents.

V. **Chapter Five** examines and compares the repellent properties of extracts of *A. ferox*, *L. leonurus* and *T. vulgaris* against *Am. hebraeum* and *Rh. appendiculatus* using different bioassays.

VI. **Chapter Six** provides data on chromatographic separation of the compounds in the extracts of *A. ferox*, *L. leonurus* and *T. vulgaris* and identifies those known in literature to be having tick repellent properties.

VII. **Chapter Seven** provides a general discussion on the findings of the current study and gives recommendations for future research.

In the next chapter, the structural, functional and behavioural adaptations of ticks which enhance a hematophagous ecto-parasitic way of life will be described.

CHAPTER 2
LITERATURE REVIEW:
STRUCTURAL AND BEHAVIOURAL ADAPTATIONS OF TICKS WHICH ENHANCES THEIR PARASITIC WAY OF LIFE

Ticks are second only to mosquitos as parasites and vectors of disease-causing agents to man and livestock (Sonenshine, 1991; Sonenshine *et al.,* 2002). This assertion implicates that ticks are well suited structurally, functionally and behaviourally for a parasitic way of life and their adaptations reflects the extent of their success as ecto-parasites. This chapter describes the structural, functional and behavioural adaptations of ticks which enhance their parasitic mode of life. An indepth understanding on these biological aspects of ticks is foundational in guiding and informing the development of any tick control strategy.

2.1 Tick Taxonomy

As indicated in the preceding chapter, there are three families of ticks, namely Ixodidae (hard ticks), Argasidae (soft ticks) and Nuttalliellidae (monotypic). The latter family has a single species, *Nutalliella namaqua*, the description of which until recently was limited to the nymphal and adult female stages only (Olivier, 1989). In the recent past, Latif *et al.* (2012) described for the first time the larval and male stages which were previously not known. The family Argasidae has the genera *Argas*, *Antricola*, *Carios*, *Ornithodoros*, and *Otobius* (Barker and Murrell, 2004; Barker and Murrell, 2008) and the Ixodidae genera include *Amblyomma*, *Anomalohimalaya*, *Bothriocroton*, *Cosmiomma*, *Cornupalpatum*, *Dermacentor*, *Haemaphysalis*, *Hyalomma*, *Ixodes*, *Margaropus*, *Nosomma*, *Rhipicentor*, and *Rhipicephalus* (Barker and Murrell, 2004; Barker and Murrell, 2008). The Ixodidae can further be sub-divided into six sub-families namely, Ixodinae (*Ixodes*), Amblyomminae (*Amblyomma* and *Aponomma*), Haemaphysalinae (*Haemaphysalis*), Hyalomminae (*Hyalomma*), Bothriocrotoninae (B*othriocronton*) and Rhipicephalinae (*Dermacentor, Cosmiomma, Margaropus, Nosomma, Anomalohimilaya, Rhipicentor* and *Rhipicephalus*) (Sonenshine *et al.,* 2002; Nicholson *et al.,* 2009). Of these approximately 80 ixodid, 25 argasid and the one Nuttalliellid tick species have been observed in South Africa (Horak, 2009). However, for the purpose of this study, focus will be exclusively on ixodid ticks.

2.2 South African Livestock Tick Species

As indicated in Table 2.1, sixteen tick species have been identified to be associated with livestock in South Africa. The most widely distributed species include *Am. hebraeum* and *Rhipicephalus (Boophilus) microplus*, found in six provinces, *Rhipicephalus (Boophilus) decoloratus* and *Rh. appendiculatus*, found in eight provinces, and *Hyalomma truncatum* and *Rhipicephalus evertsi evertsi* found in all provinces of South Africa.

Table 2.1 Distribution of livestock tick species in South Africa

Tick species	**Province**	**Reference**
Rh. (Bo.) decoloratus *Rh. appendiculatus* *Rhipicephalus evertsi evertsi*	KwaZulu-Natal	Bryson *et al.* (2002)
Am. hebraeum *Hyalomma truncatum* *Ixodus pilosus* *Rh. appendiculatus* *Rh. (Bo.) decoloratus* *Rhipicephalus (Boophilus) microplus* *Rh. evertsi evertsi* *Rhipicephalus simus*		Walker *et al.* (2003)
Am. hebraeum *Hy. truncatum* *Ixodes rubicundus* *Rh. appendiculatus* *Rh. (Bo.) decoloratus* *Rh. evertsi evertsi*	Gauteng	Walker *et al.* (2003)
Am. hebraeum *Hyalomma rufipes* *Hy. truncatum* *Rh. appendiculatus* *Rh. evertsi evertsi* *Rh. (Bo.) decoloratus* *Rh. simus*	Limpopo province	Bryson *et al.* (2002)
Am. hebraeum *Hy. truncatum* *Ix. pilosus* *Rh. appendiculatus* *Rh. (Bo.) decoloratus* *Rh. (Bo.) microplus* *Rh. evertsi evertsi* *Rh. simus* *Rhipicephalus zambeziensis*		Walker *et al.* (2003)

Table continues on the next page

Tick species	Province	Reference
Amblyomma marmoreum *Hy rufipes* *Hy. truncatum* *Ix. rubicundus*	Free State	Bryson *et al.* (2002)
Hy. truncatum *Ix. rubicundus* *Rh. appendiculatus* *Rh. (Bo.) decoloratus* *Rh. evertsi evertsi*		Walker *et al.* (2003)
Am. hebraeum *Hy. rufipes* *Hy. truncatum* *Rh. (Bo.) decoloratus* *Rh. (Bo.) microplus* *Rh. appendiculatus* *Rh. evertsi evertsi* *Rh. simus* *Rh. zambeziensis*	North West Province	Bryson *et al.* (2002)
Am. hebraeum *Hy. truncatum* *Ix. pilosus* *Rh. appendiculatus* *Rh. (Bo.) decoloratus* *Rh. (Bo.) microplus* *Rh. evertsi evertsi*		Walker *et al.* (2003)
Am. hebraeum *Hy. rufipes* *Rh. (Bo.) decoloratus* *Rh. appendiculatus* *Rh. evertsi evertsi* *Rh. simus* *Rh. zambeziensis*	Mpumalanga	Bryson *et al.* (2002)
Am. hebraeum *Hy. truncatum* *Ix. rubicundus* *Rh. appendiculatus* *Rh. (Bo.) decoloratus* *Rh. (Bo.) microplus* *Rh. evertsi evertsi* *Rh. simus*		Walker *et al.* (2003)
Hy. truncatum *Ix. pilosus* *Rh. (Bo.) decoloratus* *Rh. (Bo.) microplus* *Rh. evertsi evertsi* *Rh. simus*	Western Cape	Walker *et al.* (2003)

Table continues on the next page

Tick species	Province	Reference
Hy. truncatum *Rh. appendiculatus* *Rh. evertsi evertsi*	Northern Cape	Walker *et al.* (2003)
Am. hebraeum *Haemaphysalis silacea* *Rh. appendiculatus* *Rhipicephalus glabrosculatum*	Eastern Cape	Bryson *et al.* (2002)
Am. hebraeum *Hy. truncatum* *Ix. pilosus* *Ix. rubicundus* *Rh. appendiculatus* *Rh. (Bo.) decoloratus* *Rh. (Bo.) microplus* *Rh. evertsi evertsi* *Rh. simus*		Walker *et al.* (2003)
Haemaphysalis aciculifer *Haemaphysalis elliptica* *Hy. rufipes* *Ix. pilosus* *Rh. (Bo.) decoloratus* *Rh. (Bo.) microplus* *Rh. evertsi evertsi* *Rhipicephalus follis* *Rh. simus*		Nyangiwe *et al.* (2011)

Am. hebraeum, Rh. appendiculatus, Rh. (Bo.) decoloratus and *Rh. (Bo.) microplus* are however considered to be the most important ticks and vectors of livestock diseases in South Africa (Nyangiwe *et al.,* 2011).

The specific features, life cycles and behaviour of these livestock associated ticks will be explored below to evaluate their role in making ticks successful as parasites and vectors of disease-causing agents.

2.3 Tick Biology

2.3.1 The Tick Mouthparts

Compared to other Acari ticks are unique as they are characterised by a large body (2 – 30 mm) (Sonenshine, 1991; Klompen *et al.,* 1996), comprising of two main regions namely, idiosoma and gnathostoma or capitulum. The gnathosoma has specialised mouthparts, comprising of a single hypostome with retrograde teeth, a pair of chelicerae and a pair of palps, attached to the basis capituli (Sonenshine, 1991; Walker *et al.,* 2003), which plays a vital role in wound creation and enhancing

attachment of ticks to the hosts to ensure successful feeding. The chelicerae with their serrated edges, are used to puncture the skin and penetrate the host. The hypostome then penetrates the wound, and acts as the initial attachment mechanism. Also, the hypostome acts as a channel through which tick saliva is injected into the host and host blood is ingested by the tick during feeding (Sonenshine, 1991). The retrograde teeth on the hypostome enhance attachment, a feature which is particularly important in ixodid ticks which spend a number of days acquiring a blood-meal from their hosts. The palps do not penetrate the host but are spread away to remain outside the skin when the tick is feeding (Sonenshine, 1991).

The mouthparts are also one of the distinguishing structures among tick species and are useful during the identification of ticks. For example, as can be seen in Figures 2.1 and 2.2, the mouthparts are either long (longirostrate) as in the case of *Am. hebraeum* or short (brevirostrate) as in the case of *Rh. appendiculatus*.

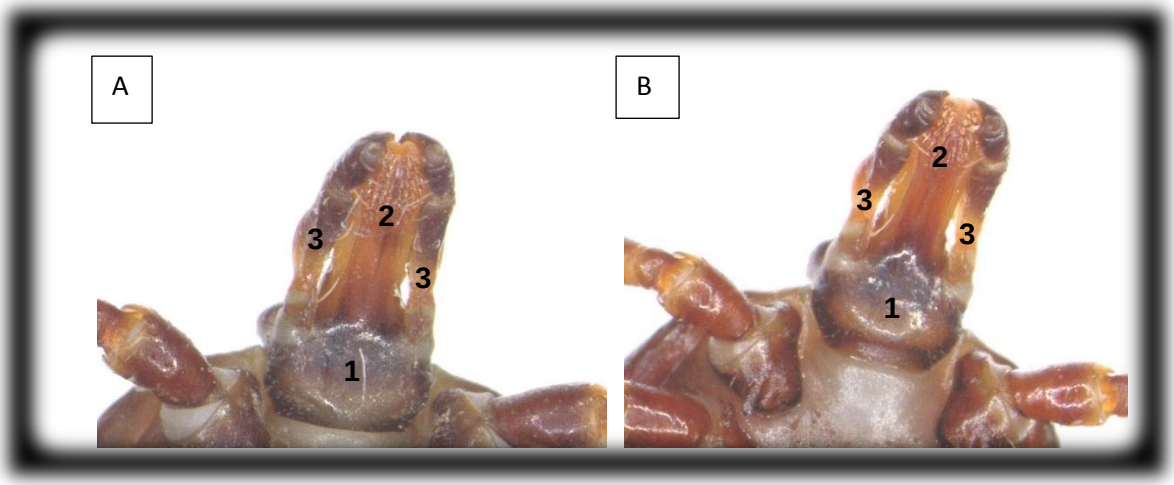

Figure 2.1 Ventral view of *Am. hebraeum* female (A) and male (B) mouthparts.
The figure shows the (1) Basis capituli, (2) Hypostome with serrated teeth, (3) Palps
Image captured by EMC Theron, July 2018, using Olympus SZX16 (Wirsam) Stereo microscope and processed using CellSense Software

The mouthparts of ticks can also cause severe damage to their hosts. For example, Walker *et al.* (2003) observed that the long mouthparts of *Amblyomma* ticks can cause scarring on teats of cattle sufficient to reduce suckling efficiency. In addition, tissue damage that result from tick infestation and the associated bacterial infections may lead to infected abscesses in cattle and sheep (Walker *et al.,* 2003; Spickett, 2013).

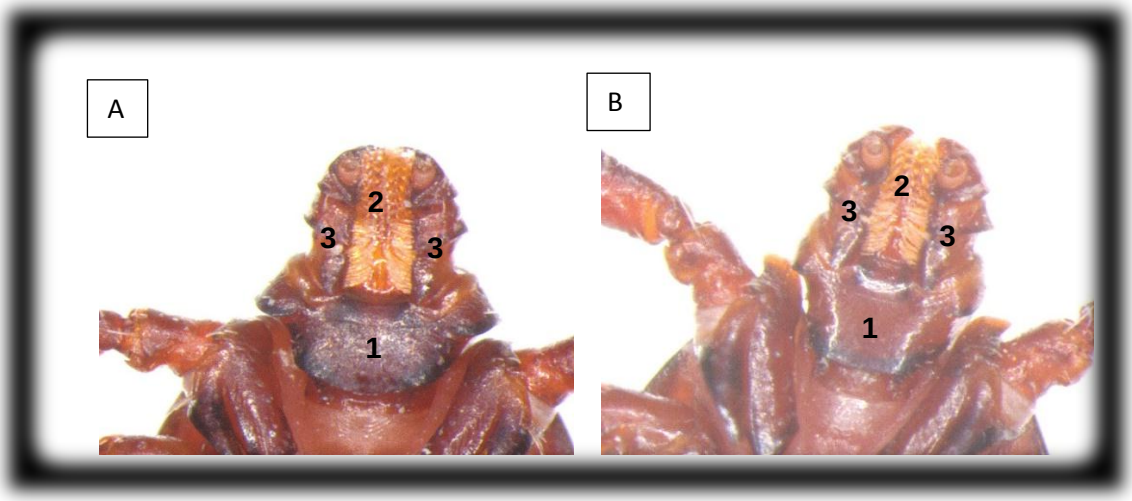

Figure 2.2 Ventral view of *Rh. appendiculatus* female (A) and male (B) mouthparts.

The figure shows the (1) Basis capituli, (2) Hypostome with serrated teeth, (3) Palps

Image captured by EMC Theron, July 2018, using Olympus SZX16 (Wirsam) Stereo microscope and processed using CellSense Software

2.3.2 Attachment to the host

In order to feed, ticks attach to the skin of their hosts using their mouthparts consisting of a pair of chelicerae, a hypostome and a pair of palps. The chelicerae and hypostome form a tube used to penetrate the skin of the host. This is possible as the chelicerae have moveable rods with sharp claws at the end that can cut a hole in the dermis and break the capillary blood vessels close to the surface of the skin, thereby forming the feeding lesion (Sonenshine, 1991; Walker *et al.,* 2003; Stafford, 2007). Larval and nymphal mouthparts are smaller, resulting in a smaller reaction from the host (Stafford, 2007). Depending on the tick the insertion of the mouthpart can take anything from 10 minutes to two hours (Stafford, 2007). A secretion, commonly known as cement, is secreted by the saliva and glues the palps to the outer epidermis and the chelicerae and hypostome to the dermis (Sonenshine *et al.,* 2002; Walker *et al.,* 2003; Stafford, 2007; Nicholson *et al.,* 2009).

2.3.3 Feeding and its role in disease transmission

All stages of ticks (larvae, nymphs and adults) are parasitic and feed only on the blood of their hosts (Walker *et al.,* 2003; Stafford, 2007; Spickett, 2013). Ticks feed on the blood and lymph that is released into the feeding lesion (Walker *et al.,* 2003; Stafford, 2007). According to Waladde and Rice (1982) feeding in ticks starts with

hunger and ends with satiation and includes a complex of sequential behavioural processes as illustrated in Figure 2.3:

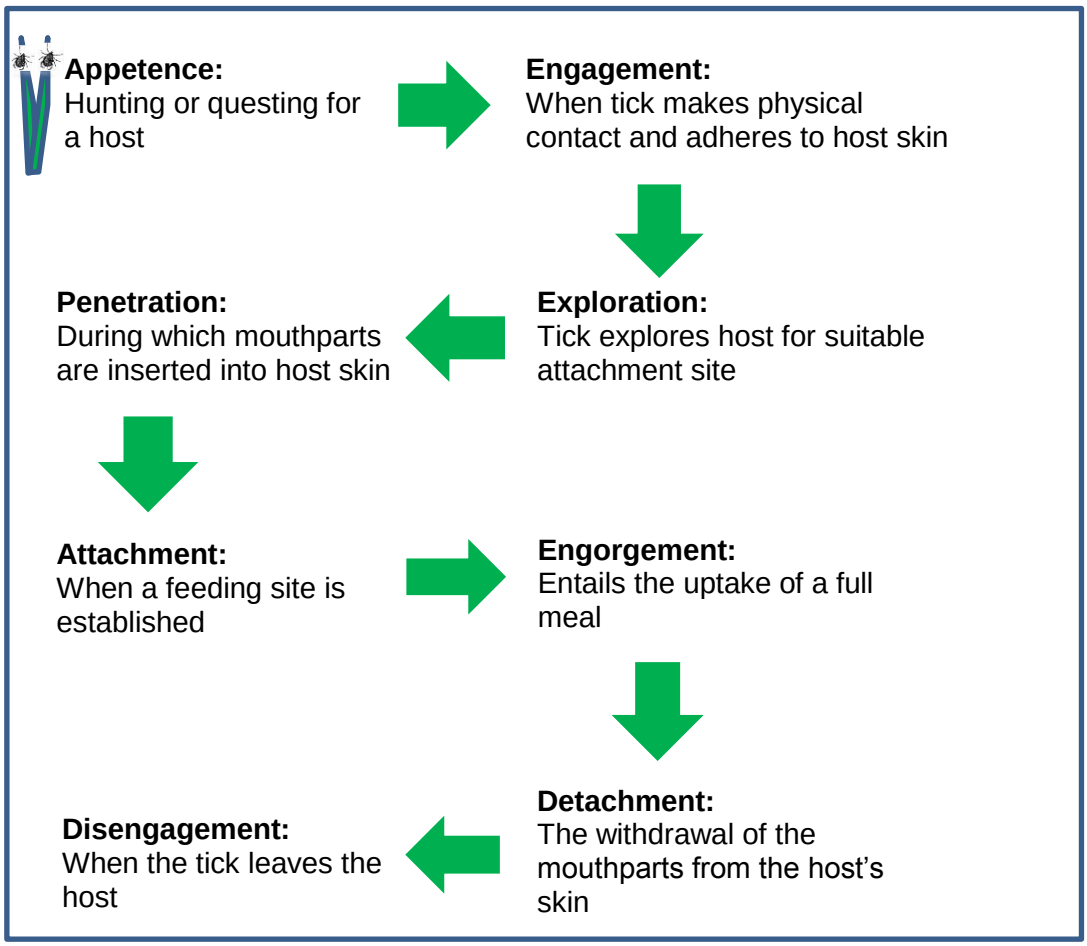

Figure 2.3 A sequence of tick behavioural processes from hunger to satiation (Adapted from: Waladde and Rice, 1982)

Ixodid ticks feed slowly as their body wall needs to grow to accommodate the expansion due to the large blood meals they ingest (Olivier, 1989; Sonenshine, 1991; Sauer *et al.,* 1995; Kuhnert, 1996; Walker *et al.,* 2003; Samish *et al.,* 2004; Rajput *et al.,* 2006; Samish *et al.,* 2008). The soft integument grows, unfolds and stretches to accommodate the changes in body size due to high volume blood ingested during feeding (Needham and Teel, 1991). Ixodid ticks ingest a small volume of blood during the first days of feeding, but during this period a number of physiological changes, including maturation of salivary glands, synthesis of procuticle in females and emission of pheromones occur (McMullen *et al.,* 1983; Kuhnert *et al.,* 1995; Kuhnert, 1996).

The feeding period of ixodid ticks comprises of three phases namely a preparatory feeding phase, during which the female inserts her mouthparts, establishes the feeding lesion and secretes cement to secure attachment to host epidermis, followed by a slow feeding phase and a rapid feeding phase (Weiss and Kaufman 2001; Kaufman, 2004; Kaufman, 2010). During the slow feeding phase, the female gradually expands to approximately ten times the unfed weight and mating also occurs during this period. In the final phase, the rapid feeding phase, the female increases in weight by an additional ten-fold, resulting in a hundred times increase from the original unfed weight, after which the female detaches from the host (Figure 2.4).

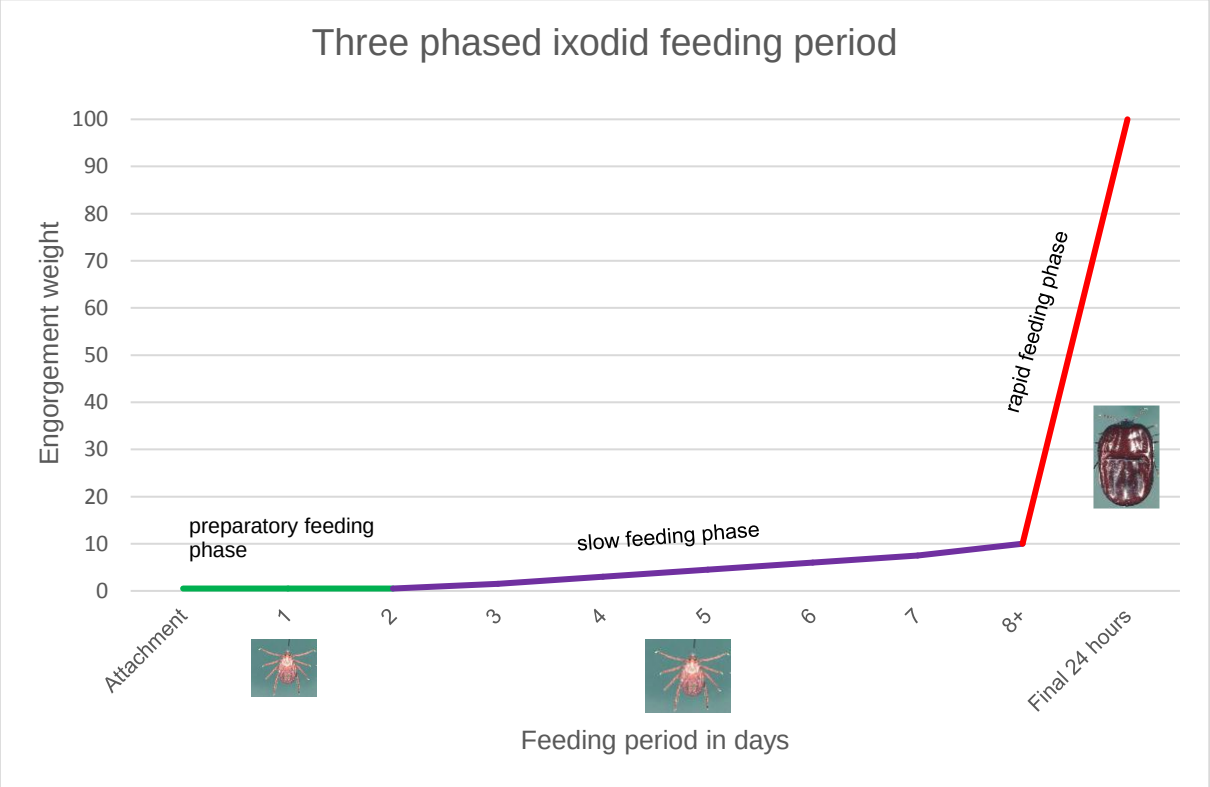

Figure 2.4 Diagrammatic illustration of ixodid ticks feeding periods
(Adapted from: Weiss and Kaufman, 2001; Kaufman, 2004; Kaufman, 2010)
(Images from: Walker *et al.,* 2003)

Furthermore, the feeding period is dependent on the life stage of the tick, the host on which they feed and the species of tick (Rajput *et al.,* 2006). Larvae have been recorded to feed between three to five days, nymphs between four and eight days and adult females between 5 and 20 days dependent on species (Olivier, 1989;

Walker *et al.,* 2003). On the other hand, the males of most species have been found not to expand like females, but feed enough to allow maturation of their reproductive organs and can take small frequent meals and remain on the host for months (Olivier, 1989; Walker *et al.,* 2003; Stafford, 2007). It has been found that species feeding on reptiles however require a longer feeding period (Olivier, 1989) compared to when feeding on mammalian hosts.

According to Sauer *et al.* (1995) and Bowman and Sauer (2004) the salivary glands of ixodid female ticks can increase up to twenty-five times in mass and protein content during feeding. Also, during feeding, the rate of secretion of salivary fluid greatly increases which allows for concentration of the blood meal by retaining blood and returning water and ions to the hosts via the salivary ducts (McMullen *et al.,* 1983). Kaufman (2010) reported that up to 2 to 3 mL of water are returned into the host. Ingestion of blood and salivation is alternated during feeding and each cycle lasts from 5 to 20 minutes (Francischetti *et al.,* 2010). It is through this process in which the tick salivates excess water back into the host that disease causing agents in the tick are transferred to the host (Francischetti *et al.,* 2010).

The volume of blood consumed by ticks range from 0.7 mL to 8.9 mL depending on species (Sonenshine *et al.,* 2002; Nicholson *et al.,* 2009). The salivary glands of mated females degenerate in about 4 days after completion of blood meal and detaching from the host (Sauer *et al.,* 2000; Kaufman, 2004), whereas the salivary glands of non-mated females above their critical weight will degenerate after eight days (Kaufman, 2004). Fluid secretion by salivary glands in ticks are controlled by nerves form the central nervous system or synganglion (Sauer *et al.,* 1995; Sauer *et al.,* 2000).

2.3.4 The role of Salivary glands

Tick saliva plays an important role in haemostasis, inflammation, immunity (Valenzuela, 2004) and the transmission of pathogens during feeding (Bowman *et al.,* 1997; Valenzuela, 2004; Kaufman, 2010). The saliva of ticks also contains anaesthetics, anti-inflammatories, immune-suppressives, anti-coagulants, anti-platelet and vasodilatory molecules, which inhibits the hosts haemostasis and ensure that blood flows continuously at the bite site (Sonenshine, 1991; Bowman *et*

al., 1997; Wikel and Bergman, 1997; Valenzuela, 2004; Stafford, 2007; Francischetti *et al.,* 2010), and also contain prostaglandins (Bowman *et al.,* 1997) which prevents platelet aggregation (Table 2.2). Salivary vasodilators in ticks are lipid derivatives, functioning by activating adenylate cyclase or guanylate cyclase which results in the formation of cyclic adenosine monophosphate or cyclic guanosine monophosphate. This results in an increase in blood flow to the bite site. Anti-inflammatory activities include anti-histaminic, anti-serotonin, anti-complement and kininase activity (Valenzuela, 2004).

Table 2.2 Chemical compounds found in tick saliva

Chemical	**Role**	**Reference**
Anti-thrombin III	Anticoagulant	Francischetti *et al.* (2010)
Apyrase	Plays a role in the hydrolysation of adenosinediphosphate (ADP) released form injured cells	Sonenshine (1991) Bowman *et al.* (1997) Francischetti *et al.* (2010)
Disintegrins	Platelet inhibitor – prevents the binding of fibrinogen to platelets	Francischetti *et al.* (2010)
Protein C	Anticoagulant	Francischetti *et al.* (2010)
Prostagladin E2	Dilate blood vessels and minimize clotting	Sonenshine (1991) Bowman *et al.* (1997)
Prostacyclin	Platelet inhibitor – prevents the binding of fibrinogen to platelets, thereby minimizing clotting, dilate blood vessels	Sonenshine (1991) Francischetti *et al.* (2010)
Tissue factor pathway inhibitor (TFPI)	Anticoagulant	Francischetti *et al.* (2010)

The salivary glands of ixodid ticks also serve as the main organs responsible for osmoregulation, whilst feeding ticks actively excrete approximately 70% excess water and ions back into their host via their mouthparts (McMullen *et al.,* 1983; Bowman and Sauer, 2004; Dantas-Torres, 2008). The salivary glands are also the development and replication site of a number of pathogens (Bowman and Sauer, 2004).

2.3.5 Life cycle and reproduction

The life cycle of an ixodid tick consists of four stages namely, the egg stage that hatches to produce a 6-legged larva. The larva will then feed and moult to produce an 8-legged nymph and finally the nymph will feed and moult into an adult tick (Harrison *et al.,* 1973; Howell *et al.,* 1983; Olivier, 1989; Sauer *et al.,* 1995; Sonenshine *et al.,* 2002; Nicholson *et al.,* 2009). Ticks emerging from hatching or moulting have soft bodies and have an inactive period of one to two weeks to allow their external body to harden (Kuhnert, 1996; Walker *et al.,* 2003; Stafford, 2007). Life cycles are generally affected by environmental factors such as light, temperature, moisture; availability of hosts and mates (Olivier, 1989; Spickett, 2013) and species of tick (Spickett, 2013). The survival and developmental stages of ticks are also dependent on the micro-climate of their environment, this include temperature, wind speed, moisture and saturation deficit (Pfäffle *et al.,* 2013).

Except for the genus *Ixodes*, mating in ixodid ticks generally occurs on the host (Needham and Teel, 1991; Sonenshine, 1991; Sonenshine *et al.,* 2002; Nicholson *et al.,* 2009). Female ixodids lay between 2000 and 20 000 eggs in a single batch before dying (Kuhnert, 1996; Walker *et al.,* 2003; Stafford, 2007). However, up to 23000 eggs have been observed in larger tick species of *Amblyomma* and *Hyalomma* (Olivier, 1989). Approximately 50 to 60% of ixodid ticks body weight at drop off is converted to eggs (Sonenshine *et al.,* 2002; Nicholson *et al.,* 2009), and the number of eggs are affected by the volume of the blood meal, the size of egg and the tick species (Sonenshine, 1991; Weiss and Kaufman, 2001). The eggs of all ticks are laid in the environment and never on the host (Sonenshine, 1991; Walker *et al.,* 2003).

During feeding, the weight of female ixodid ticks generally increase 80 to 120 times their unfed body weight, whereas the weight of argasid ticks increases only 5 to 12 times prior to egg production (Olivier, 1989). Ixodid female ticks generally do not fully engorge if not mated. If an unmated female reaches the receptive period and production of sex attractant has begun, the female might continue slow feeding and remain attached to a host for a longer period of time (Olivier, 1989). During their life cycle males will also find a host, may take several small meals, mate and die.

Males will attempt to mate with as many females whilst they are feeding (Kuhnert, 1996; Walker *et al.,* 2003; Stafford, 2007).

Ixodid ticks can follow either a one-host, two-host or three-host life cycle (Olivier, 1989; Needham and Teel, 1991; Sonenshine, 1991; Sonenshine *et al.,* 2002; Walker *et al.,* 2003; Nicholson *et al.,* 2009; Spickett, 2013). In a one-host cycle all life stages feed on the same host (Figure 2.5), only the adult will detach (Kuhnert, 1996; Sonenshine, 1991; Walker *et al.,* 2003; Stafford, 2007).

Examples of one-hosts ticks include *Rh. (Bo.) decoloratus*, also known as the blue tick and *Rh. (Bo.) microplus*, also known as the cattle tick (Howell *et al.,* 1983; Walker *et al.,* 2003). The life cycle for both species are illustrated in Figure 2.5.

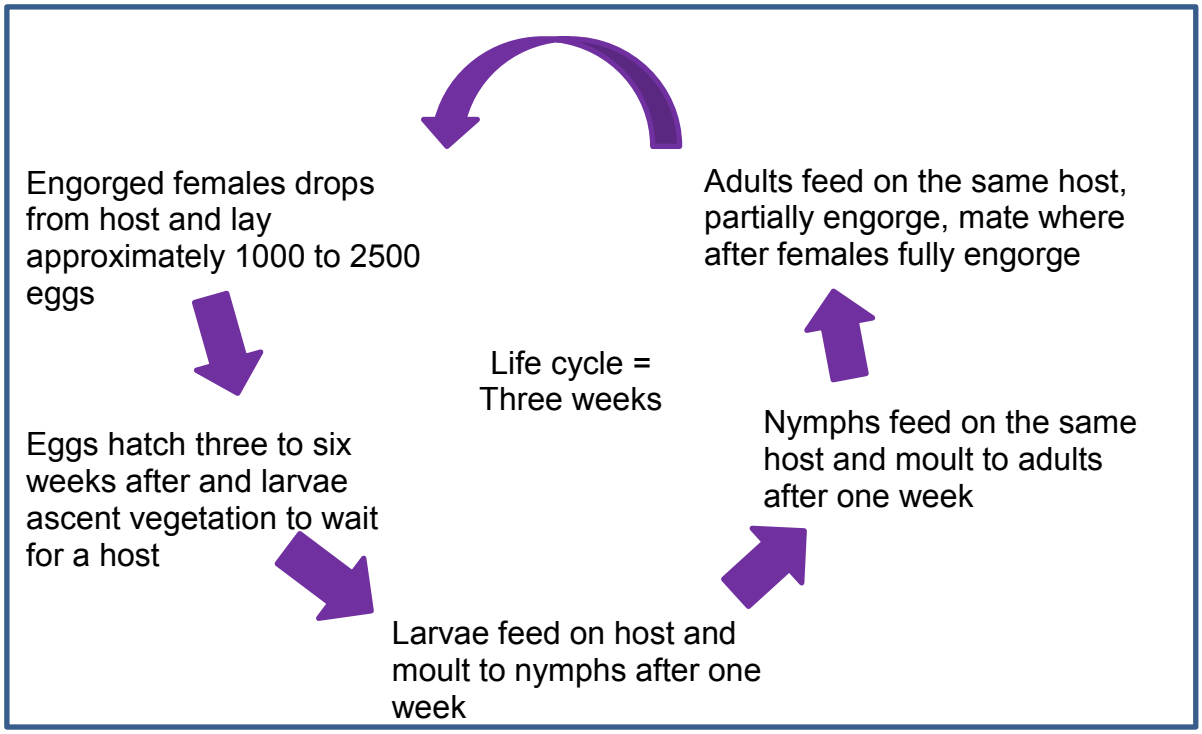

Figure 2.5 Diagrammatic illustration of the life cycle *Rh. (Bo.) decoloratus* and *Rh. (Bo.) microplus*
(Adapted from: Bedford and Graf, 1939; Howell *et al.,* 1983; Walker *et al.,* 2003; Spickett, 2013)

The female *Rh. (Bo.) microplus* generally lays approximately 500 more eggs comparted to *Rh. (Bo.) decoloratus*. Adults for both species are present during summer to early winter months (Walker *et al.,* 2003).

In a two-host cycle the eggs hatch into larvae which after development seek a host to which they attach. The larvae then feeds but do not detach. They moult and the emerging nymphs then feed on the same host before detaching and moulting to either a male or female adult tick (Kuhnert, 1996; Sonenshine, 1991; Walker *et al.,* 2003; Stafford 2007).

Examples of two-host ticks include; *Hyalomma rufipes* (previously *Hyalomma marginatum rufipes*) also known as the hairy *Hyalomma* or coarse-legged *Hyalomma, Hyalomma truncatum* also known as the shiny Hyalomma (Walker *et al.,* 2003), as illustrated in Figure 2.6 and *Rhipicephalus evertsi evertsi* is also known as the red-legged tick (Walker *et al.,* 2003).

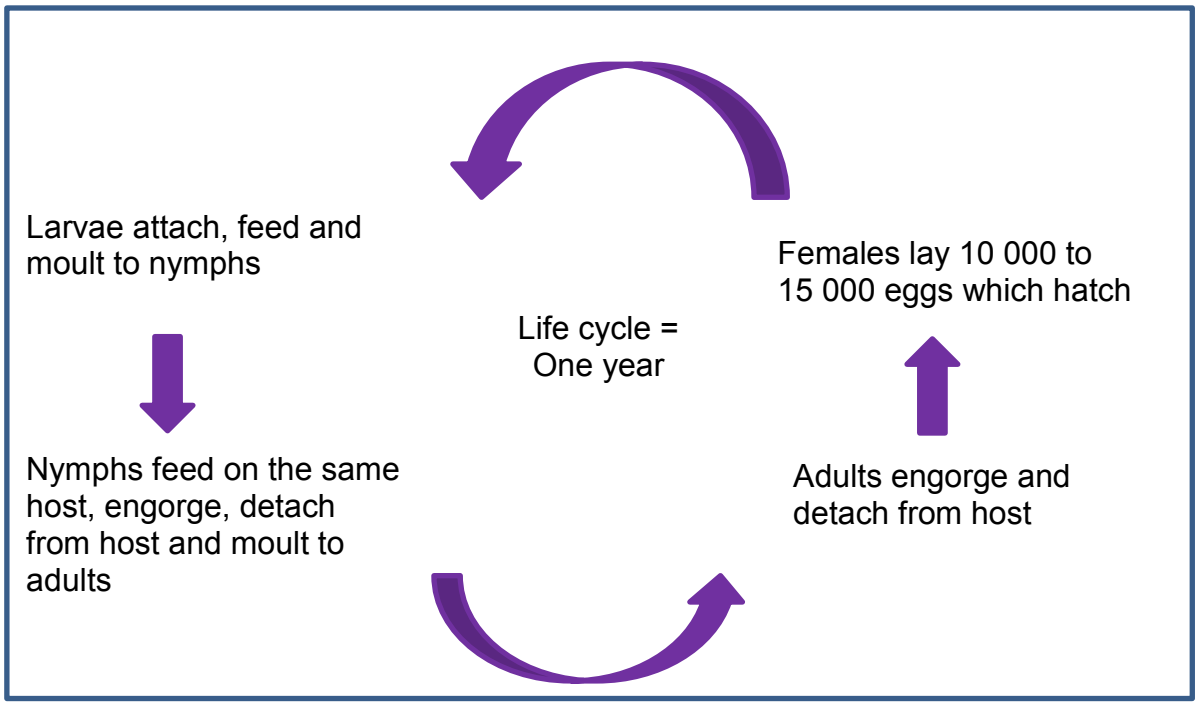

Figure 2.6 Diagrammatic illustration of the life cycle *Hy. rufipes* and *Hy. truncatum*
(Adapted from: Howell *et al.,* 1983; Walker *et al.,* 2003; Bishop *et al.,* 2004; Spickett, 2013)

Adult *Hy. rufipes* are present during the early part of the wet season whereas the immature stages are present during the dry season (Walker *et al.,* 2003), whereas adults *Hy. truncatum* are present during the late wet summer months and immature stages during the dry autumn to spring months (Walker *et al.,* 2003). *Rh. evertsi evertsi* are mainly active during the summer months but can be present throughout the year in warm regions (Howell *et al.,* 1983; Walker *et al.,* 2003).

During a typical three-host life cycle, larvae develop in the eggs until ready to hatch, find a host on which they will feed once, detach and hide in soil or vegetation where they will moult into nymphs. Nymphs will then find a host, feed once detach and hide in the soil or vegetation where they will moult to either a male or female. The female will find a host, feed until engorged, detach and lays a batch of eggs (Needham and Teel, 1991; Sonenshine, 1991; Sonenshine *et al.,* 2002; Nicholson *et al.,* 2009), the depleted female will then die.

Examples of three host ticks include *Am. hebraeum* also known as the South African bont tick (Figure 2.7), *Rh. appendiculatus* also known as the brown ear tick (Figure 2.8) (Howell *et al.,* 1983; Walker *et al.,* 2003; Spickett, 2013).

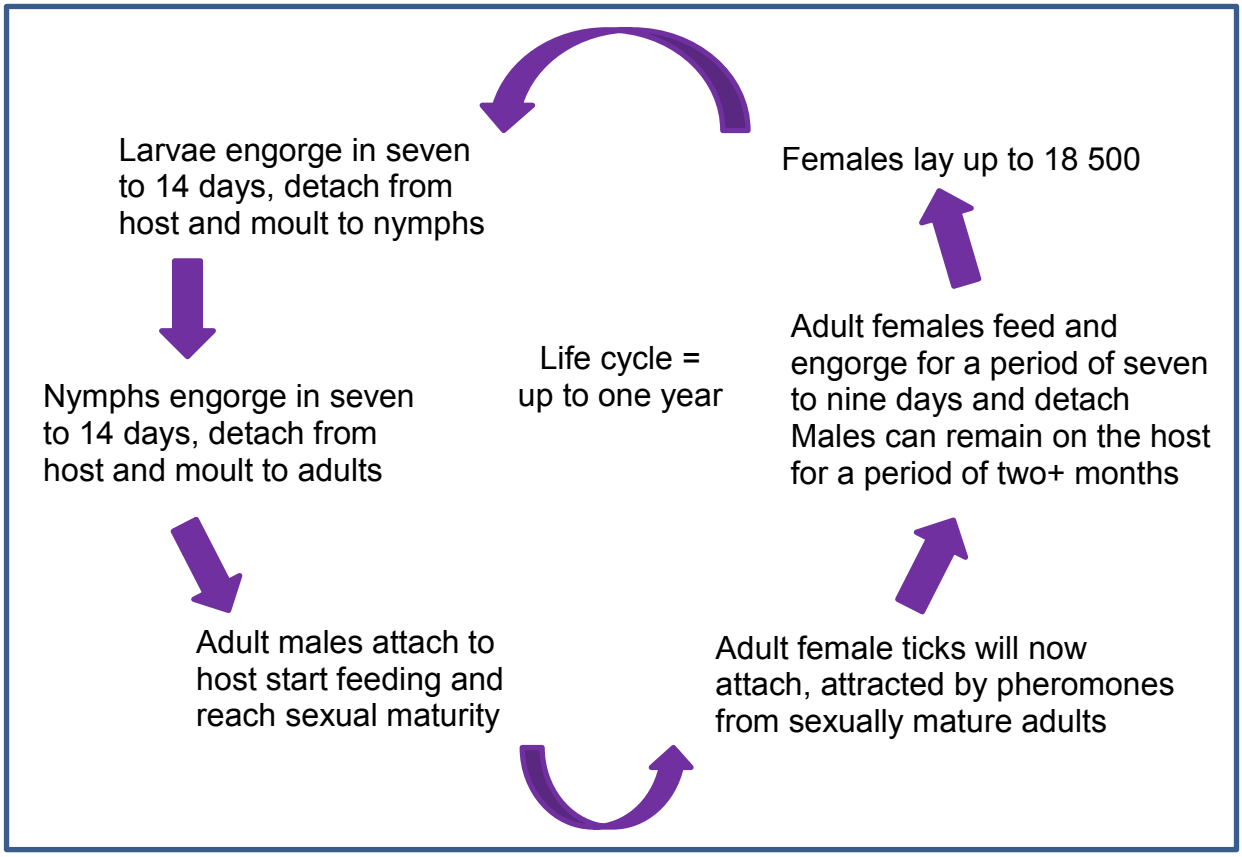

Figure 2.7 Diagrammatic illustration of the life cycle *Am. hebraeum* (Adapted from: Bedford and Graf, 1939; Howell *et al.,* 1983; Walker *et al.,* 2003; Spickett, 2013)

Adult *Rh. appendiculatus* moulting from the nymphal stage during the winter months enters a diapause period and generally start seeking a host after November (Randolph, 2004). Two types of diapause have been observed in ticks, behavioural diapause, which is the suppression of seeking hosts in unfed ticks, and a delay of

full engorgement in attached ticks; or morphogentic or developmental diapause may occur during embryogenesis, during ecdysis of engorged immatures or during ovipositioning in engorged females (Olivier, 1989).

In southern Africa the *Rh. appendiculatus* ticks occur during the rainy season, larvae during the cooler late summer to winter period and nymphs during the winter to early spring (Walker *et al.,* 2003).

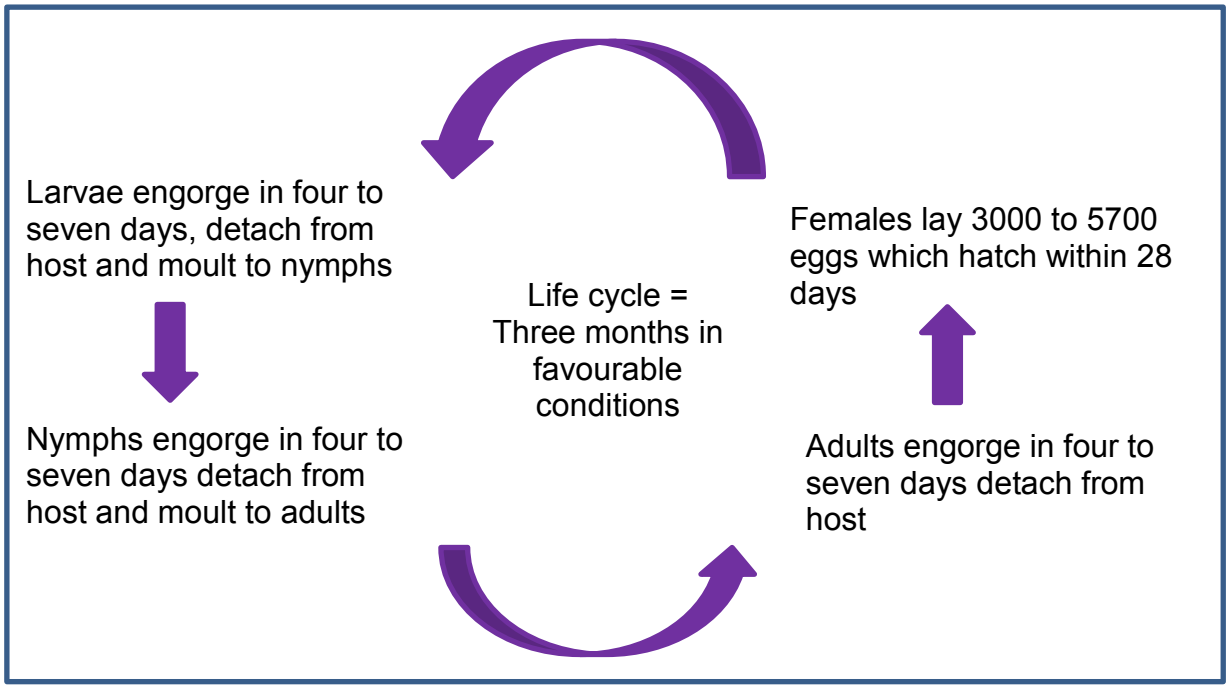

Figure 2.8 Diagrammatic illustration of the life cycle *Rh. appendiculatus* (Adapted from: Bedford and Graf, 1939; Howell *et al.,* 1983; Walker *et al.,* 2003; Spickett, 2013)

As seen in the preceding paragraphs ticks have different life cycles namely one-host, two-host or three-host. The length of time ticks spend on their hosts varies, as does the time of year ticks are present and active. All these aspects play an important role and should be taken into consideration when hosts are treated to prevent or limit tick attachment as part of a control program.

2.3.6 Off-host ecology

With the exception of one- and two-host ticks, ixodid ticks spend the majority of their lives (up to 99%) off their hosts (Pfäffle *et al.,* 2013). During the off-host phases, ticks face a challenge of evaporative water loss through body surface. Nonetheless,

the cuticle which forms part of the external integument and is excreted by the epidermis reduces evaporative water loss. The cuticle is divided into two layers, a thin outer epicuticle and thick inner procuticle and has a superficial layer of lipids that minimizes water loss through the body surface (Walker *et al.,* 2003). Also, the spiracles which are found in ixodid nymphs and adults are covered by the spiracular plates to assist with evaporative water loss regulation and ventilation (Needham and Teel, 1991). In addition, all ixodid ticks are characterised by having a sclerotized dorsal plate (Dantas-Torres, 2008), with the female hard scutum on the anterior half of dorsal surface and that of the males occupying the entire dorsal surface (Sonenshine *et al.,* 2002; Nicholson *et al.,* 2009).

The hemolymph is the main water reserve in ticks (Needham and Teel, 1991). Water can both be absorbed and lost through the cuticle. Also, water loss can occur from the respiratory membranes, rectal lumen, excretions and defecation (Hackman, 1982). Water loss in nymphal and adult ixodid ticks is minimized by the spiracles, which are covered by spiracular plate that can close to regulate ventilation. Furthermore, water loss is regulated by valves located in the anus and hygroreceptors enabling ticks to sense humidity gradients (Needham and Teel, 1991). Ticks can absorb water vapour from the atmosphere when dehydrated to a certain degree. In order to absorb water vapour the relative humidity of their habitat must be higher than the critical equilibrium humidity which range from 75 to 95%, depending on species and developmental stage (Bowman and Sauer, 2004; Kaufman, 2010).

Tick saliva also play a role in ion and water metabolism. When a tick is not attached to a host, salivary gland lobes of the type I acine (Sauer *et al.,* 1995; Bowman and Sauer, 2004; Francischetti *et al.,* 2010), can produce hygroscopic (highly salty) saliva onto the surface of the hypostome. Atmospheric moisture can then be absorbed by this saliva, sucked back into the body and hydrate the tick (Francischetti *et al.,* 2010).

Tick species are also associated with specific habitats. For example, *Am. hebraeum* requires moisture and warmth, brush and bush, and cannot survive in open grasslands (Jensensius *et al.,* 2003; Walker *et al.,* 2003). They occur in tropical and subtropical environments and generally require an annual rainfall of 300 – 800 mm,

where they are mostly active during rainy seasons, even though they are present throughout the year (Jensensius *et al.,* 2003). *Am. hebraeum* generally are found in close proximity of their hosts and aggressively attach to their hosts (Jensensius *et al.,* 2003). *Hy. rufipes* occurs widely in Africa, but its distribution is patchy and the tick is most probably more commonly found in the drier areas (Walker *et al.,* 2003). Humidity and vegetation types play a role in the distribution of *Rh. appendiculatus* (Perry *et al.,* 1990). *Rh. (Bo.) decoloratus* are found predominantly in regions with savannah and temperate climates, typically grasslands and wooded areas, but tend to be absent in drier areas (Walker *et al.,* 2003).

2.3.7 Host Finding and Attachment

Tick behaviour can either be described as, (1) exophilic or non-nidicolous, where ticks live and find their hosts in open environments, (2) endophilic or nidicolous, where ticks live in their hosts nests (Sonenshine, 1993; Walker *et al.,* 2003), or as (3) domestic, as seen in the dog tick *Rhipicephalus sanguineus*, where they have adapted to living in housing, this in known as domestic behaviour (Walker *et al.,* 2003).

Various abiotic factors play a role on when, how and where ticks start host questing (Randolph, 2004). Ticks are highly sensitive to various stimuli during host seeking. The stimuli include chemical stimuli, vibrations and host body temperature (Dantas-Torres, 2008). The presence of hosts is detected through stimuli such as carbon dioxide, ammonia, lactic acid, butyric acid, heat, shadows and vibration (Sonenshine, 1993; Stafford, 2007; Bissinger and Roe, 2010). Ticks are characterised by a specialized accumulation of sensory organs on the tarsus of the first leg known as the Haller's organ (Sonenshine, 1991; Klompen *et al.,* 1996). The Haller's organ, as seen in Figure 2.9, responds to stimuli produced by host such as ammonia, lactic acid and carbon dioxide during host finding (Sonenshine, 1991).

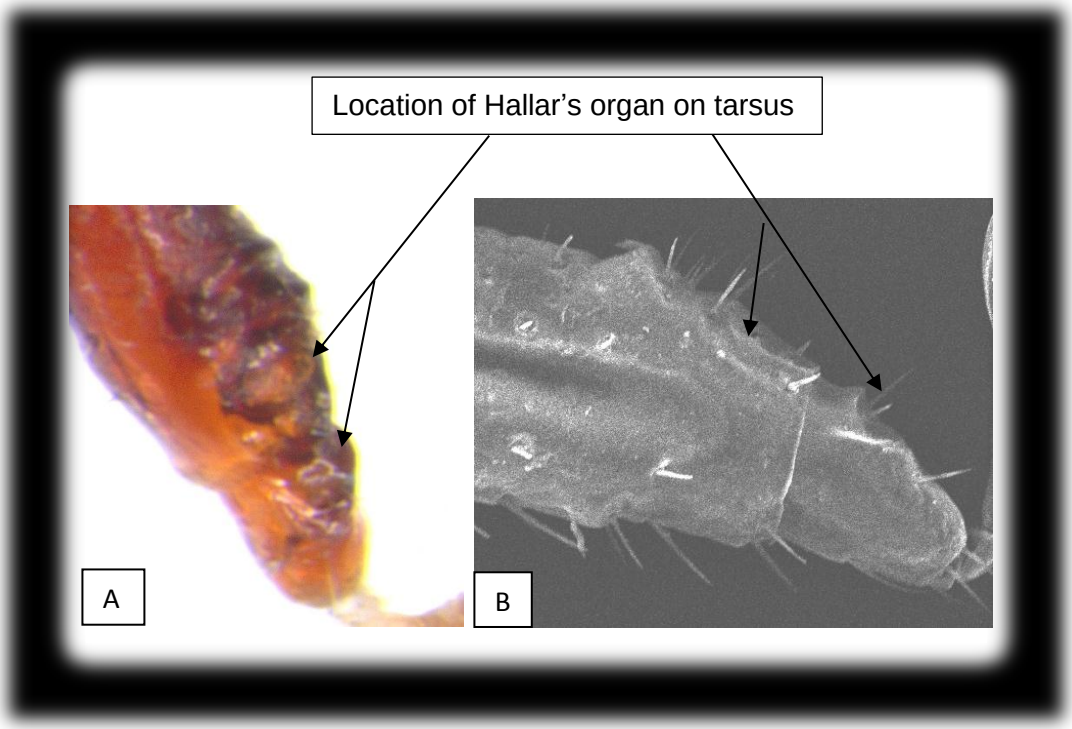

Figure 2.9 Haller's organ – *Rh. appendiculatus*

A: Stereo microscope image captured by EMC Theron, July 2018, using Olympus SZX16 (Wirsam) Stereo microscope and processed using CellSense Software

B (view from the side): Electron microscope image captured by EMC Theron, July 2018, using Carl Zeiss Merlin Gemini 2 Scanning Electron Microscope and processed using SmartEM Software

Different tick species use different methods to find hosts. In open environments, some ticks for example *Rh. appendiculatus* make use of questing. When questing the ticks will crawl onto vegetation and wait for their hosts to pass by and cling onto the host using their front legs. Once on the host a tick will crawl on the skin to find a suitable feeding site where they will then attach (Walker *et al.,* 2003; Sonenshine *et al.,* 2002; Stafford, 2007; Dantas-Torres, 2008; Nicholson *et al.,* 2009; Bissinger and Roe 2010). Adult ticks also adopt species-specific questing heights when finding hosts (Randolph, 2004). Ticks may remain in their questing state for days to weeks and only abandon host seeking due to changes in water balance (Sonenshine, 1993). Some ticks such as *Am. hebraeum* are active hunters and run across the ground after detecting that the hosts are nearby. Some species of ticks may spend their entire life cycle in their host's nest and attach to their hosts from there (Walker *et al.,* 2003; Sonenshine *et al.,* 2002; Dantas-Torres, 2008; Nicholson *et al.,* 2009). Some tick species for example *Rh. appendiculatus* might use only one host-seeking strategy in all active life stages

(larvae, nymphs and adults) whereas others might adapt their seeking strategies (Dantas-Torres, 2008), for example *Am. hebraeum* where the adults and nymphs are active hunters, but hatched larvae will wait for hosts on vegetation (Walker *et al.,* 2003).

The host-parasite relationship can be either host-specific, where the tick species will only feed on one specific host or closely related species, as can be seen in *Rhipicephalus* ticks with mammals being the prefared hosts; or opportunistic where the ticks will feed on any or various hosts, as can be seen in *Amblyomma* ticks, which feed on mammals and some reptiles such as tortoises (Sonenshine, 1993; Walker *et al.,* 2003). Table 2.3 shows preferred hosts and predilection sites of ticks.

Table 2.3 Tick species and associated hosts

Tick Species	**Preferred host**	**Attachment site**	**Reference**
Am. hebraeum	Adults: Cattle, sheep, horses, donkeys, pigs, goats, large wild ruminants and small antelopes	The hairless area of cattle located under the tail, lower perineal region, under the udder, around genitalia, and in axillae. Around the feet of sheep and goats	Howell *et al.* (1983) Jensensius *et al.* (2003) Walker *et al.* (2003) Spickett (2013)
	Immature stages: Similar to adults but also scrub hares, helmeted guinea fowls, and tortoises	Larvae can be found on the feet, legs and muzzle Nymphs can be found on the feet, legs, groin, sternum and neck	Howell *et al.* (1983) Jensensius *et al.* (2003) Walker *et al.* (2003) Spickett (2013)
Hy. rufipes	Adults: Cattle, sheep, goats, horses, wild ungulates	Hairless peri-anal region, lower perineum and genitalia	Howell *et al.* (1983) Walker *et al.* (2003)
	Immature stages: Hares and ground-frequenting birds	Entire body	

Table continues on the next page

Tick Species	Preferred host	Attachment site	Reference
Hy. truncatum	Adults: Large domestic herbivores, wild herbivores and in some cases domestic dogs	The tail switch, around anus, on lower perineum, on the legs and around the feet	Howell *et al.* (1983) Walker *et al.* (2003)
	Immature stages: Hares, rodents but will also feed on humans	Entire body	
Rh. appendiculatus	Adults: Cattle, goats, buffaloes, elands, waterbucks, nyalas, greater kudus and sable antelopes, dogs and sheep can also be infested	Ear pinna, during heavy infestations can also be found on the eyelids, horns, upper neck, in the tail-brush and around the anus	Howell *et al.* (1983) Walker *et al.* (2003) Spickett, (2013)
	Immature stages: Similar to adults but also on smaller antelopes and scrub hares	Neck and dewlaps, cheeks, eyelids, muzzle and ears	
Rh. evertsi evertsi	Adults: Horses, donkeys, cattle and sheep	Hairless area around the anus and the groin area	Howell *et al.* (1983) Walker *et al.* (2003) Spickett, (2013)
	Immature Stages: Similar to adults but also scrub hares and small antelope species	Deeper parts of the inner ear surface ear and in the outer ear canal	
Rh. simus	Adults: Cattle, sheep, goats, horses, dogs, large wild carnivores, zebras, warthogs and rhinoceroses	Tail-brush and around the feet of cattle), around the feet (sheep), tails (horses and zebras), and head and shoulders (dogs and warthogs)	Walker *et al.* (2003) Spickett, (2013)
	Immature stages: Murid rodents	Entire body	

Table continues on the next page

Tick Species	Preferred host	Attachment site	Reference
Rh. (Bo.) decoloratus	Cattle, horses, donkeys, sheep, goats and wild ungulates	Back, upper legs, neck, shoulders, dewlap and belly	Howell *et al.* (1983) Walker *et al.* (2003) Spickett, (2013)
Rh. (Bo.) microplus	Cattle, other livestock and wildlife	Belly, dewlap, shoulders and flanks	Walker *et al.* (2003)

2.4 Conclusion

This chapter looked at the taxonomy of tick species in general as well as the tick species most commonly associated with livestock in South Africa. The chapter also considered the structural and behavioural aspects of ixodid ticks which enhance their parasitic way of life. These features not only make ticks successful and important parasites, but also enhance their survival, including their ability to live off host for a significant period of time in less than favourable conditions. All of these aspects should be taken into account when potential control measures are evaluated.

Understanding the basic biology and behaviour of ticks can assist in understanding the role these small and integrate organisms play in the everyday life of other organisms. In the next chapter, the effect of ticks on veterinary, medical and economic sectors are considered in order to highlight the role ticks play as parasites and as vectors of various diseases causing agents. A more in-depth look will be taken on the role and impact ticks have on the different sectors and to demonstrate why it is important that they together with the disease-causing agents they transmit should be controlled.

CHAPTER 3
LITERATURE REVIEW:
VETERINARY, MEDICAL AND ECONOMIC IMPORTANCE OF TICKS

3.1 Veterinary, Medical and economic importance of ticks and tick-borne diseases

3.1.1 Introduction

As indicated in the preceding chapters, ticks are haematophagous ecto-parasites of a wide range of hosts. In addition to the structural, functional and behavioural adaptations enhancing ticks parasitic life, their capacity as parasites and vectors of various disease causing agents is accentuated by the fact that for ixodid ticks each of the larval, nymphal and adults stages must acquire a blood meal for further development. Also, the fact that in ticks pursuing a three-host life strategy the host type and host range of immature stages differs from those of adults scales the tick-host interactions along host-type and -range, thus widening the scope of impact. A similar account holds for ticks pursuing a two-host life strategy, though not to the extent of three-host ticks. Tick hosts suffer direct and indirect effects as a result of tick infestations. The current chapter explores the veterinary, medical and economic impacts of ticks resulting from direct and indirect effects of tick infestations.

3.1.2 Direct effects

Direct effects of ixodid ticks on their hosts result from ticks being parasites that feed on host blood and the close and prolonged contact they establish with their hosts. These effects include damage to the hide, host paralysis, anaemia, and effects on the general wellbeing of their hosts.

Direct effects of ticks on their animal hosts include the following:

I. Damage to skin

Tick bites create wounds that in some instances result in the formation of abscesses, and loss of teats or teat-lameness. Tick species characterised with long mouthparts, such as those in the genera *Amblyomma* and *Hyalomma*, are generally associated with the formation of sores, abscesses, and secondary infections. For example, foot abscesses are regularly observed in goats and

sheep infested by *Am. hebraeum* (Howell *et al.,* 1983; Walker *et al.,* 2003). The feeding sites of adult *Hy. rufipes* may lead to the formation of large lesions where severe abscesses can form, and when adult *Hy. truncatum* ticks attach to the interdigital clefts, on the feet, and fedlocks of lambs, it results in lameness in the hosts (Walker *et al.,* 2003). In dogs, where *Hy. truncatum* may attach in a cluster at one site, skin necrosis will occur (Walker *et al.,* 2003). Loss of teats and teat-lameness caused by bites also has a direct effect on the increased mortality of calves (Brossard, 1998; Minjauw and McLeod, 2003; Walker *et al.,* 2003; Jongejan and Uilenberg, 2004; Rajput *et al.,* 2006). Heavy infestations of *Rh. (Bo.) decoloratus* are characterised by damage to hides and a reduction in cattle growth rate (Walker *et al.,* 2003).

II. Paralysis and Toxicosis

The saliva of certain tick species contains paralysing toxins and as a result, induce paralysis and / or toxicosis (Walker *et al.,* 2003). *Hy. truncatum* is known to induce "sweating sickness", a generalised eczema-like condition in calves and other species of livestock in Africa (Sonenshine, 1993; Minjauw and McLeod, 2003; Walker *et al.,* 2003; Jongejan and Uilenberg, 2004; Spickett, 2013). *Rh. evertsi evertsi* is associated with "spring lamb paralysis" in the eastern Highveld of Mpumalanga and Free State, South Africa (Walker *et al.,* 2003). This paralysis is caused by a toxin found within the saliva of the female ticks (Walker *et al.,* 2003; Spickett, 2013). *Rh. simus* also produces a toxin, transmitted through saliva, which can cause paralysis in calves and lambs (Walker *et al.,* 2003), and *Rh. appendiculatus* has also been implicated in tick toxicosis (Olwoch *et al.,* 2007).

III. Tick worry

Tick bites also affect animal production and welfare negatively by causing what is generally known as 'tick worry', which includes irritation, allergic responses and blood losses, and also have debilitating effects on animal skins and / or death (Barnett, 1961; Wharton and Norris, 1980; Sonenshine *et al.,* 2002; Rajput *et al.,* 2006; Nicholson *et al.,* 2009). Tick worry can also lead to a reduction in immunity against diseases (Moyo and Masika, 2009). Instead of spending time foraging and increasing the body biomass tick infested animals

spend prolonged periods grooming themselves in an effort to remove ticks from their bodies. Undoubtedly, this has a negative impact on growth and development of livestock since the time that should be used for feeding is taken up by grooming. For example, tick infestations were associated with the reduction of weight gain, up to 4g per single engorged *Rh. appendiculatus* tick and up to 46 – 61g for *Am. variegatum* ticks (Estrada-Peña and Salman, 2013). Research performed in Australia showed a loss of 0.6g growth for each female *Rh. (Bo.) microplus* tick that completed feeding on cattle (Walker *et al.,* 2003).

IV. Anaemia

Blood loss caused by heavy infestations can result in anaemia (Brossard, 1998; Ghosh *et al.,* 2007; Rajput *et al.,* 2006; Adenubi *et al.,* 2012; Asmaa *et al.,* 2014). Heavy tick infestations have also shown to be associated with a decrease in packed cell volume, haemoglobin, serum albumin, cholesterol, triglyceride and amylase concentrations of hosts (Jonsson *et al.,* 1998).

V. General

As a result of tick infestations animals become stressed and as a result have a reduced immune system (Bedford and Graf, 1939; Walker *et al.,* 2003; Ghosh *et al.,* 2007; Asmaa *et al.,* 2014). Heavy infestations are also associated with a reduction in growth rate, such as observed with *Am. hebraeum* (Walker et al 2003).

3.1.3 Indirect effects

Indirect effects of tick infestations result from the capacity of ticks to transmit different disease-causing agents and secondary infections through wounds caused by tick bites.

I. Transmission of disease-causing agents

Globally ticks are considered to be the second to mosquitoes as vectors of disease-causing agents (Sonenshine, 1991; Sonenshine *et al.,* 2002). Pfäffle *et al.* (2013) further assets that ticks are unique and differ from other arthropod vectors, and as such create habitats for pathogens in areas where other vectors are not able to transmit diseases due to constraining abiotic factors. They are associated with transmission of protozoan (such as theilerioses and babesiosis),

rickettsial (such as anaplasmosis and heartwater), spirochaetial and viral diseases affecting both humans and animals (Tisdell *et al.,* 1999; Sonenshine *et al.,* 2002; Hlatshywayo *et al.,* 2004; Jongejan and Uilenberg, 2004).

II. Secondary infections

Secondary infections result from the wounds and damage to the hide caused by tick infestation. For example, the sores formed by *Am. hebraeum* mouthparts attract the blowfly, *Chrysomya bezziana,* the larvae of which can cause severe myiasis in the host (Walker *et al.,* 2003).

Another important aspect to consider with regard to tick borne diseases, is that even in the absence of symptoms and with clinically healthy animals, the disease is still transmitted to vectors, thereby contributing to the silent spread of the disease (Mencke, 2013). According to Tisdell *et al.* (1999) and Mapholi *et al.* (2014) tick-borne diseases have different effects on the male and female host animals, with silent oestrus periods, prevention of fertilisation, abortion, dead, weak or deformed calves being born being problems in cows, whereas problems observed in bulls include reduced mobility resulting in bulls not seeking a receptive cow, reduced libido, and / or infertility.

The major tick species responsible for transmission of diseases include *Rh. (Bo.) microplus*, *Rh. appendiculatus* and *Amblyomma* spp. (Jongejan and Uilenberg, 2004; Kröber and Guerin, 2007a; Adenubi *et al.,* 2012), with *Rh. (Bo.) microplus* characterised as one of the most widely distributed tick and vector of economically important pathogens (Shyma *et al.,* 2014).

The major veterinary important diseases transmitted by ticks include include the following:

I. Anaplasmosis

Bovine anaplasmosis (gallsickness) is caused by *Anaplasma marginale,* which replicate in the erythrocytes of cattle with, as many as 70% of erythrocytes that can be infected during an acute infection (Kocan *et al.,* 2004). Anaplasmosis has an incubation period between 20 and 40 days (Sonenshine, 1993), and symptoms of acute anaplasmosis generally include weight loss, fever, abortions,

reduction in milk production, death (Kocan *et al.,* 2004), anaemia, jaundice and blood in urine which may also be observed at times (Jonsson *et al.,* 2008). In South Africa it is estimated that approximately 35% of cattle are at risk (Spickett, 2013).

Tick species associated with the transmission of *A. marginale* include *Hy. rufipes*, *Rh. (Bo.) decoloratus, Rh. (Bo.) microplus, Rh. evertsi evertsi* and *Rh. simus* (Howell *et al.,* 1983; Walker *et al.,* 2003; Rikhotso *et al.,* 2005; Olwoch et al 2007; Spickett, 2013; Shyma *et al.,* 2014). Other *Anaplasma* transmitted by ticks include; *Anaplasma bovis*, responsible for bovine ehrlichiosis transmitted by *Rh. appendiculatus* (Walker *et al.,* 2003), and *Anaplasma centrale* transmitted by *Rh. (Bo.) decoloratus* and *Rh. (Bo.) microplus* (Howell *et al.,* 1983; Spickett, 2013).

II. Babesiosis

Tick-transmitted *Babesia* is the second most common blood transmitted parasite of mammals, with trypanosomes being the most commonly transmitted parasite (Hunfeld *et al.,* 2008). *B. bovis* and *B. bigemina* are associated with transmission of bovine babesiosis or redwater, with an incubation period generally between eight and sixteen days (Sonenshine, 1993). These protozoans infect the red blood cells of cattle. Clinical symptoms of babesiosis caused by *B. bovis* include fever, depression, haemoglobinuria (blood in urine), jaundice, anaemia, abortion, diarrhoea, muscle loss, tremors, convulsions and coma, neurological symptoms such as aggression, circling, hyperaestesia, nystagmus and paralysis may also occur. Symptoms of babesiosis caused by *B. bigemina* include haemoglobinuria, anaemia, jaundice and death (Jonsson *et al.,* 2008). Babesiosis is listed as the second most economically important tick-borne disease in South Africa, with an estimate of 35% of South African cattle population at risk (Spickett, 2013).

Tick species associated with the transmission of *B. bigemina* include *Rh. appendiculatus* (Howell *et al.,* 1983; Kettle, 1995), *Rh. (Bo.) decoloratus* (Howell *et al.,* 1983; Walker *et al.,* 2003; Tønnesen *et al.,* 2006), and *Rh. evertsi evertsi* (Rikhotso *et al.,* 2005; Nyangiwe and Horak, 2007).

B. bigemina is only transmitted by *Rh. (Bo.) decoloratus* nymphs and adults, after it has passed transovarially from the previous generation of ticks (Howell *et al.,* 1983; Walker *et al.,* 2003). *Rh. (Bo.) microplus* is associated with the transmission of both *Babesia* species (Walker *et al.,* 2003; Rikhotso *et al.,* 2005; Tønnesen *et al.,* 2006; Shyma *et al.,* 2014). Other Babesia transmitted by ticks include; *Babesia occultans* in cattle transmitted by *Hy. rufipes* (Sonenshine, 1993; Walker *et al.,* 2003), *Babesia caballi* responsible for equine piroplasmosis in horses and is transmitted by *Hy. truncatum* and *Rh. evertsi evertsi* (Walker *et al.,* 2003; Nyangiwe and Horak, 2007).

III. Theileria

Vectors of economically important *Theileria* species include *Rhipicephalus*, *Amblyomma*, *Hyalomma* and *Haemaphysalis* ticks. The most important species are *T. parva*, transmitted by *Rhipicephalus* ticks. In particular East Coast fever which is transmitted by *Rhipicephalus appendiculatus* (Howell *et al.,* 1983; Sonenshine, 1993; Walker *et al.,* 2003; Bishop *et al.,* 2004; Olwoch *et al.,* 2007; Bishop *et al.,* 2008), was responsible for big economical losses in Sub-Saharan Africa in 1989 (Bishop *et al.,* 2004). Currently management of East Coast Fever is mainly through the control of the vector using acaricides (Bishop *et al.,* 2004).

Other *Theileria* transmitted by ticks include; *T. velifera* responsible for mild diseases in cattle and is transmitted by *Am. hebraeum* (Kettle, 1995), *T. mutans* responsible for benign theileriosis in cattle is transmitted by *Am. hebraeum* and *Rh. evertsi evertsi* (Howell *et al.,* 1983; Walker *et al.,* 2003; Bishop *et al.,* 2008; Spickett, 2013), *T. taurotragi* which is associated with benign bovine theileriosis transmitted by *Rh. appendiculatus* (Sonenshine, 1993, Walker *et al.,* 2003), and *T. equi* is transmitted by *Rh. evertsi evertsi* (Walker *et al.,* 2003; Nyangiwe and Horak 2007).

IV. Ehrlichiosis

Heartwater is caused by the rickettsia, *Ehrlichia ruminantium* and is characterised with fluid accumulation in body cavities and soft tissue of the lungs, pericardial sac and brain of infected animals (Oberem and Oberem, 2011). This is due to the organism replicating in the endothelial cells

of capillaries thereby increasing permeability (Oberem and Oberem, 2011). The disease has an average incubation period of 18 days but can range between nine and twenty-nine days (Sonenshine, 1993). Symptoms include fever, respiratory distress, increased blinking, paddling motions of legs and staggering (Oberem and Oberem, 2011).

Heartwater is currently considered to be one of the most important tick-borne diseases, with 37% of beef cattle and 5% of dairy cattle at risk based on the distribution of *Am. hebraeum* in South Africa (Spickett, 2013). Ticks associated with the transmission of *E. ruminantium* include; *Am. hebraeum* in cattle, sheep, goats (Howell *et al.,* 1983; Sonenshine, 1993: Sonenshine *et al.,* 2002; Walker *et al.,* 2003; Rikhotso *et al,* 2005; Nyangiwe and Horak, 2007) and susceptible wild antelope species (Howell *et al.,* 1983; Sonenshine *et al.,* 2002; Walker *et al.,* 2003; Nyangiwe and Horak, 2007). *Am. hebraeum* is also specifically important in the role it plays in the transmission of *E. ruminantium* by the male ticks that wanders between feeding (Walker *et al.,* 2003). In a laboratory reared study to determine the susceptibility of *Am. hebraeum,* Mahan *et al.* (1995) determined that this tick species is highly susceptible to all four *E. ruminantium* strains, more than *Am. variegatum*, suggesting the tick species is a more efficient vector of the disease in animals.

V. Rickettsial diseases (other)

Rickettsia africae, responsible for African tick bite fever are transmitted by *Am. hebraeum* (Howell *et al.,* 1983; Sonenshine, 1993; Kettle, 1995; Jensensius *et al.,* 2003; Walker *et al.,* 2003), where the tick is not only the vector but also a reservoir. The infection is maintained from female adult to offspring, known as transovarial transmission, and from larval stage, to nymphyl stage to adult, known as trans-stadial transmission (Jensensius *et al.,* 2003; Walker *et al.,* 2003). *Rickettsia conorii* responsible for tick typhus is transmitted by, *Am. hebraeum* (Howell *et al.,* 1983; Sonenshine, 1993; Kettle, 1995; Jensensius *et al.,* 2003; Walker *et al.,* 2003; Spikett, 2013), *Hy. rufipes*, *Rh. appendiculatus* (Howell *et al.,* 1983; Walker *et al.,* 2003; Spickett, 2013), *Hy. truncatum* and *Rh. evertsi evertsi* (Walker *et al.,* 2003; Spickett, 2013).

VI. Crimean-Congo haemorrhagic fever

Hy. rufipes is the most widespread *Hyalomma* in Africa and is the main vector of the virus (*Nairovirus*) the causative agent of Crimean-Congo haemorrhagic fever (Sonenshine, 1993; Kettle, 1995; Sonenshine *et al.,* 2002; Walker *et al.,* 2003; Nicholson *et al.,* 2009; Spickett, 2013). *Hy. truncatum* is also associated with the transmission of Crimean Congo Haemorrhagic fever in humans, cattle, ostriches, and wildlife where it also serves as a reservoir (Spickett, 2013).

VII. Other conditions

Rh. appendiculatus are associated with leukocytropic disease in cattle relapsing from other tick-borne diseases, a condition characterised by fevers, oedema of subcutaneous tissues, swelling of palpable lymphatic glands, anorexia, nasal discharges, listlessness and weakness (Mans *et al.,* 2008).

Rh. (Bo.) decoloratus and *Rh. (Bo.) microplus* are associated with the transmission of *Borrelia theileri*, which causes spirochaetosis in cattle, sheep, goats, and horses (Howell *et al.,* 1983; Walker *et al.,* 2003).

Rh. simus is associated with the transmission of *Coxiella burneti* responsible for Q fever in animals and humans (Howell *et al.,* 1983).

It is estimated that 80% of the global cattle population, is exposed to infestations by ticks (FAO, 2004; Ghosh *et al.,* 2006; Rushton 2009; Adane *et al.,* 2012; Estrada-Peña and Salman, 2013), and even though tick species and associated tick-borne diseases differ from one region to the next, they still have a great impact on animal production (Adane *et al.,* 2012; Estrada-Peña and Salman, 2013). Ticks and tick-borne diseases have been classified as the most common and important problem affecting animals in Africa (de Castro, 1997; Minjauw and McLeod, 2003; Rushton, 2009), and in South Africa it accounts for 8.5% of the economic problems (Spickett, 2013). In questionnaires conducted amongst Nguni farmers in South Africa, disease and parasites were listed by 65% of participants as causes of mortality in cattle, with ticks listed as the most common parasite (75%) during summer months (Mapiye *et al.,* 2009). The most common problems reported by famers in the Eastern Cape, in a study conducted by Moyo and Masika (2009)

included anaplasmosis, babesiosis, ehrlichiosis, abscesses, screwworms and wounds at bite sites, necrosis also observed at bite sites specifically from bites by *Amblyomma* and *Hyalomma* due to their longer mouthparts.

Tick-borne diseases, such as anaplasmosis, babesiosis and cowdriosis (ehrlichiosis), are considered to be the second most important cause, after malnutrition of mortality amongst cattle in communal areas in the Easter Cape Province, South Africa (Masika *et al.,* 1997a), with anaplasmosis regarded as one of the most widespread diseases in South Africa. It is estimated that 99% of cattle are at risk of suffering this condition in South Africa (Moyo and Masika, 2009). In an ethnoknowledge study conducted in the Bungoma district in Kenya, Wanzala *et al.* (2012) identified major problems or diseases experienced by local farmers to include anaemia, poor performance or weak animals, wounds and skin damage, East Coast fever, anaplasmosis, heartwater, fever, gall disease, economic losses and death.

3.1.4 Zoonoses and 'One Health'

Tick-borne diseases are not only of veterinary and economic importance in livestock (Shaw, 2009), but also pets (de la Fuente *et al.,* 2015). As a result, tick-borne diseases may have a high impact on the human public health, as many of these can be transmitted to humans (Shaw, 2009; de la Fuente *et al.,* 2015). Therefore, the implementation of a 'One Health' approach, a concept where physicians, veterinarians, researchers, agencies and government unify in their effort to management zoonotic tick-borne diseases (Shaw, 2009; Day, 2011) is of critical importance.

While approximately 60% of human pathogens are zoonotic in nature, consideration of emerging and re-emerging diseases may increase the estimation to approximately 73% (Shaw, 2009). A well-known example is Lyme disease which is caused by a spirochaete *Borrelia burgdoferi* transmitted by the tick *Ixodes scapularis* (black-legged tick) which causes major problems to humans in the northern hemisphere (Schwan *et al.,* 1995; Stafford, 2007; Pfäffle *et al.,* 2013). Other tick-borne zoonotic diseases such as encephalitis and haemorrhagic fevers are responsible for high morbidity and mortality rates in humans (Jongejan and Uilenberg, 2004). From 2000 to 2010 approximately 250 000 cases

of human Lyme diseases were reported in the United States and around 50 000 per year in Europe (Dantas-Torres *et al.,* 2012). Also, the number of Lyme disease cases in the United States of America increased by 101% between 1992 and 2006 (Pfäffle *et al.,* 2013).

It is important to note that the number of tick-borne diseases affecting humans and domestic animals have increased in recent years and include anaplasmosis, babesiosis, ehrlichiosis, and Lyme borreliosis. Most of the cases of human tick-borne diseases have mainly been associated with ixodid ticks, but some cases of infestation by argasid ticks have also been reported (Dantas-Torres *et al.,* 2012). Since the 1980s the number of recognised medically important tick-borne diseases has increased. For example; since 1984, 10 new *Rickettsia* spp. responsible for human diseases have been identified (Dantas-Torres *et al.,* 2012).

Humans can also be infected via an indirect route, i.e. not being bitten by a tick. For example, humans can be infected with Crimean-Congo haemorrhagic fever through a tick bite (*Hyalomma* spp.) but also through contact with blood and / or tissue from infected animals (Swanepoel, 2012; Richards *et al.,* 2015). The disease has a fatality rate of between five and thirty percent in humans (Swanepoel, 2012). The majority of human cases are recorded in people working in the livestock industries (Swanepoel, 2012; Richards *et al.,* 2015), including farm workers exposed while performing general procedures such as castration, vaccination, attachment of ear tags or cutting of ears for identification purposes, abattoir workers and veterinary personnel (Swanepoel, 2012). In South Africa, approximately 204 cases of laboratory confirmed Crimean-Congo haemorrhagic fever have been recorded between 1981 and 2017 (Coetzee *et al.,* 2017), with the majority of incidences recorded in the Free State and Northern Cape provinces (Richards *et al.,* 2015). When taking into consideration the seroprevalence of Crimean-Congo haemorrhagic fever in cattle in South Africa the occurrence in humans are considered to be low (Swanepoel, 2012). However, outbreaks are possible as was seen during 1996 when 17 confirmed cases were recorded at an ostrich abattoir (Richards *et al.,* 2015).

3.1.5 Economic impact

As indicated in the foregoing paragraphs, tick infestations affect the growth and development of animals negatively and also through the transmission of diseases. Many countries, particularly those in the African continent experience heavy losses on an annual basis as a result of the impact of ticks on the livestock industries and the management and the control of diseases transmited by ticks.

Areas of economic impact include the following:

I. Animal growth and production

Ticks have a direct impact on the economy by affecting weight gain and milk production in cattle (Brossard, 1998; Tisdell *et al.,* 1999; Rajput *et al.,* 2006; Kiss *et al.,* 2012; Mapholi *et al.,* 2014). In a study conducted by Jonsson *et al.* (1998) it was shown that with an increasing tick infestation there was a significant decrease in milk production, with an estimated loss in milk production of 92.5 L over a 15 week period for each cow.

In Australia it was estimated that cattle ticks are responsible for a reduction ranging from 0.6g to 0.9 g of weight gain in cattle per fully engorged female tick, resulting in an approximate decrease for a single animal in weight of 600 to 900 g over the three week feeding period during low infestations and 2 kg during medium to high infestations (Estrada-Peña and Salman, 2013). In studies conducted by Norval *et al.* (1989a and 1989b) the estimated life weight gain was affected by a loss of 10 g per single fully engorged adult female *Am. hebraeum*, compared to the approximate 4 g per single fully engorged adult female *Rh. appendiculatus*.

The damage caused by tick bites also has a negative effect on the economy as it lowers the value of hides used in the manufacturing of leather (Barnett, 1961; Brossard, 1998; Tisdell *et al.,* 1999; Jongejan and Uilenberg, 2004; Adane *et al.,* 2012; Kiss *et al.,* 2012; Mapholi *et al.,* 2014).

II. Tick-borne diseases

Tick-borne diseases are associated with high economic costs. It is estimated that globally, the annual cost associated with cattle ticks and tick-borne diseases is between 13.9 and 18.7 billion US dollars (de Castro, 1997; Minjauw and

McLeod, 2003; Estrada-Peña and Salman, 2013; Mapholi *et al.,* 2014). In Africa the estimated loss as a result of tick infestation and tick-borne diseases is 160 million US dollars and approximately 92 million US dollars in South Africa (Mapholi *et al.,* 2014). However, it is believed that when costs that results from the implementation of tick control measures are added the estimation can approximate 720 million US dollars in Africa (Adenubi *et al.,* 2012). In a study conducted by Adenubi *et al.* (2012) losses suffered by small scale and traditional farmers on account of *Theleria* related diseases are estimated to be over 200 million US dollars, when in Zimbabwe heartwater, together with the costs for acaricides, milk losses and treatment approximates 6 million US dollars annually.

Tick-borne diseases of companion animals in industrialised countries also have an economic effect when pathogens infect animals such horses thereby affecting international trade and sporting events (Jongejan and Uilenberg, 2004).

III. Control

Costs relating to ticks do not only apply to controlling the actual ticks infesting a host, but also costs include costs incurred in controlling and treating tick-borne diseases. Control measures for tick-borne diseases include preventative measures such as the administration of live, attenuated or recombinant vaccines or the administration of antigens and treatment, such as administration of. antibiotics and / or other therapeutic agents (de Castro, 1997; Minjauw and McLeod, 2003; Mapholi *et al.,* 2014). Spickett (2013) reported an average annual sale of doses of vaccines against babesiosis to be 140 000, anaplasmosis 100 000 and heartwater 60 000.

Costs incurred in protecting animals by using acaricides may vary as they are dependent on the number of applications required, the application method used, the number of animals in the herd, and the chemical acaricide used (Minjauw and McLeod, 2003). For example, control of *Theileria* in central and southern Africa is estimated to be 168 million US dollars and 384.3 million US dollars in India (Adenubi *et al.,* 2012). Additional considerations in the costs must also

include investments in the infrastructure and / or equipment required for administration of acaricides, and the associated labour (Minjauw and McLeod, 2003).

According to Spickett (2013) commercial farmers in South Africa generally follow an intensive tick control program which can result in up to 37 applications of acaricides per year. Application techniques are influenced by convenience, availability and costs. For example, dipping or using a spray race are the most convenient methods used to treat large herds. However, this is not feasible for small-scale farmers, who will find hand-spraying or the use of pour-ons to be more economical. It is estimated that large dipping tanks would require between 9000 and 18000 litres to be filled, with the animal emerging soaked in about 9 litres of dip and 6 ½ litres draining back into the tank. With smaller herds, hand spraying is more ideal, and it is estimated that approximately 13 litres are required to fully saturate larger animals (Harrison *et al.,* 1973). In 1999 the estimated cost associated with dipping of cattle in South Africa ranged between US$1.60 to US$9.60 per adult for a year for between five and thirty applications. According to Minjauw and McLeod (2003) vaccination costs could range between US$3.30 to US$12.60 for an individual administration, and treatment of a cow for heartwater ranged between US$1.50 to US$3.00 per adult per year.

3.2 Conclusion

It is clear from the foregoing account that losses incurred on an annual basis as a result of tick infestations are enormous. The situation is much direr in developing countries in the African continent. However, much of the account on the economic impact of ticks is largely based on the losses incurred by commercial entities. The losses experienced by small-scale subsistence farmers are often excluded from the estimates. It is therefore important that future estimations of losses resulting from tick infestations and the diseases they transmit should also incorporate losses experienced by small-scale farmers in order to have much more inclusive estimates. In the next chapter focus will be on the tick control methods and their impact in reducing tick infestations.

CHAPTER 4
LITERATURE REVIEW:
TICK CONTROL METHODS

4.1 Introduction

It is evident from the preceding chapter that as a result of the duality of the role ticks play as parasites and vectors of diverse kinds of disease-causing agents resulting in significant loss in revenue particularly in developing countries, the need for effective tick control methods remains critical. The continued prevalence of tick-borne diseases and the sustained negative impact they cause to the livestock industry suggests that the current tick control methods are only partially effective.

Currently, tick control methods include the use of chemical acaricides for short-term methods to clean the animals and long-term methods to reduce tick burdens (de Castro, 1997; Dantas-Torres *et al.,* 2012; Kaur *et al.,* 2016). This also includes the use of repellents which according to Bissinger and Roe (2010) are chemical substances that cause an organism to move away from their source.

Most commonly the use of chemical acaricides involve their application on the host animals by dipping, spray races, hand sprays, pour-ons, injectables, hand-dressings (Barnett, 1961; Young *et al.,* 1988; Minjauw and McLeod, 2003; FAO, 2004; Rajput *et al.,* 2006) or the use of treated plastic tag or collars (Lwande *et al.,* 1999; Rajput *et al.,* 2006). The use of acaricides can also either follow a suppressive approach, which include multiple treatments at regular intervals during the height of the infestation or a threshold approach where acaricides are applied only when infestation levels exceed the acceptable level (FAO, 2004).

However, over reliance on synthetic chemicals for the control of ticks has led to a number of problems including environmental pollution, lack of target specificity, residues in products destined for human consumption, emergence of tick strains resistant to acaricides and high costs of acaricides that are out of reach by small-scale farmers.

These problems heighten the need to search for alternative tick control methods and are considered in a somewhat detailed manner in the latter paragraphs in this chapter.

According to Ghosh *et al.* (2007) and Asmaa *et al.* (2014) a successful tick control strategy should lead to the following outcomes:

I. a reduction in the number of tick infestations to the level that reduces the medically and economically important tick-borne pathogens,
II. avoid animal production losses,
III. and minimizes chemical residues.

In this chapter a critical account will be given on the chemicals used for tick control and the exploration of the related alternative tick control methods will be made.

4.2 A historical account on the use of chemicals for tick control

Figure 4.1 illustrates the history of chemical control of ticks, highlighting the chemical compounds, reasons for withdrawal as well as showing the chemicals most commonly used today.

Effective chemical control of ticks basically began with the introduction of dips in South Africa and Australia between 1893 and 1895 (Harrison *et al.,* 1973). As from the 1930's, the chemical control of ticks greatly improved with the discovery of the insecticidal properties of organochlorines including dichlorodiphenyl trichloroethane (DDT) (George *et al.,* 2004; Graf *et al.,* 2004; Ghosh *et al.,* 2007; George *et al.,* 2008; Kaur *et al.,* 2016), benzenehexachloride (BHC) (George *et al.,* 2004; George *et al.,* 2008; Kaur *et al.,* 2016), and other organochlorines (Graf *et al.,* 2004; Ghosh *et al.,* 2007). However, organochlorines previously used for treating livestock are no longer available or have been withdrawn from the market (George *et al.,* 2004; George *et al.,* 2008).

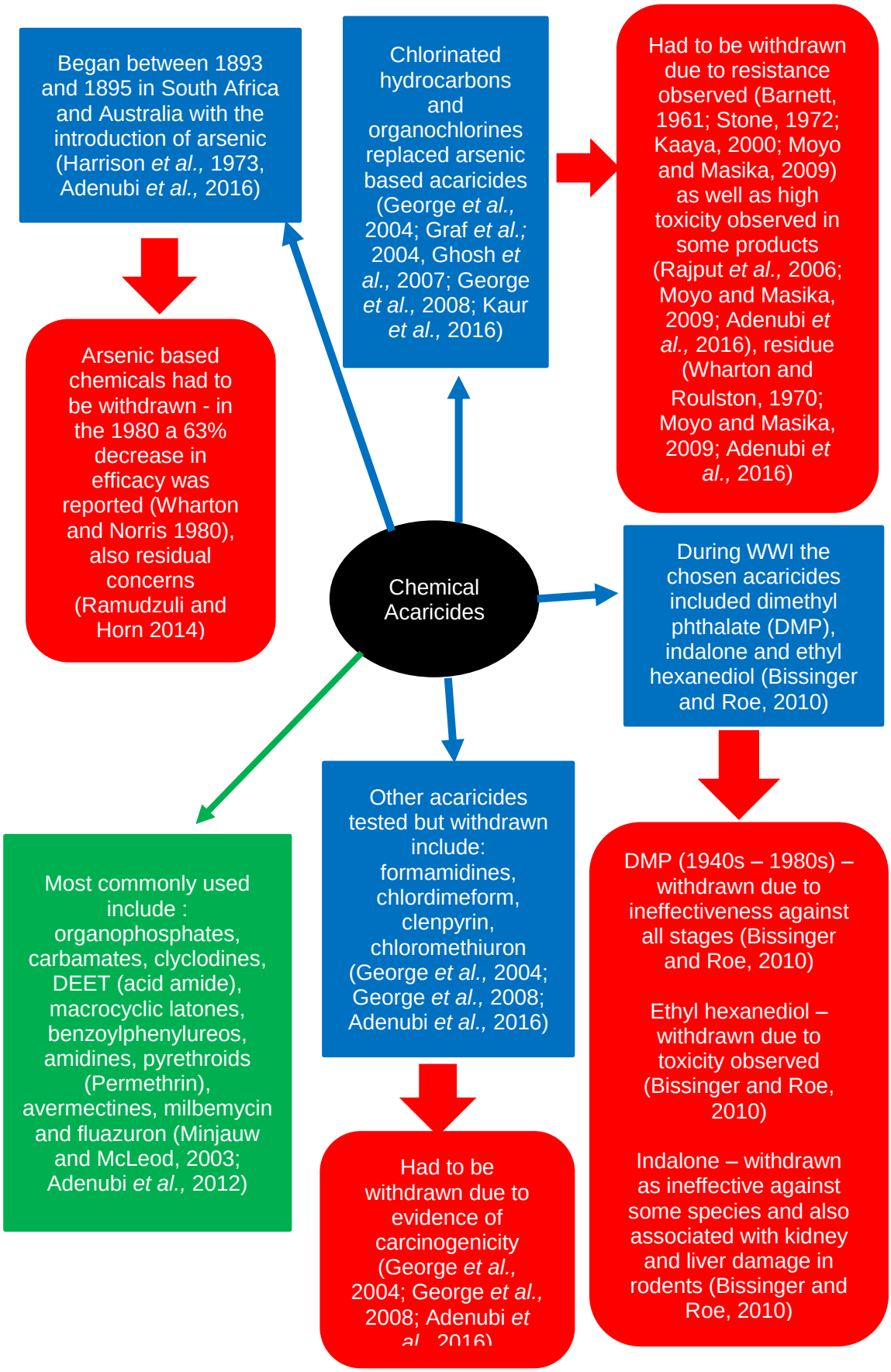

Figure 4.1 History of chemicals used for tick control

In South Africa, for example, resistance was observed against DDT five years after introduction as acaricide (Kaaya, 2000) and was also associated with residue (Wharton and Roulston, 1970). Other organic pesticides developed and recommended for use in tick control include organophosphates, carbamates, cyclodines, macrocyclic lactones, benzoylphenylureas, amidines, pyrethroids (Frisch, 1999; Minjauw and McLeod, 2003; Graf *et al.,* 2004; Peter *et al.,* 2005; Ghosh *et al.,* 2007; Kaur *et al.,* 2016), avermectines and milbemycin (Frisch, 1999), all associated with various concerns or disadvantages. Other chemicals found to have been effective against ticks also include formamidines, chlordimeform, clenpyrin and chloromethiuron although it had to be withdrawn due to evidence of carcinogenicity (George *et al.,* 2004; George *et al.,* 2008; Adenubi *et al.,* 2016). Frisch (1999) also suggested the use of growth regulators, such as fluazuron for tick control.

Currently ticks are primarily controlled by the use of synthetic acaricides such as organophosphate, pyrethriods (Minjauw and McLeod, 2003; Adenubi *et al.,* 2012), arsenicals, chlorinated hydrocarbons, carbamates, formamidines, macrocyclic lactones and insect growth regulators (Adenubi *et al.,* 2012) with amitraz, picaridin, and N,N-diethyl-m-toluamide (DEET) (Mkolo and Magano, 2007; Katz *et al.,* 2008; Bissinger and Roe, 2010) being the most commonly used.

4.3 Short comings of the current tick control methods

As indicated previously, over-reliance on chemical acaricides is associated with various problems. Table 4.1 provides a brief summary of the chemical groups and the problems that are associated with them. However, broadly speaking the problems resulting from over-reliance on synthetic chemicals for tick control include:

I. **Environmental pollution and residues in products destined for human consumption**

   Drawbacks such as environmental pollution, contamination of milk and meat products with chemical residues are noted in literature (Kaaya *et al.,* 1995; de Castro, 1997; Minjauw and McLeod, 2003; FAO, 2004). Contaminants can include agrochemicals which include veterinary medicine and pesticides, environmental contaminants which include heavy metals, persistent organic pollutants and natural toxins or processing contaminants from cooking,

processing and packaging (Cooper *et al.,* 2014). Farmers are also generally concerned with several other problems associated with dipping. These include losses caused by the stress, abortions, potential of drowning and physical injury (Rikhotso *et al.,* 2005).

II. Emergence of acaricide resistant tick strains

Harrison *et al.* (1973) first reported that ticks show a genetic potential to resist a wide range of chemicals. According to Abbas *et al.* (2012) three types of resistance might be observed in ticks. These are acquired resistance (inherited resistance), cross-resistance (resistance against different but similar-acting acaricides) and / or multiple resistance (resistance against different and different acting acaricides probably due to metabolism of acaricides). Unfortunately, ticks have built up resistance against most of the currently used chemicals (Table 4.1), thereby lowering their efficacy (Graf *et al.,* 2004).

Factors that play a role in the resistance of ticks to chemical acaricides may be due to a reduced penetration through the integument, a reduction of chemical uptake, an increase in the storage or excretion of unchanged toxins, reduced effect of the toxin in the body of the tick due to metabolic breakdown of the toxin, reduced reactivity to the chemical at the site of contact, and genetic resistance (Stone, 1972), the frequency of acaricide use, treating herds at times when tick populations are small, use of poor quality acaricides and use of acaricides with a prolonged sub-lethal curve and under-treatment of herd (FAO, 2004).

III. High costs of synthetic acaricides

In addition, chemical acaricides are expensive and as a result out of reach by poor-resourced farmers (Lwande *et al.,* 1999; Minjauw and McLeod, 2003). Development and registration of new acaricides is very expensive and can exceed US$100 million while taking up to 10 years from initial conception to availability in the market (Graf *et al.,* 2004).

Table 4.1 Common concerns and drawbacks of chemical acaricides

Concern / drawback	**Chemical**	**Reference**
Resistance	DDT, various of the organophosphates, carbamates, amidines, synthetic pyrethriods	Willadsen and Kemp (1988)
	Permethrin, cypermethrin, alpha cypermethrin, deltamethrin, cyhalothrin, flumethrin, fenvalerate, cyfluthrin, dieldrin, toxaphene, lindane, carbaryl, ethaphos, cyclophos, trichlofon, dioxathion, chlorpyriphos, ethion, chlrofenvinphos, dimethoate, amitraz	FAO (2004)
	diazinon, coumaphos	FAO (2004) Lovis *et al.* (2011)
	ivermectin	Lovis *et al.* (2011) Klafke *et al,* (2012)
Residue in tissue, fat or milk	Pyretroid	Cooper *et al.* (2014)
	DDT, BHC and cyclodienes	George *et al.* (2004) Graf *et al.* (2004) George *et al.* (2008)
	Organophosphates	Minjauw and McLeod (2003)
	Macrocyclic lactones	Minjauw and McLeod (2003)
	Benzoylphenylureas	Minjauw and McLeod (2003)
	Avermectins	Frisch (1999)
	Fluazuron	Frisch (1999)
High cattle toxicity	Arsenic	Graf *et al.,* (2004) Rajput *et al.,* (2006) Ghosh *et al.* (2007)
Persistence in the environment	All organochlorines	George *et al.* (2004) Graf *et al.* (2004) George *et al.* (2008)
	Arsenic	Ramudzuli and Horn (2014)

As a result of poor resourced farmers not being able to afford expensive acaricides, they often resort to using alternative means in an effort to reduce tick infestations on their livestock. Masika *et al.* (1997b), Moyo and Masika (2009) and Mapholi *et al.* (2014) identified the use of household disinfectants, old vehicle oil and diesel, pour-ons, manually removal, cutting off of ticks using blades and paraffin as practices that can be harmful to animal and human health. As many rural and small-

scale farmers do not dip small ruminants, these animals become reservoirs for ticks and can as such re-infect dipped cattle (Moyo and Masika, 2009). In a study conducted by Hlatshwayo and Mbati (2005) in the eastern Free State (Qwa-Qwa) it was found that 28% of resource-poor farmers use commercial acaricides, including pour on, dipping and tick grease, and about 70% follow some type of tick control, alternative methods including the use of used engine oil, Jeyes fluid and paraffin, deticking and the use of chickens are also used.

In Africa, problems associated with the use of synthetic acaricides also include changes in economic conditions which result in higher prices on imported items (Zorloni *et al.,* 2010). Also, incorrect administration methods of acaricides might assist in the development of resistance (Zorloni *et al.,* 2010). On the other hand, short comings of commonly used collars or tags include that the acaricide is not effective on ticks around the groin, udder and areas on the hindquarters (Sonenshine *et al.,* 2002; Nicholson *et al.,* 2009).

4.4 Non-chemical tick control methods

As a result of the problems arising from over-reliance on the use of synthetic chemicals for tick control, a number of alternative tick control methods have been considered. These include introduction of tick resistant cattle, exploitation of acquired resistance, vaccination, biological control and use of plant-based products.

4.4.1 Natural (acquired) resistance

Alternative non-chemical methods for tick control include the introduction of resistant cattle into an area (FAO, 2004). Cattle species, such as *Bos indicus*, have shown acquired resistance against some tick species, specifically against *Rh. (Bo.) microplus* (de Castro, 1997; Brossard, 1998; Jonsson *et al.,* 1998; Frisch, 1999; Willadsen, 2004; Ghosh *et al.,* 2006; Willadsen, 2006; Ghosh *et al.,* 2007; Willadsen, 2008; Mapholi *et al.,* 2014). Similar resistance has been observed in some *Bos taurus* breeds (Minjauw and de Castro, 2000). A study conducted by Solomon and Kaaya (1996) also highlighted an increased resistance in endemic breeds compared to cross-bred breeds.

Natural or acquired resistance has also been observed against both *Am. hebraeum* and *Rh. appendiculatus* ticks (Solomon and Kaaya, 1996). Primary response by

the host immune system to the introduction of tick saliva includes the degranulation of mast cells. Animals showing a higher resistance show mast-cell degranulation and the accumulation of eosinophils in the bite lesion (Minjauw and de Castro, 2000). Rechav (1992) reported that certain host and environmental factors affect the natural resistance of cattle to tick infestations. These include:

I. nutrition,
II. sex,
III. age of host and
IV. pregnancy.

Cross protection has also been observed where animals that express resistance against one tick species also show partial or complete resistance against other species or genus of ticks, but not present for all genera or species (Brown, 1985). Other factors that play a role in the natural resistance of cattle include hide thickness. In a study conducted by Fourie *et al.* (2013) on four different breeds it was observed that the number of ticks related directly to the thickness of the hide. Fewer ticks were found on cattle with thicker hides and sleeker coats due to these factors acting as a natural deterrent.

Cattle are considered to show resistance to ticks when they are less attractive to tick infestations or where grooming is more efficient in removing ticks (Young *et al.,* 1988; Kaur *et al.,* 2016). The manifestation of this resistance to ticks by hosts can be measured by evaluating the following parameter(Rechav, 1992; Rechav and Field, 1995; Wikel and Bergman, 1997; Brossard 1998):

I. a reduction in the number of engorged ticks
II. smaller blood meals consumed during feeding resulting in a decrease in weight of ticks
III. changes in feeding duration
IV. reduction in the number of viable eggs post feeding
V. and failure of ticks to complete their developmental cycle and mortality of ticks and eggs.

Among these parameters, reduction in engorgement weight of ticks is regarded to be the most consistent indicator of host resistance (Rechav and Field, 1995).

4.4.2 Use of Vaccines in Tick Control

Another alternative tick control method includes the use of vaccines. Vaccines are considered to be an environmentally friendly alternative in tick control methods (de la Fuente *et al.,* 2015). The role of a tick vaccine is to reduce the number of engorging females on a host, by affecting their weight and reproduction capacity, thereby affecting the subsequent generation (Mapholi *et al.,* 2014). To date various cattle tick vaccines have been developed and commercialised including TickGARD^{TM}, TickGARD plus^{TM} and Gavac^{TM} (FAO, 2004; Willadsen, 2004; Ghosh *et al.,* 2007; Willadsen, 2008; Kiss *et al.,* 2012; Schwarz *et al.,* 2012; Kaur *et al.,* 2016), based on the midgut transmembrane Mb86 pf of *Rh. (Bo). microplus* (Schwarz *et al.,* 2012; Kaur *et al.,* 2016). It is important to note that these vaccines do not boost the natural antibody titers as they are based on a concealed antigen (Schwarz *et al.,* 2012).

Field trials of cattle vaccinated showed a reduction in incidences of babesiosis together with a reduction in tick-infestations (Ghosh *et al.,* 2007). A benefit of vaccine use is that it is expected that where a single point mutation in a target molecule is sufficient to render a pesticide ineffective may not have the same effect on the effectiveness of a vaccine (Kiss *et al.,* 2012). During vaccine development the anti-tick strategy looks at proteins that play a role in feeding success, and / or tick physiology. Proteins that therefore modulate the host immune responses are targeted for investigation (Schwarz *et al.,* 2012). The Bm86-based vaccines have shown to reduce the number of engorged females, their weight, reproductive capacity, and damage tick guts (Frisch, 1999; Willadsen, 2006). The vaccine has shown to be effective against *Rh. (Bo.) microplus*, *Rh. annulatus* and *Rh. (Bo.) decoloratus*, with partial protection observed against *Hyalomma* and other *Rhipicephalus* spp. with a related phylogeny (de la Fuente *et al.,* 2015). Willadsen (2004) and Willadsen (2008) indicated that vaccines could potentially be non-contaminating, used on a variety of hosts, and cheaper to produce and register compared to chemical acaricides.

There are also disadvantages associated with anti-tick vaccines. A vaccine cannot be used as a standalone control measure due to inefficacy of a vaccine against some tick species (Kiss *et al.,* 2012) and the immediate decrease in the number of

ticks (Frisch, 1999). An additional disadvantage of vaccines is that they are sometimes effective against only a single tick species (Mapholi *et al.,* 2014).

4.4.3 Biological Control

Other alternative methods which are currently being explored include the use of biological controls such as bacteria, fungi, entomopathogenic nematodes, parasitoids and predators. Examples include *Cadecea lapagei*, a bacterium, which is pathogenic to *Rh. (Bo.) microplus*; fungi of the class Deutreromycetes; nematodes, specifically Heterorhabditidae and Steinernematidae; parasitoids, most commonly belonging to the order Hymenoptera and predators including ants, beetles and various bird species (FAO, 2004; Samish *et al.,* 2004; Samish *et al.,* 2008; Kaur *et al.,* 2016), on farms and chickens are also considered to be useful in the control of cattle ticks (Kaur *et al.,* 2016).

However, shortcomings of biological tick control methods include difficulties of application and lack of stability (Willadsen, 2006), and also only suppresses tick populations and are not able to eliminate them completely (Schwarz *et al.,* 2012).

4.4.4 Other non-chemical Control

Other non-chemical tick control methods include, pasture spelling where pastures are left unstocked to break the life-cycle of the ticks (Harrison *et al.,* 1973; Young *et al.,* 1988; Minjauw and McLeod, 2003; FAO, 2004; Ghosh *et al.,* 2006; Kaur *et al.,* 2016); the release of sterile males into the habitat (Minjauw and McLeod, 2003); grazing measurement, where pasture rotation is combined with the application of acaricides; pasture burning (Barnett, 1961; Harrison *et al.,* 1973; Mapholi *et al.,* 2014) and removal of wildlife or rodent hosts from the livestock environment (Harrison *et al.,* 1973; Mapholi *et al.,* 2014).

However, pasture spelling is not without problems. As a tick control method it cannot be kept clear of hosts as some of the tick species of importance are three-host ticks and may therefore feed on other hosts and survive for a longer time period in the pasture. Rough pastures also increase the survival of tick populations (Harrison *et al.,* 1973; Young *et al.,* 1988; Kettle, 1995). Also, burning of grasslands

does not show to be effective. A study by Harrison *et al.* (1973) in Australia showed larvae to be present in the burnt area 33 days after the fire.

4.5 Botanical use in tick control

For centuries botanical substances have been used to repel insects, ranging from burning to the application of oils on the skin (Maia and Moore, 2011). According to Bissinger and Roe (2010) repellents created from plant material are also considered to be safe as there are less harmful side effects from their regular use. Natural insecticides, such as insecticides derived from plants, were mostly used prior to the introduction of synthetic or chemical insecticides (Maia and Moore, 2011; Gonzalez-Coloma *et al.,* 2013), with synthetic repellents being introduced by World War II (Bissinger and Roe, 2010).

The advantages of utilizing botanical products as agents of tick control is that they are regarded as a safer alternative compared to chemical acaricides, as they have shown a reduced residue and are also bio-degradable. As a result they are also considered to be eco-friendly, with an estimated lower costs involved compared to the production costs of chemical acaricides and a reduced probability of resistance due to multiple active ingredients present in one plant extract (Lwande *et al.,* 1999; Mkolo *et al.,* 2011b; Kiss *et al.,* 2012; Adenubi *et al.,* 2016).

Botanical products exhibit a number of biological activities, including toxicity, repellence, and effecting growth, feeding patterns (Bissinger and Roe, 2010; Maia and Moore, 2011; Kiss *et al.,* 2012; Adenubi *et al.,* 2016), inhibiting egg development, disrupting mating communication and inhibition of the formation of chitin (Adenubi *et al.,* 2016). Magano *et al.* (2008) and Zahir *et al.* (2009) reported that botanical extracts or essential oils may therefore be an alternative acaricidal source as they constitute a rich source of bioactive compounds that are biodegradable into nontoxic products. On the other hand, chemical insecticides are generally based on a single active ingredient. Plant-derived insecticides include several compounds thereby decreasing the chances of pests developing resistance, with the main groups of plant-derived active compounds including phenylpropanoids and phenolics, terpenoids and steroids, alkaloids and nitrogenated compounds (Maia and Moore, 2011; Gonzalez-Coloma *et al.,* 2013). Therefore, with the high

costs involved in the development of newer chemically based acaricides and vaccines, due to resistance and the concerns regarding residues in produce and the environment by chemically based products, botanically based compounds offer a safer, more cost-effective and effective alternative to chemical acaricides (Abbas *et al.,* 2012).

Ethnobotanical products are most often used as suspensions poured on the animal, fumigation using smoke from the plants, dusting animal bodies with ash, and hanging the products in cattle sheds (Wanzala *et al.,* 2012). It is estimated in South Africa that up to 75% of rural livestock owners in the Eastern Cape Province use plants and plant-based remedies to treat their animals, and is wide spread due to the low costs and convenience (McGaw and Eloff, 2008).

The most commonly used botanical products used for insect control and repellence includes pyrethrum, rotenone, neem and essential oils (Isman, 2006). Kaur *et al.* (2016) noted that between 14 and 28% of the approximate 422 000 plants identified to date have been used for medicinal purposes.

Following a citation matrix search on Web of Science, Scopus and Google Scholar, a total of 45 articles (citations) were identified using the keywords 'botanical control of ticks'. Using the identified articles from the citation search as well as other articles identified, a review was done on botanicals tested for efficacy as tick repellents and / or acaricide. The review focused on botanicals tested against the commonly found ticks in South Africa. Data obtained is summarized in Table 4.2 which shows the tested plant species, associated ticks, method of preparation of plant extracts and resulting effect. Table 4.3 summarizes the reviews done as part of this study against non-livestock and non-South African tick species. The review also highlighted different treatment methods of plant material, including preparation of essential oils and extraction using various solvents that are currently used to evaluate the potential and efficacy of botanicals.

Table 4.2 Botanicals tested as repellent and / or acaricide against commonly found South African cattle ticks

Tick Species	Plant Species	Plant part	Extract/mixture	Level of effect	Reference
Am. hebraeum	*Azadirachta indica*	Seed	10% oil suspension	Weekly application of the 10% suspension result in effective tick control in goats year round	Schwalbach *et al.* (2003)
	Mentha piperita	NA	NA	As repellent a 5%, 10% and 20% v/v concentration of essential oil in dichloromethane was 100% effective	Mkolo *et al.* (2011a) Mkolo *et al.* (2011b)
	Mentha spicata	NA	NA	As repellent a 10% and 20% v/v concentration of essential oil in dichloromethane was 100% effective, the 5% concentration varied between 90-100% effective	Mkolo *et al.* (2011a)
Am. variegatum	*Azadirachta indica*	NA	Oil	Deter larval and nymph attachment, inhibit feeding, reduce fecundity and egg hatching and moulting of larvae and nymphs	Zimmerman *et al.* (1984)
	Gynandropsis (Cleome) gynandra	Aerial parts	Hydrodistilled	A 0.1µl composition showed a 98.9% repellency effect and a 0.01µl composition a 89.8% repellency effect	Lwande *et al.* (1999)
		Leaves	NA	All stages avoid the leaves and continuous exposure resulted in death	Kaaya (2000)
	Margaritaria discoidea	Bark	Aqueous	High mortality in nymphs	Kaaya (2000)
		Dry bark and wood	Oily hexane	6.25% concentration caused 100% mortality in nymphs after 10 min exposure	Kaaya *et al.* (1995) Kaaya (2000)
Am. variegatum	*Tephrosia vogelli*	Leaves	Aqueous	Average mortality observed 71.67%	Ndava *et al.* (2018)
			Acetone	Average mortality observed 91.16%	

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Tick Species	Plant Species	Plant part	Extract/mixture	Level of effect	Reference
Hy. rufipes	*Allium sativum*	Bulbs	Ethanol	No mortality observed in contact toxicity bioassay within one hour and showed 15% mortality after 24 hours	Nchu *et al.* (2005)
		Bulbs	Acetone	No mortality observed in contact toxicity bioassay within one hour and showed 100% mortality after 24 hours	Nchu *et al.* (2005)
			Dichloromethane	In a contact toxicity bioassay 100% of ticks were killed within one hour	
		Bulbs	Dichloromethane	Using a repellency bioassay 40.08 to 86.96% of ticks were repelled with varying concentrations	Nchu *et al.* (2016)
	Eucalyptus glaboidea	Leaves	Dichloromethane	A 20% v/w concentration showed a high repellency (>70%) for first 30 minutes with a decline thereafter, a 30% v/w concentration showed high repellency (>75%) for up to 90 minutes whereafter a decline was observed, the 40% concentration showed >90% repellency for up to 50 minutes where after test was stopped	Magano *et al.* (2011a)
	Lavendula angustifolia	Leaves, soft branches and inflorescences	Hydrodistilled	High repellency (70 - 100%) was observed in 5%, 10% and 20% v/v concentrations, however 5% concentration was only effective as high repellent for 40 minutes whereas 10% and 20% was 120 minutes	Mkolo and Magano (2007) Magano *et al.* (2011a)
	Lippia javanica	Leaves, branches and flowers	Hydrodistilled	Varying repellency (33.3 - 100%) was observed in 2.7%, 5.3% and 10.7% v/v concentrations	Magano *et al.* (2011b)

Table continues on the next page

Tick Species	Plant Species	Plant part	Extract/mixture	Level of effect	Reference
Hy. rufipes	*Nicotiana tabacum*	Leaves	Ethyl acetate	Mixed repellency were observed for 20% and 30% v/w concentration for the first 40 minutes of the test with a low repellency (<65%) after 40 minutes, a 40% v/w concentration showed a high repellency (>75%) for first 30 minutes, and decrease (<60%) from 40 minutes	Magano *et al.* (2011a)
	Senna italica subsp. *arachoides*	Roots	Chloroform	No effect observed	Magano *et al.* (2008)
			Ethyl acetate	A 15g/50mL extract resulted in a 100% mortality after 24h	
			Dichloromethane	No effect observed	
			Hexane	No effect observed	
			Methanol	No effect observed	
			Water	An aqueous extract ingested by rabbits resulted in a reduction of feeding performance of ticks	
	Tagetes minuta	Leaves, branches and flowers	Hydrodistilled	A 0.107mL/mL concentration of essential oil resulted in a 93.61% repellency	Thembo *et al.* (2010)
		Leaves, branches and flowers	Hydrodistilled	A 0.107mL/mL concentration of essential oil resulted in a 93.61% repellency, and delay of moulting in 60% of nymphs	Nchu *et al.* (2012)
Hy. truncatum	*Azadirachta indica*	Seed	10% oil suspension	Weekly application of the 10% suspension result in effective tick control in goats year round	Schwalbach *et al.* (2003)

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Tick Species	Plant Species	Plant part	Extract/mixture	Level of effect	Reference
Rh. appendiculatus	*Allium sativum*	Cloves	Aqueous	Efficacy of concentration sprayed on cattle during in vivo study: 10% - 74% mortality, 25% - 83% mortality and 50% - 98% mortality after 24 hours	Ndava *et al.* (2018)
	Cassia didymobotrya	Dried plant	Hexane	Average repellency of 81.32%	Opiro *et al.* (2013)
			Dichloromethane	Average repellency of 83.80%	
			Methanol	Average repellency of 87.67%	
	Cissus adenocucaulis	Dried plant	Hexane	Average repellency of 47.90%	Opiro *et al.* (2013)
			Dichloromethane	Average repellency of 46.45%	
			Methanol	Average repellency of 52.61%	
	Cissus quadrangularis	Dried plant	Acetone	No effect	Luseba *et al.* (2016)
			Dichloromethane	No Effect	
			Methanol	No Effect	
	Cleome monophylla	Aerial parts	Hydrodistilled	In a tick climbing assay a 0.1µl of oil showed a 89.9% repellency	Ndungu *et al.* (1995)
	Euphorbia hirta	Dried plant	Hexane	Average repellency of 42.16%	Opiro *et al.* (2013)
			Dichloromethane	Average repellency of 41.64%	
			Methanol	Average repellency of 45.54%	
	Euphorbia ingens	Dried plant	Acetone	No effect	Luseba *et al.* (2016)
			Dichloromethane	No Effect	
			Methanol	No Effect	
	Gynandropsis (Cleome) gynandra	Leaves and complete plant	Fresh	Fresh leaves (plant) act as repellent	Malonza *et al.* (1992)
		Leaves and complete plant	Fresh	Acaricidal properties observed during glass tube assays (olfactometer assay)	
		Aerial parts	Hydrodistilled	A 0.1µl composition showed a 98.9% repellency effect and a 0.01µl composition a 89.8% repellency effect	Lwande *et al.* (1999)

Table continues on the next page

Tick Species	Plant Species	Plant part	Extract/mixture	Level of effect	Reference
Rh. appendiculatus	*Gynandropsis (Cleome) gynandra*	Leaves	NA	All stages avoid the leaves and continuous exposure resulted in death	Kaaya (2000)
	Kigelia africana	Dried plant	Hexane	Average repellency of 70.80%	Opiro *et al.* (2013)
			Dichloromethane	Average repellency of 72.29%	
			Methanol	Average repellency of 76.38%	
	Lippia javanica	Dried plant	Acetone	90% repellency	Luseba *et al.* (2016)
			Dichloromethane	Not reported	
			Methanol	Not reported	
	Margaritaria discoidea	Bark	Aqueous	High mortality in nymphs and adults	Kaaya *et al.* (1995) Kaaya (2000)
		Dry bark and wood	Oily hexane	6.25% concentration caused 100% mortality in adult and nymphs after 10 min exposure	
	Melinis minutiflora	Grass	Climbing assay - green grass	Larvae, nymphs and adults avoided climbing on grass	Mwangi *et al.* (1995b)
			Climbing assay - sun dried grass	A lower percentage of larvae, nymphs and adults were observed climbing on grass dried in shade compared to grass dried in the sun	
			Climbing assay - shade dried grass		
			Climbing assay - in study plot	No larvae were observed to climb to the top of *M. minutiflora*, 4.3% nymphs and 3.8% of adults did climb to the top of the grass	
			Steam distillation - extract mixed with petroleum jelly	Using the extract resulted in a prolonged feeding period of all instars fed on rabbits, only the feeding period was affected, moulting and engorgement were similar to control	

Table continues on the next page

Tick Species	Plant Species	Plant part	Extract/mixture	Level of effect	Reference
Rh. appendiculatus	*Nicotiana tabacum*	Leaves mixed with 'Magadi soda' ('kupertaba')	Aqueous	10%, 50% and 100% suspensions killed all stages, prevented completion of feeding, suppressed ovipositioning, reduced hatching of eggs, reduced attachment	Dipeolu and Ndungu (1991)
	Ocimum suave	Leaves	Oil in liquid paraffin	LC_{50} at 0.024%	Kaaya (2000)
			Steam distillation	100% mortality observed with 0.2%, 2.0% and 20.0% concentration using larval package test with LC50 observed at 0.024% concentration	Mwangi *et al.* (1995b)
			Steam distillation	Using a feeding bioassay, 2% killed 95% of larvae and 5% and 10% were highly effective in killing larvae and nymphs (>75%) and 100% (>75%) mortality in adults	Mwangi *et al.* (1995b)
	Tephrosia vogelli	Leaves	Aqueous	Average mortality observed 32.16%	Ndava *et al.* (2018)
			Acetone	Average mortality observed 51.67%	
Rhipicephalus species	*Allium sativum*	Cloves	Aqueous	Efficacy of concentration sprayed on cattle during in vivo study: 10% - 74% mortality, 25% - 83% mortality and 50% - 98% mortality after 24 hours	Mgocheki (2017)
Rh. (Bo.) decoloratus	*Aloe ferox*	Powedered aloe juice	Medicated game pellets	None	Fourie *et al.* (2005)
	Azadirachta indica		Oil	Deter larval and nymph attachment, inhibit feeding, reduce fecundity and egg hatching and moulting of larvae and nymphs	Kaaya (2000)
		Seed	Powder mixed with pellets	Inhibition of larval attachment and development	

Table continues on the next page

Tick Species	Plant Species	Plant part	Extract/mixture	Level of effect	Reference
Rh. (Bo.) microplus	*Acacia nilotica*	Bark	Methanol	LC_{99} after 24 hours 29.2%	Sindhu *et al.* (2012)
	Acacia pennatula	Leaves	70% Acetone	Larval mortality of 54.8% observed and no effect on egg laying ability	Fernández-Salas *et al.* (2011)
	Achyranthes aspera	Leaves	Acetone	89% mortality at 2000 ppm on larvae	Zahir *et al.* (2009)
			Chloroform	79% mortality at 2000 ppm on larvae	
			Ethyl acetate	100% mortality at 2000 ppm on larvae	
			Hexane	82% mortality at 2000 ppm on larvae	
			Methanol	93% mortality at 2000 ppm on larvae	
	Aegle marmelos	Leaves	Methanol	100% mortality at 2000 ppm	Elango and Rahuman (2011)
			Hexane	67% mortality at 2000 ppm	
			Chloroform	60% mortality at 2000 ppm	
			Ethyl acetate	54% mortality at 2000 ppm	
			Acetone	59% mortality at 2000 ppm	
	Andropogon gayanus	Grass	Fresh	Anti-tick properties against larvae	Kaaya (2000)
			Fresh	Anti-tick properties against larvae	Fernandez-Ruvalcaba *et al.* (2004)
			Fresh	Anti-tick properties against larvae (with mean number of larvae 502 14 days post infestation of plot infested with 40 000 larvae	Thompson *et al.* (1978)
			Fresh	Repellency only effective in mature plants (older than 6 months) on larvae	Cruz-Vazquez and Rubalcaba (2000)
	Andrographis lineata	Leaves	Methanol	100% mortality at 2000 ppm	Elango and Rahuman (2011)
			Hexane	86% mortality at 2000 ppm	
			Chloroform	61% mortality at 2000 ppm	
			Ethyl acetate	71% mortality at 2000 ppm	
			Acetone	86% mortality at 2000 ppm	

Table continues on the next page

Tick Species	Plant Species	Plant part	Extract/mixture	Level of effect	Reference
Rh. (Bo.) microplus	*Andrographis paniculata*	Leaves	Methanol	90% mortality at 2000 ppm	Elango and Rahuman (2011)
			Hexane	60% mortality at 2000 ppm	
			Chloroform	73% mortality at 2000 ppm	
			Ethyl acetate	100% mortality at 2000 ppm	
			Acetone	63% mortality at 2000 ppm	
	Anisomeles malabarica	Leaves	Acetone	76% mortality at 2000 ppm on larvae	Zahir *et al.* (2009)
			Chloroform	71% mortality at 2000 ppm on larvae	
			Ethyl acetate	74% mortality at 2000 ppm on larvae	
			Hexane	69% mortality at 2000 ppm on larvae	
			Methanol	100% mortality at 2000 ppm on larvae	
	Azadirachta indica		Hydrodistilled	When used in combination *A. indica, N. tabacum, C procera* and *T. ammi* acaricidal activity	Zaman *et al.* (2012)
	Buxus papillosa		Methanol	LC_{99} after 24 hours 20.8%	Sindhu *et al.* 2012
	Calotropis procera		Hydrodistilled	When used in combination *A. indica, N. tabacum, C procera* and *T. ammi* acaricidal activity	Zaman *et al.* (2012)
	Cocculus hirsutus	Leaves	Methanol	70% mortality at 2000 ppm	Elango and Rahuman (2011)
			Hexane	79% mortality at 2000 ppm	
			Chloroform	66% mortality at 2000 ppm	
			Ethyl acetate	100% mortality at 2000 ppm	
			Acetone	78% mortality at 2000 ppm	
	Cuminum cyminym	Seeds	Steam distillation	100% mortality observed with 1.25%, 2.5%, 5%, 10% and 20% concentrations	Martinez-Velazquez *et al.* (2011)
	Eclipta prostrata	Leaves	Methanol	100% mortality at 2000 ppm	Elango and Rahuman (2011)
			Hexane	72% mortality at 2000 ppm	
			Chloroform	51% mortality at 2000 ppm	
			Ethyl acetate	66% mortality at 2000 ppm	
			Acetone	59% mortality at 2000 ppm	

Table continues on the next page

Tick Species	Plant Species	Plant part	Extract/mixture	Level of effect	Reference
Rh. (Bo.) microplus	*Fumaria parviflora*		Methanol	LC_{99} after 24 hours 11.9%	Sindhu *et al.* (2012)
	Gloriosa superba	Flowers	Acetone	95% mortality at 2000 ppm on larvae	Zahir *et al.* (2009)
			Chloroform	81% mortality at 2000 ppm on larvae	
			Ethyl acetate	75% mortality at 2000 ppm on larvae	
			Hexane	73% mortality at 2000 ppm on larvae	
			Methanol	100% mortality at 2000 ppm on larvae	
		Seeds	Acetone	80% mortality at 2000 ppm on larvae	
			Chloroform	65% mortality at 2000 ppm on larvae	
			Ethyl acetate	79% mortality at 2000 ppm on larvae	
			Hexane	74% mortality at 2000 ppm on larvae	
	Gloriosa superba	Seeds	Methanol	88% mortality at 2000 ppm on larvae	Zahir *et al.* (2009)
	Leucaena leucocephala	Leaves	70% Acetone	Larval mortality of 66.79% observed and no effect on egg laying ability of adults	Fernández-Salas *et al.* (2011)
	Lysiloma latisiliquum	Leaves	70% Acetone	Larval mortality of 56.0% observed and no effect on egg laying ability of adults	Fernández-Salas *et al.* (2011)
	Melinis minutiflora	Grass	Fresh	Antitick properties against larvae	Fernandez-Ruvalcaba *et al.* (2004)
			Fresh	Antitick properties against larvae (with mean number of larvae 520 14 days post infestation of plot infested with 40 000 larvae	Thompson *et al.* (1978)
			Fresh	Antitick properties against larvae	Kaaya (2000)
	Melia azedarach	Fruit	Hexane	3.31 to 67.5% mortality after 24 hours (0.015 to 0.25% concentration), 2.3 to 66.9% after 72 hours and 61.9 to 98% after 168 hours	Borges *et al.* (2003)

Table continues on the next page

Tick Species	Plant Species	Plant part	Extract/mixture	Level of effect	Reference
Rh. (Bo.) microplus	*Melia azedarach*	Fruit	Chloroform	0.2 to 11.7% mortality after 24 hours (0.015 to 0.25% concentration), 3.3 to 12.4% after 72 hours and 32.4 to 99.4% after 168 hours	Borges *et al.* (2003)
			Ethanol	3.1 to 2.8% mortality after 24 hours (0.015 to 0.25% concentration), 0.8 to 25.5% after 72 hours and 0 to 38.7% after 168 hours	
	Nicotiana tabacum		Hydrodistilled	When used in combination *A. indica, N. tabacum, C procera* and *T. ammi* acaricidal activity	Zaman *et al.* (2012)
	Ocimum basilicum	Leaves	Steam distillation	No mortality observed with 1.25%, 2.5%, 5%, 10% and 20% concentrations	Martinez-Velazquez *et al.* (2011)
	Pimenta dioica	Berries	Steam distillation	13.64% mortality observed with 1.25% and 100% mortality observed with 2.5%, 5%, 10% and 20% concentrations	Martinez-Velazquez *et al.* (2011)
	Piscidia piscipula	Leaves	70% Acetone	Larval mortality of 88.14% observed and no effect on egg laying ability of adults	Fernández-Salas *et al.* (2011)
	Psidium guajava	Leaves	Acetone	76% mortality at 2000 ppm on larvae	Zahir *et al.* (2009)
			Chloroform	66% mortality at 2000 ppm on larvae	
			Ethyl acetate	68% mortality at 2000 ppm on larvae	
			Hexane	62% mortality at 2000 ppm on larvae	
			Methanol	72% mortality at 2000 ppm on larvae	

Table continues on the next page

Tick Species	Plant Species	Plant part	Extract/mixture	Level of effect	Reference
Rh. (Bo.) microplus	*Ricinus communis*	Leaves	Acetone	94% mortality at 2000 ppm on larvae	Zahir *et al.* (2009)
			Chloroform	83% mortality at 2000 ppm on larvae	
			Ethyl acetate	83% mortality at 2000 ppm on larvae	
			Hexane	74% mortality at 2000 ppm on larvae	
			Methanol	100% mortality at 2000 ppm on larvae	
	Solannum trilobatum	Leaves	Acetone	72% mortality at 2000 ppm on larvae	Zahir *et al.* (2009)
			Chloroform	73% mortality at 2000 ppm on larvae	
			Ethyl acetate	74% mortality at 2000 ppm on larvae	
			Hexane	65% mortality at 2000 ppm on larvae	
			Methanol	74% mortality at 2000 ppm on larvae	
		Seeds	Acetone	68% mortality at 2000 ppm on larvae	
			Chloroform	63% mortality at 2000 ppm on larvae	
			Ethyl acetate	71% mortality at 2000 ppm on larvae	
			Hexane	56% mortality at 2000 ppm on larvae	
			Methanol	62% mortality at 2000 ppm on larvae	
	Stylosanthes hamata	Stems	Methanol	79% repellency effect on larvae	Castrejón *et al.* (2003)
			Chloroform	74% repellency effect on larvae	
			Hexane	87% repellency effect on larvae	
			Acetone	71% repellency effect on larvae	
			Water	5% repellency effect on larvae	
		Leaves	Methanol	75% repellency effect on larvae	
			Chloroform	73% repellency effect on larvae	
			Hexane	70% repellency effect on larvae	
			Acetone	76% repellency effect on larvae	
			Water	4% repellency effect on larvae	
		Whole plant	Methanol	82% repellency effect on larvae	
			Chloroform	80% repellency effect on larvae	
			Hexane	79% repellency effect on larvae	
			Acetone	76% repellency effect on larvae	
			Water	6% repellency effect on larvae	

Table continues on the next page

Tick Species	Plant Species	Plant part	Extract/mixture	Level of effect	Reference
Rh. (Bo.) microplus	*Stylosanthes humilis*	Stems	Methanol	77% repellency effect on larvae	Castrejòn *et al.* (2003)
			Chloroform	78% repellency effect on larvae	
			Hexane	86% repellency effect on larvae	
			Acetone	70% repellency effect on larvae	
			Water	8% repellency effect on larvae	
		Leaves	Methanol	82% repellency effect on larvae	
			Chloroform	86% repellency effect on larvae	
			Hexane	86% repellency effect on larvae	
			Acetone	75% repellency effect on larvae	
			Water	5% repellency effect on larvae	
		Whole plant	Methanol	68% repellency effect on larvae	
			Chloroform	78% repellency effect on larvae	
			Hexane	92% repellency effect on larvae	
			Acetone	74% repellency effect on larvae	
			Water	7% repellency effect on larvae	
	Stylosanthes scabra	Plant	NA	Increased mortality of *larvae* as well as reduced host seeking and attachment	Zimmerman *et al.* (1984)
	Tagetes erecta	Leaves	Methanol	77% mortality at 2000 ppm	Elango and Rahuman (2011)
			Hexane	59% mortality at 2000 ppm	
			Chloroform	74% mortality at 2000 ppm	
			Ethyl acetate	60% mortality at 2000 ppm	
			Acetone	55% mortality at 2000 ppm	
	Tagetes minuta		Hydrodistilled	20% concentration of oil showed >95% mortality in larvae and adults	Andreotti *et al.* (2014)
	Trachyspermum ammi		Hydrodistilled	When used in combination *A. indica, N. tabacum, C procera* and *T. ammi* acaricidal activity	Zaman *et al.* (2012)
Rh. evertsi	*Azadirachta indica*	Seed	10% oil suspension	Weekly application of the 10% suspension result in effective tick control in goats year round	Schwalbach *et al.* (2003)

Table 4.3 Botanicals showing repelling and / or acaricidal effects against other tick species

Tick Species	Plant Species	Reference
Rh. (Bo.) annulatus	*Allium cepa*	Aboelhadid *et al.* (2013)
	Allium sativum	
	Artocarpus altilis	
	Silybum mariamum	Abdel-Shafy *et al.* (2006)
Rhipicephalus pulchellus	*Allium sativum*	Nchu *et al.* (2005)
	Calpurnia aurea	Zorloni *et al.* (2010)
Rh. sanguineus	*Tagetes minuta*	Andreotti *et al.* (2014)
	Tagetes patula	Politi *et al.* (2012)
	Vitex agnus castus	Mehlhorn *et al.* (2005)
Rh. haemaphysaloides	*Nicotiana tabacum*	Choudhary *et al.* (2004)
Haemaphysalis bispinosa	*Aegle marmelos*	Elango and Rahuman (2011)
	Andrographis lineat	
	Andrographis paniculata	
	Cocculus hirsutus	
	Eclipta prostrata	
	Tagetes erecta	
Ixodes ricinus	*Artemmisia abrotanum*	Tunón *et al.* (2006)
	Dianthus caryophyllum	
	Essential oils from citronella, cloves and lily of the valley	Thorsell *et al.* (2006)
	Artemisia absinthium	Jaenson *et al.* (2005)
	Ledum palustre	
	Myrica gale	
	Thododendron tomentosum	
Hy. dromedarii	*Artemisia herba-alba*	Abdel-Shafy *et al.* (2006)
	Artemisia monosperma	
	Euphorbia aegyptiaca	
	Francoeuria crispa	
	Mesembbryanthemus forsskale	
	Reaumuria hirtella	
Am. cajennense	*Tagetes minuta*	Andreotti *et al.* (2014)
Ticks in general	*Cordia curassavic*	Lans *et al.* (2000)
	Ocimum basilicum	Del Fabbro and Nazzi (2008)
	Commiphora holtziana	Kaaya (2000) Birkett *et al.* (2008)

Pavela *et al.* (2016) compiled a comprehensive list of botanicals showing repellent or acaricidal effects following a literature search conducted using various databases, and identified 238 plant species traditionally used against tick species

globally as part of ethno-botanical practices. From the list prepared by Pavela *et al* (2016), the plant species listed in Table 4.4, have been identified as used for tick control in South Africa.

Table 4.4 Botanicals used in South Africa as part of ethno-botanical approaches to tick control

Acanthospermum hispidum	*Cassia abbreviate*
Cassia sophera	*Cassytha filiformis*
Cissus quadrangularis	*Cyclospermum leptophullum*
Diospyros lycioides	*Elephantorrhiza elephantine*
Erythrina lysistemon	*Helichrysum kraussii*
Jatropha curcas	*Ochna holstii*
Peltophorum africamum	*Philenoptera violacea*
Prunus persica	*Pterocarpus angolensis*
Rauvolia coffra	*Rothmannia capensis*
Solanum aculeastrum	*Spirostachys africanus*
Synadenium cupulare	*Tagetes minuta*
Terminalia sericea	*Vernonia colorata*
Xanthocercis zambesiaca	*Ziziphus mucronata*

Alternative uses include the direct effect of plants, specifically grass species, as observed with *Stylosanthes humilis* and *Stylosanthes hamata* (grasses) having an anti-tick effect on *Rh. (Bo.) microplus* larvae (Castrejón *et al.,* 2003; Habeeb, 2010). *Stylosanthes scabra showed an increased mortality of Rh. (Bo.) microplus larvae* and *Am. variegatum* larvae and nymphs as well as reduced host seeking and attachment (Zimmerman *et al.,* 1984). *Melinis minutiflora* (molasses grass) showed repellent properties against *Rh. (Bo.) microplus* (Thompson *et al.,* 1978; Fernandez-Ruvalcaba *et al.,* 2004), in South America (Kaaya, 2000), and South Africa (Mwangi *et al.,* 1995a). *Andropogon gayanus* (Gamba grass) expressed anti-tick properties against *Rh. (Bo.) microplus* larvae (Thompson *et al.* 1978, Cruz-Vazquez and Rubalcaba, 2000; Kaaya, 2000; Fernandez-Ruvalcaba *et al.,* 2004); and *G. gynandra* (L.) Brig plants, and also showing efficacy in repelling all stages of *Rh. appendiculatus* (Malonza *et al.,* 1992; Kaaya, 2000). However, a major drawback which is associated with grasses such as *Stylosanthes* spp., *M. minutiflora* and *Andropogon gaynus* is their low nutritional value to cattle (Abbas *et al.,* 2012; Kaur *et al.,* 2016) and as a result additional high nutritional feed should be supplied to the animals.

4.6 Discussion

Considering the health and economic impact ticks and tick-borne diseases have on humans and animals, particularly when these are viewed against the backdrop of the problems that are inherent in the current tick control methods, there is an increased need to search for alternative control means of tick control which are effective and exclude pollution, contamination of the environment and animal products, and being accessible even to resource poor farmers.

It is therefore suggested that an integrated tick management program, a program that involves the use of several methods, must be implemented to promote better control methods (Willadsen, 2006; Stafford, 2007). Kaaya (2000), for example, suggested the incorporation of anti-tick grasses and plants as part of an integrated tick control program, whereas Abbas *et al.* (2012) also suggested potential methods to manage and reduce resistance against acaricides including monitoring the use of acaricide and efficacy of these acaricides against ticks or a regular basis rotating the acaricides used, by using a mixture of different acaricides, following a vaccination programme, including a nutritional management programme, the use of botanicals, improving the genetic resistance in cattle and managing the environment such as pasture burning and pasture rotation.

4.7 Bioassays used for testing anti-tick properties of botanicals

Different tests have been used to determine the efficacy of plant-based products against ticks. These tests can be conducted either using *in vivo* methods (in the presence of a hosts); or using *in vitro* methods, in the presence of host stimuli or in the absence of a host and / or host stimuli. However, the *in vitro* methods in the absence of a host are commonly preferred since they exclude the pain suffered by hosts as a result of tick infestation. The following bioassays have been previously used to determine the efficacy of plant-based products against ticks:

4.7.1 Field studies

An advantage of field tests is the inclusion of natural hosts of the tick species in the tests. However, field tests must be conducted during the correct season and in the correct area since the environmental conditions cannot be manipulated as in the case of laboratory studies. In a natural environment the following parameters

should be taking into consideration: weather can affect the tests, not all ticks will approach a host at the same time and will not attach in a controlled manner, behaviour of the host must also be recorded which might be difficult with animals, host features might also impact on the experiment such as length of hair and the effect of the repellent on the host (Dautel, 2004).

4.7.2 Laboratory experiments

In these bioassays, laboratory animals, such as rabbits, guinea pigs, mice, and in some cases humans (Dautel, 2004) are used as test subjects. The benefit of these types of bioassays is that the effect of the plant extract on ticks is studied under relatively favourable conditions for ticks. However, there are several disadvantages which accompany the in vivo studies. To start with, there may be studies where natural or preferred hosts might not be available (Dautel, 2004). Also, experimental animals may require to be kept separately in specifically designed sheds to ensure disease-free conditions. Inevitably, this may increase the monetary costs incurred on the study (Zaman *et al.,* 2012). Additional costs and challenges may result from the maintenance and care of test animals, including feeding and treatments, as well as ethical considerations with regards to conducting tests using animal hosts.

4.7.2.1 Feeding bioassay

Feeding deterrent bioassays involves attaching a container in which there are ticks on a treated skin area of the host (Mkolo *et al.,* 2011a). However, this bioassay induces stress and pain suffered by host animals and imposes additional costs resulting from maintenance and care of animals.

4.7.2.2 Fingertip bioassay

During the fingertip bioassay, the test material is applied around second phalanx of the forefinger. The finger is held horizontally and ticks are transferred to the dorsal surface of the untreated distal segment of the finger. The finger is then tilted vertically with the tip pointing down and the location of ticks are recorded (Opiro *et al.,* 2013). While this bioassay is advantageous in the sense of studying tick behaviour on a host, it exposes humans to the risk of tick bites.

4.7.2.3 Moving-object bioassay

In this bioassay heat and movement is used as stimuli, a slowly rotating vertical drum's surface is heated to 35 - 36°C. The drum has an elevated surface that serves as attachment site. A horizontal rod is used for the ticks to approach the drum, and to attach to the rotating attachment site. For repellency tests the attachment site is treated and results can then be recorded if ticks approach the drum, distance effect of the repellent, or if the tick attach, remains on or drops off from attachment site, to determine the effectiveness of a contact repellent. The advantage is that the bioassay can be used to screen pure substances, to determine the compounds, and different formulations and products can be tested under standardised conditions. Disadvantages include that the authors indicated that it is not advisable to conduct several tests in parallel thereby making mass screenings impossible, and the bioassay is confined to tick species that follow an ambush strategy when host-seeking (Dautel, 2004).

4.7.2.4 Larval Package Test (LPT)

During the LPT filter paper is impregnated with the test extract and allowed to dry. The filter paper is then folded to form a packet and the larvae are then placed in the packet to evaluate the effect of the extract (FAO, 2004; Shyma *et al.,* 2014). The disadvantage of this method includes a time-delay of five to six weeks required for completion of the test (FAO, 2004; Jonsson *et al.,* 2007; Castro-Jener *et al.,* 2009; Lovis *et al.,* 2011; Klafke *et al.,* 2012; Adenubi *et al.,* 2016).

4.7.2.5 Adult Immersion Test (AIT)

During the AIT engorged female ticks are immersed in solution for a period of time, and the comparison of rate of oviposition between a treated and untreated group, egg weight and viability are analysed. The mortality of females is also determined. Limiting factors in this bioassay include the number of ticks that can be used (Klafke *et al.,* 2012; Shyma *et al.,* 2014) and the number of days (7 days) it takes to get results (Lovis *et al.,* 2011).

4.7.2.6 Larval Immersion Test (LIT)

The LIT is an alternative to the AIT. The LIT follows the same protocol as the AIT but allows for more specimens to be used per assay (Klafke *et al.,* 2012).

4.7.2.7 Petri dish bioassay

The Petri dish bioassay consists of a tick walking area with treated and untreated areas. Repellence can be determined by the number of ticks entering the area treated with repellent compared to the control area. The advantages of this bioassay are its simplicity and low costs. In addition, it can be used with all tick species, and conditions can be controlled. Disadvantages of the bioassay includes the low demand on the repellent, i.e. the effect of the presence of a host does not play a role and therefore the true effect of the repellent cannot be determined, implying that even weak repellents might show results (Dautel, 2004; Anisuzzaman *et al.,* 2005).

4.7.2.8 Tick climbing bioassay

The tick climbing bioassay consists of rods being placed vertically to observe the ambush strategy followed by some tick species to determine if a repellent will alter the height climbed on treated and untreated rods. The disadvantages of the bioassay are the difficulty when determining the effects of weak repellents and that the method is also time consuming and requires continuous monitoring (Dautel, 2004).

4.7.2.9 Fumigant toxicity / Growth inhibition assay bioassay

In the fumigant toxicity bioassay, a filter paper containing an essential oil is added to glass vials. A second glass vial containing ticks is covered with mesh which is placed upside down on the vial containing the filter paper and the fumes are allowed to saturate the atmosphere in the vials. The effect of the essential oil on ticks is thus determined (Mkolo *et al.,* 2011a; Nchu *et al.,* 2012).

4.7.2.10 Syringe bioassays

The syringe test method can be used to test the effect of compounds on hatched eggs. Eggs are left to hatch in syringe, following which test extracts are added and placed in an incubator to evaluate the effect on emerging larvae (Sindhu *et al.,* 2012).

4.7.2.11 Capillary tube feeding bioassay

An alternative method to using live animals for tick disease investigation is to feed ticks using capillary tubes. During the capillary tube feeding bioassay, ticks are attached between two microscope slides using double sided tape. The microscope slides are placed in a petri dish. Capillary tubes are then filled with test material and fixed over the mouthparts of the ticks and rested at an angle against the side of the petri dish (Broadwater *et al.,* 2002; Kocan *et al.,* 2005; Abel *et al.,* 2008). Advantages of the system are that ticks can be exposed to a pathogen without infecting a host. It also allows for testing changes in the composition of meal content (Kocan *et al.,* 2005). The disadvantage of this bioassay is that a limited quantity of blood is ingested and as a result the ticks must be pre-fed on a natural host before their mouthparts can be fixed to the capillary tubes (Young *et al.,* 1996). Furthermore, such ticks cannot feed to repletion and as a result after a feeding period using capillary tubes ticks must be placed on an animal to complete their life cycle (Young *et al.,* 1996; Abel *et al.,* 2008).

4.7.2.12 Glass plate devices

As alternative to an *in vivo* study in order to test the effects of a repellent on ticks, i.e. their behaviour and movement, Kröber *et al.* (2013) evaluated the effect of using a heated glass plate. The study compared the behaviour of nymphs using the treated heated glass plate with the behaviour of nymphs on treated human skin. The responses were identical for the two studies making this method a good alternative to use as initial test to determine the effect a repellent will have on the behaviour of ticks.

4.8 Conclusion

This chapter took a closer look at the different options currently available for tick control and also looked at the advantages and disadvantages of these methods. Chemical control is still the most commonly used method globally, in spite of synthetic chemical repellents being associated with several concerns and drawbacks which reinforces the need to find alternative tick control methods, such as botanicals with tick repellent properties. The review highlighted the shift in focus from chemical to botanical research as avenue for tick control.

In order to evaluate the efficacy of a potential repellent and / or acaricide of a new product, the product must be subjected to testing, as seen in the preceding discussions. A number of bioassays have been used to examine the anti-tick properties of a number of plant extracts. All bioassays have their individual advantages and disadvantages, all of which have to be taken into account when selecting an appropriate bioassay for the type of research to be conducted.

For the purpose of this study two of the most commonly used *in vitro* repelling bioassays, namely the petri dish and climbing repellent bioassay were used to test the efficacy of *A. ferox*, *L. leonurus* and *T. vulgaris* as potential tick repellents. The disadvantages associated with each of the bioassays were taken into consideration in the modification of the bioassays, in order to improve them and add value to future tick research using these bioassays. The next chapter will focus on the materials and methods used and the results obtained for the two modified bioassays.

CHAPTER 5
METHODOLOGY, RESULTS AND DISCUSSION
IN VITRO BIOASSAYS

From the discussions in the preceding chapters it is clear that ticks are considered amongst the most successful parasites associated with a significant impact, not only on the health of their hosts, but also on the economy as a whole. This reality appeals for innovative tick control methods that are environmentally friendly, safe and cost effective.

In Chapter Four of this study, various bioassays that have been previously used to test for potential anti-tick products were explored. Among them, the petri dish and tick climbing bioassays appear to be mostly preferred by many researchers. The benefits in using these two bioassays arise from the exclusion of host animals in the tests thus avoiding exposing animals to unnecessary stress and pain and also from the simplicity of the methods which permits easy replication.

The slightly modified versions of the traditional petri dish and tick climbing bioassays were used in this study to determine the repellent effects of botanical extracts prepared from *A. ferox*, *L. leonurus* and *T. vulgaris* on *Am. hebraeum* and *Rh. appendiculatus* adult ticks. The modifications in these bioassays were an effort to improve the exclusion of the influence by the plant extracts against tick behaviour either in the control area of a petri dish bioassay or on the control rod of a climbing bioassay and to examine the influence of attractants on tick behaviour in the presence of repellents.

The outline for this Chapter is as follows:

I. **Section A** describes the selection of the study material, preparation of botanical extracts and the design and procedure of the modified petri dish and climbing bioassays used in this study.
II. **Section B** includes the results and brief review on the results obtained from the two bioassays.
III. **Section C** focuses on the discussion and basic conclusions made on the efficacy of the changes in the bioassays as well as the potential of the selected plant species as tick repellents.

Section A – Methodology

5.1 Study material selection

5.1.1 Tick species

As indicated in Chapter Three of this thesis, *Am. hebraeum* and *Rh. appendiculatus* are among ticks of veterinary, medical and economic importance in South Africa.

Only the adult stages of *Am. hebraeum* and *Rh appendiculatus* were used in this study. Following the South African government legislation on the use of parasites for research, Section 20 approval 12/11/1/1/23 (Appendix A) was secured and laboratory reared disease-free *Am. hebraeum* adults (Figure 5.1) were obtained from Onderstepoort Veterinary Institute (Pretoria, Gauteng, South Africa). The colony originated in March 2014 from Gauteng, and was maintained by breeding larvae on rabbits and nymphs and adults on cattle.

Figure 5.1 *Amblyomma hebraeum* – Female (A) and Male (B)

Image captured by EMC Theron, July 2018, using Olympus SZX16 (Wirsam) Stereo microscope and processed using CellSense Software

Also, following government legislation for use of parasites in research, Section 20 approval 12/11/1/1/23 (Appendix A) was secured and laboratory reared disease-free *Rh. appendiculatus* adults (Figure 5.2) were obtained from ClinVet (Bloemfontein, Free State, South Africa). The colony originated in March 2016 at the Malalane Research Unit in Mpumalanga, South Africa, and since then was bred on rabbits.

Figure 5.2 *Rhipicephalus appendiculatus* – Female (A) and Male (B)

Image captured by EMC Theron, July 2018, using Olympus SZX16 (Wirsam) Stereo microscope and processed using CellSense Software

5.1.2 Plant species

The following three plant species were included in the study:

- *A. ferox* (yellow powder from leaf exudate),
- *L. leonurus* (leaves), and
- *T. vulgaris* (leaves and stalks).

As shown in Chapter Four of this study, a wide variety of botanicals have been tested for anti-tick properties and some incorporated in tick control practices. However, McGaw and Eloff (2008) cautions about the important aspects which are to be considered when selecting plants for treatment in animals and/or humans. These aspects include the determination of the bioactivity and safety of the selected plants, especially if the use of these plants is to be promoted and developed for commercial use, (McGaw *et al.,* 2007). For the purpose of this study only locally sourced plant species which are traditionally used as remedies for various animal health conditions were included.

Aloe ferox

The use of Aloe as a remedy against various aliments has been recorded over a number of centuries worldwide. *A. ferox* (Cape aloe) is found in the Southern, Western and Eastern Cape provinces of South Africa. The green epidermis is rich in fibre when the yellow exudate is rich in aloin (Mwale and Masika, 2010). The inner white flesh is rich in minerals, vitamins, amino acids, polysaccharides, enzymes and lipids (Mwale and Masika, 2010). *A. ferox* is also well known for its antiseptic, cleaning moisturising and anti-inflammatory properties, and is used by

farmers to control gastro-intestinal parasites in chickens (Mwale and Masika, 2010), and was also listed as one of the entho-veterinary medicine used by communities and small-scale farmers in the Eastern Cape region, South Africa (Mapiye *et al.,* 2009).

In addition, *A. ferox* is traditionally used to prevent tick infestations in poultry (Dold and Cocks, 2001) and goats (Sanhokwe *et al.,* 2016). According to Moyo and Masika (2009) *A. ferox* is one of the two plant species most commonly used as anti-tick agents by small scale farmers in the Eastern Cape Province (South Africa). However, contrary to these views a study conducted by Fourie *et al.* (2005) on the anti-tick properties *A. ferox* showed no activity against *Rh. (Bo.) decoloratus.* As a result of the contradictory outcomes on the effects of *A. ferox* extracts, it became necessary to further explore its extracts for anti-tick properties in this study in an effort to validate its use.

The yellow powder of *A. ferox* (Figure 5.3) used in this study was obtained from Organic Aloe (Pty) Ltd., Albertina, Western Cape, South Africa. The powder was prepared from the spray-dried sap collected from leaves. *A. ferox* harvested by Organic Aloe (Pty) Ltd. grows naturally in the wild as well as plantations which are not agriculturally maintained to ensure that the plant material remains free from use of pesticides or herbicides. The leaves were harvested throughout the year. During harvesting only the bottom row of leaves were harvested, and each individual plant was only harvested every second or third year (Organic Aloe, 2017).

Figure 5.3 *Aloe ferox* plant (A) and the yellow powder derived from the leaf exudate (B)

Leonotis leonurus

Traditionally, *L. leonurus* (Lion's Tail / Wild dagga) species has been used for headaches, coughs, colds, dysentery and in the treatment of snakebites, and was shown to have antibacterial and anti-inflammatory properties (Fennell *et al.,* 2004, Zizameleni Farming, 2018a). This plant also forms part of the ethno-veterinary medicine used by communities and small-scale farmers in the Easter Cape Province, South Africa (Mapiye *et al.,* 2009).

Using a climbing repellency bioassay, Luseba *et al.* (2016) reported a 90% repellency against *Rh. appendiculatus* by acetone extract of *L. leonurus* and Wanzala *et al.* (2012) also reported on the use of *L. nepetifolia* by livestock farmers in the Bungoma district in Kenya. These two reports suggest that the genus *Leonotis* may be endowed with elements that have anti-tick properties. In an effort to validate the use of *L. leonurus* for tick control, it was included in this study to determine its activity against *Am. hebraeum* and *Rh. appendiculatus* ticks.

Dried *L. leonurus* leaves (Figure 5.4), used in this study were obtained from Mountain Herb Estate, Hartebeespoort Dam, North West Province, South Africa. The plants originated from Zizameleni Farming (Pty) Ltd., Brits, North West Province, South Africa. The leaves were harvested during the summer of 2018, processed during March 2018, allowed to air dry before packaging in heat-sealed Zip lock pouches (Zizameleni Farming, 2018a).

Figure 5.4 *Leonotis leonurus* plant (A) and dried leave material (B)

Figure 5.4 A – Adapted from SANBI [accessed 21 December 2018]

Thymus vulgaris

As a medicinal herb, *T. vulgaris* (common or garden thyme) has been traditionally used to treat gastrointestinal ailments, coughs, dermatitis, minor wounds, menstruation and associated cramps and also it is believed to have sedative, antiseptic, antifungal, antibacterial and antipyretic properties (WHO, 1999; Zizameleni Farming, 2018b).

In addition, *T. vulgaris* has been reported to be effective in repelling flies (*Musca domestica*), mites (Dermatophagoides spp. and *Dermanyssus gallinae*), mosquitoes (*Culex pipiens pallens* and *Culex quinquefasciatus*) and mealworms (*Alphitobius diaperinus*) (Kim *et al.,* 2004; Park *et al.,* 2005; Pavela, 2007; Pavela *et al.,* 2009; Lee *et al.,* 2010; Maia and Moore, 2011; Zoubiri and Baaliouamer, 2011; Szczepanik *et al.,* 2012). Based on the repellent properties of *T. vulgaris* against mites and mosquitoes as indicated above it became reasonable to suspect *T. vulgaris* of having anti-tick properties, hence its inclusion in this study.

Dried *T. vulgaris* leaves and fine stalks (Figure 5.5), used in this study were obtained from Mountain Herb Estate, Hartebeespoort Dam, North West Province, South Africa. The plants originated from Zizameleni Farming (Pty) Ltd., Brits, North West Province, South Africa. The plant material was harvested during the summer of 2017, processed during March 2018 allowed to air dry, and packed in heat-sealed Zip lock pouches (Zizameleni Farming, 2018b).

Figure 5.5 *Thymus vulgaris* plant (A) and dried leaf material (B)
Figure 5.5 A – Adapted from Kuete (2017)

5.2 Botanical Extracts Preparation

Extracts were prepared from the plant material to determine if they demonstrated any biological activity against the adult stages of *Am. hebraeum* and *Rh. appendiculatus*. A mortar and pestle were used to further grind dried *L. leonurus* and *T. vulgaris* material into finer powders. *A. ferox* powder was already fine from the supplier and as a result did not require any further grinding. The powders from the three plant species were extracted using acetone, methanol and ethanol as solvents at a weight per volume ratio (w/v) of 1:10. The w/v ratio used was based on the ratios described by Eloff *et al.* (2011). According to Azmir *et al.* (2013) acetone is associated with extraction of flavonoids; ethanol with tannins, polyphenols, flavonol, terpenoids, and alkaloids; and methanol with anthocyanin, terpenoids, saponins, tannins, flavones and polyphenols.

The mixtures of plant material and solvents were covered and placed on the IKA KS130 Basic Orbital Shaker (AEC Amercham) and allowed to shake for one hour at 80 rpm and then left standing overnight. Subsequently, filtration was done using Whatmann No 1 filter paper for each of the mixtures. The collected filtrate was then left in an extraction hood to allow the solvent to evaporate. The resultant yield from each of the filtrates was weighed (Table 5.1), using a Shimadzu, UW4200H analytical balance. The dried extract was re-constituted using the same solvent used for extraction process at a 1:1 w/v ratio to prepare crude extracts. Each crude extract was diluted accordingly with the same solvent to prepare the final concentrations used in the bioassays (Table 5.2).

Table 5.1 Yields of plant material following extraction with different solvents

Plant Species (10g)	Solvent (100mL)	Approximate Yield per extract (g)
A. ferox	Acetone	6.44g
A. ferox	Ethanol	6.83g
A. ferox	Methanol	7.10g
L. leonurus	Acetone	0.43g
L. leonurus	Ethanol	0.93g
L. leonurus	Methanol	1.30g
T. vulgaris	Acetone	0.48g
T. vulgaris	Ethanol	0.67g
T. vulgaris	Methanol	1.92g

Table 5.2 Preparation of different plant extract concentrations

Plant material	Solvent	Final concentration
0.25mL	9.75mL	2.5%
0.5mL	9.5mL	5%
0.75mL	9.25mL	7.5%
1mL	9mL	10%
2mL	8mL	20%

5.3 Modified bioassays

As seen in Chapter Four, the petri dish and tick climbing bioassays are routinely used in laboratories to determine the potential repellent effects of plant extracts on ticks. For the purpose of this study the traditional models of these bioassays were modified and the modifications tested as alternative and improved models for use during laboratory analysis of potential repellents. The modified bioassays are fully described in the following paragraphs. As indicated previously, the modifications were an effort to ensure exclusion of the influence of the plant extracts on the behaviour of ticks on the control side of the bioassays and also to examine the influence of the attractants on tick behaviour in the presence of repellents.

Commercialised repellents namely Bayticol 2% EC Dip (Bayer health Care, Bayer (Pty) Ltd Animal Health Division) and F10® wound spray with insecticide (Health and Hygiene (Pty) Ltd) were used to test the efficacy of the modified models of the bioassays and also served as positive controls against which the anti-tick properties of *A. ferox*, *L. leonurus* and *T. vulgaris* extracts were compared.

5.3.1 Petri Dish Choice Chamber bioassays

In Chapter Four the traditional petri dish bioassay was described as consisting of a single chamber subdivided (without partitioning) into a tick walking area with treated and untreated areas. Tick repellency was determined by recording the number of ticks entering the treated area compared to the control area. However, in spite of the bioassay having various advantages, including simplicity, low costs, versatility as all tick species can be included using this bioassay, and the ability to control external conditions (Dautel, 2004; Anisuzzaman *et al.,* 2005), the influence of volatile plant extracts on tick behaviour may easily extent throughout the entire chamber due to lack of partitioning between different areas. The petri dish bioassay

also only allows for two areas, the control and treatment areas to be observed. In an effort to avoid or at the least reduce this limitation, a two chambered version (a petri dish choice chamber version) including control and treatment chambers joined by a neutral area was for the first time designed and used in this study. The petri dish choice chamber bioassay therefore includes a third area, the neutral zone, from where ticks can be placed to move in either direction. (Figure 5.6).

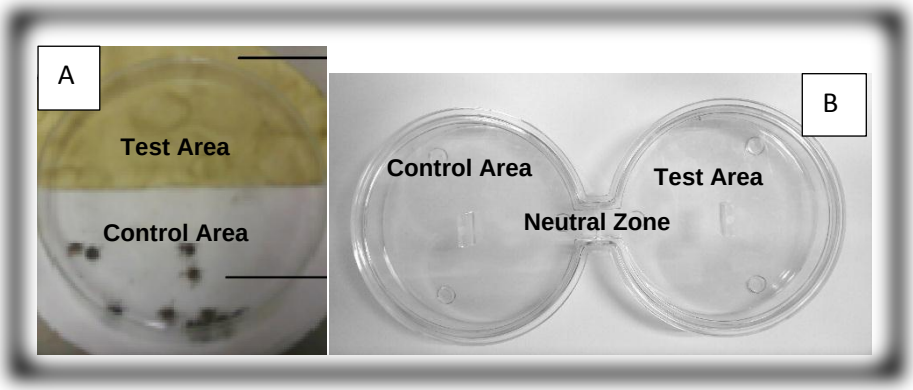

Figure 5.6 The traditional petri dish bioassay (A) and the modified petri dish choice chamber bioassay (B)
Figure 5.6 A – Adapted from Mkolo *et al.* (2011)

Commercial repellent

To test the efficacy of the modified method known repellent products were used to observe the behaviour of both tick species. The first petri dish choice chamber was set up using Bayticol 2% EC Dip (Bayer Health Care, Bayer (Pty) Ltd Animal Health Division) and distilled water as control, and similarly the second contained F10® wound spray with insecticide (Health and Hygiene (Pty) Ltd) and distilled water used as a control.

The bioassays were set up by treating two pieces of filter paper (6.5 cm x 8 cm) with 400 µl of 100% Bayticol 2% EC Dip (Bayer Health Care, Bayer (Pty) Ltd Animal Health Division) and F10® wound spray with insecticide (Health and Hygiene (Pty) Ltd) respectively as the test products and second pieces of filter paper of the same size treated with 400 µl distilled water. The filter papers were allowed to air dry and respectively attached, to the bottom of the chambers using double sided sticky tape. In the choice chamber, the control was set up on the left side and the test product

on the right side. The neutral zone was the middle area which excluded a filter paper (Figure 5.7).

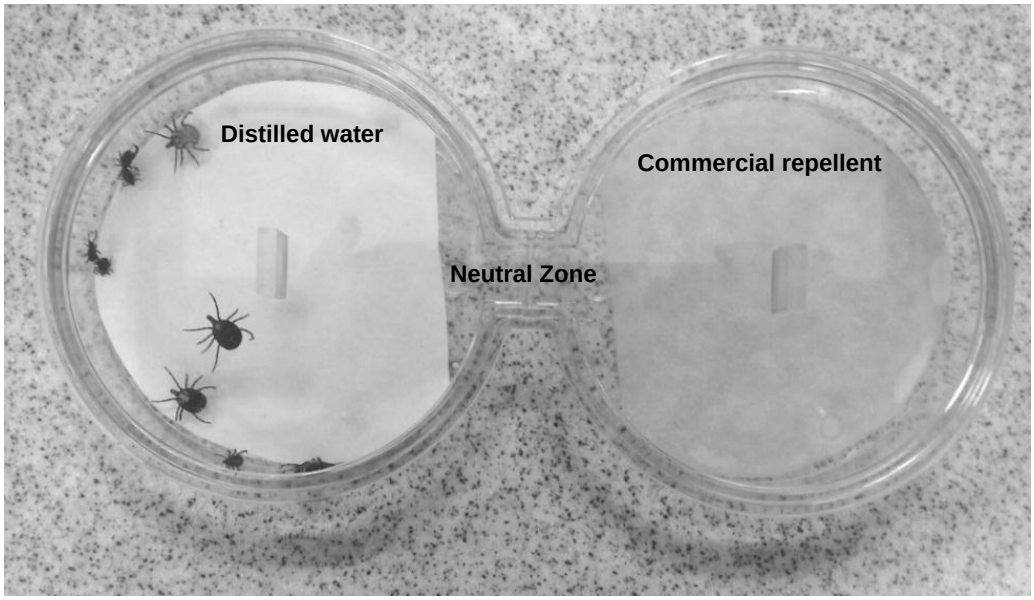

Figure 5.7 Petri dish choice chamber bioassay with control (distilled water) and treatment (commercial repellent) chambers

Three male and three female ticks of each species were placed in the neutral zone. Ticks were allowed to acclimatize for 10 minutes after being introduced to the neutral area. Ticks that had moved from the neutral area were taken back after the acclimatization period. The movement and location of ticks was observed every five minutes for one hour. Tick location was recorded in the results sheet (Appendix B). After each five minutes interval, ticks were returned to the neutral zone.

Ticks observed on the control area of the petri dish choice chamber (on the filter paper, on the side of the choice chamber or the lid) were counted as “control”. Similarly ticks observed on the treated side of the petri dish choice chamber (on the filter paper, on the side of the choice chamber or the lid) were counted as “treatment”. Ticks observed on the remainder area (not covered by filter paper) were counted as being in the neutral zone. As the ticks moved into either the control or treatment area their location was recorded. However, these ticks were not used for the determination of the percentage repellency.

Botanical Extracts

A similar procedure as described above was followed, except that plant extracts were used and the control included the solvents used to prepare the extracts. In particular for each plant extract, a piece of filter paper (6.5 cm x 8 cm) treated with 400 µl of the solvent used to prepare that extract served as a control, and a second filter paper of the same size was treated with 400 µl of the plant extract. The different concentrations of the plant extracts tested are indicated in Table 5.3. As it was unknown what effect (if any) the extracts would have the start concentrations were selected as 5% and 10% with the third individually selected for each plant species based on the observations made during the Petri Dish Choice Chamber bioassays for the first two selected concentrations. Tests for each of the different plant extracts including the solvent were conducted in triplicate. After each test run, newly treated filter papers were used to set up for the next run.

Table 5.3 Plant extracts tested using the petri dish choice chamber bioassay

Plant material	Solvents	Concentration
A. ferox	Acetone, Ethanol, Methanol	5%
		10%
		20%
L. leonurus	Acetone, Ethanol, Methanol	5%
		7.5%
		10%
T. vulgaris	Acetone, Ethanol, Methanol	2.5%
		5%
		10%

The same procedure followed for the commercial repellent testing was used to observe the behaviour of tick species for each individual concentration of a plant extract (Figure 5.8). The bioassays were set up separately for each of the two species tested to simplify observation and counting of ticks. Ticks which were in the control chamber were considered to be repelled.

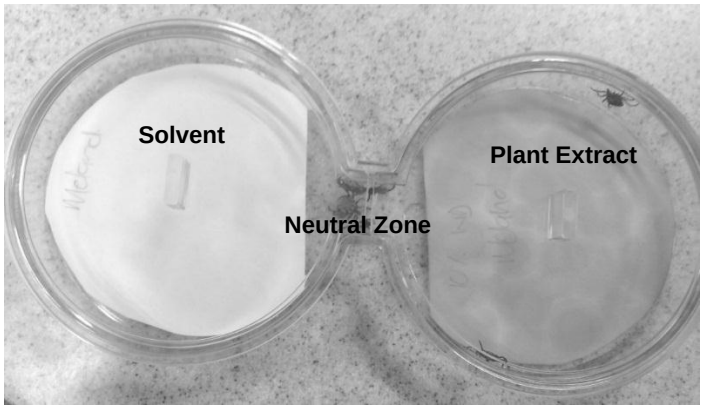

Figure 5.8 Petri dish choice chamber bioassay showing control (solvent) and treatment (plant extract) chambers

5.3.2 Tick Climbing bioassays

In Chapter Four the traditional tick climbing bioassay was described as consisting of rods placed vertically to observe the ambush strategy followed by some tick species and in an effort to determine if a repellent will alter the height climbed on treated rods compared to those that are untreated (Dautel 2004). However, a limitation with this bioassay is that the close proximity between the treated and untreated rods is often not considered and if the two rods are close to each other ticks on the control rods may be influenced by the volatiles from plant extracts on the treated rods. In this study (Experiment One) the bioassay was modified to exclude this possibility by using partitions between the control and treatment rods. The bioassay is also commonly performed in the absence of attractants such as host body heat and CO_2, which in nature influence tick questing behaviour. In this study (Experiment Two) the traditional bioassay was conducted in the presence of attractants (host body heat, CO_2, and the circulation of ambient air by a moving board over the experimental setup) were included in the bioassay to determine their influence on the tick climbing behaviour in the presence of repellents. Each of these experiments is discussed in detail in the following paragraphs:

5.3.2.1 Experiment One – Effects of plant extracts on the climbing questing behaviour of *Rh. appendiculatus* using a modified tick climbing bioassay

I. Design of the modified tick climbing bioassay

Except for the separation of the control rod from the treatment rod by partitioning done in this study, the bioassay followed that described by Mkolo and Magano (2007). In brief, the bioassay these authors followed entail a vertically positioned glass rod held in position by polystyrene in a glass beaker. The polystyrene also served as a platform on which ticks were placed. The setup was included in a water container, with water filled to the level of the polystyrene platform to discourage ticks from wandering away from the platform. Two separate apparatus which served as control and treatment respectively were positioned close to each other without partitioning.

In this study, a combined unit with fully partitioned compartments was designed. Two polystyrene containers with the volumes, 39 cm x 30 cm x 15 cm and 18 cm x 27 cm x 15 cm were glued together to create one unit. Three dividers of which each were 25 cm x 19 cm were inserted approximately 5 cm from the bottom of the containers, to create four individually separated compartments. The apparatus permitted running three treatment tests and one control test simultaneously. Four 50 mL plastic sample containers were used for insertion of wooden rods. For each plastic container, a 4mm diameter hole was drilled in the bottom of the sample plastic container, to allow for the insertion the 21 cm wooden rod. The lid of each container was glued to the bottom of the polystyrene container, using Tombo Mono Multi Liquid glue. The container also served as the tick platform as well (Figure 5.9). The polystyrene container was filled with water to just below the tick platform during testing.

Figure 5.9 Modified tick climbing bioassay apparatus

II. Efficacy of commercial repellents

To test the efficacy of the modified tick climbing bioassay the same known tick repellent products used for the petri dish choice chamber efficacy test were used to observe the climbing questing behaviour of *Rh. appendiculatus*. The bioassay was set up by treating a piece of filter paper (1 cm x 5 cm) with a 1:400 dilution of Bayticol 2% EC Dip (Bayer Health Care, Bayer (Pty) Ltd Animal Health Division), which was attached to the top of a 21 cm long wooden rod, and a second piece of filter paper (1 cm x 5 cm) with distilled water on it, attached directly below on the first rod. The second rod had filter paper (1 cm x 5 cm) with F10® wound spray with insecticide (Health and Hygiene (Pty) Ltd) and a second similar filter paper with distilled water on it. For each of the tests a control rod treated only with distilled water on both pieces of filter paper were included (Figure 5.10). The filter papers were allowed to air dry before they were fixed on the rods. Tests were conducted in triplicate. After each test run newly treated filter papers were used to set up for the next run. Ticks which were not on the treated filter paper were considered to be repelled.

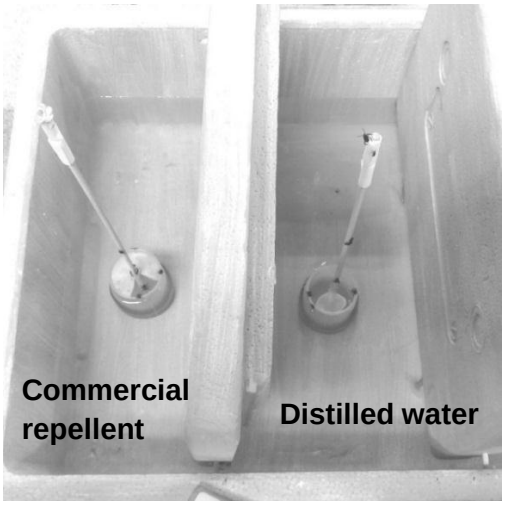

Figure 5.10 Modified tick climbing bioassay showing the treatment and control setups

The tick climbing bioassay was performed by observing the behaviour of five male and five female *Rh. appendiculatus* adult ticks per test product. After adding the ticks to the platforms they were allowed to acclimatize for 15 minutes. The movement of ticks was observed every 15 minutes for the first hour and 30 minutes for the second hour and their location recorded on the results sheet (Appendix B). After each interval the ticks were returned to an untreated area just above the tick platform on the wooden rod. Ticks that were not climbing were recorded as being “below filter paper”.

III. Efficacy of botanical extracts

The procedure followed to test the repellency of different extracts was similar to that described above. A piece of filter paper (1 cm x 5 cm) was treated with 150 µl of solvent or extract and allowed to air dry before it was attached to the top of a 21 cm long wooden rod using double sided tape. A second piece of filter paper (1 cm x 5 cm) treated with distilled water was attached directly below that with the solvent / extract. The extracts tested using this bioassay is indicated in Table 5.4. Tests for each of the different plant extracts including the solvent were conducted in triplicate. After each test run newly treated filter papers were used to set up for the next run.

Table 5.4 Plant extract and the concentrations tested using the modified tick climbing bioassay

Plant material	Solvent	Concentration
A. ferox	Acetone	20%
	Ethanol	20%
	Methanol	20%
L. leonurus	Acetone	10%
	Ethanol	10%
	Methanol	10%
T. vulgaris	Acetone	10%
	Ethanol	10%
	Methanol	10%

The tick climbing bioassay was performed by observing the behaviour of five male and five female *Rh. appendiculatus* adult ticks per extract and solvent. The same experimental procedure as followed for the commercial repellent was implemented in order to observe the behaviour of the ticks during the experiment (Figure 5.11).

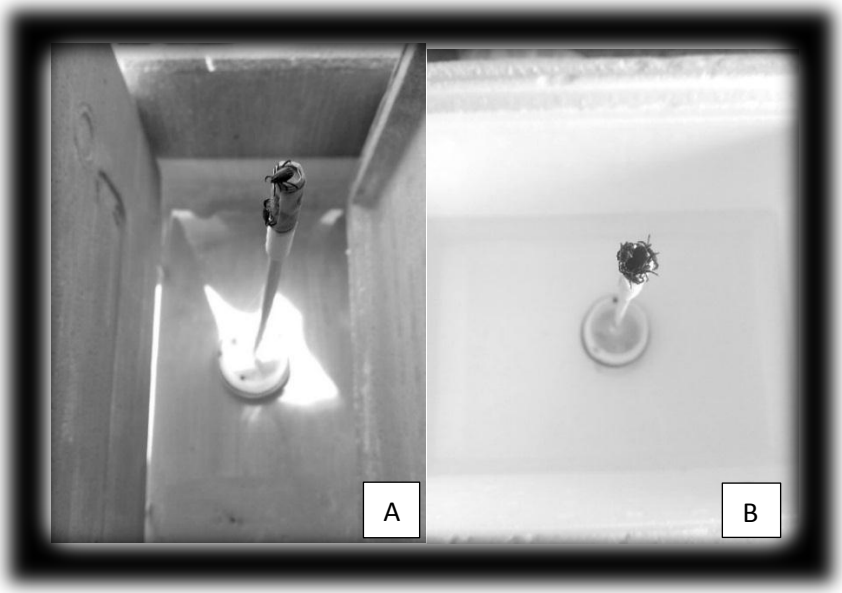

Figure 5.11 Modified tick climbing bioassay showing (A) treatment (plant extract) and (B) control (solvent)

5.3.2.2 Experiment Two – Effect of attractant on tick climbing behaviour in the presence of repellents

I. Design of the modified tick climbing bioassay

In order to determine if attractants will have an effect on the behaviour of ticks when conducting a tick climbing bioassay, three attractants namely heat at 37^0C, mimicking mammalian host body temperature, CO_2 mimicking CO_2 produced by mammalian host when breathing and circulation of ambient air by a moving board over the experimental setup were included in the bioassay.

A polystyrene container (32 cm x 32 cm x 15 cm) was used to hold four 50 mL plastic sample containers, spaced at 5 cm apart. Similar to the tick climbing bioassay as described in experiment one above, the lids were secured to the bottom of the polystyrene container with Tombo Mono Multi Liquid glue. For each of the sample plastic container, a 4 mm diameter hole was drilled at the bottom to allow for the insertion of the 21 cm wooden rod. The polystyrene container was filled with water to just below the tick platform during the test. The apparatus was setup to allow for three test rods and one control rod simultaneously.

Ambient air in the container was heated at 37^oC by a foil heating pad (Figure 5.12) to mimic mammalian host body temperature. Carbon dioxide mimicking the CO_2 produced by host animals when breathing, was produced by regularly adding dry ice cubes to water on a Stuart hotplate set at 150^oC to ensure continuous production. To create a continuous constant air movement in the experimental setup, a cardboard attached to battery operated rotating cogwheels was used (Figure 5.13).

II. Efficacy of commercial repellents

To test the efficacy of the modified tick climbing bioassays the same known acaricidal and repellent products used for the tick climbing bioassay as previously described, were used to observe the behaviour of *Rh. appendiculatus*. The first rod was set up using a 1:400 dilution of Bayticol 2% EC Dip (Bayer Health Care, Bayer (Pty) Ltd Animal Health Division), and

the second contained F10® wound spray with insecticide (Health and Hygiene (Pty) Ltd) and distilled water. For each of the tests a control rod treated only with distilled water was included. The effect of attractants were tested first individually and also in combination to evaluate the effect on tick questing (Figures 5.12 and 5.13).

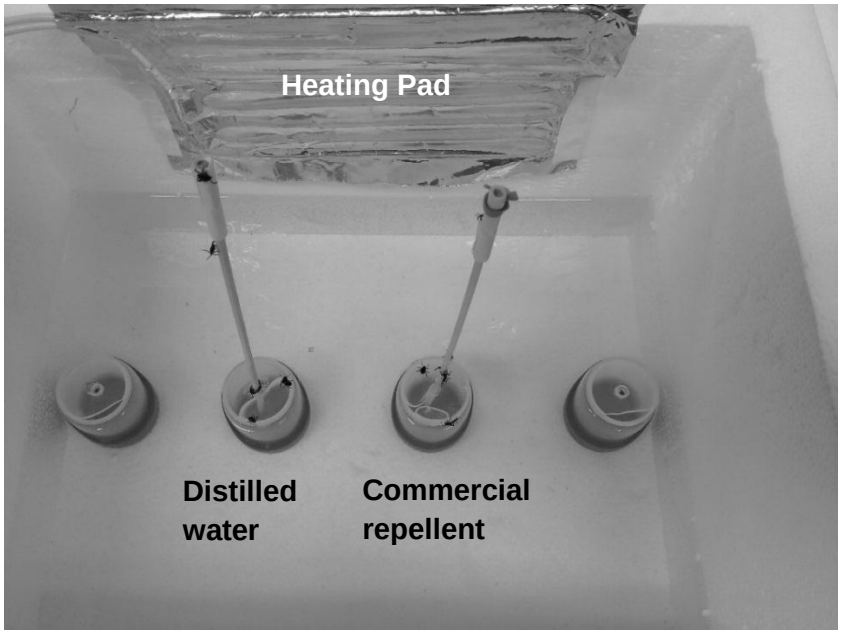

Figure 5.12 A modified tick climbing bioassay showing a heating pad that heated air at 37°C

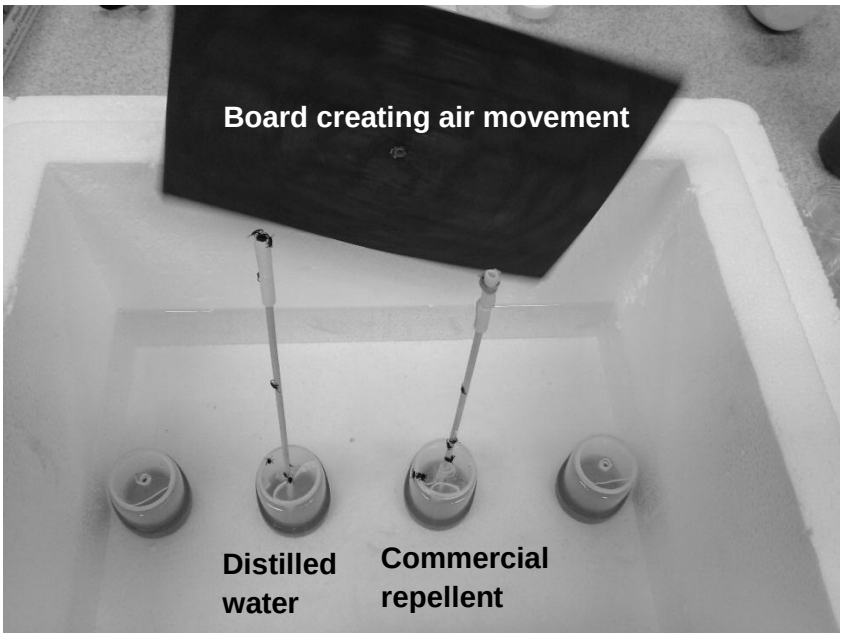

Figure 5.13 A modified tick climbing bioassay showing a board that constantly moved to create air movement of the experimental setup

The tick climbing bioassay was performed by observing the behaviour of five male and five female *Rh. appendiculatus* adult ticks per test product. After adding the ticks to the tick platforms, they were allowed to acclimatize for 15 minutes before being moved to an untreated area at the base of the wooden rod to promote climbing. The movement of ticks was observed every 15 minutes for one hour and the location recorded on the results sheet (Appendix B). After each interval the ticks were returned to an untreated area just above the tick platform on the wooden rod. Ticks that were not climbing were recorded as “below filter paper”.

III. Efficacy of botanical extracts

To test different plant extracts, the same procedure as described for the tests on commercialised chemical repellents was used. For each individual plant extract tested a piece of filter paper (1 cm x 5 cm) was treated with 150 µl solvent or plant extract and allowed to air dry before it was attached to the top of a 21 cm long wooden rod using double sided tape. A second piece of filter paper (1cm x 5cm) treated with distilled water was attached directly below the solvent or plant extract treated filter paper. The plant extracts tested using this bioassay are indicated in Table 5.5. Tick repellency tests for each of the different plant extracts including the solvent were conducted in triplicate.

Table 5.5 Plant extract tested using an attractant included tick climbing bioassay

Plant material	Solvent	Concentration
A. ferox	Acetone	20%
	Ethanol	20%
	Methanol	20%
L. leonurus	Acetone	10%
	Ethanol	10%
	Methanol	10%
T. vulgaris	Acetone	10%
	Ethanol	10%
	Methanol	10%

The questing behaviour of five male and five female *Rh. appendiculatus* adult ticks on both control and treatment rods was observed. The tick attractants used to stimulate the questing behaviour of ticks included heat at 37^{o}C

mimicking mammalian host body temperature, CO_2 representing CO_2 produced by the host, and a moving board which assisted with air circulation over the experimental setup. The set up included the solvent used on the first rod, and botanical extracts on the remaining three rods. The same experimental procedure used during the testing of commercial repellents was followed to observe the movement of ticks towards or away from the plant extracts. After each test run newly treated filter papers were used to set up the next run (Figure 5.14).

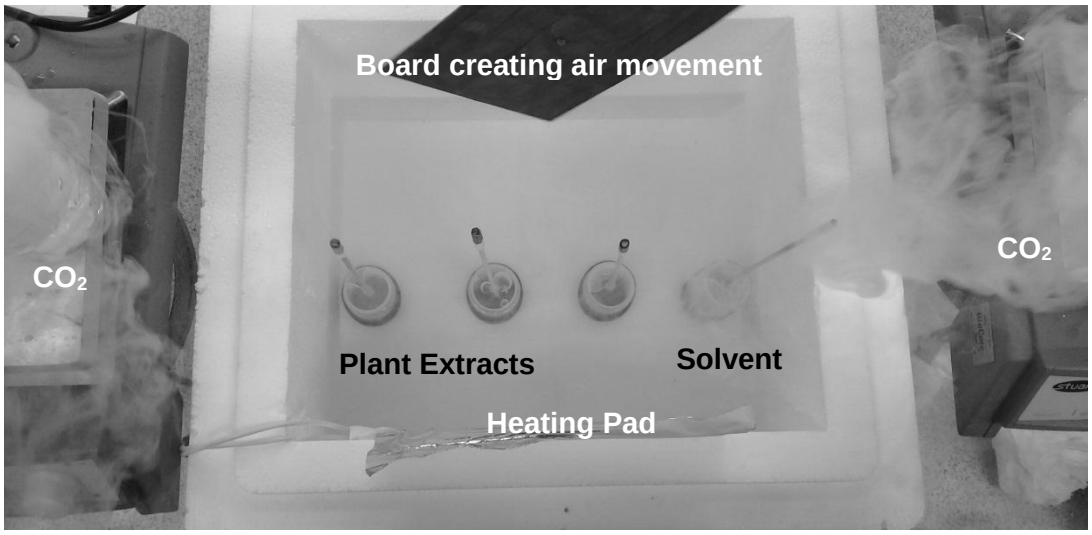

Figure 5.14 A modified tick climbing bioassay showing, a heating pad, CO_2 production and a board whose movement induced air movement

5.3.2.3 Experiment Three – Test for repellency using the preference tick climbing bioassay

I. Design of the modified tick climbing bioassay

As an alternative to the traditional tick climbing bioassay, a preference tick climbing bioassay was designed. This bioassay was conducted to evaluate the preference of adult *Rh. appendiculatus* ticks in approaching rods variedly treated as treatments and controls. The design was motivated by the observation described by Mwangi *et al.,* (1995b) where the approach behaviour of *Rh. appendiculatus* were tested towards specific grass species.

A polystyrene container (18 cm x 27 cm x 15 cm) was used to hold a 50 mL plastic sample container, similar to the tick climbing bioassay described previously. The lid was secured to the bottom of the polystyrene container with Tombo Mono Multi Liquid glue, two 4mm holes were drilled in the bottom of the sample container, 1cm apart, to allow for the insertion of two 21 cm wooden rods (Figure 5.15).

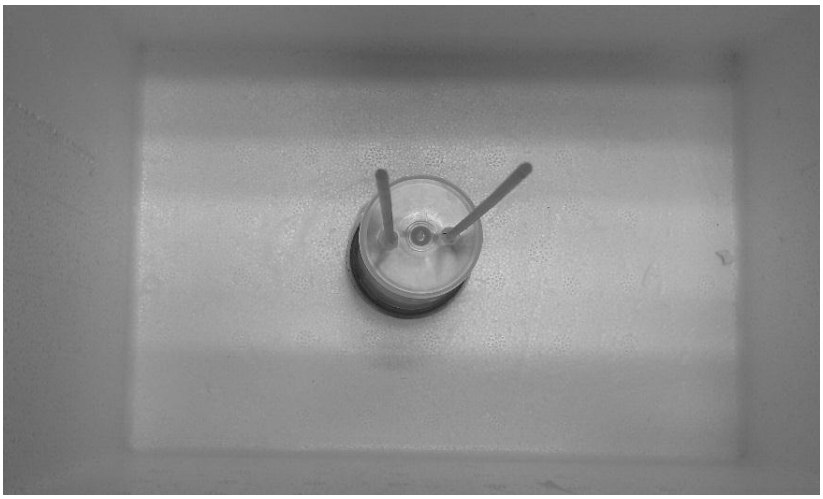

Figure 5.15 Preference tick climbing bioassay apparatus

II. Efficacy of commercial repellents

For the preference tick climbing bioassay, two control tests were conducted. During the first test the wooden rods were treated first with only distilled water to evaluate if ticks would approach both rods, as illustrated in Figure 5.16. Rods were treated by submerging each rod for 30 seconds in distilled water before allowed to air dry and insertion in the containers. A second control test was performed using the commercial repellents used for the tick climbing bioassay as previously described. One rod was dipped in Bayticol 2% EC Dip (Bayer Health Care, Bayer (Pty) Ltd Animal Health Division) and the control rod was dipped in distilled water, a second test rod was dipped in F10® wound spray with insecticide (Health and Hygiene (Pty) Ltd) and the control rod dipped in distilled water. The polystyrene container was filled with water to just below the tick platform to prevent escape of ticks from the container.

III. Efficacy of botanical extracts

For the preference tick climbing bioassay, the wooden rods were treated with either the botanical extract or solvent by submerging them for 30 seconds. The rods were then air dried and inserted in the containers. The polystyrene container was filled with water to just below the tick platform to discourage ticks from leaving the platform. Anti-tick properties of each of the different plant extracts (Table 5.6) were tested and the test were replicated three times.

Table 5.6 Plant extract tested using the preference tick climbing bioassay

Plant material	Solvent	Concentration
A. ferox	Acetone	20%
L. leonurus	Ethanol	10%
T. vulgaris	Acetone	10%

The preference tick climbing bioassay was performed by observing the behaviour of five male and five female *Rh. appendiculatus* adult ticks against each of the extracts indicated in Table 5.6. The test as demonstrated in Figure 5.16 were conducted and replicated three times.

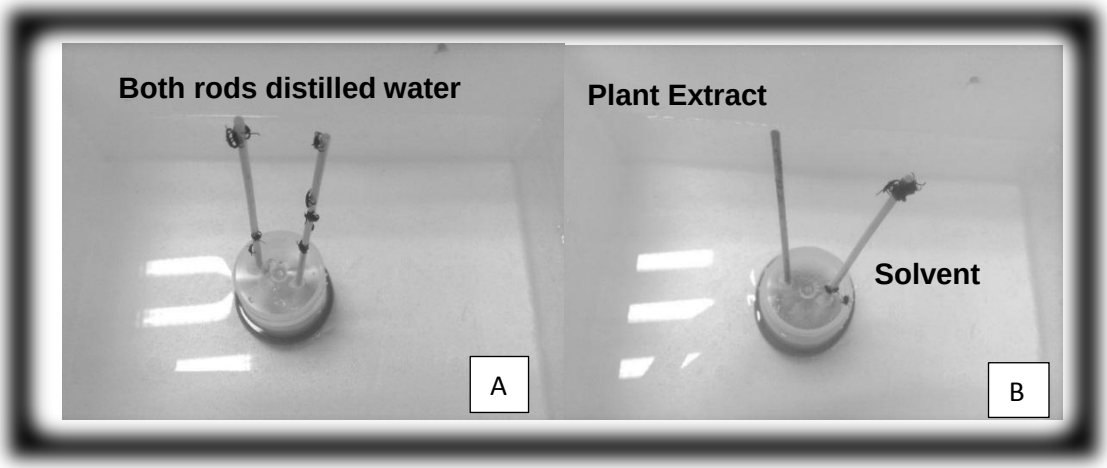

Figure 5.16 Preference tick climbing bioassay showing (A) Control setup with distilled water and (B) Treatment setup with plant extract /solvent

5.4 Data and Statistical Analysis

The percentage repellencies in all bioassays used to determine the efficacy of the extracts for individual and multiple species were calculated using the formular described by Jantan and Zaki (1999) which is as follows:

$$\% \text{ Repellency} = 100 - \left[\frac{\text{Number of ticks on treated area or rod}}{\text{Number of ticks on control area or rod}} \times 100 \right]$$

As the choice chamber bioassay has a neutral zone which has been excluded from the repellency percentage equation, the conclusions with regard to the most repellent extracts took both the behaviour as well as percentage repellency into account.

For the tick climbing bioassays the percentage repellency included the ticks observed on the treated and neutral filter paper as “number of ticks on treated area” in the equation. The percentage repellency for the preference tick climbing bioassay included the number of ticks observed on the control rod with the number of ticks not climbing as “number of ticks on control rod” in the equation.

Three extracts per solvent were tested using the choice chamber bioassay. The percentage repellency calculated for each tick species as well as the combined percentage repellency values were used to determine the half maximal effective concentration (EC_{50}) for each of acetone, methanol and ethanol plant extracts. The EC_{50} was determined by using Microsoft Excel (2016).

The IBM SPSS Statistics program version 25 and Microsoft Excel (2016) were used for all statistical analysis. To determine any significant difference between different parameters multivariant ANOVA (analysis of variance) followed by T-test were performed. Significance of difference was tested at 5% level of significance. A p-value less than (<) 0.05 indicated a significant difference between the parameters compared.

Section B – Results

5.5 Petri Dish Choice Chamber bioassays

5.5.1 Efficacy of Petri Dish Choice Chamber bioassay

The results on mean percentage tick repellency of the commercial repellents obtained using the choice chamber petri dish bioassay are summarised in Table 5.7.

The results demonstrate that most ticks preferred the control chamber over the test chamber. Significant differences ($P < 0.05$) were observed in the percentage of ticks observed in the control chamber compared to the Bayticol 2% EC Dip test chamber (Table 5.7). Only after 20 minutes did some ticks move into the test chamber. The mean percentage repellency of the Bayticol 2% EC dip against *Am. hebraeum* adult ticks was 56.10% and that against *Rh. appendiculatus* adult ticks was 76.74%. The estimated combined mean percentage repellency of Bayticol 2% EC dip against these two tick species infesting an individual host as it may be the case in nature would be 66.67%.

It should be noted that the Bayticol 2% EC Dip was used without dilution in the petri dish choice chamber bioassay. It was observed that from 35th minute all ticks started showing sluggish movements and from the 40th minute some of the ticks did not show any movement at all. After an hour a 92% mortality was recorded followed by 100 % mortality in 24 hours.

For the F10® wound spray with insecticide, the mean percentage repellency against *Am. hebraeum* adult ticks was 42.86% and that recorded against *Rh. appendiculatus* was 55.56 %. The estimated combined mean percentage repellency of F10® wound spray against these two tick species infesting an individual host would be 50.88 %. The results and significant differences between the percentage repellencies against ticks in the treatment and control areas demonstrated that the F10® wound spray with insecticide had a greater effect on *Rh. appendiculatus* ($P = 0.045$) compared to *Am. hebraeum* ($P = 0.075$) adult ticks. No significant difference ($P > 0.05$) was found between mean percentage repellencies of the two commercial tick repellents (Table 5.7).

Table 5.7 Tick movement and percentage repellency petri dish choice chamber – distilled water / commercial repellent

	Am. hebraeum				*Rh. appendiculatus*				Combined			
Time	**dH_2O**	**NZ**	**Test**	**% Repel**	**dH_2O**	**NZ**	**Test**	**% Repel**	**dH_2O**	**NZ**	**Test**	**% Repel**
Bayticol												
5 min	6.00	0.00	0.00	100.00	6.00	0.00	0.00	100.00	12.00	0.00	0.00	100.00
10 min	6.00	0.00	0.00	100.00	6.00	0.00	0.00	100.00	12.00	0.00	0.00	100.00
15 min	6.00	0.00	0.00	100.00	6.00	0.00	0.00	100.00	12.00	0.00	0.00	100.00
20 min	6.00	0.00	0.00	100.00	5.00	0.00	1.00	80.00	11.00	0.00	1.00	90.91
25 min	5.00	0.00	1.00	80.00	3.00	0.00	3.00	0.00	8.00	0.00	4.00	50.00
30 min	3.00	0.00	3.00	0.00	5.00	0.00	1.00	80.00	8.00	0.00	4.00	50.00
35 min	2.00	0.00	4.00	-100.00	4.00	0.00	2.00	50.00	6.00	0.00	6.00	0.00
40 min	3.00	0.00	3.00	0.00	4.00	2.00	0.00	100.00	7.00	2.00	3.00	57.14
45 min	1.00	2.00	3.00	-200.00	2.00	3.00	1.00	50.00	3.00	5.00	4.00	-33.33
50 min	1.00	3.00	2.00	-100.00	2.00	3.00	1.00	50.00	3.00	6.00	3.00	0.00
55 min	1.00	3.00	2.00	-100.00	0.00	5.00	1.00	0.00*	1.00	8.00	3.00	-200.00
60 min	1.00	5.00	0.00	100.00	0.00	6.00	0.00	0.00*	1.00	11.00	0.00	100.00
Mean %	56.94	18.06	25.00	56.10	59.72	26.39	13.89	76.74	58.33	22.22	19.44	66.67
T-Test	0.044				0.022				0.036			
F10®												
5 min	2.00	4.00	0.00	100.00	4.00	1.00	1.00	75.00	6.00	5.00	1.00	83.33
10 min	2.00	3.00	1.00	50.00	4.00	2.00	0.00	100.00	6.00	5.00	1.00	83.33
15 min	3.00	2.00	1.00	66.67	2.00	4.00	0.00	100.00	5.00	6.00	1.00	80.00
20 min	2.00	2.00	2.00	0.00	3.00	3.00	0.00	100.00	5.00	5.00	2.00	60.00
25 min	3.00	2.00	1.00	66.67	4.00	0.00	2.00	50.00	7.00	2.00	3.00	57.14
30 min	3.00	1.00	2.00	33.33	5.00	0.00	1.00	80.00	8.00	1.00	3.00	62.50
35 min	2.00	3.00	1.00	50.00	3.00	2.00	1.00	66.67	5.00	5.00	2.00	60.00
40 min	1.00	3.00	2.00	-100.00	3.00	0.00	3.00	0.00	4.00	3.00	5.00	-25.00
45 min	0.00	4.00	2.00	0.00*	4.00	0.00	2.00	50.00	4.00	4.00	4.00	0.00
50 min	0.00	6.00	0.00	0.00*	2.00	1.00	3.00	-50.00	2.00	7.00	3.00	-50.00
55 min	1.00	5.00	0.00	100.00	1.00	4.00	1.00	0.00	2.00	9.00	1.00	50.00
60 min	2.00	4.00	0.00	100.00	1.00	3.00	2.00	-100.00	3.00	7.00	2.00	33.33
Mean %	29.17	54.17	16.67	42.86	50.00	27.78	22.22	55.56	39.58	40.97	19.44	50.88
T-Test	0.075				0.045				0.063			
P value				0.386				0.335				0.851

*Percentage repellency where no ticks were observed in control area is recorded as 0.00%
% Repel: % Repellency; NZ: Neutral Zone

5.5.2 Botanical Extract Testing – *Aloe ferox*

As indicated in Table 5.8, nine *A. ferox* extracts, three per solvent type, were tested to determine their effect on the movement of *Am. hebraeum* and *Rh. appendiculatus* adult ticks within the petri dish choice chamber. Mean percentage repellencies of *A. ferox* extracts against *Am. hebraeum* and *Rh. appendiculatus* adult ticks and a combined mean percentage repellency effect are described below.

Am. hebraeum

Data on mean percentage repellency of different extracts of *A. ferox* against *Am. hebraeum* ticks are summarised and illustrated in Table 5.8 and Figures 5.17 and 5.18 respectively. The *A. ferox* extracts showed a varying degree of repellency against *Am. hebraeum* adult ticks ranging between -93.55% as observed for the 5% concentration methanol extract and 79.07% as expressed by the 10% concentatration acetone extract. Several extracts expressed a percentage repellency less than 50%, including the 5% concentration of acetone extract, 5% and 20% concentration of ethanol extract and 5% and 10% concentrations of methanol extract. Both the 5% concentration of ethanol extract and 5% concentration of methanol extract showed no effect (0.00% and -93.55% respectively) on repelling *Am. hebraeum* adult ticks. When comparing the mean percentage repellency of the positive control (F10®) with the mean percentage repellency of the extracts, some *A. ferox* extracts yielded a percentage repellency equal or greater than that of positive control (42.86%). These extracts included 10% concentration of acetone extract with 59.94% tick repellency, 20% concentration of acetone extract with 79.07% tick repellency, 10% concentration of ethanol extract with 67.86% tick repellency, 20% concentration of ethanol extract with 46.86% tick repellency and 20% concentration of methanol extract with 59.18% tick repellency.

Table 5.8 Percentage of ticks in the different areas of the petri dish choice chamber and percentage repellency of *A. ferox*

	Am. hebraeum				***Rh. appendiculatus***				**Combined**			
	Control	**Neutral Zone**	**Treatment**	**% Repel**	**Control**	**Neutral Zone**	**Treatment**	**% Repel**	**Control**	**Neutral Zone**	**Treatment**	**% Repel**
5% *A. ferox* Acetone	19.44	67.13	13.43	30.95	40.28	29.17	30.56	24.14	29.86	48.15	21.99	26.36
10% *A. ferox* Acetone	23.61	65.28	11.11	52.94	37.96	38.89	23.15	39.02	30.79	52.08	17.13	44.36
20% *A. ferox* Acetone	39.81	51.85	8.33	79.07	36.11	38.89	25.00	30.77	37.96	45.37	16.67	56.10
5% *A. ferox* Ethanol	19.91	60.19	19.91	0.00	13.43	50.93	35.65	-165.52	16.67	55.56	27.78	-66.67
10% *A. ferox* Ethanol	25.93	65.74	8.33	67.86	31.48	25.46	43.06	-36.76	28.70	45.60	25.69	10.48
20% *A. ferox* Ethanol	21.76	66.67	11.57	46.81	51.39	22.22	26.39	48.65	36.57	44.44	18.98	48.10
5% *A. ferox* Methanol	14.35	57.87	27.78	-93.55	14.81	58.80	26.85	-81.25	14.58	58.33	27.31	-87.30
10% *A. ferox* Methanol	19.44	67.13	13.43	30.95	40.28	29.17	30.56	24.14	29.86	48.15	21.99	26.36
20% *A. ferox* Methanol	22.69	68.06	9.26	59.18	43.98	27.78	28.24	35.79	33.33	47.92	18.75	43.75
Positive Control (F10®)	29.17	54.17	16.67	42.86	50.00	27.78	22.22	55.56	39.58	40.97	19.44	50.88

Green – Most effective concentration per parameter

Light Red – Negative repellence

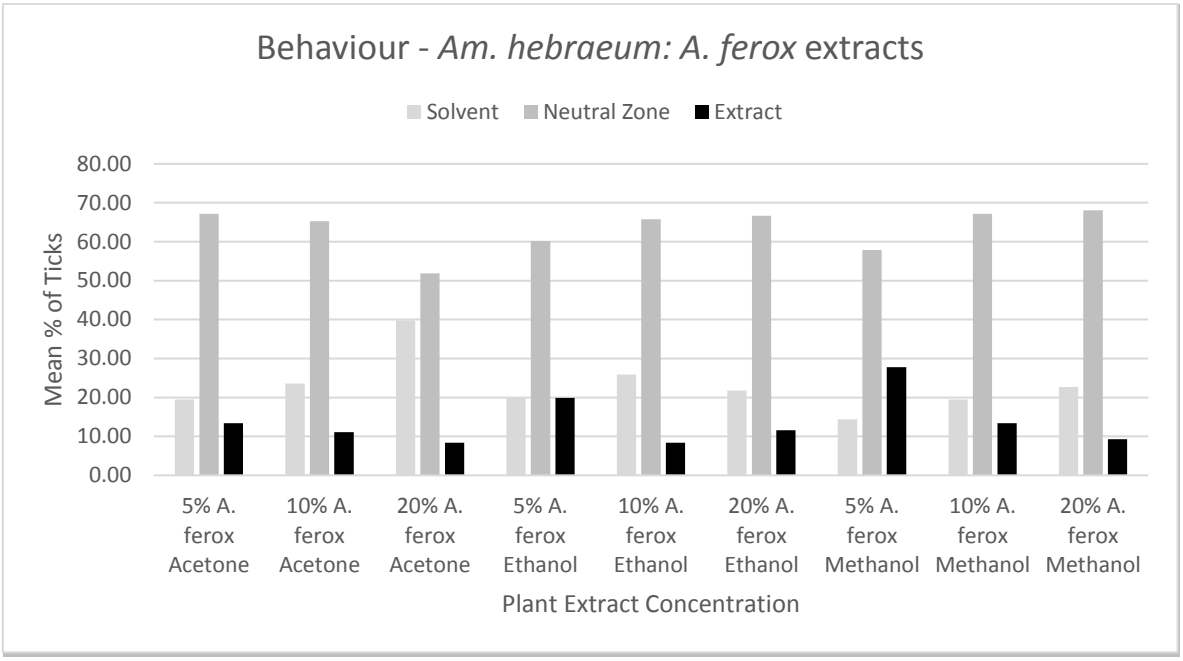

Figure 5.17 Response of *Am. hebraeum* ticks to different concentrations of *A. ferox* using a petri dish choice chamber bioassay

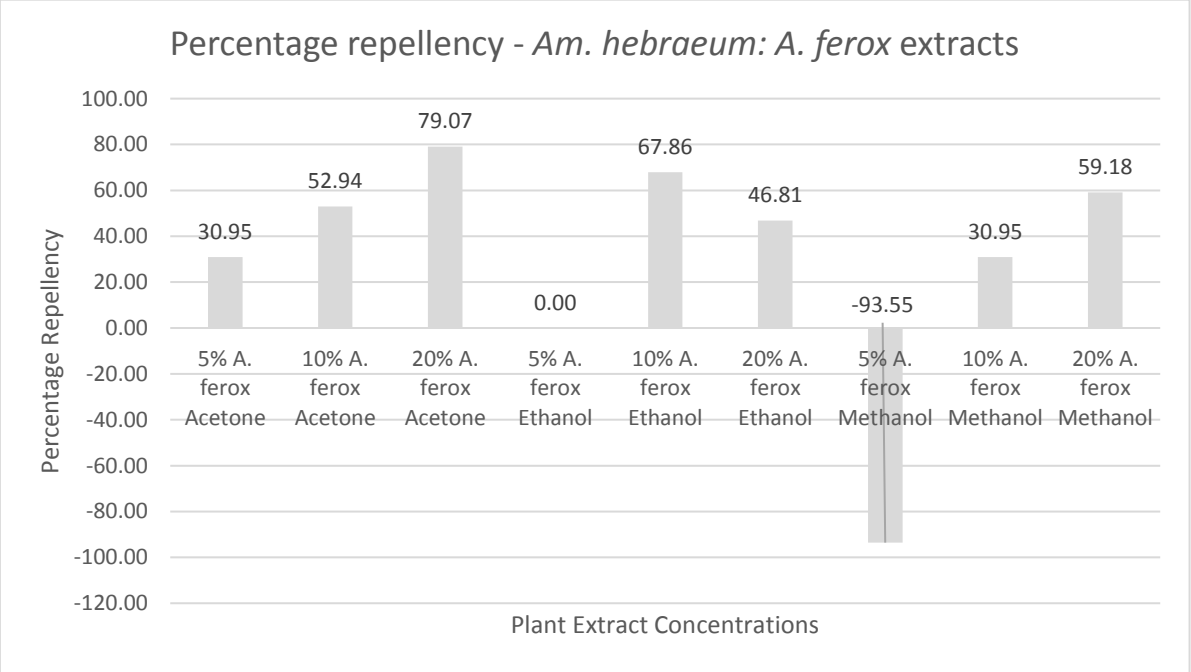

Figure 5.18 Percentage repellency of different concentrations of *A. ferox* extracts against *Am. hebraeum* adults using a petri dish choice chamber bioassay

The statistical comparisons of different repellencies are summarised in Table 5.9 below. Significant differences in mean percentage repellencies are highlighted in light red.

Table 5.9 Differences between the repellencies of different concentrations and types of extracts of *A. ferox* against *Am. hebraeum* adults

Parameter	Statistical Difference
Acetone	
% repellency: *A. ferox* 5% and *A. ferox* 10% (Acetone)	0.435
% repellency: *A. ferox* 5% and *A. ferox* 20% (Acetone)	0.034
% repellency: *A. ferox* 10% and *A. ferox* 20% (Acetone)	0.050
% repellency: *A. ferox* 5% (Acetone) and F10® (positive control)	0.277
% Mean: *A. ferox* 5% (Acetone) and Negative control	0.129
% repellency: *A. ferox* 10% (Acetone) and F10® (positive control)	0.735
% Mean: *A. ferox* 10% (Acetone) and Negative control	0.049
% repellency: *A. ferox* 20% (Acetone) and F10® (positive control)	0.015
% Mean: *A. ferox* 20% (Acetone) and Negative control	0.021
Ethanol	
% repellency: *A. ferox* 5% and *A. ferox* 10% (Ethanol)	0.004
% repellency: *A. ferox* 5% and *A. ferox* 20% (Ethanol)	0.086
% repellency: *A. ferox* 10% and *A. ferox* 20% (Ethanol)	0.060
% repellency: *A. ferox* 5% (Ethanol) and F10® (positive control)	0.119
% Mean: *A. ferox* 5% (Ethanol) and Negative control	0.500
% repellency: *A. ferox* 10% (Ethanol) and F10® (positive control)	0.052
% Mean: *A. ferox* 10% (Ethanol) and Negative control	0.029
% repellency: *A. ferox* 20% (Ethanol) and F10® (positive control)	0.838
% Mean: *A. ferox* 20% (Ethanol) and Negative control	0.063
Methanol	
% repellency: *A. ferox* 5% and *A. ferox* 10% (Methanol)	0.001
% repellency: *A. ferox* 5% and *A. ferox* 20% (Methanol)	0.002
% repellency: *A. ferox* 10% and *A. ferox* 20% (Methanol)	0.396
% repellency: *A. ferox* 5% (Methanol) and F10® (positive control)	0.002
% Mean: *A. ferox* 5% (Methanol) and Negative control	0.059
% repellency: *A. ferox* 10% (Methanol) and F10® (positive control)	0.424
% Mean: *A. ferox* 10% (Methanol) and Negative control	0.129
% repellency: *A. ferox* 20% (Methanol) and F10® (positive control)	0.706
% Mean: *A. ferox* 20% (Methanol) and Negative control	0.039
Comparison	
% repellency: *A. ferox* 20% (Acetone) and *A. ferox* 20% (Ethanol)	0.016
% repellency: *A. ferox* 20% (Acetone) and *A. ferox* 20% (Methanol)	0.020
% repellency: *A. ferox* 20% (Ethanol) and *A. ferox* 20% (Methanol)	0.592

The statistical comparison of percentage repellencies showed the 5% and 10% concentrations of acetone plant extracts, 10% and 20% concentrations of ethanol plant extracts and 10% and 20% concentrations of methanol plant extracts to have no significant differences ($P > 0.05$) when compared with the repellent effects of a commercial repellent. The 20% concentration of acetone plant extract showed a significantly higher ($P < 0.05$) repellency against *Am. hebraeum* ticks when

compared with the commercial repellent whereas the 5% concentration of ethanol and 5% concentration of methanol showed no repelling effect ($P > 0.05$). The statistical comparison also showed a significant difference ($P < 0.05$) between the percentage repellencies of 10% and 20% concentrations of acetone plant extracts, 10% concentration of ethanol plant extract and 20% concentration of methanol plant extract and the negative control. This observation suggest that these plant extracts had a repelling effect against *Am. hebraeum* adult ticks. Although the percentage repellency of the 20% concentration of ethanol plant extract was lower than the percentage repellency expressed by the 10% concentration of ethanol plant extract the difference was not significant ($P = 0.060$). A significant difference ($P < 0.05$) was observed between the 20% concentration of the acetone plant extract and the 20% concentration of the plant extracts of the other two solvents. Notably, the acetone extract expressed a significantly higher ($P < 0.05$) percentage repellency compared to the ethanol and methanol extracts.

In summary, it is reasonable to suggest that plant extracts that showed a lower percentage repellency to that of the commercial repellent (42.86% repellency) demonstrated a limited or no repellency. These included the 5% concentration of acetone plant extract (30.95% repellency), 5% concentration of ethanol plant extract (0.00% repellency), 5% concentration of methanol plant extract (-93.55% repellency) and 10% concentration of methanol extract (30.95% repellency). The 10% concentration of acetone plant extract (52.94% repellency), 20% concentration of methanol plant extract (59.18% repellency) and 10% (67.86% repellency) and 20% (46.81% repellency) concentrations of ethanol plant extracts all expressed a percentage repellency higher than the percentage repellency of the commercial repellent. However, it should be noted that these repellencies were less than 70% repellency, suggesting a medium strength repellency against *Am. hebraeum.* The 20% concentration of acetone plant extract yielded the highest percentage repellency observed against *Am. hebraeum* ticks with a 79.07% repellency. When comparing the percentage repellencies between the 20% concentrations of the different solvent plant extracts, both the percentage repellencies of the ethanol and methanol extracts were significantly lower ($P < 0.05$) compared to the percentage repellency of the acetone extract.

Rh. appendiculatus

Data for mean and repellencies of different extracts of *A. ferox* against adults of *Rh. appendiculatus* ticks are summarised and illustrated in Table 5.8 and Figures 5.19 and 5.20 respectively. The *A. ferox* extracts showed a varying degree of repellency against *Am. hebraeum* adult ticks ranging from -165.52% as observed for the 5% concentration of ethanol plant extract to 48.65% as expressed by the 10% concentration of ethanol plant extract. All plant extracts expressed a percentage repellency less than 50%. The 5% concentration of ethanol plant extract (with -165.52% repellency), 10% concentration of ethanol plant extract (with -36.76% repellency) and 5% concentration of methanol plant extract (with -81.25% repellency) showed to have no effect on repelling *Rh. appendiculatus* adult ticks. When comparing the percentage repellency of the positive control (F10®) with the percentage repellency of the extracts, none of the *A. ferox* extracts yielded a percentage repellency equal to or greater than that of the positive control (55.56%).

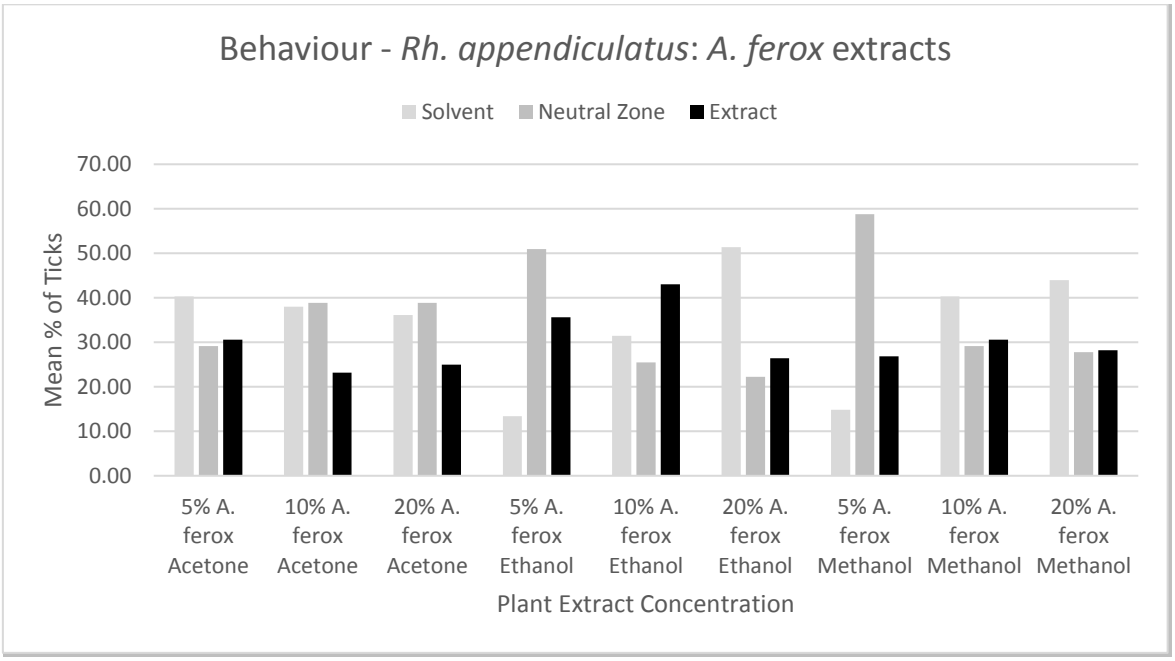

Figure 5.19 Response of *Rh. appendiculatus* ticks to different concentrations of *A. ferox* using a petri dish choice chamber bioassay

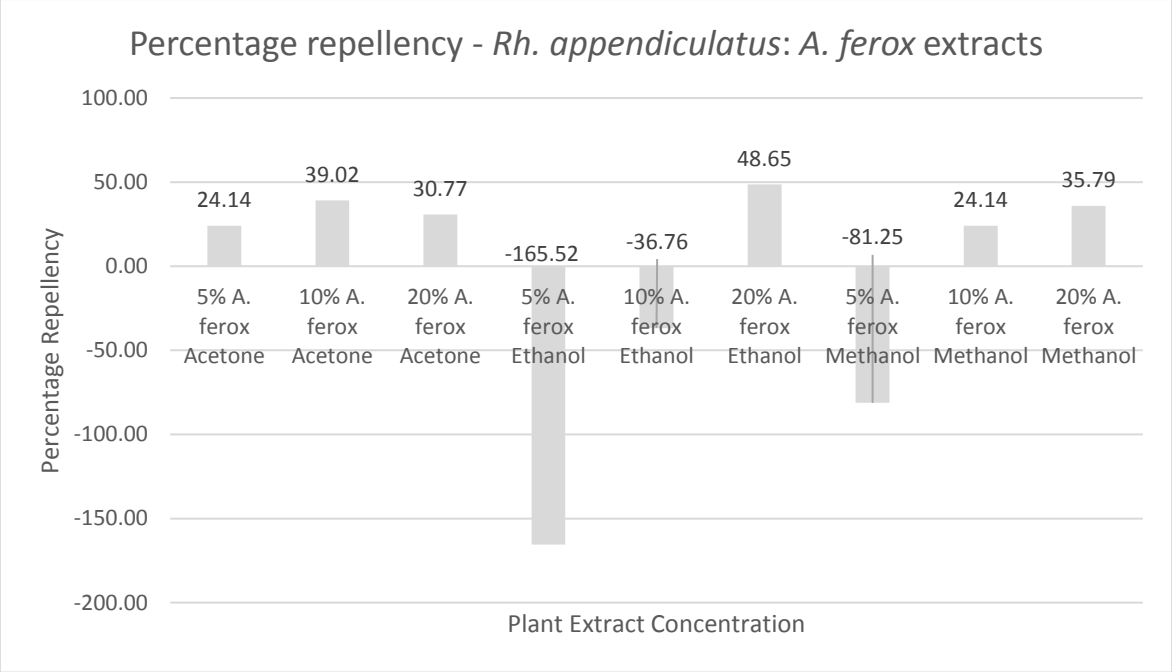

Figure 5.20 Percentage repellency of different concentration of *A. ferox* extracts against *Rh. appendiculatus* adults using a petri dish choice chamber bioassay

The statistical comparison of different repellencies are summarised in Table 5.10 below. Significant differences of percentage repellencies are highlighted in light red.

Table 5.10 Significance of difference between the repellency of different concentrations and types of extracts of *A. ferox* against *Rh. appendiculatus* adults

Parameter	Statistical Difference
Acetone	
% repellency: *A. ferox* 5% and *A. ferox* 10% (Acetone)	0.586
% repellency: *A. ferox* 5% and *A. ferox* 20% (Acetone)	0.956
% repellency: *A. ferox* 10% and *A. ferox* 20% (Acetone)	0.851
% repellency: *A. ferox* 5% (Acetone) and F10® (positive control)	0.378
% Mean: *A. ferox* 5% (Acetone) and Negative control	0.180
% repellency: *A. ferox* 10% (Acetone) and F10® (positive control)	0.697
% Mean: *A. ferox* 10% (Acetone) and Negative control	0.089
% repellency: *A. ferox* 20% (Acetone) and F10® (positive control)	0.393
% Mean: *A. ferox* 20% (Acetone) and Negative control	0.120
Ethanol	
% repellency: *A. ferox* 5% and *A. ferox* 10% (Ethanol)	0.021
% repellency: *A. ferox* 5% and *A. ferox* 20% (Ethanol)	0.000
% repellency: *A. ferox* 10% and *A. ferox* 20% (Ethanol)	0.012
% repellency: *A. ferox* 5% (Ethanol) and F10® (positive control)	0.000
% Mean: *A. ferox* 5% (Ethanol) and Negative control	0.035

Table continues on the next page

Parameter	Statistical Difference
Ethanol (continue)	
% repellency: *A. ferox* 10% (Ethanol) and F10® (positive control)	0.016
% Mean: *A. ferox* 10% (Ethanol) and Negative control	0.157
% repellency: *A. ferox* 20% (Ethanol) and F10® (positive control)	0.937
% Mean: *A. ferox* 20% (Ethanol) and Negative control	0.059
Methanol	
% repellency: *A. ferox* 5% and *A. ferox* 10% (Methanol)	0.000
% repellency: *A. ferox* 5% and *A. ferox* 20% (Methanol)	0.000
% repellency: *A. ferox* 10% and *A. ferox* 20% (Methanol)	0.371
% repellency: *A. ferox* 5% (Methanol) and F10® (positive control)	0.000
% Mean: *A. ferox* 5% (Methanol) and Negative control	0.069
% repellency: *A. ferox* 10% (Methanol) and F10® (positive control)	0.364
% Mean: *A. ferox* 10% (Methanol) and Negative control	0.180
% repellency: *A. ferox* 20% (Methanol) and F10® (positive control)	0.726
% Mean: *A. ferox* 20% (Methanol) and Negative control	0.103
Comparison	
% repellency: *A. ferox* 20% (Acetone) and *A. ferox* 20% (Ethanol)	0.303
% repellency: *A. ferox* 20% (Acetone) and *A. ferox* 20% (Methanol)	0.457
% repellency: *A. ferox* 20% (Ethanol) and *A. ferox* 20% (Methanol)	0.598

The statistical comparison of the repellent effects of 5% and 10% concentrations of acetone plant extract, 20% concentration of ethanol plant extract and 10% and 20% concentrations of methanol plant extract showed to have no significant differences ($P > 0.05$) when compared with the repellent effects of a commercial repellent. The repellent effects of 5% and 10% concentrations of ethanol plant extract and 5% concentration of methanol plant extract showed significant differences ($P < 0.05$) when compared with the commercial repellent. The statistical analysis also showed no significant difference ($P > 0.05$) between all extracts with the exception of the 5% concentration of ethanol plant extract, and the negative control, suggesting that the extracts of *A. ferox* had low repelling effects against *Rh. appendiculatus* adult ticks. The 20% concentrations of the three different solvents all had a similar effect in repelling *Rh. appendiculatus* adult ticks ($P > 0.05$).

In summary, data presented suggest that the plant extract that expressed a relatively higher percentage repellency against *Rh. appendiculatus* was the 20% concentration of ethanol plant extract of *A. ferox*. However, based on the observations made and the comparison between percentage repellency of the commercial repellent with the percentage repellencies of the botanical extracts, none of the extracts expressed a strong repelling effect against *Rh. appendiculatus*.

Combined data

Data for mean and repellency of different extracts of *A. ferox* as multi-species repellent is summarised and illustrated in Table 5.8 and Figure 5.21. As observed for the individual species, the *A. ferox* extracts showed a varying degree of repellency as multi-species repellent ranging from -87.30% as observed for the 5% concentration of methanol plant extract to 56.10% as expressed by the 20% concentatration of acetone plat extract. Except for the 20% concentration of acetone plant extract (with 56.10% repellency), all extracts expressed a percentage repellency less than 50%. The 5% concentration of ethanol plant extract (with negative 66.67% repellency) and 5% concentration of methanol plant extract (with negative 87.30% repellency) showed to have no effect on repelling multiple tick species. When comparing the percentage repellencies of the positive control (F10®) with the percentage repellency of the extracts, only the 20% concentration of acetone plant extract yielded a percentage repellency equal or greater than that of positive control (50.88%).

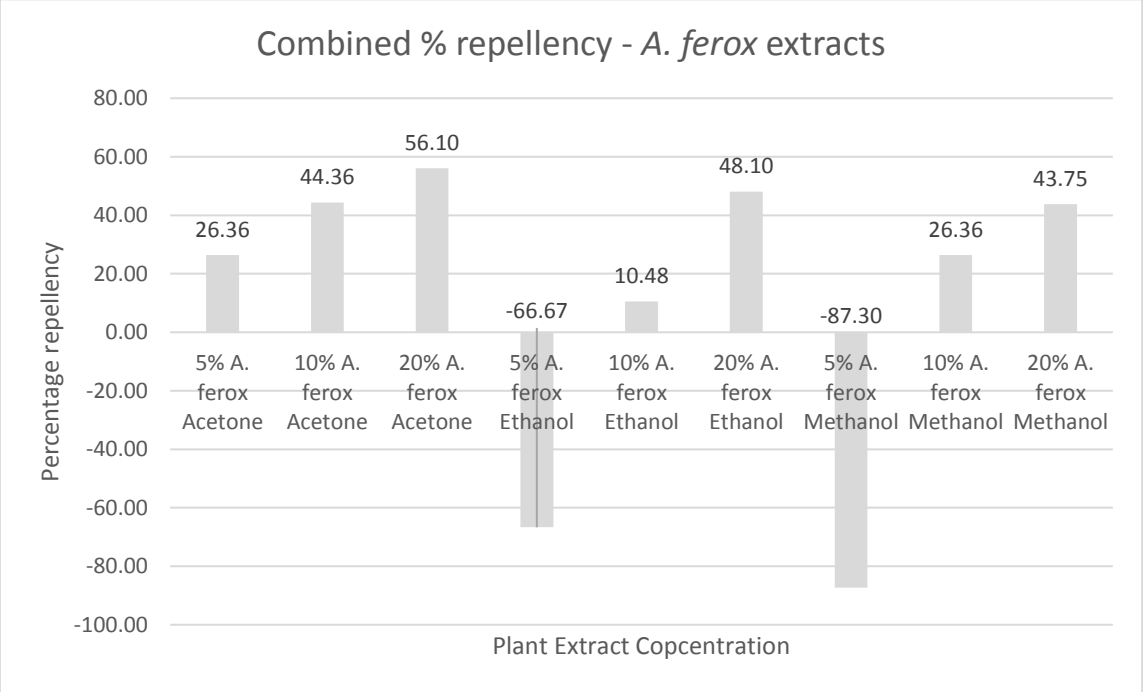

Figure 5.21 Percentage repellency of different concentrations of *A. ferox* extracts as multi species repellent using a petri dish choice chamber bioassay

The statistical comparison of different repellencies are summarised in Table 5.11 below. Significant differences in percentage repellencies are highlighted in light red.

Table 5.11 Significance of difference between the repellency of different concentrations and types of extracts of *A. ferox* against multiple tick species

Parameter	Statistical Difference
Acetone	
% repellency: *A. ferox* 5% and *A. ferox* 10% (Acetone)	0.430
% repellency: *A. ferox* 5% and *A. ferox* 20% (Acetone)	0.047
% repellency: *A. ferox* 10% and *A. ferox* 20% (Acetone)	0.344
% repellency: *A. ferox* 5% (Acetone) and F10® (positive control)	0.300
% Mean: *A. ferox* 5% (Acetone) and Negative control	0.161
% repellency: *A. ferox* 10% (Acetone) and F10® (positive control)	0.939
% Mean: *A. ferox* 10% (Acetone) and Negative control	0.070
% repellency: *A. ferox* 20% (Acetone) and F10® (positive control)	0.238
% Mean: *A. ferox* 20% (Acetone) and Negative control	0.044
Ethanol	
% repellency: *A. ferox* 5% and *A. ferox* 10% (Ethanol)	0.001
% repellency: *A. ferox* 5% and *A. ferox* 20% (Ethanol)	0.000
% repellency: *A. ferox* 10% and *A. ferox* 20% (Ethanol)	0.039
% repellency: *A. ferox* 5% (Ethanol) and F10® (positive control)	0.000
% Mean: *A. ferox* 5% (Ethanol) and Negative control	0.085
% repellency: *A. ferox* 10% (Ethanol) and F10® (positive control)	0.044
% Mean: *A. ferox* 10% (Ethanol) and Negative control	0.339
% repellency: *A. ferox* 20% (Ethanol) and F10® (positive control)	0.854
% Mean: *A. ferox* 20% (Ethanol) and Negative control	0.060
Methanol	
% repellency: *A. ferox* 5% and *A. ferox* 10% (Methanol)	0.019
% repellency: *A. ferox* 5% and *A. ferox* 20% (Methanol)	0.012
% repellency: *A. ferox* 10% and *A. ferox* 20% (Methanol)	0.283
% repellency: *A. ferox* 5% (Methanol) and F10® (positive control)	0.016
% Mean: *A. ferox* 5% (Methanol) and Negative control	0.064
% repellency: *A. ferox* 10% (Methanol) and F10® (positive control)	0.642
% Mean: *A. ferox* 10% (Methanol) and Negative control	0.161
% repellency: *A. ferox* 20% (Methanol) and F10® (positive control)	0.792
% Mean: *A. ferox* 20% (Methanol) and Negative control	0.072
Comparison	
% repellency: *A. ferox* 20% (Acetone) and *A. ferox* 20% (Ethanol)	0.382
% repellency: *A. ferox* 20% (Acetone) and *A. ferox* 20% (Methanol)	0.135
% repellency: *A. ferox* 20% (Ethanol) and *A. ferox* 20% (Methanol)	0.775

The 5% and 10% concentrations of ethanol plant extract and 5% concentration of methanol plant extract showed significantly lower repelling effects when compared with the commercial repellent ($P < 0.05$). The rest of the extracts expressed repellent effects that were not significantly different ($P > 0.05$) to those of a commercial repellent. The statistical analysis also showed no significant difference between all extracts, with the exception of the 20% concentration of acetone plant extract, and the negative control, suggesting a limited repelling effect of these extracts against multiple tick species. Comparison of 20% concentrations of the

acetone, methanol and ethanol extracts of *A. ferox* showed no significant repellent differences against *Am. hebraeum* adult ticks (P > 0.05).

In summary, data presented in this study suggest that *A. ferox* extracts had a greater repellent effect against *Am. hebraeum* ticks compared to *Rh. appendiculatus* ticks. The combined repellency of the extracts on both tick species had a lower efficacy due to the reduced effect observed in *Rh. appendiculatus*. When comparing the percentage repellencies of the different concentration of plant extracts with the percentage repellency expressed by the commercial repellent (50.88%), only the 20% concentration of acetone plant extract yielded a slightly higher percentage repellency (56.10%) compared to the commercial repellent. All other extracts yielded a percentage repellency lower than that of the commercial repellent and can therefore be considered to show a limited repellency effect against *Am. hebraeum* and *Rh. appendiculatus* ticks.

EC_{50}

The EC_{50} for each of the acetone, methanol and ethanol extracts of *A. ferox* against *Am. hebraeum* were calculated using Microsoft Excel (2016) as indicated in Table 5.12.

Table 5.12 EC_{50} for *A. ferox* extracts against *Am. hebraeum*

	Am. hebraeum	***Rh. appendiculatus***	**Combined**
	Acetone		
EC_{50}	8.83%	Not Calculated*	Not Calculated*
	Ethanol		
EC_{50}	14.17%	Not Calculated*	Not Calculated*
	Methanol		
EC_{50}	15.91%	Not Calculated*	Not Calculated*

*As a result of *A. ferox* extracts having expressed poor repellency effects against *Rh. appendiculatus* EC_{50} was not calculated.

5.5.3 Botanical Extract Testing – *Leonotis leonurus*

As indicated in Table 5.13, nine *L. leonurus* extracts, were tested to determine the response of *Am. hebraeum* and *Rh. appendiculatus* adult ticks. A combination mean for the response and location of both species, as well as percentage

repellency were determined to evaluate the effect of *L. leonurus* extracts as potential tick repellents.

Am. hebraeum

Data on mean and percentage repellency of different extracts of *L. leonurus* against *Am. hebraeum* ticks are summarised and illustrated in Table 5.13 and Figures 5.22 and 5.23 respectively. The *L. leonurus* extracts showed a varying degree of repellency against *Am. hebraeum* adult ticks ranging from 25.64% as observed for the 5% concentration of ethanol plant extract to 92.56% as expressed by the 10% concentration of ethanol plant extract. Both the 5% and 7.5% concentrations of ethanol plant extract expressed a percentage repellency less than 50%. When comparing the percentage repellency of the positive control (F10®) with the percentage repellency of the plant extracts it is reasonable to suggest that plant extracts yielding a percentage repellency equal or greater than the positive control (42.86%) might be considered as potential repellents for ticks. These included all the acetone (5%: 50.00%, 7.5%: 57.33%, 10%: 63.19), 10% ethanol (92.56%) and all methanol (5%: 51.32%, 7.5%: 63.83%, 10%: 82.81%) *L. leonurus* extracts.

Table 5.13 Percentage of ticks in the different areas of the petri dish choice chamber and percentage repellency of *L. leonurus*

	Am. hebraeum				***Rh. appendiculatus***				**Combined**			
	Control	**Neutral Zone**	**Treatment**	**% Repel**	**Control**	**Neutral Zone**	**Treatment**	**% Repel**	**Control**	**Neutral Zone**	**Treatment**	**% Repel**
5% *L. leonurus* Acetone	25.93	61.11	12.96	50.00	22.69	47.22	30.09	-32.65	24.31	54.17	21.53	11.43
7.5% *L. leonurus* Acetone	34.72	50.46	14.81	57.33	20.83	59.26	19.91	4.44	27.78	54.86	17.36	37.50
10% *L. leonurus* Acetone	29.17	60.19	10.65	63.49	48.15	26.85	25.00	48.08	38.66	43.52	17.82	53.89
5% *L. leonurus* Ethanol	18.06	68.52	13.43	25.64	35.65	36.57	27.78	22.08	26.85	52.55	20.60	23.28
7.5% *L. leonurus* Ethanol	25.00	60.19	14.81	40.74	39.35	37.50	23.15	41.18	32.18	48.84	18.98	41.01
10% *L. leonurus* Ethanol	56.02	39.81	4.17	92.56	49.54	35.19	15.28	69.16	52.78	37.50	9.72	81.58
5% *L. leonurus* Methanol	35.19	47.69	17.13	51.32	42.59	37.50	19.91	53.26	38.89	42.59	18.52	52.38
7.5% *L. leonurus* Methanol	43.52	40.74	15.74	63.83	42.13	39.35	18.52	56.04	42.82	40.05	17.13	60.00
10% *L. leonurus* Methanol	29.63	65.28	5.09	82.81	46.76	38.43	14.81	68.32	38.19	51.85	9.95	73.94
Positive Control (F10®)	29.17	54.17	16.67	42.86	50.00	27.78	22.22	55.56	39.58	40.97	19.44	50.88

Green – Most effective concentration per parameter

Light Red – Negative repellent

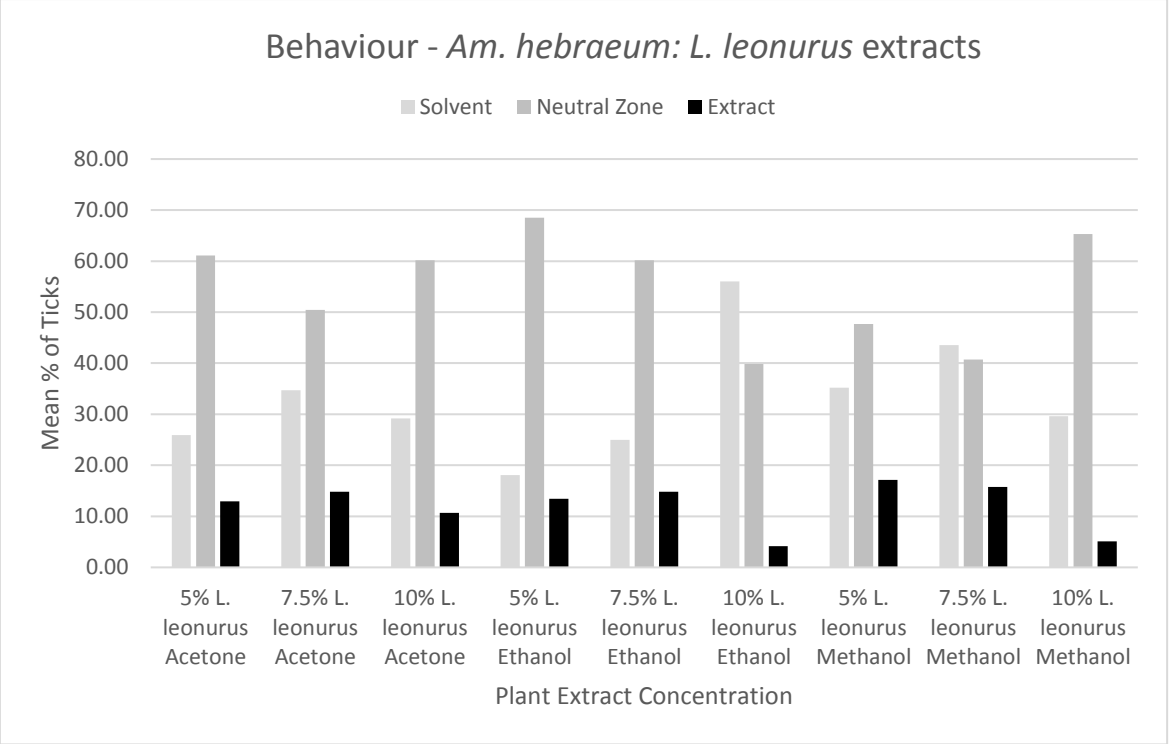

Figure 5.22 Response of *Am. hebraeum* ticks to different concentrations of *L. leonurus* using a petri dish choice chamber bioassay

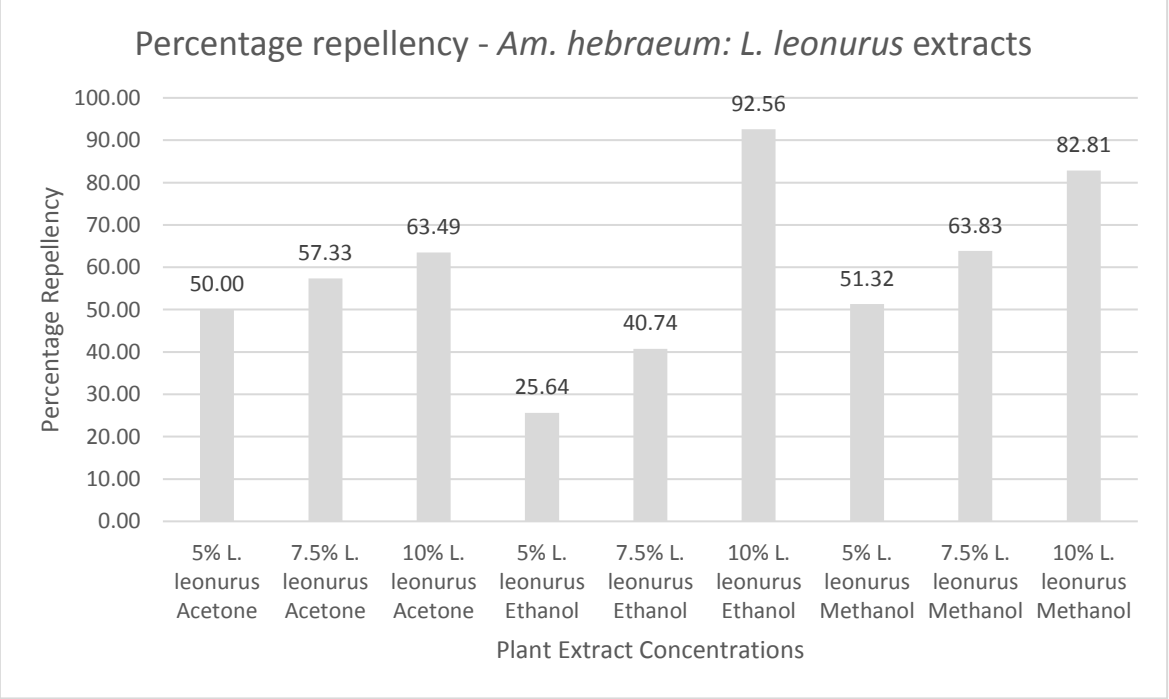

Figure 5.23 Percentage repellency of different concentrations of *L. leonurus* extracts against *Am. hebraeum* adults using a petri dish choice chamber bioassay

The statistical comparison of different repellencies are summarised in Table 5.14 below. Significant differences in percentage repellencies are highlighted in light red.

Table 5.14 Significance of difference between the repellency of different concentrations and types of extracts of *L. leonurus* against *Am. hebraeum* adults

Parameter	Statistical Difference
Acetone	
% repellency: *L. leonurus* 5% and *L. leonurus* 7.5% (Acetone)	0.398
% repellency: *L. leonurus* 5% and *L. leonurus* 10% (Acetone)	0.147
% repellency: *L. leonurus* 7.5% and *L. leonurus* 10% (Acetone)	0.408
% repellency: *L. leonurus* 5% (Acetone) and F10® (positive control)	0.847
% Mean: *L. leonurus* 5% (Acetone) and Negative control	0.055
% repellency: *L. leonurus* 7.5% (Acetone) and F10® (positive control)	0.460
% Mean: *L. leonurus* 7.5% (Acetone) and Negative control	0.042
% repellency: *L. leonurus* 10% (Acetone) and F10® (positive control)	0.142
% Mean: *L. leonurus* 10% (Acetone) and Negative control	0.034
Ethanol	
% repellency: *L. leonurus* 5% and *L. leonurus* 7.5% (Ethanol)	0.761
% repellency: *L. leonurus* 5% and *L. leonurus* 10% (Ethanol)	0.000
% repellency: *L. leonurus* 7.5% and *L. leonurus* 10% (Ethanol)	0.002
% repellency: *L. leonurus* 5% (Ethanol) and F10® (positive control)	0.326
% Mean: *L. leonurus* 5% (Ethanol) and Negative control	0.167
% repellency: *L. leonurus* 7.5% (Ethanol) and F10® (positive control)	0.614
% Mean: *L. leonurus* 7.5% (Ethanol) and Negative control	0.082
% repellency: *L. leonurus* 10% (Ethanol) and F10® (positive control)	0.001
% Mean: *L. leonurus* 10% (Ethanol) and Negative control	0.015
Methanol	
% repellency: *L. leonurus* 5% and *L. leonurus* 7.5% (Methanol)	0.276
% repellency: *L. leonurus* 5% and *L. leonurus* 10% (Methanol)	0.041
% repellency: *L. leonurus* 7.5% and *L. leonurus* 10% (Methanol)	0.167
% repellency: *L. leonurus* 5% (Methanol) and F10® (positive control)	0.644
% Mean: *L. leonurus* 5% (Methanol) and Negative control	0.053
% repellency: *L. leonurus* 7.5% (Methanol) and F10® (positive control)	0.152
% Mean: *L. leonurus* 7.5% (Methanol) and Negative control	0.033
% repellency: *L. leonurus* 10% (Methanol) and F10® (positive control)	0.027
% Mean: *L. leonurus* 10% (Methanol) and Negative control	0.019
Comparison	
% repellency: *L. leonurus* 10% (Acetone) and *L. leonurus* 10% (Ethanol)	0.001
% repellency: *L. leonurus* 10% (Acetone) and *L. leonurus* 10% (Methanol)	0.222
% repellency: *L. leonurus* 10% (Ethanol) and *L. leonurus* 10% (Methanol)	0.016

The statistical comparison of percentage repellencies demonstrated no significant differences ($P > 0.05$) between all concentrations of acetone plant extracts, 5% and 7.5% concentrations of ethanol plant extracts and 5% and 7.5% concentrations of methanol plant extracts and that of the commercial repellent. The 10% concentration of ethanol plant extract and 10% concentration of methanol plant extract showed a significantly higher ($P < 0.05$) percentage repellency against

Am. hebraeum ticks when compared with that of the commercial repellent. The statistical analysis also showed a significant difference ($P < 0.05$) between the 7.5% and 10% concentrations of acetone plant extract, 10% concentration of ethanol plant extract and 7.5% and 10% concentrations of methanol plant extract and that of the negative control. This suggests that *L. leonurus* has repellent properties against *Am. hebraeum* adult ticks. A significant difference was observed between the 10% concentrations of the ethanol and acetone as well as the ethanol and methanol extracts as the ethanol extract expressed a significantly higher ($P < 0.05$) percentage repellency compared to the acetone and methanol extracts.

In summary, it is reasonable to suggest that the plant extracts with percentage repellencies that are lower than the commercial repellent (with 42.86% repellency) showed a limited repellent effect. These included the 5% concentration of ethanol plant extract (with 25.64% repellency), and 7.5% concentration of ethanol plant extract (with 40.74% repellency). All other extracts expressed a percentage repellency higher than the percentage repellency of the commercial repellent. Data obtained in this study further suggest that *L. leonurus* extracts expressed a medium repellent strength against *Am. hebraeum* ticks with a less than 65% repellency. Extracts that showed this level of repellency include all acetone plant extracts, as well as the 5% and 7.5% concentration of methanol plant extracts. The 10% concentrations of ethanol plant extract (with 92.56% repellency) and methanol (with 82.82% repellency) extracts both expressed strong repelling effects against *Am. hebraeum* ticks.

Rh. appendiculatus

Data on mean and percentage repellency of different extracts of *L. leonurus* against *Rh. appendiculatus* ticks are summarised and illustrated in Table 5.13 and Figures 5.24 and 5.25 respectively. The *L. leonurus* extracts showed a varying degree of repellency against *Rh. appendiculatus* adult ticks ranging from -32.65% as observed for the 5% concentration of acetone plant extract to 69.16% as expressed by the 10% concentration of ethanol plant extract. Several extracts expressed a percentage repellency less than 50%. These included all acetone extracts and 5% and 7.5% concentrations of ethanol plant extract. The 5% concentration of acetone plant extract (with -32.65% repellency) showed to have no effect in repelling

Rh. appendiculatus adult ticks. By comparing the percentage repellency of the positive control (F10®) with the percentage repellency of the extracts it is reasonable to suggest that the extracts yielding a percentage repellency equal or greater than the positive control (55.56%) may be considered as having anti-tick properties. These included the 10% concentration of ethanol plant extract (with 69.16% repellency), 7.5% concentration of methanol plant extract (with 56.04% repellency) and 10% concentration of methanol plant extract (with 68.32% repellency).

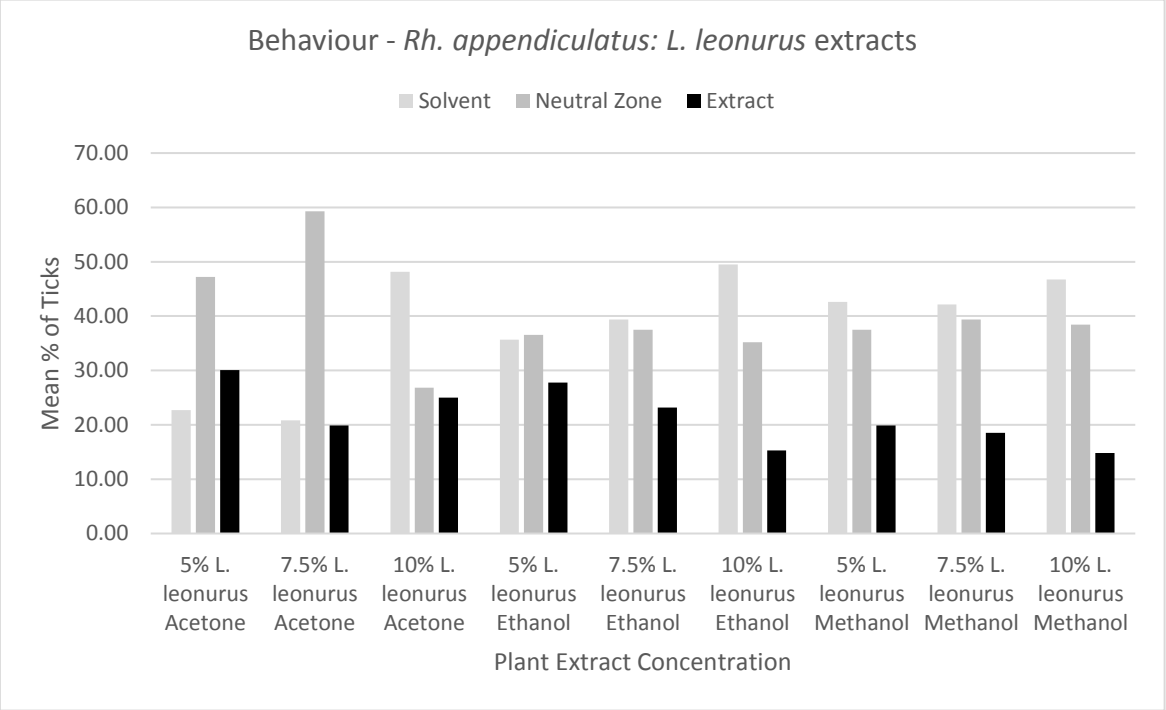

Figure 5.24 Response of *Rh. appendiculatus* ticks to different concentrations of *L. leonurus* using a petri dish choice chamber bioassay

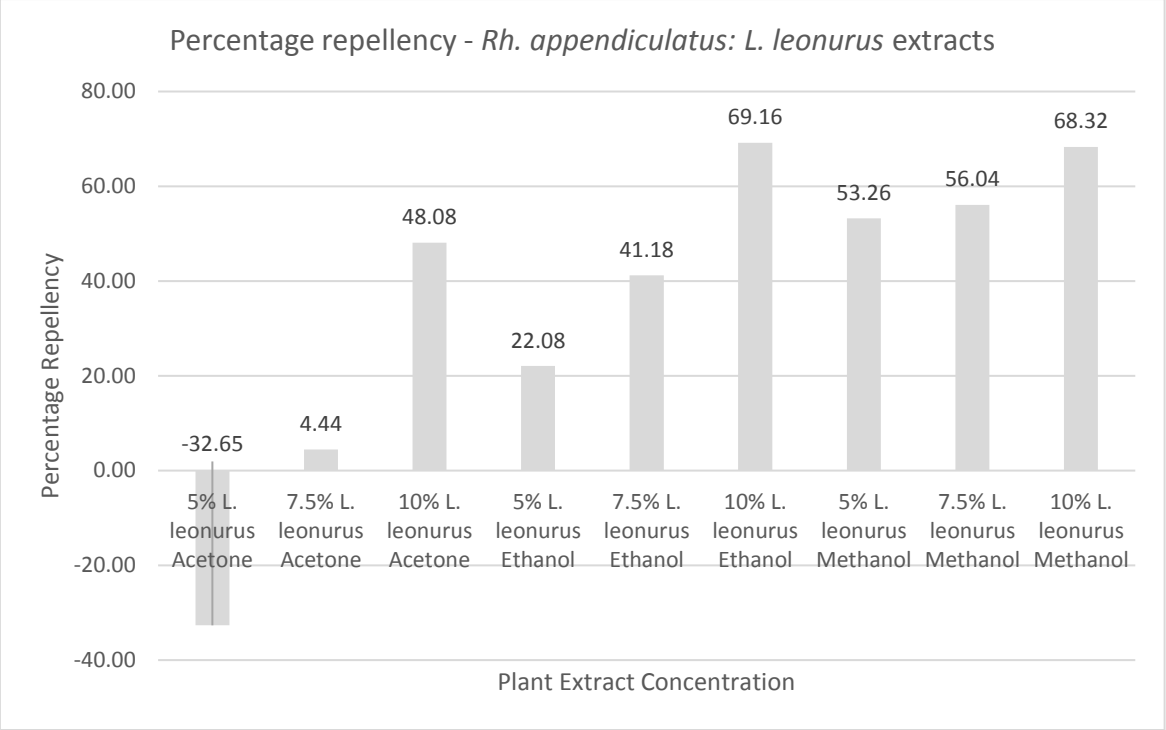

Figure 5.25 Percentage repellency of different concentrations of *L. leonurus* extracts against *Rh. appendiculatus* adults using a petri dish choice chamber bioassay

The statistical comparison of different repellencies are summarised in Table 5.15 below. Significant differences in percentage repellencies are highlighted in light red.

Table 5.15 Significance of difference between the repellency of different concentrations and types of extracts of *L. leonurus* against *Rh. appendiculatus* adults

Parameter	Statistical Difference
Acetone	
% repellency: *L. leonurus* 5% and *L. leonurus* 7.5% (Acetone)	0.088
% repellency: *L. leonurus* 5% and *L. leonurus* 10% (Acetone)	0.003
% repellency: *L. leonurus* 7.5% and *L. leonurus* 10% (Acetone)	0.007
% repellency: *L. leonurus* 5% (Acetone) and F10® (positive control)	0.008
% Mean: *L. leonurus* 5% (Acetone) and Negative control	0.175
% repellency: *L. leonurus* 7.5% (Acetone) and F10® (positive control)	0.038
% Mean: *L. leonurus* 7.5% (Acetone) and Negative control	0.431
% repellency: *L. leonurus* 10% (Acetone) and F10® (positive control)	0.886
% Mean: *L. leonurus* 10% (Acetone) and Negative control	0.060
Ethanol	
% repellency: *L. leonurus* 5% and *L. leonurus* 7.5% (Ethanol)	0.133
% repellency: *L. leonurus* 5% and *L. leonurus* 10% (Ethanol)	0.004

Table continues on the next page

Parameter	Statistical Difference
Ethanol	
% repellency: *L. leonurus* 7.5% and *L. leonurus* 10% (Ethanol)	0.048
% repellency: *L. leonurus* 5% (Ethanol) and F10® (positive control)	0.242
% Mean: *L. leonurus* 5% (Ethanol) and Negative control	0.198
% repellency: *L. leonurus* 7.5% (Ethanol) and F10® (positive control)	0.909
% Mean: *L. leonurus* 7.5% (Ethanol) and Negative control	0.081
% repellency: *L. leonurus* 10% (Ethanol) and F10® (positive control)	0.250
% Mean: *L. leonurus* 10% (Ethanol) and Negative control	0.028
Methanol	
% repellency: *L. leonurus* 5% and *L. leonurus* 7.5% (Methanol)	0.630
% repellency: *L. leonurus* 5% and *L. leonurus* 10% (Methanol)	0.034
% repellency: *L. leonurus* 7.5% and *L. leonurus* 10% (Methanol)	0.036
% repellency: *L. leonurus* 5% (Methanol) and F10® (positive control)	0.599
% Mean: *L. leonurus* 5% (Methanol) and Negative control	0.049
% repellency: *L. leonurus* 7.5% (Methanol) and F10® (positive control)	0.385
% Mean: *L. leonurus* 7.5% (Methanol) and Negative control	0.044
% repellency: *L. leonurus* 10% (Methanol) and F10® (positive control)	0.145
% Mean: *L. leonurus* 10% (Methanol) and Negative control	0.029
Comparison	
% repellency: *L. leonurus* 10% (Acetone) and *L. leonurus* 10% (Ethanol)	0.128
% repellency: *L. leonurus* 10% (Acetone) and *L. leonurus* 10% (Methanol)	0.124
% repellency: *L. leonurus* 10% (Ethanol) and *L. leonurus* 10% (Methanol)	0.692

The statistical comparison showed that there were no significant differences ($P > 0.05$) between the percentage repellencies of all plant extracts, except the 5% and 7.5% concentrations of acetone plant extract and the commercial repellent. The percentage repellencies of 5% and 7.5% concentrations of acetone plant extracts were significantly lower ($P < 0.05$) compared to that of the commercial repellent. The statistical analysis also showed a significant difference ($P < 0.05$) between the percentage repellency of the 10% concentration of ethanol plant extract and 7.5% and 10% concentrations of methanol plant extract and the negative control, suggesting that these extracts had repellent effects against *Rh. appendiculatus* adult ticks. No significant differences ($P > 0.05$) occurred among the repellencies of the 10% concentrations of the plant extracts of the three different solvents against *Rh. appendiculatus* adult ticks.

Data obtained from this study indicates that only the 10% concentration of ethanol plant extract (with 69.16% repellency), 7.5% concentration of methanol plant extract (with 56.04% repellency) and 10% concentration of methanol plant extract (with 68.32% repellency) yielded a percentage repellency higher that the commercial repellent (with 55.56%). All other concentrations of plant extracts yielded a percentage repellency lower than that of the commercial repellent and can therefore

be considered to have showed limited or no repellency against *Rh. appendiculatus*. Comparing the 10% concentrations of the different solvent extracts, no significant differences were observed between the percentage repellencies of the extracts. The 10% concentration of acetone plant extract yielded a percentage repellency lower than the commercial repellent suggesting that it does not express a strong repelling effect against *Rh. appendiculatus* ticks. However, both the 10% concentrations of ethanol and methanol plant extracts expressed a percentage repellency higher than the commercial repellent, with a significant effect on *Rh. appendiculatus* ticks.

Combined data

Data for mean and repellency of different extracts of *L. leonurus* as multi-species repellents is summarised and illustrated in Table 5.13 and Figure 5.26. As observed for the individual species, the *L. leonurus* extracts showed a varying degree of repellency as multi-species repellent ranging from 11.43% as observed for the 5% concentration of acetone plant extract to 81.58% as expressed by the 10% concentration of ethanol plant extract. The 5% and 7.5% concentration of acetone plant extract and 5% and 7.5% concentration of ethanol plant extracts expressed a percentage repellency less than 50%. The 10% concentration of acetone plant extract, 5% and 7.5% concentrations of methanol plant extract all expressed a percentage repellency between 50% and 60%. The 10% concentration of methanol plant extract and 10% concentration of ethanol plant extract recorded high efficacy with percentage repellencies greater than 70%. The 10% concentration of ethanol plant extract recorded the highest efficacy as a repellent with a percentage repellency of 81.58%. In a comparison of the percentage repellency of the positive control (F10®) with the percentage repellency of the extracts, extracts yielding a percentage repellency equal or greater than the positive control (50.88%) might be considered as potential repellents for ticks. These included the 10% concentration of acetone plant extract, 7.5% and 10% concentrations of ethanol plant extract and 7.5% and 10% concentrations of methanol plant extract.

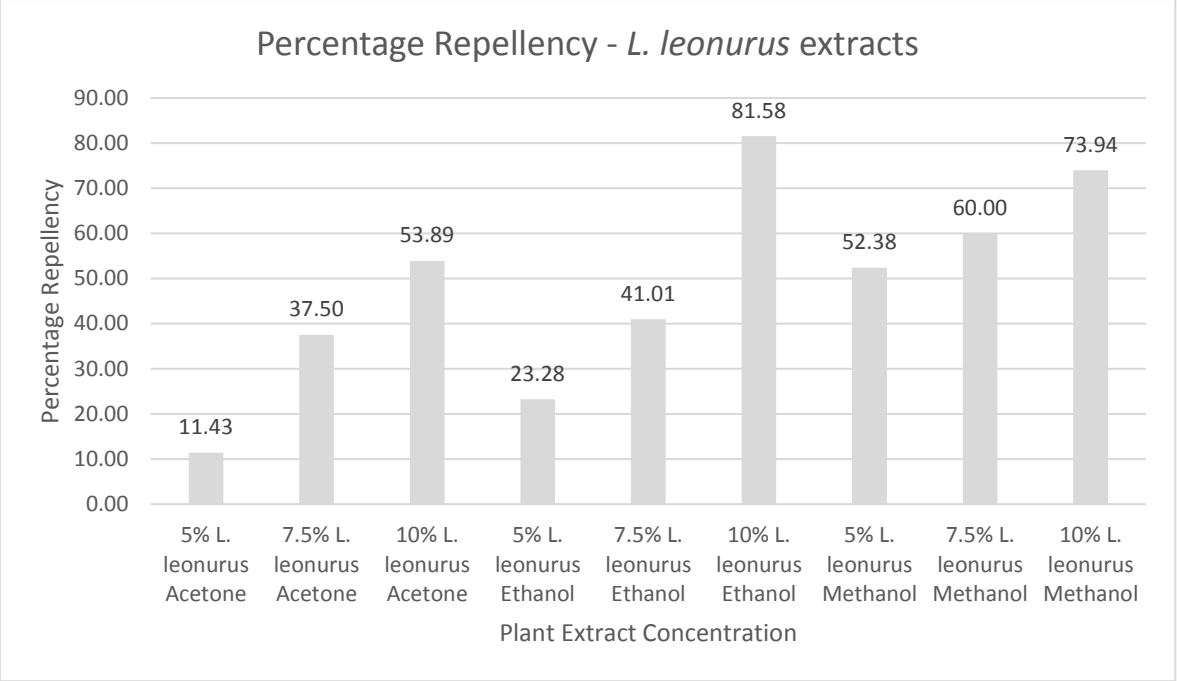

Figure 5.26 Percentage repellency of different concentrations of *L. leonurus* extracts as multi species repellent using a petri dish choice chamber bioassay

The statistical comparison of different repellencies are summarised in Table 5.16 below. Significant differences in percentage repellencies are highlighted in light red.

Table 5.16 Significance of difference between the repellency of different concentrations and types of extracts of *L. leonurus* against multiple tick species

Parameter	Statistical Difference
Acetone	
% repellency: *L. leonurus* 5% and *L. leonurus* 7.5% (Acetone)	0.081
% repellency: *L. leonurus* 5% and *L. leonurus* 10% (Acetone)	0.007
% repellency: *L. leonurus* 7.5% and *L. leonurus* 10% (Acetone)	0.003
% repellency: *L. leonurus* 5% (Acetone) and F10® (positive control)	0.071
% Mean: *L. leonurus* 5% (Acetone) and Negative control	0.326
% repellency: *L. leonurus* 7.5% (Acetone) and F10® (positive control)	0.777
% Mean: *L. leonurus* 7.5% (Acetone) and Negative control	0.095
% repellency: *L. leonurus* 10% (Acetone) and F10® (positive control)	0.202
% Mean: *L. leonurus* 10% (Acetone) and Negative control	0.048
Ethanol	
% repellency: *L. leonurus* 5% and *L. leonurus* 7.5% (Ethanol)	0.158
% repellency: *L. leonurus* 5% and *L. leonurus* 10% (Ethanol)	0.000
% repellency: *L. leonurus* 7.5% and *L. leonurus* 10% (Ethanol)	0.000

Table continues on the next page

Parameter	Statistical Difference
Ethanol	
% repellency: *L. leonurus* 5% (Ethanol) and F10® (positive control)	0.266
% Mean: *L. leonurus* 5% (Ethanol) and Negative control	0.187
% repellency: *L. leonurus* 7.5% (Ethanol) and F10® (positive control)	0.889
% Mean: *L. leonurus* 7.5% (Ethanol) and Negative control	0.081
% repellency: *L. leonurus* 10% (Ethanol) and F10® (positive control)	0.001
% Mean: *L. leonurus* 10% (Ethanol) and Negative control	0.020
Methanol	
% repellency: *L. leonurus* 5% and *L. leonurus* 7.5% (Methanol)	0.262
% repellency: *L. leonurus* 5% and *L. leonurus* 10% (Methanol)	0.008
% repellency: *L. leonurus* 7.5% and *L. leonurus* 10% (Methanol)	0.023
% repellency: *L. leonurus* 5% (Methanol) and F10® (positive control)	0.293
% Mean: *L. leonurus* 5% (Methanol) and Negative control	0.050
% repellency: *L. leonurus* 7.5% (Methanol) and F10® (positive control)	0.073
% Mean: *L. leonurus* 7.5% (Methanol) and Negative control	0.040
% repellency: *L. leonurus* 10% (Methanol) and F10® (positive control)	0.007
% Mean: *L. leonurus* 10% (Methanol) and Negative control	0.024
Comparison	
% repellency: *L. leonurus* 10% (Acetone) and *L. leonurus* 10% (Ethanol)	0.000
% repellency: *L. leonurus* 10% (Acetone) and *L. leonurus* 10% (Methanol)	0.013
% repellency: *L. leonurus* 10% (Ethanol) and *L. leonurus* 10% (Methanol)	0.096

The statistical comparison of percentage repellency showed that all extracts, excluding the 10% extracts of all three solvents, had no repellent effects that differed significantly to that of the commercial repellent ($P > 0.05$). The 10% concentrations of acetone, methanol and ethanol extracts of *L. leonurus* showed a higher percentage repellency compared to that of the commercial repellent ($P < 0.05$). The statistical analysis also showed a significant difference ($P < 0.05$) between the 10% concentrations of acetone and ethanol plant extracts and 7.5% and 10% concentrations of methanol plant extracts and the negative control suggesting that these extracts had tick repellent properties. A significant difference was observed between the 10% concentrations of the ethanol and acetone plant extracts as well as the 10% concentration of acetone and methanol plant extracts ($P < 0.05$) as the ethanol extract expressed a significantly higher percentage repellency compared to the acetone and methanol extracts.

Data obtained in this study suggests that *L. leonurus* extracts had a greater effect against *Am. hebraeum* ticks compared to *Rh. appendiculatus* ticks. The combined repellency of the extracts on both species had a lower efficacy due to the reduced effect observed in the repellency against *Rh. appendiculatus*. When comparing the percentage repellencies of the different concentration of plant extracts with the percentage repellency expressed by the commercial repellent (with 50.88%

repellency), extracts that yielded a percentage repellency lower than the commercial repellent can be considered to show a limited repellency. These included the 5% (with 11.43% repellency) and 7.5% (with 37.50% repellency) concentration of acetone plant extracts, and 5% (with 23.28% repellency) and 7.5% (with 41.01% repellency) concentration of ethanol plant extracts. The 10% (with 53.89% repellency) concentration of acetone plant extract, and 5% (with 52.38% repellency) and 7.5% (with 60.00% repellency) concentration of methanol extracts all expressed a percentage repellency that is higher than the percentage repellency of the commercial repellent. This observation suggests that these concentrations of *L. leonurus* extracts expressed a medium repellency against multiple species with a percentage repellency that is less than 65% repellency. The 10% concentrations of ethanol (with 81.58% repellency) and methanol (with 73.94% repellency) extracts both expressed higher repellent effects against *Am. hebraeum* and *Rh. appendiculatus* ticks.

EC_{50}

The EC_{50} of the acetone, methanol and ethanol extracts of *L. leonurus* were calculated for the individual tick species as well as a combined repellency using Microsoft Excel (2016) as indicated in Table 5.17.

Table 5.17 EC_{50} for *L. leonurus* extracts against *Am. hebraeum* and *Rh. appendiculatus*

	Am. hebraeum	***Rh. appendiculatus***	**Combined**
	Acetone		
EC_{50}	5.04%	10.52%	9.31%
	Ethanol		
EC_{50}	6.98%	7.88%	7.33%
	Methanol		
EC_{50}	4.02%	4.63%	4.83%

5.5.4 Botanical Extract Testing – *Thymus vulgaris*

As indicated in Table 5.18, nine *T. vulgaris* extracts, were tested to determine the response of *Am. hebraeum* and *Rh. appendiculatus* adult ticks. A combination mean for the response and location of both species, as well as percentage repellency were also determined to evaluate the effect of *T. vulgaris* extracts as tick repellent.

Am. hebraeum

Data on mean and percentage repellency of different extracts of *T. vulgaris* against *Am. hebraeum* ticks are summarised and illustrated in Table 5.18 and Figures 5.27 and 5.28. The *T. vulgaris* extracts showed a high degree of repellency against *Am. hebraeum* adult ticks, with only the 2.5% concentration of acetone plant extract yielding a percentage repellency (54.41%) that is lower than 60%. All other plant extracts yielded percentage repellencies greater than 65%. The 10% concentration of plant extracts of all three solvents as well as the 5% concentration of methanol plant extract demonstrated a percentage repellency that was higher than 90%. The 10% concentration of ethanol plant extract recorded the highest efficacy as a repellent with a percentage repellency of 99.25%. Excect for the 5% concentration of acetone plant extract, 2.5% concentration of ethanol plant extract and 2.5% concentration of methanol plant extract all other extracts of *T. vulgaris* yielded a percentage repellency that is higher than that of the commercial repellent (with 42.86% repellency). This suggests that *T. vulgaris* extracts were more effective as repellents against *Am. hebraeum* ticks.

Table 5.18 Percentage of ticks in the different areas of the choice chamber and percentage repellency of *T. vulgaris*

	Am. hebraeum				***Rh. appendiculatus***				**Combined**			
	Control	**Neutral Zone**	**Treatment**	**% Repel**	**Control**	**Neutral Zone**	**Treatment**	**% Repel**	**Control**	**Neutral Zone**	**Treatment**	**% Repel**
2.5% *T. vulgaris* Acetone	31.48	54.17	14.35	54.41	34.72	48.61	16.67	52.00	33.10	51.39	15.51	53.15
5% *T. vulgaris* Acetone	34.26	54.63	11.11	67.57	34.72	52.78	12.50	64.00	34.49	53.70	11.81	65.77
10% *T. vulgaris* Acetone	70.37	26.85	2.78	96.05	65.28	32.87	1.85	97.16	67.82	29.86	2.31	96.59
2.5% *T. vulgaris* Ethanol	35.19	53.70	11.11	68.42	34.26	51.39	14.35	58.11	34.72	52.55	12.73	63.33
5% *T. vulgaris* Ethanol	38.43	51.39	10.19	73.49	45.83	42.59	11.57	74.75	42.13	46.99	10.88	74.18
10% *T. vulgaris* Ethanol	61.57	37.96	0.46	99.25	66.67	26.39	6.94	89.58	64.12	32.18	3.70	94.22
2.5% *T. vulgaris* Methanol	38.89	49.07	12.04	69.05	33.80	53.70	12.50	63.01	36.34	51.39	12.27	66.24
5% *T. vulgaris* Methanol	56.94	38.43	4.63	91.87	51.85	29.17	18.98	63.39	54.40	33.80	11.81	78.30
10% *T. vulgaris* Methanol	65.28	32.87	1.85	97.16	53.24	34.72	12.04	77.39	59.26	33.80	6.94	88.28
Positive Control (F10®)	29.17	54.17	16.67	42.86	50.00	27.78	22.22	55.56	39.58	40.97	19.44	50.88

Green – Most effective concentration per parameter

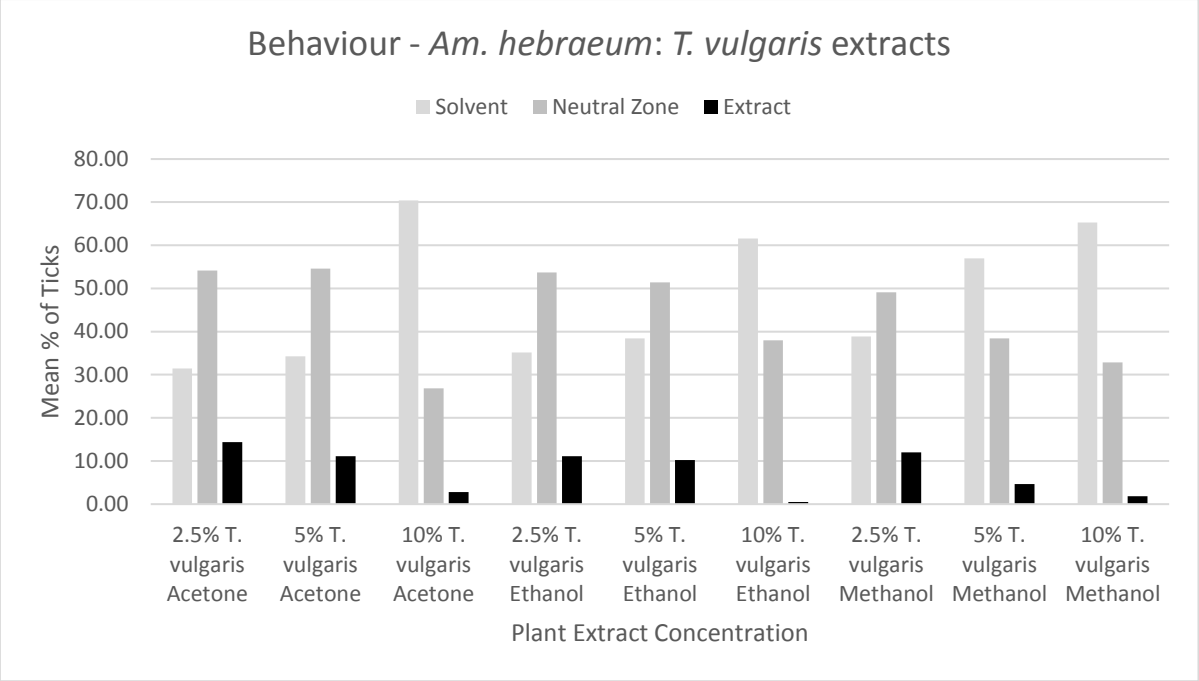

Figure 5.27 Response of *Am. hebraeum* ticks to different concentrations of *T. vulgaris* using a petri dish choice chamber bioassay

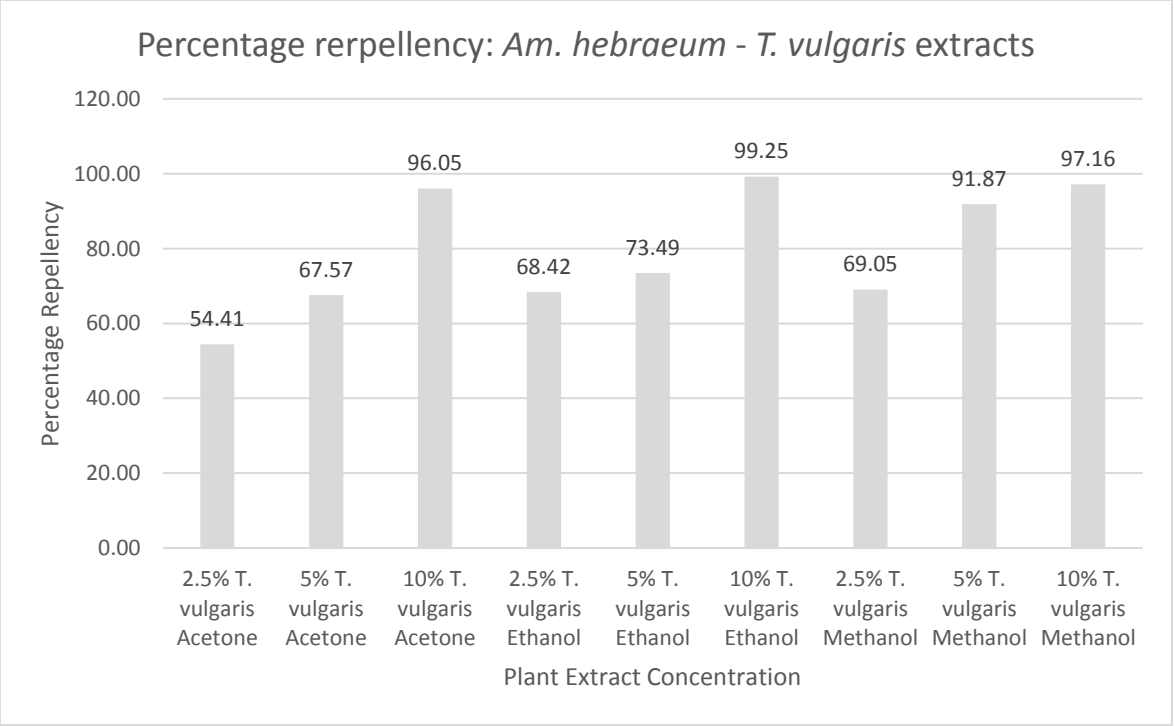

Figure 5.28 Percentage repellency of different concentrations of *T. vulgaris* extracts against *Am. hebraeum* adults using a petri dish choice chamber bioassay

The statistical comparison of different repellencies are summarised in Table 5.19 below. Significant differences in percentage repellencies are highlighted in light red.

Table 5.19 Significance of difference between the repellency of different concentrations and types of extracts of *T. vulgaris* against *Am. hebraeum* adults

Parameter	Statistical Difference
Acetone	
% repellency: *T. vulgaris* 2.5% and *T. vulgaris* 5% (Acetone)	0.064
% repellency: *T. vulgaris* 2.5% and *T. vulgaris* 10% (Acetone)	0.000
% repellency: *T. vulgaris* 5% and *T. vulgaris* 10% (Acetone)	0.000
% repellency: *T. vulgaris* 2.5% (Acetone) and F10® (positive control)	0.460
% Mean: *T. vulgaris* 2.5% (Acetone) and Negative control	0.047
% repellency: *T. vulgaris* 5% (Acetone) and F10® (positive control)	0.089
% Mean: *T. vulgaris* 5% (Acetone) and Negative control	0.029
% repellency: *T. vulgaris* 10% (Acetone) and F10® (positive control)	0.001
% Mean: *T. vulgaris* 10% (Acetone) and Negative control	0.014
Ethanol	
% repellency: *T. vulgaris* 2.5% and *T. vulgaris* 5% (Ethanol)	0.904
% repellency: *T. vulgaris* 2.5% and *T. vulgaris* 10% (Ethanol)	0.000
% repellency: *T. vulgaris* 5% and *T. vulgaris* 10% (Ethanol)	0.001
% repellency: *T. vulgaris* 2.5% (Ethanol) and F10® (positive control)	0.123
% Mean: *T. vulgaris* 2.5% (Ethanol) and Negative control	0.028
% repellency: *T. vulgaris* 5% (Ethanol) and F10® (positive control)	0.036
% Mean: *T. vulgaris* 5% (Ethanol) and Negative control	0.024
% repellency: *T. vulgaris* 10% (Ethanol) and F10® (positive control)	0.001
% Mean: *T. vulgaris* 10% (Ethanol) and Negative control	0.013
Methanol	
% repellency: *T. vulgaris* 2.5% and *T. vulgaris* 5% (Methanol)	0.000
% repellency: *T. vulgaris* 2.5% and *T. vulgaris* 10% (Methanol)	0.000
% repellency: *T. vulgaris* 5% and *T. vulgaris* 10% (Methanol)	0.050
% repellency: *T. vulgaris* 2.5% (Methanol) and F10® (positive control)	0.069
% Mean: *T. vulgaris* 2.5% (Methanol) and Negative control	0.028
% repellency: *T. vulgaris* 5% (Methanol) and F10® (positive control)	0.003
% Mean: *T. vulgaris* 5% (Methanol) and Negative control	0.015
% repellency: *T. vulgaris* 10% (Methanol) and F10® (positive control)	0.001
% Mean: *T. vulgaris* 10% (Methanol) and Negative control	0.013
Comparison	
% repellency: *T. vulgaris* 10% (Acetone) and *T. vulgaris* 10% (Ethanol)	0.098
% repellency: *T. vulgaris* 10% (Acetone) and *T. vulgaris* 10% (Methanol)	0.536
% repellency: *T. vulgaris* 10% (Ethanol) and *T. vulgaris* 10% (Methanol)	0.317

The statistical comparison of percentage repellency showed the 2.5% concentrations of plant extracts of all three solvents and 5% concentration of ethanol plant extract not to be significantly different ($P > 0.05$) to that of the commercial repellent. All other plant extract concentrations showed higher repellency against *Am. hebraeum* ticks when compared with the commercial repellent ($P < 0.05$). The statistical analysis also showed a significant difference

($P < 0.05$) between the plant extracts and the negative control, suggesting that all *T. vulgaris* extracts had a repelling effect on *Am. hebraeum* adult ticks. Comparing among the percentage repellencies of 10% concentrations of the plant extracts of the three different solvents against *Am. hebraeum* adult ticks showed no significant differences ($P > 0.05$).

In summary, data obtained in this study suggests that *T. vulgaris* has anti-tick properties particularly when considering that most of its extracts demonstrated percentage repellencies that are significantly higher than that of the commercial repellent at 42.86%. The 10% concentration of ethanol plant extract showed the highest percentage repellency at 99.25%.

Rh. appendiculatus

Data on mean and percentage repellency of different extracts of *T. vulgaris* against *Rh. appendiculatus* ticks are summarised and illustrated in Table 5.18 and Figures 5.29 and 5.30. The *T. vulgaris* extracts showed a high degree of repellency against *Rh. appendiculatus* adult ticks, with only the 2.5% concentration acetone (52.00%) and 2.5% concentration ethanol (58.11%) extracts yielding a percentage repellency less than 60%. All other extracts yielding a percentage repellency that is more than 60%. The 10% concentrations of all three solvents extracts recorded high efficacy with percentage repellencies which were greater than 75%. The 10% concentration acetone extract recorded the highest efficacy as a repellent with a percentage repellency of 97.16%. When comparing the percentage repellency of the positive control (F10®) with the percentage repellency of the extracts, only the 2.5% concentration acetone (52.00%) and 2.5% concentration ethanol (58.11%) extracts yielded a percentage repellency lower than the commercial repellent (55.56%).

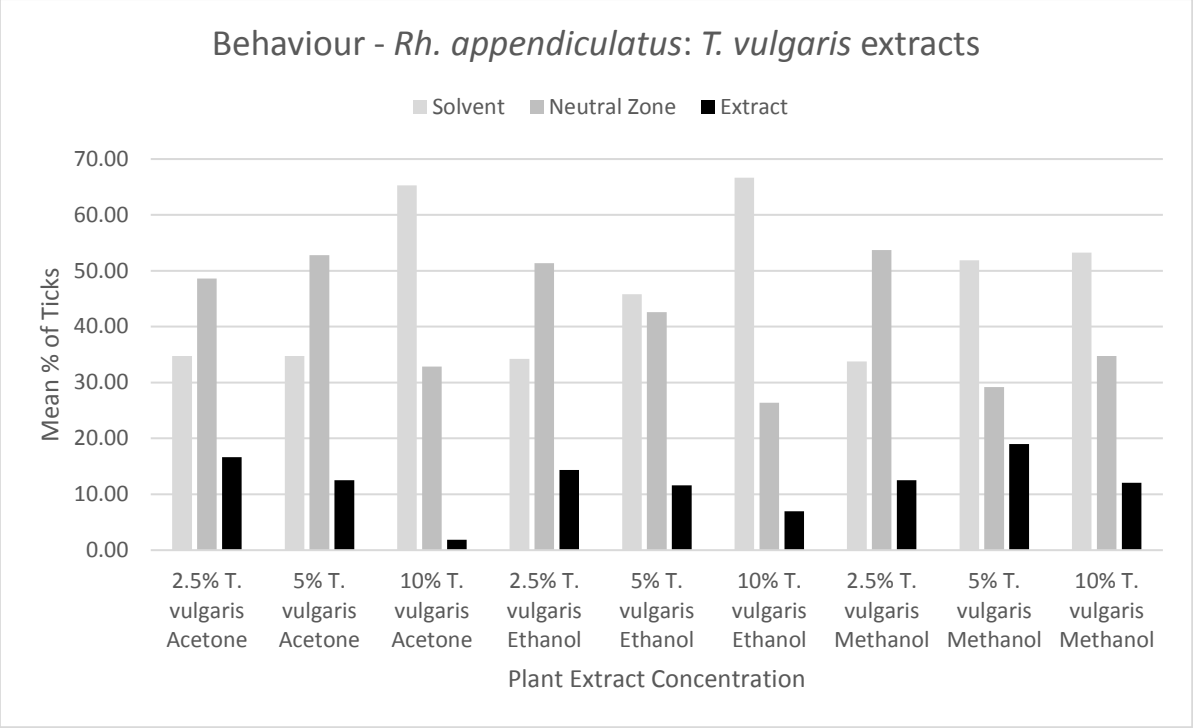

Figure 5.29 Response of *Rh. appendiculatus* ticks to different concentrations of *T. vulgaris* using a petri dish choice chamber bioassay

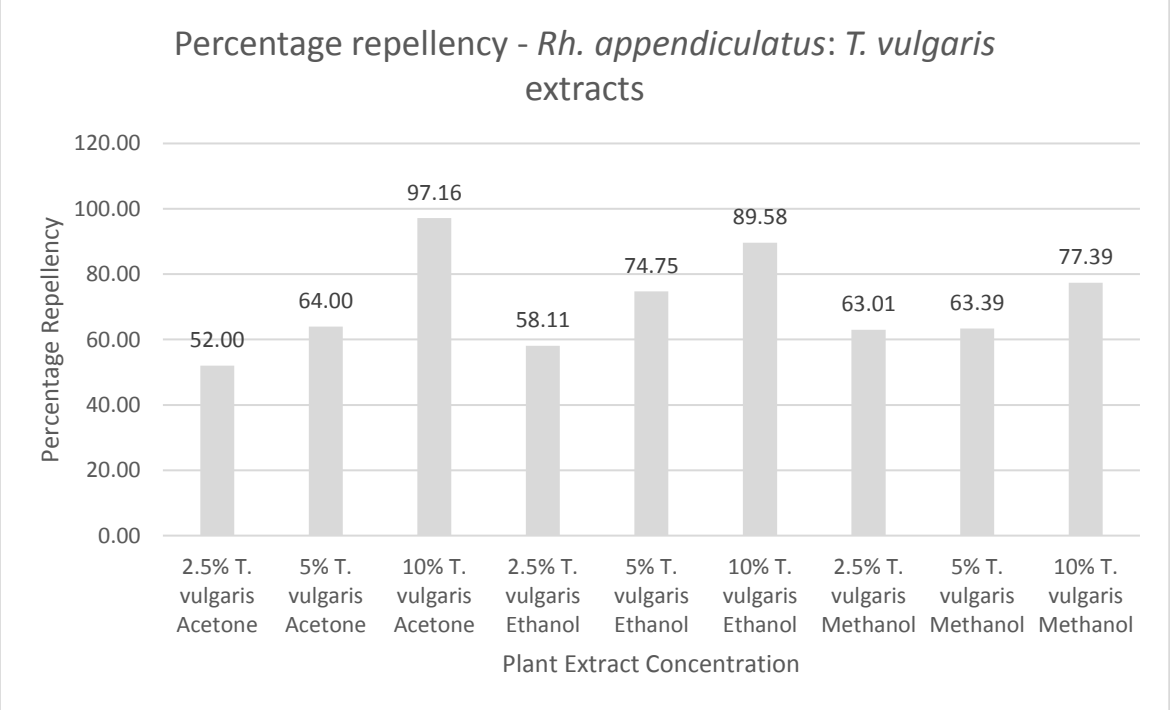

Figure 5.30 Percentage repellency of different concentrations of *T. vulgaris* extracts against *Rh. appendiculatus* adults using a petri dish choice chamber bioassay

The statistical comparison of different repellencies are summarised in Table 5.20 below. Significant differences in percentage repellencies are highlighted in light red.

Table 5.20 Significance of difference between the repellency of different concentrations and types of extracts of *T. vulgaris* against *Rh. appendiculatus* adults

Parameter	Statistical Difference
Acetone	
% repellency: *T. vulgaris* 2.5% and *T. vulgaris* 5% (Acetone)	0.311
% repellency: *T. vulgaris* 2.5% and *T. vulgaris* 10% (Acetone)	0.000
% repellency: *T. vulgaris* 5% and *T. vulgaris* 10% (Acetone)	0.000
% repellency: *T. vulgaris* 2.5% (Acetone) and F10® (positive control)	0.700
% Mean: *T. vulgaris* 2.5% (Acetone) and Negative control	0.051
% repellency: *T. vulgaris* 5% (Acetone) and F10® (positive control)	0.367
% Mean: *T. vulgaris* 5% (Acetone) and Negative control	0.033
% repellency: *T. vulgaris* 10% (Acetone) and F10® (positive control)	0.003
% Mean: *T. vulgaris* 10% (Acetone) and Negative control	0.014
Ethanol	
% repellency: *T. vulgaris* 2.5% and *T. vulgaris* 5% (Ethanol)	0.010
% repellency: *T. vulgaris* 2.5% and *T. vulgaris* 10% (Ethanol)	0.000
% repellency: *T. vulgaris* 5% and *T. vulgaris* 10% (Ethanol)	0.011
% repellency: *T. vulgaris* 2.5% (Ethanol) and F10® (positive control)	0.524
% Mean: *T. vulgaris* 2.5% (Ethanol) and Negative control	0.041
% repellency: *T. vulgaris* 5% (Ethanol) and F10® (positive control)	0.069
% Mean: *T. vulgaris* 5% (Ethanol) and Negative control	0.024
% repellency: *T. vulgaris* 10% (Ethanol) and F10® (positive control)	0.011
% Mean: *T. vulgaris* 10% (Ethanol) and Negative control	0.016
Methanol	
% repellency: *T. vulgaris* 2.5% and *T. vulgaris* 5% (Methanol)	0.669
% repellency: *T. vulgaris* 2.5% and *T. vulgaris* 10% (Methanol)	0.031
% repellency: *T. vulgaris* 5% and *T. vulgaris* 10% (Methanol)	0.035
% repellency: *T. vulgaris* 2.5% (Methanol) and F10® (positive control)	0.222
% Mean: *T. vulgaris* 2.5% (Methanol) and Negative control	0.034
% repellency: *T. vulgaris* 5% (Methanol) and F10® (positive control)	0.320
% Mean: *T. vulgaris* 5% (Methanol) and Negative control	0.034
% repellency: *T. vulgaris* 10% (Methanol) and F10® (positive control)	0.050
% Mean: *T. vulgaris* 10% (Methanol) and Negative control	0.022
Comparison	
% repellency: *T. vulgaris* 10% (Acetone) and *T. vulgaris* 10% (Ethanol)	0.014
% repellency: *T. vulgaris* 10% (Acetone) and *T. vulgaris* 10% (Methanol)	0.000
% repellency: *T. vulgaris* 10% (Ethanol) and *T. vulgaris* 10% (Methanol)	0.009

The statistical comparison of percentage repellency of the 2.5% and 5% concentrations of the extracts of all three solvents and the 10% concentration of methanol plant extract showed lack of significant differences ($P > 0.05$) when compared with that of the commercial repellent (F10®). The 10% concentrations of both acetone and ethanol plant extracts showed the highest percentage repellencies (97.16% and 89.58% respectively) and were found to be significantly

higher ($P < 0.05$) than the repellency of the commercial repellent (F10®) against *Rh. appendiculatus* adults. In general, the acetone extracts showed a significantly high reppelency ($P < 0.05$) against *Rh. appendiculatus* adults when compared with the negative control.

When comparing the percentage repellencies of the different concentration of plant extracts with the percentage repellency expressed by the commercial repellent (55.56%), only the 5% concentration of acetone plant extract yielded a lower percentage repellency than the commercial repellent suggesting that this plant extract demonstrated a limited tick repellency effect. All other extracts expressed a percentage repellency higher than the percentage repellency of the commercial repellent. Significant differences ($P < 0.05$) in repellency were among the 10% concentrations of the extracts of the three different solvents with the acetone plant extract expressing the highest repellency (97.16 %) compared to the ethanol (89.58%) and methanol (77.39%) plant extracts. These observations suggest that *T. vulgaris* extracts had strong repellent properties against *Rh. appendiculatus* adult ticks.

Combined data

Data on mean and percentage repellency of different extracts of *T. vulgaris* as multi-species repellent is summarised and illustrated in Table 5.18 and Figure 5.31. The *T. vulgaris* extracts showed a high degree of repellency against both tick species, with only the 2.5% concentration acetone (53.15%) extract yielding a percentage repellency less than 60% with all other extracts yielding a percentage repellency greater than 60%. The 10% concentrations of all three solvents extracts recorded high efficacy with percentage repellencies greater than 75%. The 10% concentration acetone extract recorded the highest efficacy as a repellent with a percentage repellency of 96.59%. When comapring the percentage repellency of the positive control (F10®) with the percentage repellencies of the plant extracts, the plant extracts yielded a percentage repellency equal or greater than the positive control (50.88%). This observation further reinforces the view that *T. vulgaris* extracts are endowed with repellent anti-tick properties.

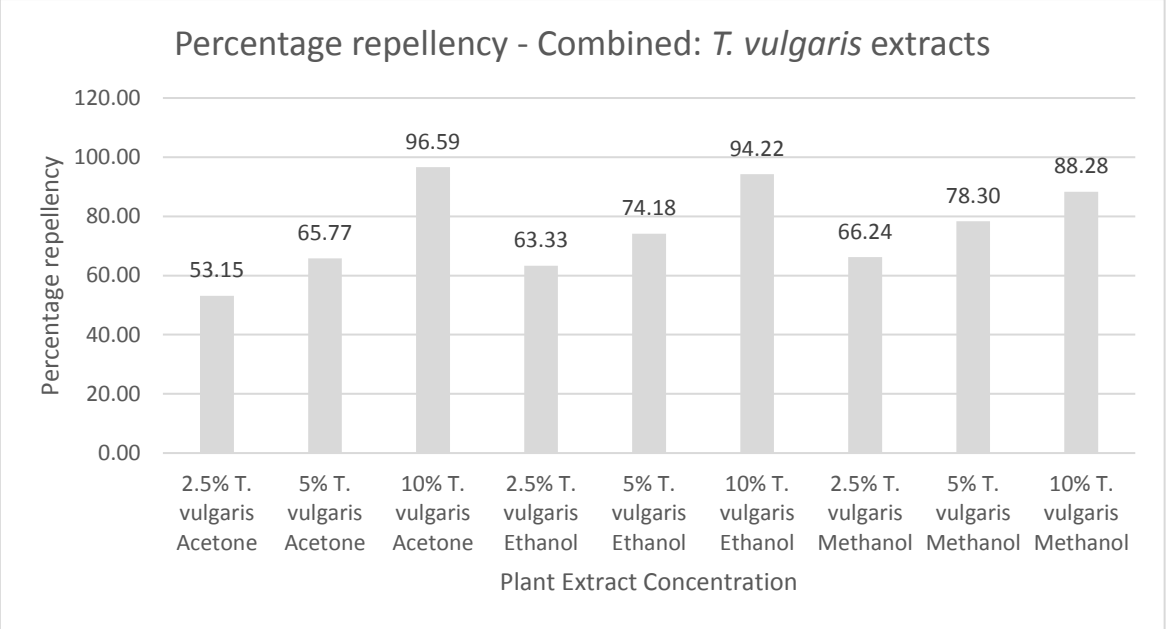

Figure 5.31 Percentage repellency of different concentrations of *T. vulgaris* extracts as multi species repellent using a petri dish choice chamber bioassay

The statistical comparison of different repellencies are summarised in Table 5.21 below. Significant differences in percentage repellencies are highlighted in light red.

Table 5.21 Significance of difference between the repellency of different concentrations and types of extracts of *T. vulgaris* against multiple tick species

Parameter	Statistical Difference
Acetone	
% repellency: *T. vulgaris* 2.5% and *T. vulgaris* 5% (Acetone)	0.104
% repellency: *T. vulgaris* 2.5% and *T. vulgaris* 10% (Acetone)	0.000
% repellency: *T. vulgaris* 5% and *T. vulgaris* 10% (Acetone)	0.000
% repellency: *T. vulgaris* 2.5% (Acetone) and F10® (positive control)	0.539
% Mean: *T. vulgaris* 2.5% (Acetone) and Negative control	0.049
% repellency: *T. vulgaris* 5% (Acetone) and F10® (positive control)	0.117
% Mean: *T. vulgaris* 5% (Acetone) and Negative control	0.031
% repellency: *T. vulgaris* 10% (Acetone) and F10® (positive control)	0.000
% Mean: *T. vulgaris* 10% (Acetone) and Negative control	0.014
Ethanol	
% repellency: *T. vulgaris* 2.5% and *T. vulgaris* 5% (Ethanol)	0.094
% repellency: *T. vulgaris* 2.5% and *T. vulgaris* 10% (Ethanol)	0.000
% repellency: *T. vulgaris* 5% and *T. vulgaris* 10% (Ethanol)	0.000
% repellency: *T. vulgaris* 2.5% (Ethanol) and F10® (positive control)	0.200
% Mean: *T. vulgaris* 2.5% (Ethanol) and Negative control	0.034
% repellency: *T. vulgaris* 5% (Ethanol) and F10® (positive control)	0.029

Table continues on the next page

Parameter	Statistical Difference
Ethanol (continue)	
% Mean: *T. vulgaris* 5% (Ethanol) and Negative control	0.024
% repellency: *T. vulgaris* 10% (Ethanol) and F10® (positive control)	0.000
% Mean: *T. vulgaris* 10% (Ethanol) and Negative control	0.016
Methanol	
% repellency: *T. vulgaris* 2.5% and *T. vulgaris* 5% (Methanol)	0.032
% repellency: *T. vulgaris* 2.5% and *T. vulgaris* 10% (Methanol)	0.000
% repellency: *T. vulgaris* 5% and *T. vulgaris* 10% (Methanol)	0.001
% repellency: *T. vulgaris* 2.5% (Methanol) and F10® (positive control)	0.063
% Mean: *T. vulgaris* 2.5% (Methanol) and Negative control	0.031
% repellency: *T. vulgaris* 5% (Methanol) and F10® (positive control)	0.007
% Mean: *T. vulgaris* 5% (Methanol) and Negative control	0.021
% repellency: *T. vulgaris* 10% (Methanol) and F10® (positive control)	0.001
% Mean: *T. vulgaris* 10% (Methanol) and Negative control	0.016
Comparison	
% repellency: *T. vulgaris* 10% (Acetone) and *T. vulgaris* 10% (Ethanol)	0.139
% repellency: *T. vulgaris* 10% (Acetone) and *T. vulgaris* 10% (Methanol)	0.000
% repellency: *T. vulgaris* 10% (Ethanol) and *T. vulgaris* 10% (Methanol)	0.029

The statistical comparison of percentage repellency showed that the 2.5% concentrations of plant extracts of all three solvents and 5% concentration of acetone plant extract were not significantly different ($P > 0.05$) with that of the commercial repellent (F10®). All other concentrations of plant extracts showed a significantly ($P < 0.05$) higher repellency when compared with the commercial repellent ($P < 0.05$). A significant difference ($P < 0.05$) was also found between the plant extracts and the negative control, suggesting that all *T. vulgaris* extracts would have a repelling effect on adult ticks of multiple species. The 10% concentrations of acetone and ethanol would have a similar effect in repelling adult ticks of multiple species ($P > 0.05$), with a significant difference occuring ($P < 0.05$) when compared with the repelling effects of 10% concentration of methanol extract.

From the observations and results as described above the following summary can be made. In comparing the percentage repellencies of the different concentration extracts with the percentage repellency expressed by the commercial repellent (50.88%), all extracts yielded percentage repellencies higher than the commercial repellent. Therefore, all the *T. vulgaris* extracts expressed repellency against multiple tick species, with the 10% acetone extract as the most effective extract as repellent.

EC_{50}

The EC_{50} of the acetone, methanol and ethanol extracts of *T. vulgaris* were calculated for the individual tick species as well as a combined repellent using Microsoft Excel (2016) as indicated in Table 5.22. The observed results (percentage repellencies) and calculated EC_{50} indicates that for ethanol and methanol extracts a concentration less than 2.5% would be effective in repelling 50% of the individual tick species as well as multi-species repellent.

Table 5.22 EC_{50} for *T. vulgaris* extracts against *Am. hebraeum* and *Rh. appendiculatus*

	Am. hebraeum	***Rh. appendiculatus***	**Combined**
	Acetone		
EC_{50}	2.35%	2.62%	2.49%
	Ethanol		
EC_{50}	1.28%	1.73%	1.47%
	Methanol		
EC_{50}	0.85%	0.89%	0.89%

5.6 Tick Climbing bioassays

5.6.1 Experiment One – Effects of plant extracts on the climbing questing behaviour of *Rh. appendiculatus* using a modified tick climbing bioassay

5.6.1.1 Efficacy of tick climbing bioassay

Data on mean percentage tick repellency of the commercial repellents against adults of *Rh. appendiculatus* are summarised in Table 5.23. Ticks observed on the neutral filter paper were included with the ticks on the treatment filter paper as repelled, as the treatment had an effect on the full questing height of the ticks, and the ticks observed on the neutral filter paper did not climb to full questing height.

Also, data obtained from this bioassay, demonstrated that *Rh. appendiculatus* ticks were located across the full length of the rods having distilled water filter paper. However, only 3.33% of ticks were observed to climb to full questing height and less than 15% of ticks quested on the neutral filter paper located below the filter paper treated with the commercial repellent.

The results indicated in Table 5.23, show significant repellent effects (P < 0.05) of Bayticol 2% EC dip (P = 0.035) and F10® wound spray (P = 0.037) against *Rh. appendiculatus* adults. However, it appears that the repellent strengths of these two commercial repellents against adults of *Rh. appendiculatus* were similar as no significant differences (P > 0.05) was observed between the two chemicals.

Table 5.23 Location and Percentage repellency of Commercial repellent and Distilled water against *Rh. appediculatus* ticks using a tick climbing bioassay

	Treated Filter paper	**Neutral filter paper**	**Below filter paper**	**% Repellency**	**Treated Filter paper**	**Neutral filter paper**	**Below filter paper**	**% Repellency**
	Bayticol				**dH_2O**			
15 min	0.00	0.00	10.00	100.00	3.00	2.00	5.00	0.00
30 min	0.00	0.00	10.00	100.00	4.00	2.00	4.00	-50.00
45 min	0.00	1.00	9.00	88.90	4.00	3.00	3.00	-133.30
60 min	0.00	2.00	8.00	75.00	5.00	3.00	2.00	-300.00
90 min	1.00	1.00	8.00	75.00	5.00	2.00	3.00	-133.30
120 min	1.00	2.00	7.00	57.10	6.00	3.00	1.00	-800.00
Mean %	3.33	10.00	86.67	84.60	45.00	25.00	30.00	-133.30
T-Test	0.035				0.385			
	F10®				**dH_2O**			
15 min	1.00	1.00	9.00	77.80	2.00	4.00	4.00	-50.00
30 min	0.00	0.00	10.00	100.00	5.00	2.00	3.00	-133.30
45 min	0.00	1.00	9.00	88.90	4.00	3.00	3.00	-133.30
60 min	0.00	3.00	7.00	57.10	5.00	3.00	2.00	-300.00
90 min	0.00	1.00	9.00	88.90	4.00	3.00	3.00	-133.30
120 min	1.00	2.00	7.00	57.10	5.00	3.00	2.00	-300.00
Mean %	3.33	13.33	85.00	80.40	41.67	30.00	28.33	-152.90
T-Test	0.037				0.322			
P-Value	1.000		0.817					

5.6.1.2 Botanical extract testing – *Aloe ferox*

The three most effective *A. ferox* extracts from the choice chamber bioassay were tested to determine the response of *Rh. appendiculatus* adult ticks using the tick climbing bioassay. Based on the movement of *Rh. appendiculatus* ticks in response to the stimuli from the *A. ferox* extracts, the following observations as summarised and illustrated in Table 5.24, Figures 5.32 and 5.33 were made.

Table 5.24 Location and Percentage repellency of *A. ferox* against *Rh. appendiculatus* using a tick climbing bioassay

	Treated Filter paper	**Neutral filter paper**	**Below filter paper**	**% Repellency**
Acetone Control	55.00	18.89	26.11	-182.98
20% Acetone	6.67	15.00	78.33	72.34
Ethanol Control	55.00	8.89	36.11	-76.92
20% Ethanol	26.11	5.00	68.89	54.84
Methanol Control	51.11	13.33	35.56	-81.25
20% Methanol	11.11	10.00	78.89	73.24
Positive Control (F10®)	3.33	13.33	85.00	80.40

Ticks continuously moved across the length of the rod and the highest number of ticks occurred on the control filter paper with distilled water suggesting that ticks avoided the filter papers with plant extracts. The *A. ferox* extracts showed varying degrees of repellency against *Rh. appendiculatus* adult ticks ranging between 54.84% as observed for the 20% concentration ethanol and 73.24% as expressed by the 20% concentration methanol plant extracts. When comparing the repellency of the plant extracts with that of a commercial repellent, only the 20% *A. ferox* acetone (with 72.34% percentage repellency) and 20% *A. ferox* methanol (with 73.24% repellency) extracts showed significant effect as repellents, comparable to the repellent effects of the commercial repellent.

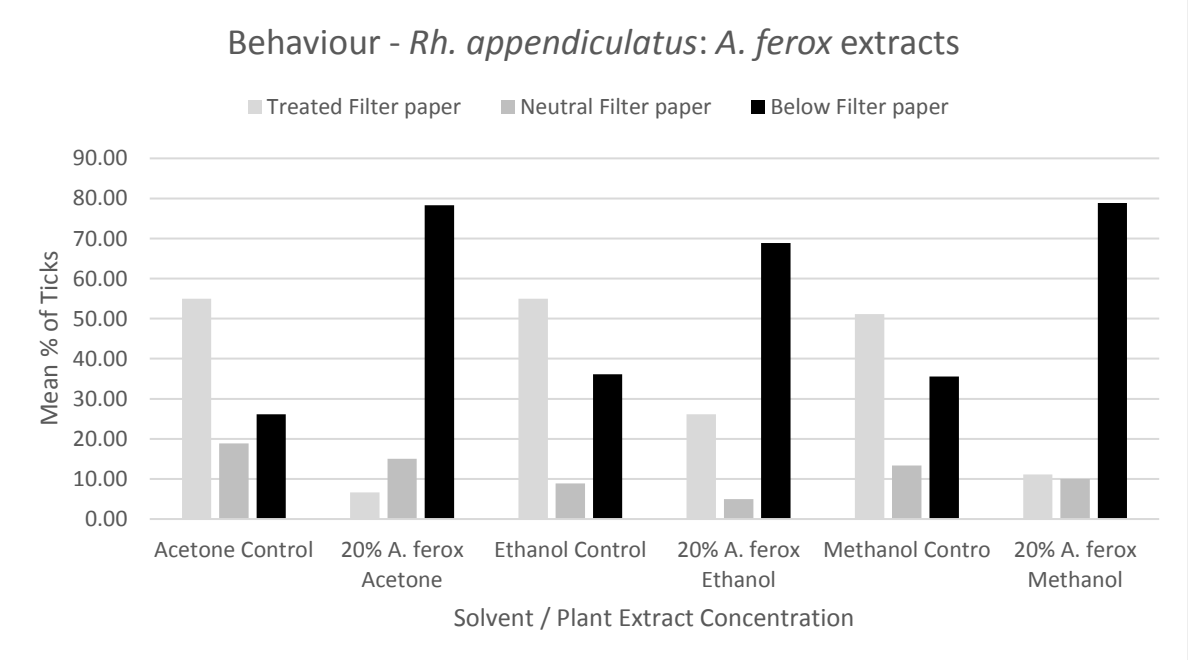

Figure 5.32 Response of *Rh. appendiculatus* to different *A. ferox* extracts using a tick climbing bioassay

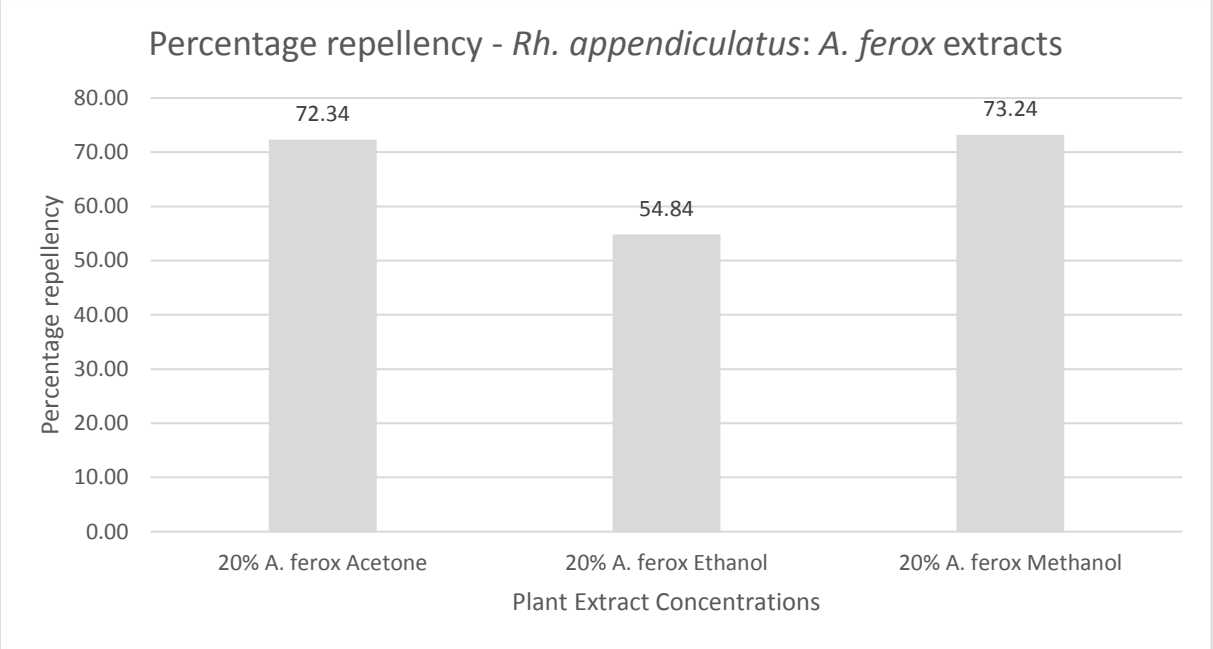

Figure 5.33 Percentage repellency of different *A. ferox* extracts against *Rh. appendiculatus* adults using a tick climbing bioassay

The statistical comparison of different repellencies are summarised in Table 5.25 below. Significant differences in percentage repellencies are highlighted in light red.

Table 5.25 Significance of difference between repellency of different types of *A. ferox* extracts against *Rh. appendiculatus* adults using a tick climbing bioassay

Parameter	Statistical Difference
Acetone	
% repellency: *A. ferox* 20% (Acetone) and F10® (positive control)	0.367
% repellency: *A. ferox* 20% (Acetone) and Negative control	0.000
Ethanol	
% repellency: *A. ferox* 20% (Ethanol) and F10® (positive control)	0.021
% repellency: *A. ferox* 20% (Ethanol) and Negative control	0.000
Methanol	
% repellency: *A. ferox* 20% (Methanol) and F10® (positive control)	0.421
% repellency: *A. ferox* 20% (Methanol) and Negative control	0.000
Comparison	
% repellency: *A. ferox* 20% (Acetone) and *A. ferox* 20% (Ethanol)	0.049
% repellency: *A. ferox* 20% (Acetone) and *A. ferox* 20% (Methanol)	0.864
% repellency: *A. ferox* 20% (Ethanol) and *A. ferox* 20% (Methanol)	0.038

Comparison between percentage repellencies of the 20% concentration of acetone and methanol plant extracts and that of the commercialised repellent found no significant differences ($P > 0.05$). The 20% concentration of ethanol plant extract showed a decreased effect on the movement of *Rh. appendiculatus* ticks when

compared with the commercialised repellent ($P < 0.05$). Also, significant differences ($P < 0.05$) occurred between all the plant extracts and the negative control. The 20% concentrations of acetone and ethanol plant extracts and 20% concentration of ethanol and methanol plant extracts expressed a significant difference ($P < 0.05$) due to the lower percentage repellency observed from the ethanol extract.

Compared to the 20% concentration of acetone (with 72.34 % repellency) and the methanol extracts (with 73.24 % repellency) which demonstrated percentage repellencies greater than 70 %, it is reasonable to regard 20% ethanol extract of *A. ferox* (with 54.84 % repellency) as having had a medium repellent effect against *Rh. appendiculatus* adults.

5.6.1.3 Botanical extract testing – *Leonotis leonurus*

The three most effective *L. leonurus* extracts from the choice chamber bioassay were tested to determine the response of *Rh. appendiculatus* adult ticks, from the movement of *Rh. appendiculatus* ticks in response to the stimuli from the *L. leonurus* extracts, the following observations were made, as recorded and illustrated in Table 5.26, Figures 5.34 and 5.35.

Table 5.26 Location and Percentage repellency of *L. leonurus* against *Rh. appendiculatus* using a tick climbing bioassay

	Treated Filter paper	**Neutral filter paper**	**Below filter paper**	**% Repellency**
Acetone Control	55.00	18.89	26.11	-182.98
10% Acetone	11.11	19.44	69.44	56.00
Ethanol Control	55.00	8.89	36.11	-76.92
10% Ethanol	21.11	8.33	70.56	58.27
Methanol Control	51.11	13.33	35.56	-81.25
10% Methanol	15.00	20.00	65.00	46.15
Positive Control (F10®)	3.33	13.33	85.00	80.40

The *L. leonurus* extracts showed varying degrees of repellency against *Rh. appendiculatus* adult ticks ranging between 46.15% as observed for the 10% concentration methanol and 58.27% as expressed by the 10% concentration ethanol extract. When comparing repellencies of the extracts (56 % acetone, 58.27% ethanol and 46.15 % methanol) with that of the commercial repellent, none

of the extracts showed a percentage repellency similar to the high percentage repellency (80.40%) expressed by the commercial repellent.

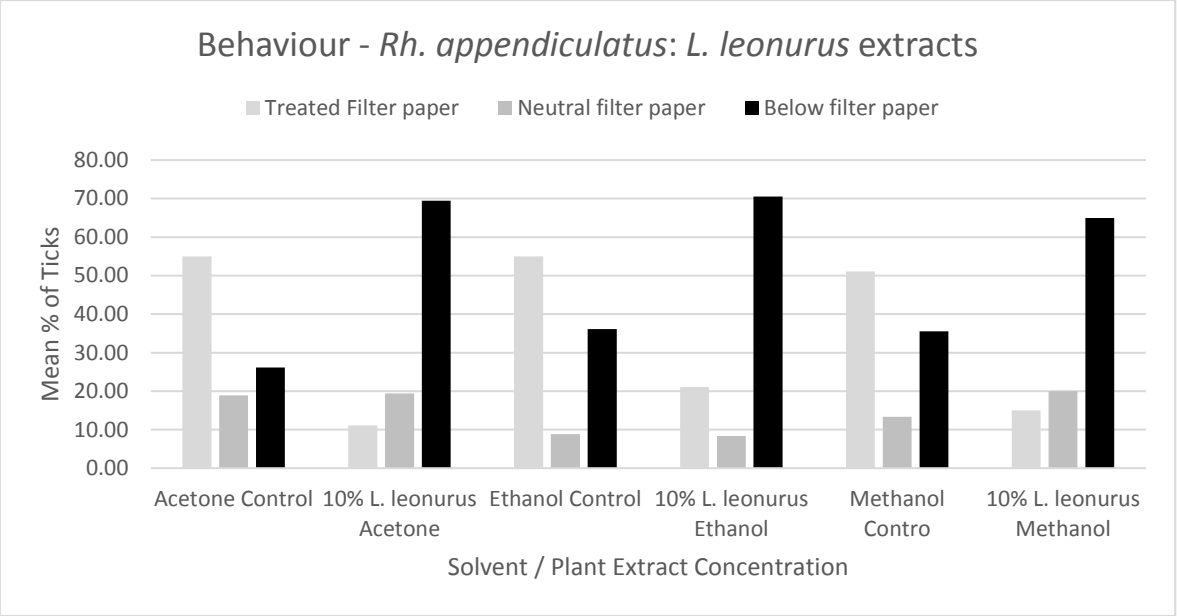

Figure 5.34 Response of *Rh. appendiculatus* to different *L. leonurus* extracts using a tick climbing bioassay

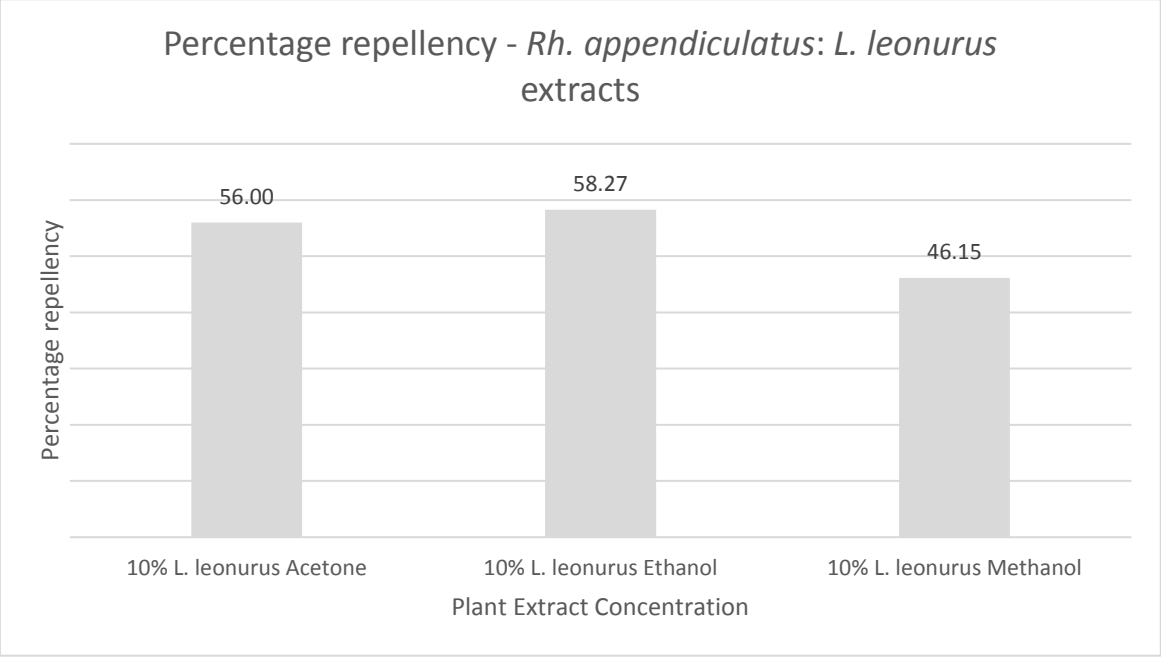

Figure 5.35 Percentage repellency of different *L. leonurus* extracts against *Rh. appendiculatus* adults using a tick climbing bioassay

The statistical comparison of different repellencies are summarised in Table 5.27 below. Significant differences in percentage repellencies are highlighted in light red.

Table 5.27 Significance of difference between repellency of different types of *L. leonurus* extracts against *Rh. appendiculatus* adults using a tick climbing bioassay

Parameter	Statistical Difference
Acetone	
% repellency: *L. leonurus* 10% (Acetone) and F10® (positive control)	0.027
% repellency: *L. leonurus* 10% (Acetone) and Negative control	0.000
Ethanol	
% repellency: *L. leonurus* 10% (Ethanol) and F10® (positive control)	0.089
% repellency: *L. leonurus* 10% (Ethanol) and Negative control	0.000
Methanol	
% repellency: *L. leonurus* 10% (Methanol) and F10® (positive control)	0.002
% repellency: *L. leonurus* 10% (Methanol) and Negative control	0.000
Comparison	
% repellency: *L. leonurus* 10% (Acetone) and *L. leonurus* 10% (Ethanol)	0.972
% repellency: *L. leonurus* 10% (Acetone) and *L. leonurus* 10% (Methanol)	0.406
% repellency: *L. leonurus* 10% (Ethanol) and *L. leonurus* 10% (Methanol)	0.510

Comparison of percentage repellency showed no significant differences ($P > 0.05$) between the 10% concentration of ethanol plant extract and the commercial repellent. However, the 10% concentrations of acetone and methanol plant extracts showed a significantly low ($P < 0.05$) repellent effect against *Rh. appendiculatus* adults compared to the commercialised repellent. Also, a significant difference ($P < 0.05$) occurred between all the plant extracts and the negative control.

In summary, the 10% concentrations of *L. leonurus* extracts expressed repellency effects that are less than 60% against adults of *Rh. appendiculatus*. The ethanol extract has shown to have a relatively stronger repelling effect against *Rh. appendiculatus* ticks compared to the 10% contrations of acetone and methanol extracts.

5.6.1.4 Botanical extract testing – *Thymus vulgaris*

The three most effective *T. vulgaris* extracts from the petri dish choice chamber bioassay were tested to determine the response of *Rh. appendiculatus* adult ticks. Based on the movement of *Rh. appendiculatus* ticks in response to the stimuli from the *T. vulgaris* extracts, the following observations as recorded and illustrated in Table 5.28 and Figures 5.36 and 5.37 were made.

Table 5.28 Location and Percentage repellency of *T. vulgaris* against *Rh. appendiculatus* using a tick climbing bioassay

	Treated Filter paper	Neutral filter paper	Below filter paper	% Repellency
Acetone Control	55.00	18.89	26.11	-182.98
10% Acetone	0.56	5.00	94.44	94.12
Ethanol Control	55.00	8.89	36.11	-76.92
10% Ethanol	2.22	8.89	88.89	87.50
Methanol Control	51.11	13.33	35.56	-81.25
10% Methanol	2.22	14.44	83.33	80.00
Positive Control (F10®)	3.33	13.33	85.00	80.40

The *T. vulgaris* extracts showed a high degree of repellency against *Rh. appendiculatus* adult ticks ranging from 80.00% as observed for the 10% concentration of methanol extract and 94.12% as expressed by the 10% concentration of acetone extract. When comparing the repellency of the extracts with that of the commercial repellent, only the 10% *T. vulgaris* methanol extract (80.00%) expressed a percentage repellency slightly lower compared to the commercial repellent (80.40%).

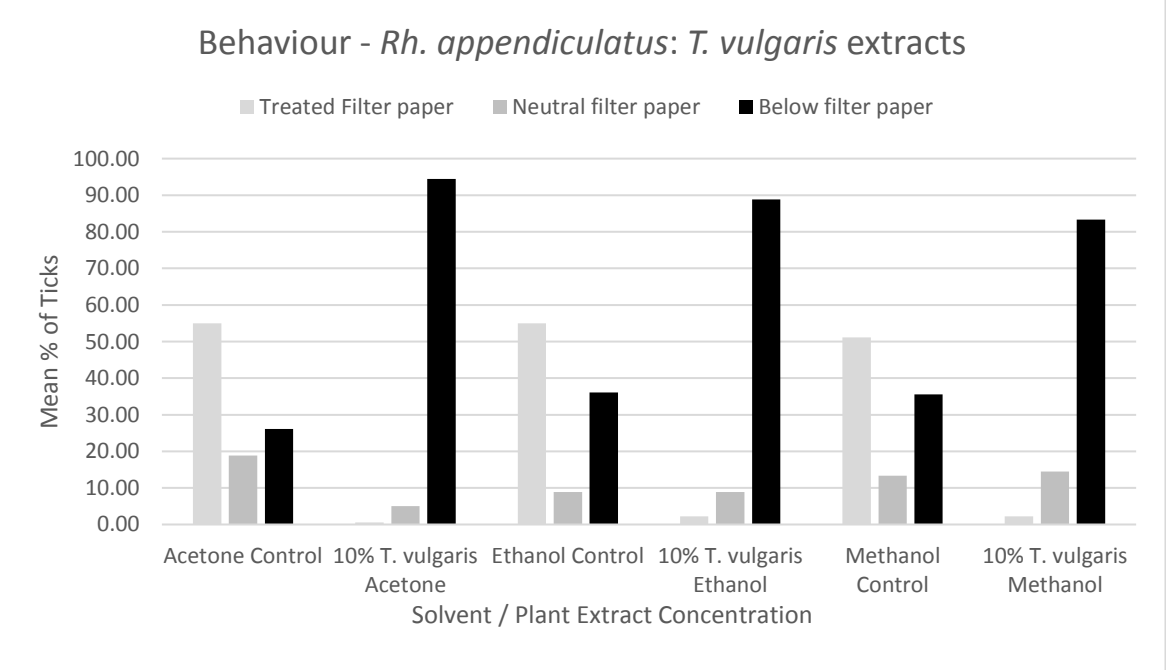

Figure 5.36 Response of *Rh. appendiculatus* to different *T. vulgaris* extracts using a tick climbing bioassay

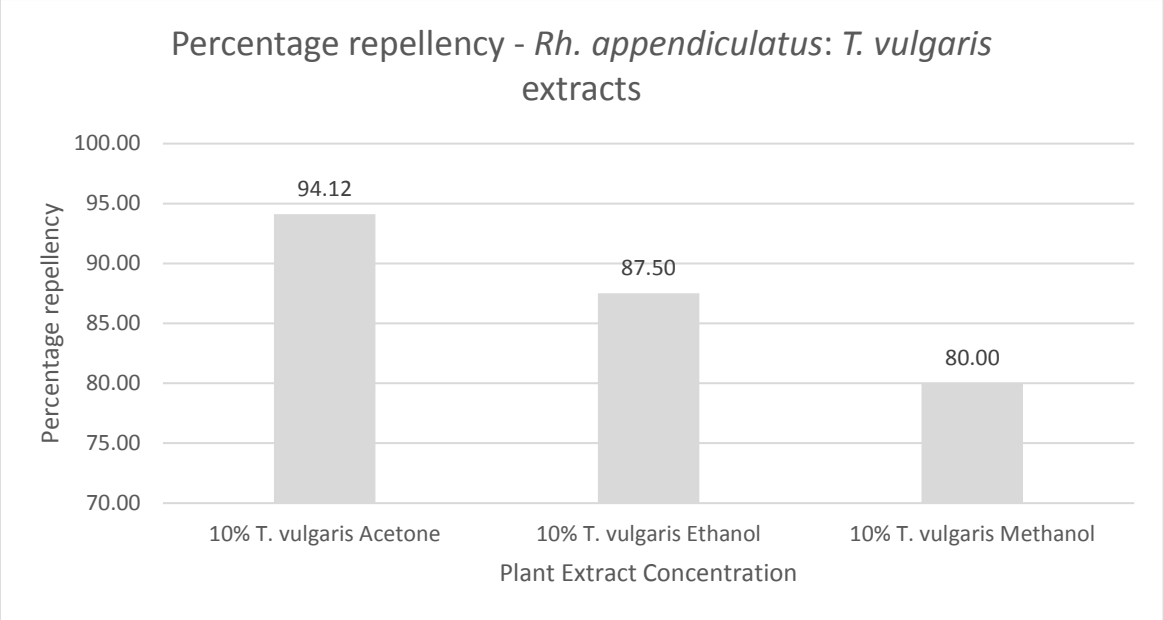

Figure 5.37 Percentage repellency of different *T. vulgaris* extracts against *Rh. appendiculatus* adults using a tick climbing bioassay

The statistical comparison of different repellencies are summarised in Table 5.29 below. Significant differences in percentage repellencies are highlighted in light red.

Table 5.29 Significance of difference between repellency of different types of *T. vulgaris* extracts against *Rh. appendiculatus* adults using a tick climbing bioassay

Parameter	Statistical Difference
Acetone	
% repellency: *T. vulgaris* 10% (Acetone) and F10® (positive control)	0.031
% repellency: *T. vulgaris* 10% (Acetone) and Negative control	0.000
Ethanol	
% repellency: *T. vulgaris* 10% (Ethanol) and F10® (positive control)	0.414
% repellency: *T. vulgaris* 10% (Ethanol) and Negative control	0.000
Methanol	
% repellency: *T. vulgaris* 10% (Methanol) and F10® (positive control)	0.888
% repellency: *T. vulgaris* 10% (Methanol) and Negative control	0.000
Comparison	
% repellency: *T. vulgaris* 10% (Acetone) and *T. vulgaris* 10% (Ethanol)	0.200
% repellency: *T. vulgaris* 10% (Acetone) and *T. vulgaris* 10% (Methanol)	0.001
% repellency: *T. vulgaris* 10% (Ethanol) and *T. vulgaris* 10% (Methanol)	0.359

Comparison of percentage repellency showed that only the 10% concentration of acetone extract expressed a percentatge repellency that is significantly higher ($P < 0.05$) than that of the commercialised repellent. However, significant differences occurred between all the plant extracts and the negative control. The

10% concentrations of acetone and ethanol and 10% concentrations of ethanol and methanol plant extracts showed no significant differences ($P > 0.05$) in repelling adults of *Rh. appendiculatus*. A significant difference ($P < 0.05$) was noted between the 10% concentration of acetone and methanol extracts.

In summary, all extracts of *T. vulgaris* showed high repelling effects on against adults of *Rh. appendiculatus* ticks. The acetone extract expressed the highest repellency effect (94.12%) against adults of *Rh. appendiculatus*.

5.6.2 Experiment Two – Plant extract and controls including attractants

5.6.2.1 Efficacy of attractant included tick climbing bioassay

The results on mean percentage tick repellency of the commercial repellents in the presence of various attractants against adults of *Rh. appendiculatus* are summarised in Table 5.30. Ticks observed on the neutral filter paper were regarded as repelled as the treatment had an effect on the full questing height of the ticks, and the ticks observed on the neutral filter paper did not climb to full questing height.

The results obtained (Tables 5.30), shows that no significant differences ($P > 0.05$) in percentage repellencies recorded under different attractants. Also, as shown in Table 5.31, no significant differences ($P > 0.05$) were found between the individual and combined attractants and the ticks' positions on the rods. However, significant differences ($P < 0.05$) were obtained for F10® wound spray (Movement: $P = 0.038$, Heat: $P = 0.038$, CO_2: $P = 0.042$ and Combined: $P = 0.033$) (Table 5.30), supporting the observations made that *Rh. appendiculatus* avoided the commercial repellents.

Table 5.30 Location and Percentage repellency of commercial repellent and distilled water against *Rh. appendiculatus* ticks using a tick climbing bioassay including attractants

	F10®				dH_2O			
	Treated Filter paper	**Neutral filter paper**	**Below filter paper**	**% Repellency**	**Treated Filter paper**	**Neutral filter paper**	**Below filter paper**	**% Repellency**
Movement								
15 min	0.00	0.00	10.00	100.00	4.00	1.00	5.00	0.00
30 min	0.00	2.00	9.00	77.80	4.00	2.00	4.00	-50.00
45 min	0.00	2.00	8.00	75.00	4.00	3.00	3.00	-133.33
60 min	1.00	2.00	7.00	57.10	5.00	3.00	2.00	-300.00
Mean %	2.50	15.00	85.00	79.40	42.50	22.50	35.00	-85.71
T-Test	0.038				0.441			
Heat								
15 min	0.00	3.00	7.00	57.10	5.00	1.00	4.00	-50.00
30 min	1.00	0.00	9.00	88.90	4.00	2.00	4.00	-50.00
45 min	0.00	1.00	9.00	88.90	6.00	3.00	1.00	-800.00
60 min	1.00	1.00	8.00	75.00	4.00	4.00	2.00	-300.00
Mean %	5.00	12.50	82.50	78.80	47.50	25.00	27.50	-163.64
T-Test	0.038				0.287			
CO_2								
15 min	0.00	1.00	9.00	88.89	2.00	1.00	7.00	57.14
30 min	0.00	3.00	7.00	57.14	7.00	0.00	3.00	-133.33
45 min	0.00	2.00	8.00	75.00	5.00	0.00	5.00	0.00
60 min	0.00	3.00	7.00	57.14	7.00	0.00	3.00	-133.33
Mean %	0.00	22.50	77.50	70.97	52.50	2.50	45.00	-22.22
T-Test	0.042				0.221			
Combined								
15 min	0.00	2.00	8.00	75.00	6.00	0.00	4.00	-50.00
30 min	0.00	0.00	10.00	100.00	4.00	0.00	6.00	33.33
45 min	0.00	0.00	10.00	100.00	7.00	0.00	3.00	-133.33
60 min	0.00	2.00	8.00	75.00	4.00	3.00	3.00	-133.33
Mean %	0.00	10.00	90.00	88.89	52.50	7.50	40.00	-50.00
T-Test	0.033				0.312			

Table 5.31 Comparison between repellency of F10® against *Rh. appendiculatus* adults in the presence of different attractants

Source	**Dependent Variable**	**Type III Sum of Squares**	**df**	**Mean Square**	**F**	**Sig.**
Movement and Heat						
Corrected Model	TreatedFilterPaper	.125	1	.125	.429	0.537
	BelowFilterPaper	.125	1	.125	.097	0.766
Movement and CO_2						
Corrected Model	TreatedFilterPaper	.125	1	.125	1.000	0.356
	BelowFilterPaper	1.125	1	1.125	.871	0.387
Heat and CO_2						
Corrected Model	TreatedFilterPaper	.125	1	.125	.435	0.577
	BelowFilterPaper	.125	1	.125	.099	0.675
Combined, Movement, Heat and CO_2						
Corrected Model	TreatedFilterPaper	.687	3	.229	1.571	0.248
	BelowFilterPaper	3.250	3	1.083	.897	0.471

As seen in Table 5.32, similar percentage repellencies were observed in percentage repellencies obtained from the traditional tick climbing bioassay without attractants included and the a similar one including attractants.

Table 5.32 Comparison between percentage repellencies obtained from the traditional tick climbing bioassay and attractant included tick climbing bioassay

	F10®				dH_2O			
	Treated Filter paper	Neutral filter paper	Below filter paper	% Repellency	Treated Filter paper	Neutral filter paper	Below filter paper	% Repellency
Traditional Tick Climbing bioassay with divisions								
	F10®				dH_2O			
Mean %	3.33	13.33	85.00	80.40	41.67	30.00	28.33	-152.90
Attractant included Tick Climbing Bioassay								
	F10®				dH_2O			
Mean %	0.00	10.00	90.00	88.89	52.50	7.50	40.00	-50.00

5.6.2.2 Botanical extract testing – *Aloe ferox*

The three most effective *A. ferox* extracts from the choice chamber bioassay were tested to determine the response of *Rh. appendiculatus* adult ticks. From the movement of *Rh. appendiculatus* ticks in response to the stimuli from the *A. ferox* extracts; in the presence of heat, CO_2 and air movement. Data obtained are summarised and illustrated in Table 5.33, Figures 5.38 and 5.39.

Table 5.33 Location and Percentage repellency of *A. ferox* against *Rh. appendiculatus* using a tick climbing bioassay including attractants

	Treated Filter paper	Neutral filter paper	Below filter paper	% Repellency
Acetone Control	44.17	32.50	23.33	-228.57
20% Acetone	28.33	30.00	41.67	-40.00
Ethanol Control	41.67	27.50	30.83	-124.32
20% Ethanol	21.67	16.67	61.67	37.84
Methanol Control	49.17	27.50	25.00	-206.67
20% Methanol	31.67	17.50	50.83	3.28
Positive Control (F10®)	0.00	10.00	90.00	88.89

Data obtained in this study shows that most ticks quested on the control filter papers. The *A. ferox* extracts showed varying degrees of repellency against *Rh. appendiculatus* adult ticks ranging from -40.00% as observed for the 20%

concentration of acetone extract to 37.84% as expressed by the 20% concentration ethanol extract. When comparing the extracts with the commercial repellent (F10®), it was found that the commercial repellent had a significantly higher repellency ($P < 0.05$) against *Rh. appendiculatus* compared to the *A. ferox* extracts.

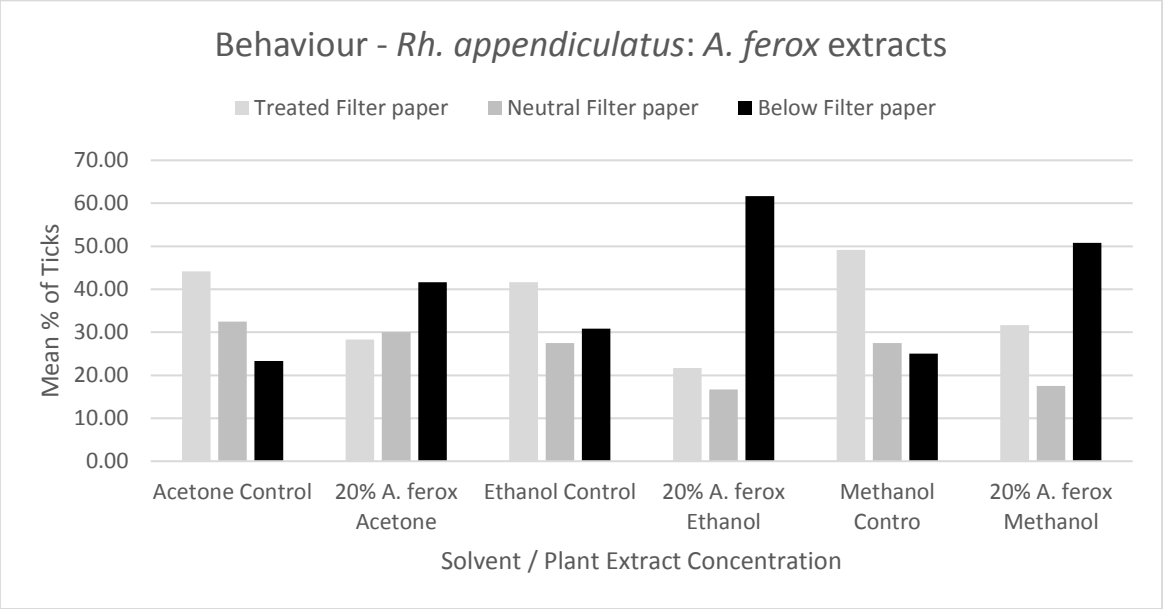

Figure 5.38 Response of *Rh. appendiculatus* to different *A. ferox* extracts using a tick climbing bioassay including attractants

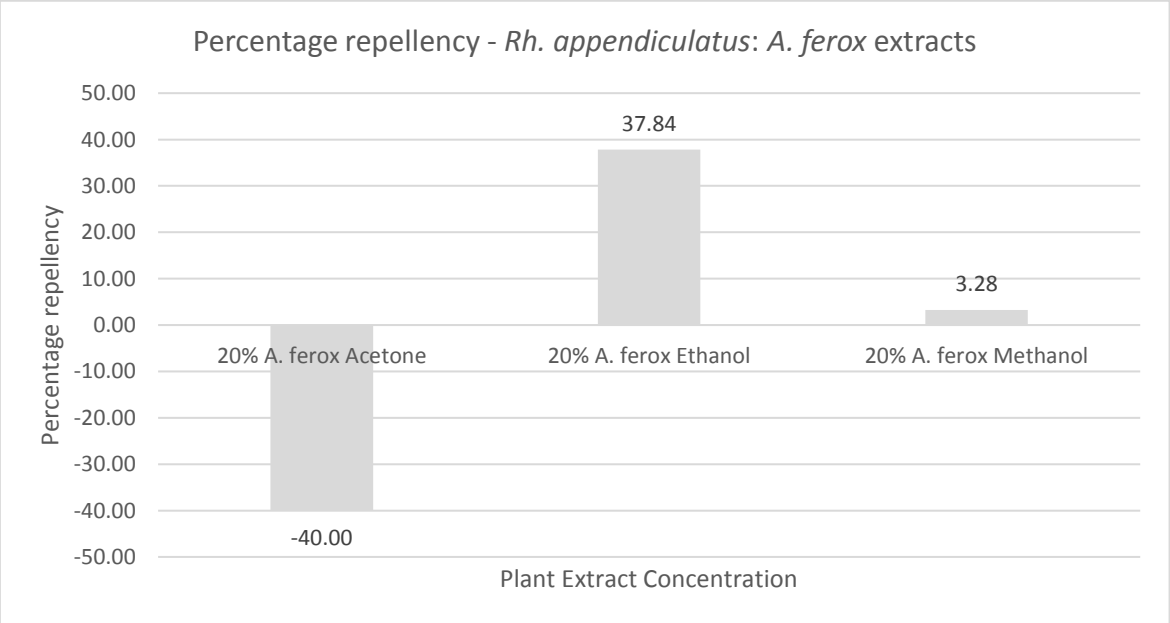

Figure 5.39 Percentage repellency of different *A. ferox* extracts against *Rh. appendiculatus* adults using a tick climbing bioassay including attractants

The statistical comparison of different repellencies are summarised in Table 5.34 below. Significant differences in percentage repellencies are highlighted in light red.

Table 5.34 Significance of difference between repellency of different types of *A. ferox* extracts against *Rh. appendiculatus* adults using a tick climbing bioassay including attractants

Parameter	Statistical Difference
Acetone	
% repellency: *A. ferox* 20% (Acetone) and F10® (positive control)	0.009
% repellency: *A. ferox* 20% (Acetone) and Negative control	0.000
Ethanol	
% repellency: *A. ferox* 20% (Ethanol) and F10® (positive control)	0.000
% repellency: *A. ferox* 20% (Ethanol) and Negative control	0.000
Methanol	
% repellency: *A. ferox* 20% (Methanol) and F10® (positive control)	0.000
% repellency: *A. ferox* 20% (Methanol) and Negative control	0.000
Comparison	
% repellency: *A. ferox* 20% (Acetone) and *A. ferox* 20% (Ethanol)	0.028
% repellency: *A. ferox* 20% (Acetone) and *A. ferox* 20% (Methanol)	0.190
% repellency: *A. ferox* 20% (Ethanol) and *A. ferox* 20% (Methanol)	0.022

All plant extracts showed a significantly low ($P < 0.05$) effect on the movement of *Rh. appendiculatus* ticks compared to the commercial repellent (F10®). The 20% concentration of all extracts of *A. ferox* showed a significant difference ($P < 0.05$) when compared with the negative control. No significant differences ($P > 0.05$) were found between the percentage repellency of the 10% concentrations of acetone and methanol extracts. Nonetheless, significant differences ($P < 0.05$) were found between the 10% concentrations of acetone and ethanol plants extract and 10% concentration ethanol and methanol plant extracts.

In summary, data presented in this study demonstrates that *A. ferox* extracts had a generally weak repellency (< 40%) against *Rh. appendiculatus* ticks.

5.6.2.3 Botanical extract testing – *Leonotis leonurus*

The three most effective *L. leonurus* extracts from the choice chamber bioassay were tested to determine the response of *Rh. appendiculatus* adult ticks, from the movement of *Rh. appendiculatus* ticks in response to the stimuli from the *L. leonurus* extracts; in the presence of heat, CO_2 and movement of air. Data obtained are summarised and illustrated in Table 5.35 and Figures 5.40 and 5.41.

Table 5.35 Location and Percentage repellency of *L. leonurus* against *Rh. appendiculatus* using a tick climbing bioassay including attractants

	Treated Filter paper	Neutral filter paper	Below filter paper	% Repellency
Acetone Control	44.17	32.50	23.33	-228.57
10% Acetone	10.83	23.33	65.83	48.10
Ethanol Control	41.67	27.50	30.83	-124.32
10% Ethanol	11.67	14.17	74.17	65.17
Methanol Control	49.17	27.50	25.00	-206.67
10% Methanol	10.00	18.33	70.83	60.00
Positive Control (F10®)	0.00	10.00	90.00	88.89

During the observation period ticks continuously moved across the length of the rod, results from all controls however showed the highest percentage of ticks on the control treated filter paper, and therefore a comparison can be made with the extract treated filter paper. The *L. leonurus* extracts showed varying degrees of repellency against *Rh. appendiculatus* adult ticks ranging between 48.10% as observed for the 10% concentration acetone to 74.13% as expressed by the 10% concentration ethanol extract. When the repellent effects of the extracts were compared with that of the commercial repellent, only the 10% ethanol (74.17%) and 10% methanol (70.83%) extracts showed a repellent effect greater than 70%.

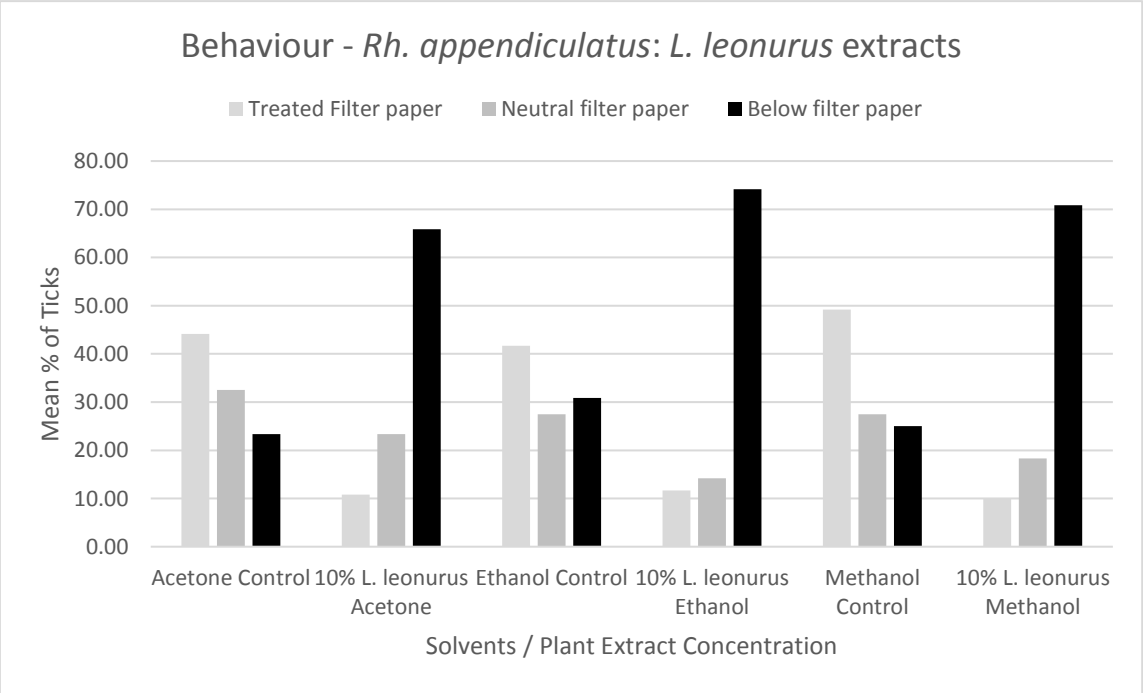

Figure 5.40 Response of *Rh. appendiculatus* to different *L. leonurus* extracts using a tick climbing bioassay including attractants

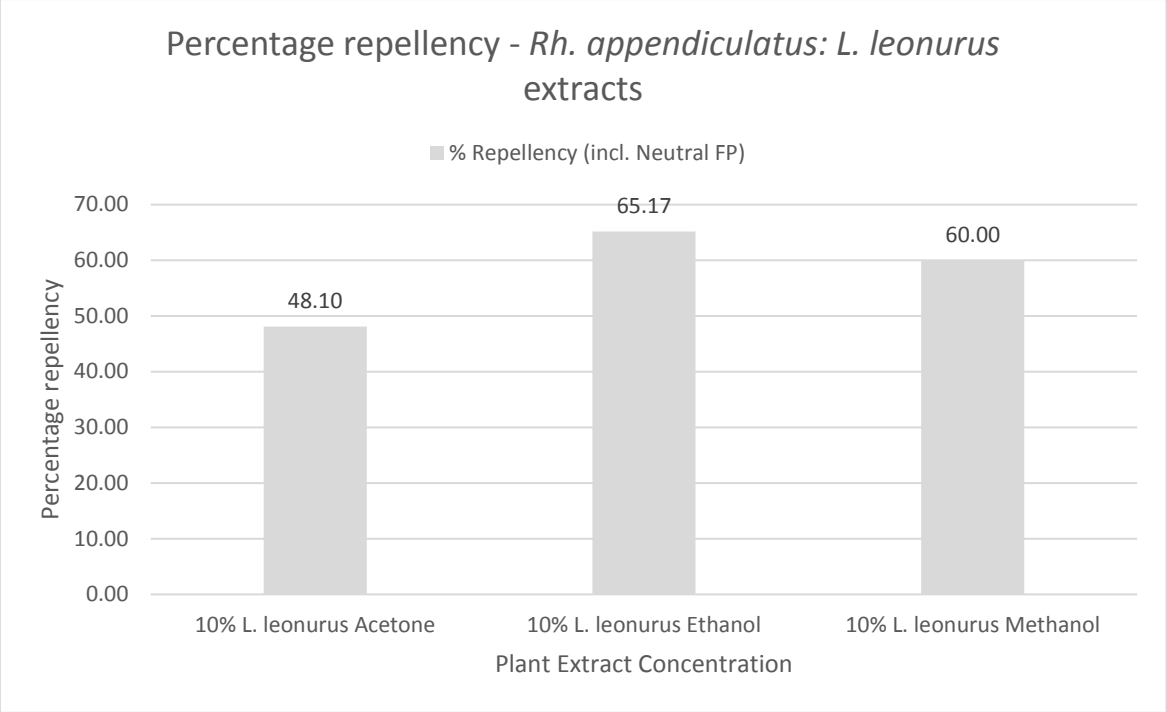

Figure 5.41 Percentage repellency of different *L. leonurus* extracts against *Rh. appendiculatus* adults using a tick climbing bioassay including attractants

The statistical comparison of different repellencies are summarised in Table 5.36 below. Significant differences in percentage repellencies are highlighted in light red.

Table 5.36 Significance of difference between repellency of different types of *L. leonurus* extracts against *Rh. appendiculatus* adults using a tick climbing bioassay including attractants

Parameter	Statistical Difference
Acetone	
% repellency: *L. leonurus* 10% (Acetone) and F10® (positive control)	0.001
% repellency: *L. leonurus* 10% (Acetone) and Negative control	0.000
Ethanol	
% repellency: *L. leonurus* 10% (Ethanol) and F10® (positive control)	0.022
% repellency: *L. leonurus* 10% (Ethanol) and Negative control	0.000
Methanol	
% repellency: *L. leonurus* 10% (Methanol) and F10® (positive control)	0.001
% repellency: *L. leonurus* 10% (Methanol) and Negative control	0.000
Comparison	
% repellency: *L. leonurus* 10% (Acetone) and *L. leonurus* 10% (Ethanol)	0.090
% repellency: *L. leonurus* 10% (Acetone) and *L. leonurus* 10% (Methanol)	0.150
% repellency: *L. leonurus* 10% (Ethanol) and *L. leonurus* 10% (Methanol)	0.550

Comparison of percentage repellency showed that all the plant extracts had a significantly lower ($P < 0.05$) repellent effect compared with the commercialised repellent. Also, all significant differences ($P < 0.05$) were found between all the plant extracts and the negative control. However, no significant differences ($P > 0.05$) in percentage repellency were found among the 10% concentrations of the acetone, ethanol and methanol extracts of *L. leonurus*.

In summary, the 10 % acetone extract has shown a relatively medium strength (< 50%) repellent effect against adults of *Rh. appendiculatus*. The ethanol and methanol extracts of *L. leonurus* have shown a repelling effect that is greater than 70%.

5.6.2.4 Botanical Extract Testing – *Thymus vulgaris*

The three most effective *T. vulgaris* extracts from the choice chamber bioassay were tested to determine the response of *Rh. appendiculatus* adult ticks to the stimuli from the *T. vulgaris* extracts in the presence of heat, CO_2; and air movement. Data obtained are summarised and illustrated in Table 5.37 and Figures 5.42 and 5.43.

Table 5.37 Location and Percentage repellency of *T. vulgaris* against *Rh. appendiculatus* using a tick climbing bioassay including attractants

	Treated Filter paper	**Neutral filter paper**	**Below filter paper**	**% Repellency**
Acetone Control	44.17	32.50	23.33	-228.57
10% Acetone	5.00	10.00	85.00	82.35
Ethanol Control	41.67	27.50	30.83	-124.32
10% Ethanol	5.00	15.00	80.00	75.00
Methanol Control	49.17	27.50	25.00	-206.67
10% Methanol	6.67	8.33	85.00	82.35
Positive Control (F10®)	0.00	10.00	90.00	88.89

The *T. vulgaris* extracts showed a high degree of repellency against *Rh. appendiculatus* adult ticks ranging from 75.00% as observed for the 10% concentration of ethanol extract to 82.35% as expressed by both the 10% concentration acetone and methanol extracts. When comparing the percentage repellency of the extracts with that of the commercialised repellent, no significant differences ($P > 0.05$) were found. However, all extracts expressed a percentage

repellency that was slightly lower compared to that of the commercial repellent (88.89%).

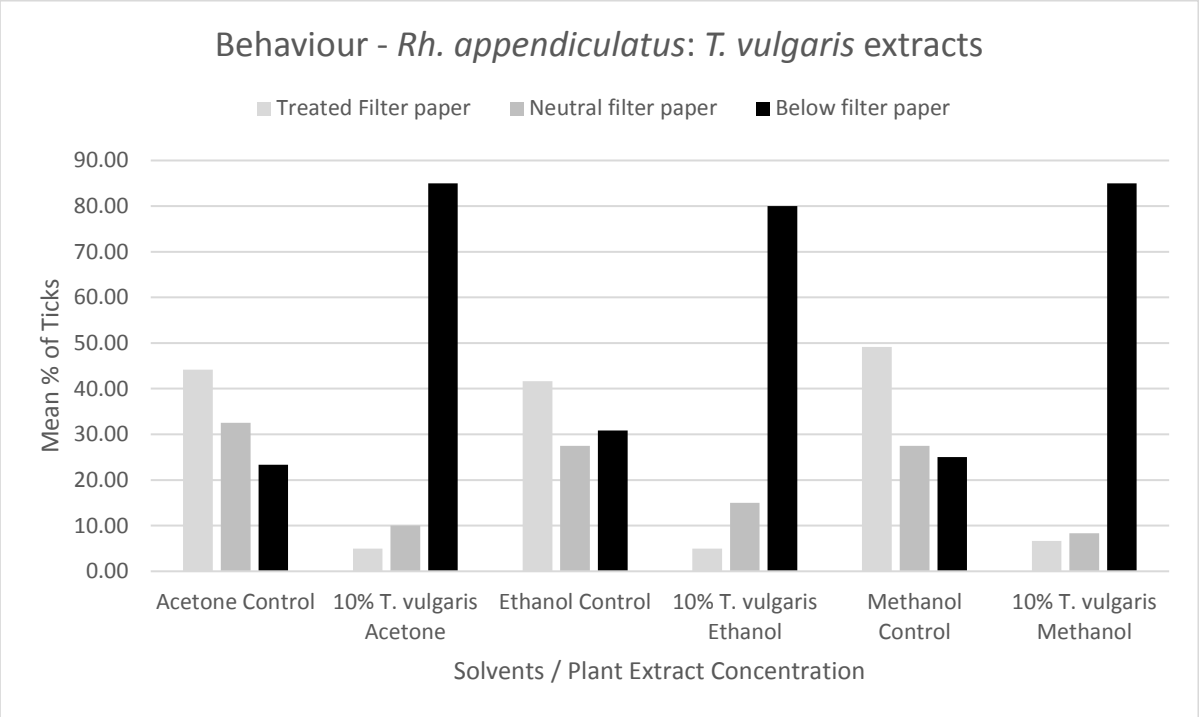

Figure 5.42 Response of *Rh. appendiculatus* to different *T. vulgaris* extracts using a tick climbing bioassay including attractants

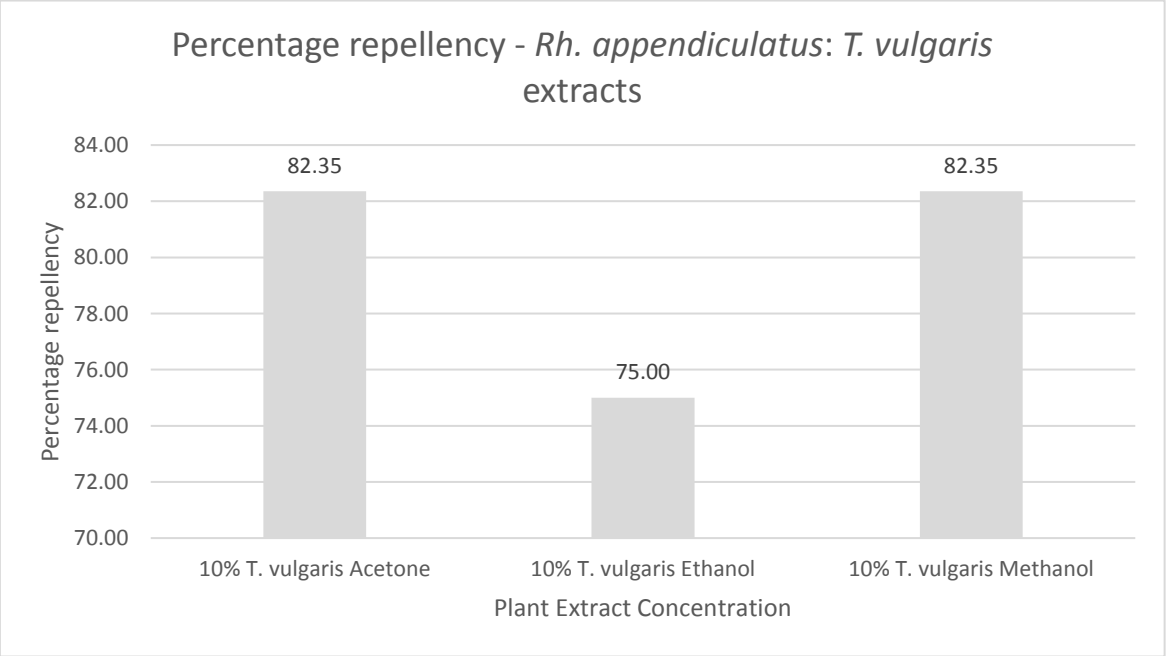

Figure 5.43 Percentage repellency of different *T. vulgaris* extracts against *Rh. appendiculatus* adults using a tick climbing bioassay including attractants

The statistical comparison of different repellencies are summarised in Table 5.38 below. Significant differences in percentage repellencies are highlighted in light red.

Table 5.38 Significance of difference between repellency of different types of *T. vulgaris* extracts against *Rh. appendiculatus* adults using a tick climbing bioassay including attractants

Parameter	Statistical Difference
Acetone	
% repellency: *T. vulgaris* 10% (Acetone) and F10® (positive control)	0.400
% repellency: *T. vulgaris* 10% (Acetone) and Negative control	0.000
Ethanol	
% repellency: *T. vulgaris* 10% (Ethanol) and F10® (positive control)	0.098
% repellency: *T. vulgaris* 10% (Ethanol) and Negative control	0.000
Methanol	
% repellency: *T. vulgaris* 10% (Methanol) and F10® (positive control)	0.062
% repellency: *T. vulgaris* 10% (Methanol) and Negative control	0.000
Comparison	
% repellency: *T. vulgaris* 10% (Acetone) and *T. vulgaris* 10% (Ethanol)	0.206
% repellency: *T. vulgaris* 10% (Acetone) and *T. vulgaris* 10% (Methanol)	0.931
% repellency: *T. vulgaris* 10% (Ethanol) and *T. vulgaris* 10% (Methanol)	0.121

Comparison of percentage repellency of all extracts and the commercialised repellent showed no significant differences ($P > 0.05$). However, significant differences ($P < 0.05$) were found between all the plant extracts and the negative control. Furthermore, no significant differences ($P > 0.05$) were found among the 10% concentrations of the extracts of the three different solvents against adults of *Rh. appendiculatus*.

In summary, all extracts of *T. vulgaris* tested in this study showed high repelling effects against adults of *Rh. appendiculatus*. The acetone and methanol extracts expressed the strongest repelling effect (82.35 %) against adults of *Rh. appendiculatus* ticks.

5.6.3 Experiment Three – Test for repellency using the preference selection tick climbing bioassay

5.6.3.1 Efficacy of Preference Tick Climbing bioassay

The efficacy and possibility of the bioassay was first tested by using two rods dipped in distilled water and the behaviour regarding rod selection observed to ensure that both rods would be approached for climbing (Table 5.39). A mean of 35.83% of

ticks were observed on Rod 1, 29.17% on Rod 2 and 35.00% as not climbing during the one hour observation period. During the bioassay ticks were not placed on the rod at the beginning or during intervals to promote climbing, as a result an additional observation (Not Climbing) was also included.

Table 5.39 Location and Percentage repellency of distilled water against *Rh. appendiculatus* using a preference tick climbing bioassay

	Rod 1 (dH_2O)	**Rod 2 (dH_2O)**	**Not Climbing**
15 min	3.67	2.67	3.67
30 min	3.33	3.33	3.33
45 min	3.33	2.33	4.33
60 min	4.00	3.33	2.67
Mean %	35.83	29.17	35.00
T-Test	0.356		
P-Value	0.064	0.896	0.405

Comparison between Rod 1 and Rod 2 showed no significant differences ($P > 0.05$). In the second control test performed using commercial repellents, ticks avoided climbing the repellent treated rods (0.00%) and were observed mainly on the neutral rod or not climbing, resulting in a 100% percentage repellency, as seen in Table 5.40.

Table 5.40 Location and Percentage repellency of a commercialised repellent against *Rh. appendiculatus* using a preference tick climbing bioassay

	Bayticol				**F10®**			
	Treated Rod	**Neutral Rod**	**Not Climbing**	**% Repellency**	**Treated Rod**	**Neutral Rod**	**Not Climbing**	**% Repellency**
15 min	0.00	7.00	3.00	100.0	0.00	6.00	4.00	100.0
30 min	0.00	8.00	2.00	100.0	0.00	4.00	6.00	100.0
45 min	0.00	6.00	4.00	100.0	0.00	5.00	5.00	100.0
60 min	0.00	4.00	6.00	100.0	0.00	4.00	6.00	100.0
Mean %	0.00	62.50	37.50	100.0	0.00	47.50	52.50	100.0
T-test	0.003				0.002			
P-Value	-	0.176	0.176		-	0.176	0.176	

Significantly high ($P < 0.05$) repellency effects against *Rh. appendiculatus* were found for both the Bayticol 2% EC dip ($P = 0.003$) and F10® wound spray ($P = 0.002$). However, no significant differences ($P > 0.05$) were found between the two commercialised repellent products tested.

5.6.3.2 Botanical Extract Testing – *Aloe ferox*

Using a preference tick climbing bioassay, the most effective extract as identified using the petri dish and tick climbing bioassays, was tested to determine the response of *Rh. appendiculatus* adult ticks. The behaviour of *Rh. appendiculatus* ticks in response to the stimuli from the 20% concentration of acetone extract of *A. ferox* was observed. Data obtained are recorded and illustrated in Table 5.41 and Figure 5.44.

Table 5.41 Location and Percentage repellency of *A. ferox* against *Rh. appendiculatus* using a preference tick climbing bioassay

	Treated Rod	Control Rod	Not Climbing	% Repellency
15 min	0.00	6.00	4.00	100.00
30 min	0.67	4.67	4.67	92.86
45 min	1.33	4.00	4.67	84.62
60 min	0.67	4.67	4.67	92.86
Mean %	6.67	48.33	45.00	92.86
Positive Control (F10®)	0.00	47.50	52.50	100.00
T-Test	0.004			
P-value				0.017

During the observation period ticks were observed to avoid the *A. ferox* dipped rod and would rather approach the control rod to climb and quest. Over the three test runs 55% of ticks were observed to climb and quest, with 48.33% of ticks observed to prefer the control rod, therefore showing a 92.86% repellency. This level of repellency was slightly lower to 100% repellency found for the commercialised repellent.

A significant difference ($P < 0.05$) in percentage repellency was found when comparing the percentage repellency of the plant extract with that of the commercialised repellent ($P = 0.017$).

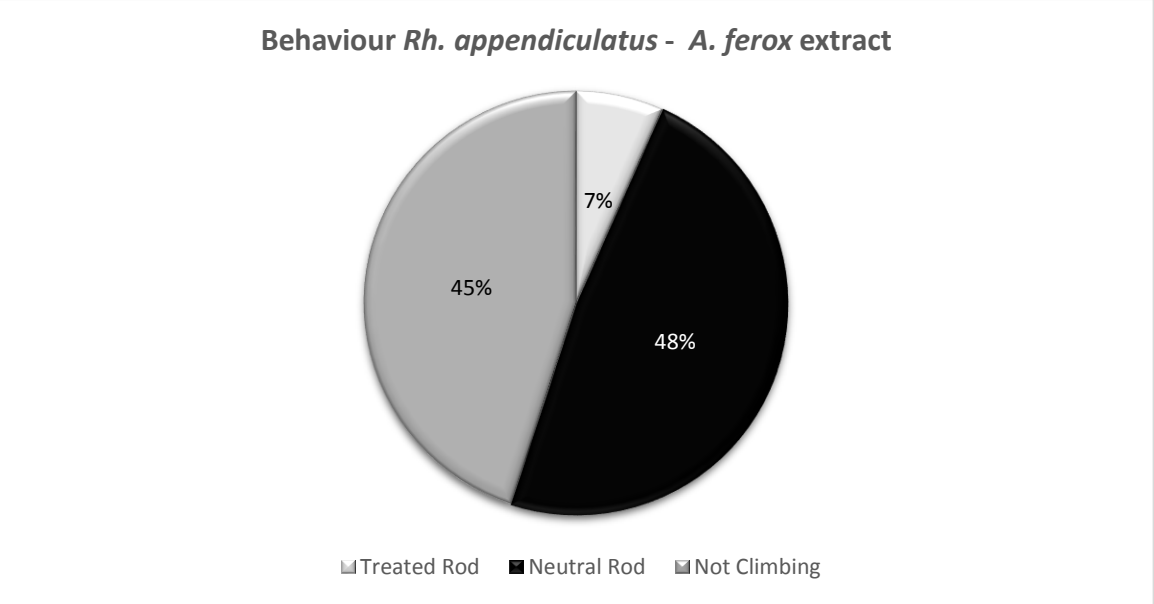

Figure 5.44 Response of *Rh. appendiculatus* to different *A. ferox* extracts using a preference tick climbing bioassay

5.6.3.3 Botanical extract testing – *Leonotis leonurus*

Using the preference tick climbing bioassay, the 10% *L. leonurus* ethanol extract was tested to determine the response of *Rh. appendiculatus* adult ticks. This extract was prefared since it demonstrated a high percentage repellency against adults of *Rh. appendiculatus* in both the petri dish choice chamber and the tick climbing bioassays. The behaviour of *Rh. appendiculatus* ticks in response to the stimuli from the 10% oncentration of the ethanol extract of *L. leonurus*, was studied. Data obtained are summarised and illustrated in Table 5.42 and Figure 5.45.

Table 5.42 Location and Percentage repellency of *L. leonurus* against *Rh. appendiculatus* using a preference tick climbing bioassay

	Treated Rod	Control Rod	Not Climbing	% Repellency
15 min	0.33	3.67	6.00	96.55
30 min	0.67	4.67	4.67	92.86
45 min	0.33	5.67	4.00	96.55
60 min	1.00	5.00	4.00	88.89
Total	5.83	47.50	46.67	93.81
Positive Control (F10®)	0.00	47.50	52.50	100.00
T-Test	0.003			
P-value				0.002

During the observation period ticks were observed to avoid the *L. leonurus* dipped rod and would rather approach the control rod to climb and quest. Over the three test runs 53.33% of ticks were observed to climb and quest, with 47.50% of ticks observed to prefer the control rod, therefore showing a 93.81% repellency. This percentage repellency level is slightly lower when compared to the 100% repellency found from the commercialised repellent (100%).

A significant difference ($P < 0.05$) was found when comparing the percentage repellency of the extract with that of the commercial repellent. Also, a significant difference ($P < 0.05$) was found between the repellencies of the treatment and control rods suggesting that *Rh. appendiculatus* avoided the rods treated with the plant extracts.

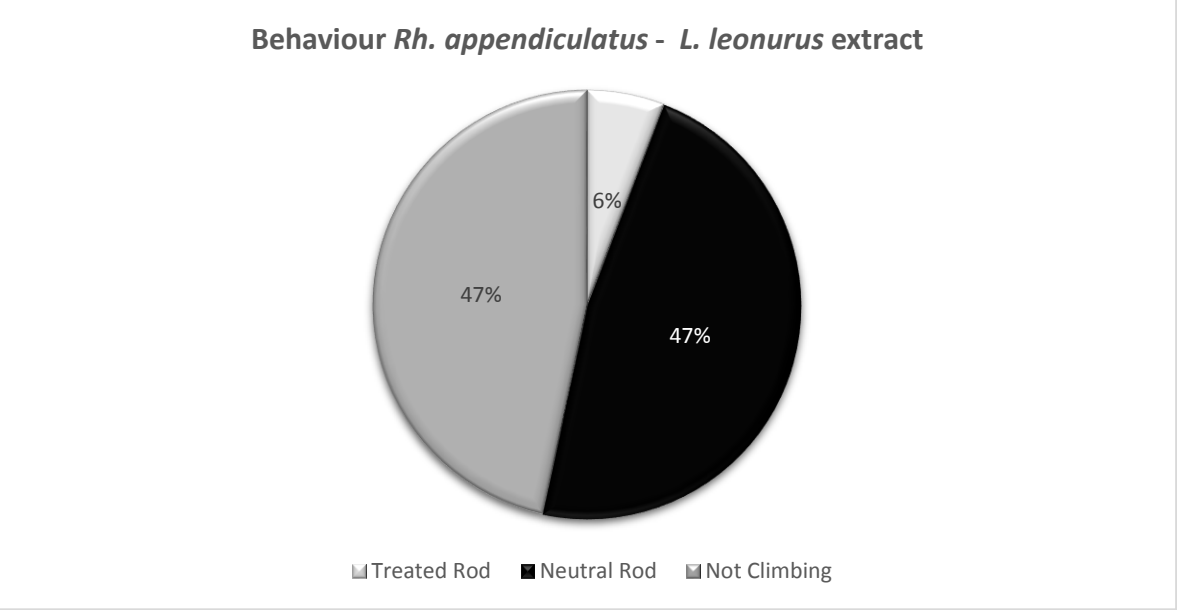

Figure 5.45 Response of *Rh. appendiculatus* to different *L. leonurus* extracts using a preference tick climbing bioassay

5.6.3.4 Botanical Extract Testing – *Thymus vulgaris*

Using a preference tick climbing bioassay, the most effective extract as identified using the petri dish and tick climbing bioassays, was tested to determine the response of *Rh. appendiculatus* adult ticks. The behaviour of *Rh. appendiculatus* ticks in response to the stimuli from the 10% *T. vulgaris* acetone extract was studied. Data obtained are summarised and illustrated in Table 5.43 and Figure 5.46.

Table 5.43 Location and Percentage repellency of *T. vulgaris* against *Rh. appendiculatus* using a preference tick climbing bioassay

	Treated Rod	Control Rod	Not Climbing	% Repellency
15 min	0.00	4.30	5.70	100.00
30 min	0.70	3.70	5.70	92.90
45 min	0.30	4.70	5.00	96.60
60 min	1.00	5.30	3.70	88.90
Total	5.00	45.00	50.00	94.70
Positive Control (F10®)	0.00	47.50	52.50	100.00
T-Test	0.003			
P-value				0.020

During the observation period, ticks avoided the rod dipped in the extract of *T. vulgaris* and would rather approach the control rod to climb and quest. Over the three test runs 50% of ticks were observed to climb and quest, with 45% of ticks observed to prefer the control rod, therefore showing a 94.70% repellency. This level of repellency was slightly lower compared to 100% repllency found for the commercialised repellent.

A significant difference ($P < 0.05$) was found when comparing the percentage repellency of the plant extract with that of the commercial repellent. Also, a significant difference ($P < 0.05$) was found between the repellencies of the treatment and control rods suggesting that adults of *Rh. appendiculatus* avoided the rod treated with the plant extracts.

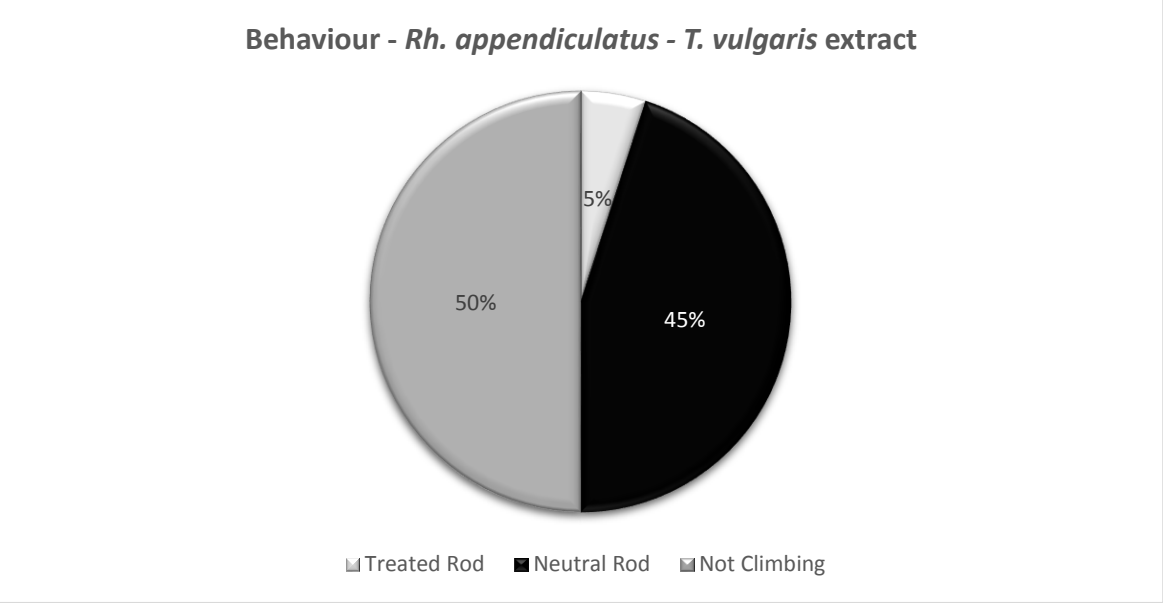

Figure 5.46 Response of *Rh. appendiculatus* to different *T. vulgaris* extracts using a preference tick climbing bioassay

Section C – Discussion

5.7 Conclusion

5.7.1 Commercial repellents

Data obtained in this study, using the petri dish choice chamber bioassay, suggest that Bayticol 2% was significantly effective as a repellent to both *Am. hebraeum* and *Rh. appendiculatus*. The repellent effects however, as demonstrated by the percentage repellency, appeared to have been much more pronounced on *Rh. appendiculatus* than on *Am. hebraeum*. As for F10®, a significant repellent effect was found only against *Rh. appendiculatus*. Although the basis for this difference in effect cannot be accounted for in this study it has been previously recorded that different tick species respond differently to the effects of the same repellent even if the concentration is the same. Caroll *et al.,* (2004), using a petri dish bioassay, and Carrol *et al.,* (2007), using a fingertip bioassay, found that the nymphs of *Ixodus scapularis* and *Amblyomma americanum* behaved and responded differently to the same concentration of DEET and compounds isolated from *Callicarpa americana*, respectively. As indicated by Dautel (2004), the percentage repellency of a test product when using the petri dish bioassay can be significantly higher due to the small test area. The inclusion of a neutral zone, as was the case with the modified petri dish choice bioassay used in this study, was chosen in an attempt to obtain additional data on the percentage of ticks not affected by a treatment product as these ticks would not move away from the treatment chamber, thereby reducing the possibility of calculating an increased percentage repellency.

Data obtained in this study, using the tick climbing bioassay (Experiment 1 of tick climbing bioassay), corroborated the observations made for the petri dish choice chamber bioassay by showing that Bayticol 2% had a slightly higher repelling effect on *Rh. appendiculatus* when compared with F10®. Although it is generally known that ticks questing behaviour is influenced by CO_2 and the body heat produced by animals (Sonenshine, 1993; Stafford, 2007; Bissinger and Roe, 2010), the traditional tick climbing bioassay as often used does not incorporate these. This shortfall may be limiting and may result with data that does not approximate the actual natural behaviour of ticks. In this study (Experiment 2 of tick climbing

bioassay) the traditional bioassay was conducted in the presence of these host attractants to determine whether the inclusion of attractants will influence the questing behaviour of ticks. Data from the comparison between the percentage repellency of F10® in the presence of host attractants and in the absence of host attractants yielded a similar, not significantly different, percentage repellency. Similarly, in observations made with regards to the repelling effects of different plant extracts, the inclusion of host attractants appeared not have played a significant role in determining a closer to *in vivo* result or observation than in the absence of host attractants.

It is known that certain grass species have a repelling effect on ticks (Thompson *et al.,* 1978; Zimmerman *et al.,* 1984; Mwangi *et al.,* 1995a; Cruz-Vazquez and Rubalcaba, 2000; Kaaya, 2000; Castrejón *et al.,* 2003; Fernandez-Ruvalcaba *et al.,* 2004). Using this knowledge and in an approach similar to the experiment conducted by Mwangi *et al.* (1995b), the potential of a treatment product to have an effect on tick species can be determined. Data obtained from Experiment 3 of tick climbing bioassay indicated that when ticks were faced with a choice between a rod that was immersed in a plant extract and that which was immersed in the sovent (control), they preferred to quest on the control rods. This bioassay demonstrated high repellencies because most ticks completely avoided the rods treated with plant extracts. Although data obtained from the three bioassays generally corroborates each other, the differences observed may be a result of the differences in the design and sensitivity of the bioassays.

The following paragraphs provide a discussion on each of the plant species tested for tick repellent properties in this study.

5.7.2 *Aloe ferox*

Data from the petri dish choice chamber bioassay indicated that *A. ferox* had a relatively low repellent effects against both *Rh*. *appendiculatus* and *Am. hebraeum*. Except for the 10% and 20% concentrations of acetone plant extracts, 20% concentration of ethanol plant extract and 20% concentration of methanol plant extracts tested against *Am. hebraeum*, all other extracts had a low repellency. The results from the Tick Climbing bioassay (Experiment 1 of tick climbing tick bioassay)

showed the 20% concentrations acetone and methanol extracts to have an effect on the full questing height of *Rh. appendiculatus* adult ticks with percentage repellencies similar to that of the commercial repellent. However, when taking into consideration data from the tick climbing bioassay including host attractants (Experiment 2 of tick climbing bioassay) a significant decrease in the repelling effect of *A. ferox* plant extracts were observed, with low percentage repellencies recorded for all three 20% concentration plant extracts tested. The data from this study therefore indicated that the inclusion of attractants promoted ticks ascending the extract treated rods, thereby resulting in a reduced efficacy compared to the observations of the tick climbing bioassay without attractants. Previously, Fourie *et al.* (2005) reported on the lack of anti-tick properties in *A. ferox* when they demonstrated its lack of activity against *Rh. (Bo.) decoloratus.* Although some concentrations of *A. ferox* demonstrated a relatively high repellency, the data obtained is not of a strength to contradict the findings by Fourie and his co-workers. Findings from this study and the observations as reported by Fourie *et al.* (2005) therefore raises a concern on the true efficacy of *A. ferox* as an anti-tick agent particularly when considering the widespread traditional use of *A. ferox* as an anti-tick agent (Moyo and Masika, 2009).

5.7.3 *Leonotis leonurus*

L. leonurus showed potential as repellent with various concentrations of plant extracts having a repelling effect on both *Am. hebraeum* and *Rh. appendiculatus*. However, the effect on *Rh. appendiculatus* was observed to be lower when compared with *Am. hebraeum*. For *Am. hebraeum*, seven of the nine extracts tested showed to have a repelling effect higher than that of the commercial repellent, including all acetone and methanol concentration plant extracts as well as the 10% concentration of ethanol plant extract. As for *Rh. appendiculatus* only three of the nine extracts tested, including the 10% concentration of ethanol plant extract and 7.5% and 10% concentrations of methanol plant extracts, showed to have a percentage repellency higher than the commercial repellent. Therefore, suggesting that these extracts show a significant potential as anti-tick agent. Even though the three extracts tested during the tick climbing bioassay (Experiment 1 of tick climbing bioassay) did not express percentage repellencies similar to the commercial repellent it can be concluded that the 10% concentrations of ethanol and methanol

plant extracts has a potential to act as anti-tick agent with percentage repellencies of 58.27%. It is also important to note that data from the tick climbing bioassay including host attractants (Experiment 2 of tick climbing bioassay) showed in the case of the 10% concentration of ethanol plant extract and 10% concentration of methanol plant extract to have a percentage repellency higher than that of Experiment 1, with a decrease in the percentage repellency of Experiment 2 compared to Experiment 1 for the 10% concentration of acetone plant extract. This again highlights the effect the inclusion of host attractants might have on the effect of a treatment product closer to the effect it might have during an *in vivo* bioassay set-up. In the study conducted by Luseba *et al.* (2016), the authors reported a 90% repellency using a tick climbing bioassay for an acetone extract of *L. leonurus*, even though the results for this study reported a lower percentage repellency for acetone extracts, across the different approaches followed, it cannot be shown to be significantly different from the results reported by Luseba *et al.* (2016) as the concentration of the extract used by these researcher were not reported. Results from this study however do support that *L. leonurus* express potential to be used as anti-tick agent.

5.7.4 *Thymus vulgaris*

Data from this study, using the petri dish choice chamber bioassay, showed all *T. vulgaris* extracts included in this study to yield a percentage repellency greater than that of the commercial repellent against *Am. hebraeum*. As for *Rh. appendiculatus*, all *T. vulgaris* extracts, except the 2.5% concentration of acetone and 2.5% concentration ethanol plant extracts showed a percentage repellency higher than the commercial repellent against. Data obtained from both tick climbing bioassays (Experiment 1 and 2 of the tick climbing bioassay) expressed high degrees of repellency against *Rh. appendiculatus* adult ticks discouraging the tick from climbing to full questing height, with only a slight decrease observed in the percentage repellencies observed in the presence of host attractants. The results from this study support the expectation of *T. vulgaris* as a repellent based on the effect of *T. vulgaris* on other arthropods (Kim *et al.,* 2004; Park *et al.,* 2005; Pavela, 2007; Pavela *et al.,* 2009; Lee *et al.,* 2010; Maia and Moore, 2011; Zoubiri and Baaliouamer, 2011; Szczepanik *et al.,* 2012) and

indicated significant effect as anti-tick agent against multiple species using different bioassays.

5.7.5 Summary

When comparing the four different bioassays utilized to observe the questing behaviour of ticks it is important to note that various external factors play a role during bioassays, as a result, a range of percentage repellencies were observed for some of the extracts tested. The discussion will focus on the results for the petri dish choice chamber as multiple-species repellent and results for *Rh. appendiculatus* for the different tick climbing bioassays. These factors include:

I. Extracts will not have a similar effect on different species, it is therefore important to perform additional bioassays where possible.
II. The choice chamber bioassay is in a closed environment and therefore the strength of the extract on the treated filter paper would remain longer and therefore have a longer impact on ticks.
III. During the preference tick climbing bioassay ticks were not placed on a rod to promote climbing / questing as a result a larger number of ticks were observed as “Not climbing” this can result in a higher percentage repellency compared to the traditional tick climbing assay where ticks are encouraged to climb by placing them on the rod during time intervals.
IV. The preference tick climbing bioassay is not an optimal bioassay to follow to determine the percentage repellency of a potential treatment product, however, can be used to determine if ticks will avoid a specific product.
V. Although the inclusion of attractants in the tick climbing bioassay did not register noticeable significant differences when compared with similar results without attractants, it is recommended that future research including this bioassay should include attractants since this arrangement approximates the real natural situation.

Therefore, data obtained from this study positions *T. vulgaris* as having stronger tick repellent properties compared to *A. ferox* and *L. leonurus*. Also, data from this study showed that *A. ferox* is a relatively weaker tick repellent compared to the other two tick species. This is an interesting observation particularly when considering that traditional livestock keepers believe that *A. ferox* reduces tick loads on animals. The

adults of *Am. hebraeum* and *Rh. appendiculatus* were variedly repelled by the same extracts of the plant species exermined in this study, an outcome that further reinforces the view that different tick species respond differently to repellents.

CHAPTER 6
METHODOLOGY, RESULTS AND DISCUSSION
CHEMICAL ANALYSIS

It is well known that some plant species contain secondary metabolites that show anti-pest properties and/or remedial effects against various diseases. In Chapter 5 of this study, it was demonstrated that some extracts of *A. ferox*; *L. leonurus*, and *T. vulgaris* have repellent effects against *Am. hebraeum* and *Rh. appendiculatus* ticks. According to Maia and Moore (2011), the compounds contained in plants can be chemically grouped as primary alkaloids, terpenoids, phenolics, proteinase inhibitors and growth regulators. To determine the presence of biological active compounds in plant extracts tested in the *in vitro* repellency bioassays in this study, thin layer chromatography (TLC) and gas chromatography and mass spectroscopy (GC-MS) were performed in order to address Objective Three of the study (as indicated in Chapter One).

Section A – Methods

6.1 Thin Layer Chromatographic analysis

A 20 x 20 cm glass-backed silica gel plate (Merck) was cut in half (10 x 10 cm). A fine pencil line was made 1 cm from the top and 1 cm from the bottom. The plant extracts were spotted on the bottom line, 1 cm apart. Two plates were prepared for two general mobile phases performed as part of initial analysis in the College of Agriculture and Environmental Sciences Laboratories and as supported by SiliCycle (2018), as indicated in Table 6.1 for polar compounds. Each of the extracts tested using the petri dish bioassay was subjected to TLC analysis to determine the number of components in the extract prior to analysis using gas chromatography. The plate was placed in the mobile phase, Dichloromethane (DCM) : Methanol, and allowed to run until the mobile phase reached the top of the TLC plate. Subsequently, the plate was allowed to air dry. Observations of bands were made by using a freshly prepared vanillin-ethanol-sulphuric acid solution (colouring reagent) and heat dried.

Table 6.1 TLC Mobile phase preparation

Plate number	Methanol	DCM
1	5mL	95mL
2	20mL	80mL

6.2 Gas Chromatographic analysis

In order to identify and characterise compounds in the plant extracts used in this study, GC-MS analysis was done. According to Schauer *et al.* (2005) and Lisec *et al.* (2006) the main compound classes that can be identified using Gas Chromatography x Gas Chromatography Time of Flight Mass Spectrometer (GCxGC-TOF-MS) include phosphates, phosphorylated intermediates, nitrogen compounds, amino acids, organic acids, sugars, sugar alcohols and fatty acids (lipophilic compounds).

In this study the Pegasus 4D GCxGC-TOF-MS (Leco, South Africa), using the Agilent 30 m x 0.32 mm x 0.25 µm HP-5 primary column, was used for GC-MS analysis in order to determine active compounds in all plant extracts used during the *in vitro* bioassays. Helium was used as the carrier gas with a flow rate set at 1mL/min, using a split inlet mode. The criteria for data analysis was set to include a minimum of ten library selections per peak and a minimum of 50 – 60% similarity before a name was assigned using the libraries (mainlib and replib) included in the software. The oven temperature and ramp rate parameters were individually set for each solvent based on the boiling point of the solvent as indicated in the Table 6.2 below following the recommendations by the manufacturer (Leco, South Africa).

Table 6.2 GC Oven temp/ramp rate parameters

Rate	Target temperature	Duration
Acetone		
Initial	40°C	20 seconds
5.00°C/min	300°C	5 minutes
Ethanol		
Initial	60°C	20 seconds
5.00°C/min	300°C	5 minutes
Methanol		
Initial	50°C	20 seconds
5.00°C/min	300°C	5 minutes

In order to determine whether the identified compounds had repellent or arthropocidal properties they were compared with those in literature to have such properties. In particular compounds reported by Bissinger and Roe (2010), Boulogne *et al.* (2012), Pino *et al.* (2013), Kegley *et al.* (2016), and Hikal *et al.* (2017), were alphabetically listed in Table 6.3 below, which served as a reference for this study. The work by these authors were preferred based on a literature search focusing on meta-data research articles specifically listing compounds with acaricidal and / or insecticidal properties.

Table 6.3 Biologically active plant compounds showing repelling and / or insecticidal effects

A	
absinthin	3,7-dimethyl-6-octen-1ol acetate
anabasine	anabasine sulphate
apiole	ascardidol
asimicine	10-hydroxy-asimicine
β-asarone	avermectin
azadirachtin	β-amyrine
B	
borneol	bisabolangelone
C	
α-caryophyllene	callicarpenal
carvacrol	carvone
β-citronellol	citronellal
cnidiadin	α-copaene
coumarin	capsaicin II
capsicum	1,8-cineole
cinerin I	cinerin II
cinnamaldehyde	trans-cinnamaldehyde
allyl isothiocyanate	p-methoxycinnamaldehyde
cinnamyl alcohol	citral
β-cyclocitral	*m*-cymene
(-)-.alpha.cubebene	cucurbitacin
24-methylene-3,22-dihydroxycholesterol	coniferyl aldehyde
D	
decanal	dodecanoic acid
2-dodecanone	deguelin
desmodium	dihydroazadirachtin
decanoic acid	
E	
estragole	eugenol
isoeugenol	methyleugenol
methyl isoeugenol	eucalyptol
G	
gedunine	glaucarubinone
geraniol	*trans*-geraniol
trans-geranylacetone	

Table continues on the next page

H	
helenalin	himachalol
humulene	
I	
iridomyrmecin	(-)-isolongifolenone
indole	ivermectin
J	
jasmolin I	jasmolin II
jasmine	methyl jasmonate
K	
karanjin	
L	
limonene	limonene-oxide
linalool	geranyl-linalool
M	
matrine	menthol
myristicin	menthone
myrtenal	24-methylenecycloarta-3-ol
p-menthane-3,8-diol	
N	
nonanal	nerol
nerolidol	neriifolin
nootkatone	nonaoic acid
nicotine	nicotine sulphate
O	
octanoic acid	ocimene
octanal	oxymatrine
P	
phenylethyl propionate	picrotoxinin
alpha-pinene	beta-pinene
pinene	2-phenylethanol
pinosylvin	pyrethrin I
piperitenone-oxide	pulegone
pyrethrin II	perythrins
Q	
quassia	qaussin
R	
rotenone	ryanodine
ryania	
T	
thymol	trimethoxybenzene
1-α-trepiniol	4-trepineol
thujone	
U	
2-undecanone	3-undecanone
V	
valencene-13-ol	verbenol
1-verbenone	
Z	
Zingiberene	

As found by Bissinger and Roe, (2010), Boulogne *et al.* (2012), Pino *et al.* (2013), Kegley *et al.* (2016), and Hikal *et al.* (2017)

Section B – Results

6.3 Thin Layer Chromatographic analysis

The TLC analysis done in this study revealed that all the extracts of *A. ferox*, *L. leonurus* and *T. vulgaris* contained a number of compounds (Figures 6.1 and 6.2). The loading sequence was done as indicated in Table 6.4.

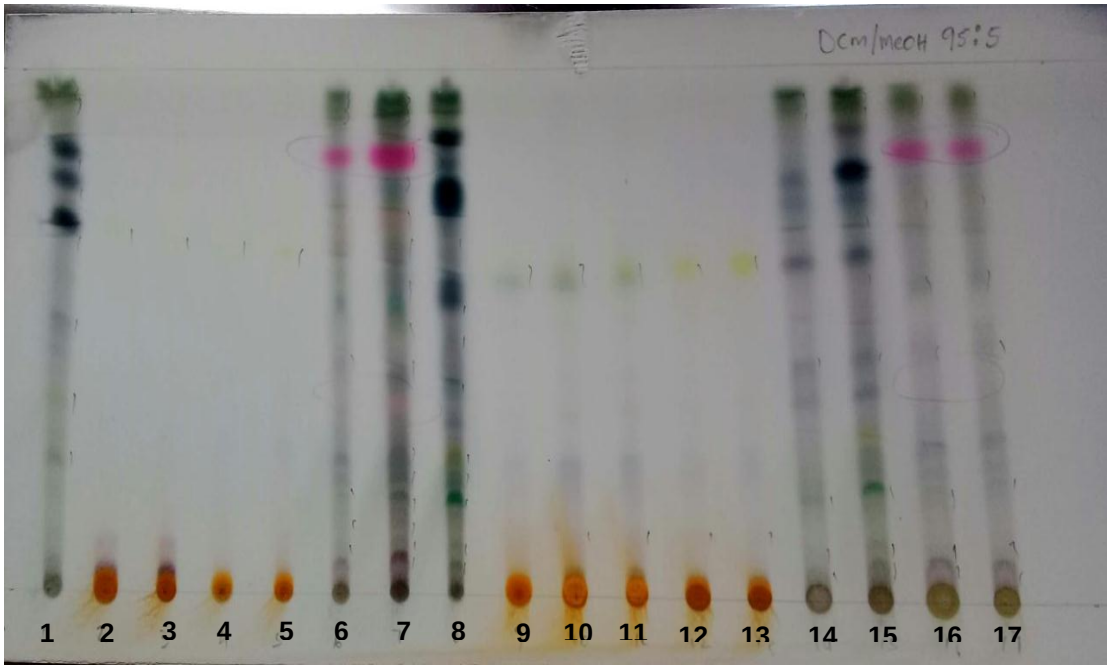

Figure 6.1 A DCM : Methanol (9.5 : 0.5) mobile phase TLC Plate

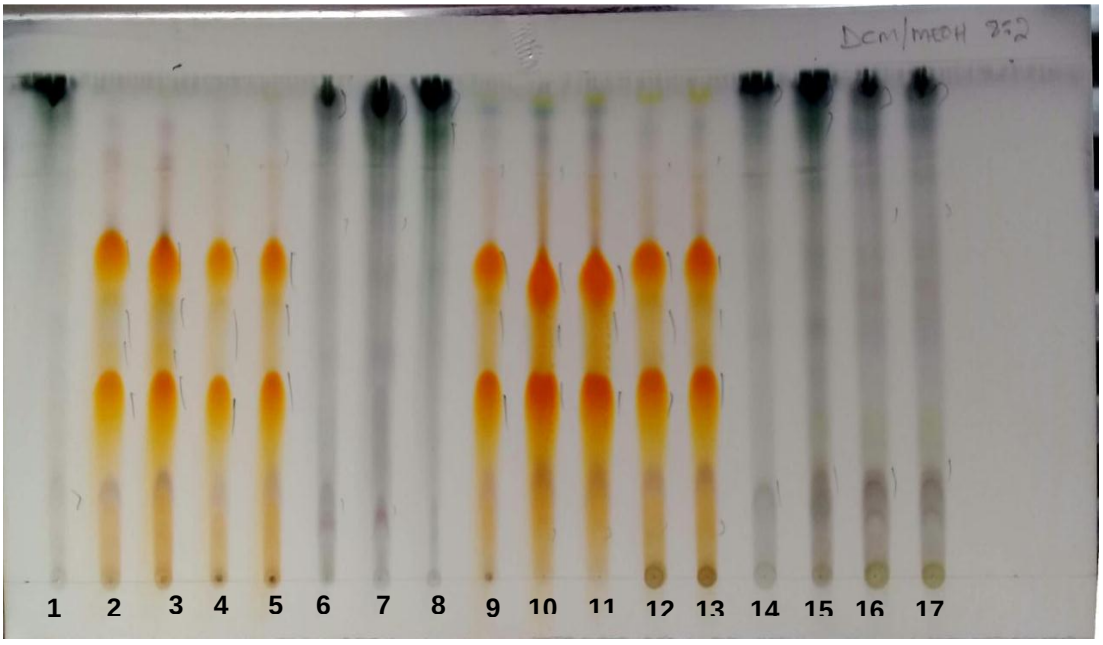

Figure 6.2 A DCM : Methanol (8 : 2) mobile phase TLC Plate

Table 6.4 Loading sequence used on TLC Plates

Plant Species & Concentration	**Solvent used for extraction**	**Line number**
L. leonurus 10%	Ethanol	1
A. ferox 10%	Ethanol	2
A. ferox 20%	Ethanol	3
A. ferox 10%	Ethanol	4
A. ferox 20%	Ethanol	5
T. vulgaris 10%	Ethanol	6
T. vulgaris 10%	Acetone	7
L. leonurus 10%	Acetone	8
A. ferox 5%	Acetone	9
A. ferox 10%	Acetone	10
A. ferox 20%	Acetone	11
A. ferox 10%	Methanol	12
A. ferox 20%	Methanol	13
L. leonurus 5%	Methanol	14
L. leonurus 10%	Methanol	15
T. vulgaris 5%	Methanol	16
T. vulgaris 10%	Methanol	17

The bands in all three plant species were similar regardless of the concentrations (Figure 6.1). However, the bands of *A. ferox* did not show high motility and as a result the mobile phase was amended to the proportion (8 : 2) DCM : Methanol.

As indicated in Chapter 5, various solvents are associated with the extraction of different phytochemical groups, these groups are associated with different colours when a visual observation is made of a TLC plate. Phenols are associated with a blue or green colour, flavonoids with a bright yellow colour, tannins with a bluish black or greenish black coulr and terpenoids with a reddish brown colour (Ganatra *et al*., 2012). Based on the visual observations of the TLC plates it is expected that tannins, phenols and terpenoids are present in the ethanol and methanol extracts of *L. leonurus* and *T. vulgaris*.

6.4 Gas Chromatographic analysis – Results

As indicated earlier, compounds identified in this study from extracts which showed the high percentage repellency values were referenced against those in literature, including their variations or combinations, that are known to be having anti-arthropod properties (Table 6.3).

Data on active compounds identified in this study are listed in decreasing order of area percentages (Tables 6.5 to 6.7, 6.9 to 6.11 and 6.13 to 6.15) and Total Ion Chromatographs (TIC) (Figures 6.3 to 6.11). Only data associated with anti-arthropod activities were considered for discussion in this study.

Compounds identified from *A. ferox* extracts

Compouds identified to have anti-arthropod properties are listed in Tables 6.5 to 6.7 and illustrated in Figures 6.3 to 6.5. Of the three solvents used in the extraction of *A. ferox* in this study, ethanol extracted the highest percentage area (5.36%) of compounds reported to have anti-arthropod properties, followed by acetone (1.88%) and methanol (1.38%). However, in terms of the number of compounds identified acetone extracted 26 different compounds known to have anti-arthropod properties followed by 21 extracted by methanol and 16 extracted by ethanol.

Table 6.5 Compounds with arthropod repellent properties identified from the 20% concentration of the acetone extract of *A. ferox*

Peak #	R.T. (s)	Name	Unique Mass	Area %
238	1655	p-Coumaric acid, trans	78	0.70
148	1004	2,7-Octadiene-1,6-diol, 2,6-dimethyl-	71	0.28
55	529.4	2-Furanmethanol, 5-ethenyltetrahydro-à,à,5-trimethyl-, cis-	59	0.15
93	787.5	Benzene, 2-methoxy-4-methyl-1-(1-methylethyl)-	149	0.12
260	1823	Hexadecanoic acid, 2-hydroxy-, methyl ester, acetate	82	0.12
131	918.9	11-Methyldodecanol	84	0.10
39	448.6	Benzene, 1-methyl-3-(1-methylethyl)-	119	0.06
73	599	Phenylethyl Alcohol	92	0.04
154	1039	1-Undecanol	83	0.04
85	722.6	L-à-Terpineol	93	0.04
127	905.6	11-Methyldodecanol	69	0.03
217	1466	p-Cymene-2,5-diol	151	0.03
65	554.8	trans-Linalool oxide (furanoid)	59	0.03
263	1877	1-Dodecanol, 3,7,11-trimethyl-	71	0.02
255	1797	Oleic Acid	140	0.02
145	974.7	2,7-Octadiene-1,6-diol, 2,6-dimethyl-	67	0.02
153	1031	Cyclopropanetetradecanoic acid, 2-octyl-, methyl ester	99	0.01
81	687.8	2H-Pyran-3-ol, 6-ethenyltetrahydro-2,2,6-trimethyl-	68	0.01
274	2350	1H-Cyclopenta[c]coumarin, 2,3-dihydro-7-pentyloxy-	115	0.01
222	1502	Aromadendrene oxide-(1)	106	0.01
69	576	Linalool	93	0.01
269	2175	2H-1-Benzopyran-2-one, 5,7-dimethoxy-	206	0.01
276	2377	1H-Cyclopenta[c]coumarin, 2,3-dihydro-7-pentyloxy-	272	0.01
295	2678	Furo[3,2-c]coumarin, 2-methyl-3-phenyl-	276	0.00*
194	1312	Cyclopropanetetradecanoic acid, 2-octyl-, methyl ester	95	0.00*
108	823.4	trans-á-Ocimene	93	0.00*
*Compound present at trace levels				1.88

Table 6.6 Compounds with arthropod repellent properties identified from the 20% concentration of the ethanol extract of *A. ferox*

Peak #	R.T. (s)	Name	Unique Mass	Area %
183	1416	p-Coumaric acid, trans	83	1.32
182	1411	p-Coumaric acid	90	1.13
179	1405	p-Hydroxycinnamic acid, ethyl ester	121	1.09
181	1409	4-Methyl-6,7-methylenedioxycoumarin	204	1.01
104	770.3	2,7-Octadiene-1,6-diol, 2,6-dimethyl-	71	0.54
111	807.8	2H-Pyran-3-ol, 6-ethenyltetrahydro-2,2,6-trimethyl-	68	0.13
49	402	Phenylethyl Alcohol	92	0.04
59	477.4	(3R,6S)-2,2,6-Trimethyl-6-vinyltetrahydro-2H-pyran-3-ol	68	0.02
197	1557	n-Hexadecanoic acid	60	0.02
225	2156	1H-Cyclopenta[c]coumarin, 2,3-dihydro-7-pentyloxy-	230	0.01
154	1188	Octanoic acid, pentafluorobenzyl ester	160	0.01
218	1936	2H-1-Benzopyran-2-one, 5,7-dimethoxy-	178	0.01
186	1465	Phenol, 2-methoxy-4-propyl-	166	0.01
223	2120	1H-Cyclopenta[c]coumarin, 2,3-dihydro-7-pentyloxy-	174	0.01
224	2124	1H-Cyclopenta[c]coumarin, 2,3-dihydro-7-pentyloxy-	202	0.00*
88	681.8	Phenol, 2-methyl-5-(1-methylethyl)-	135	0.00*
*Compound present at trace levels				5.36

Table 6.7 Compounds with arthropod repellent properties identified from the 20% concentration of the methanol extract of *A. ferox*

Peak #	R.T. (s)	Name	Unique Mass	Area %
200	1535	p-Coumaric acid	62	0.42
49	435.2	2-Furanmethanol, 5-ethenyltetrahydro-à,à,5-trimethyl-, cis-	42	0.24
199	1530	p-Coumaric acid, trans	176	0.24
47	434	trans-Linalool oxide (furanoid)	94	0.22
119	888.7	2,7-Octadiene-1,6-diol, 2,6-dimethyl-	71	0.14
201	1545	p-Coumaric acid, trans	151	0.04
215	1679	n-Hexadecanoic acid	60	0.03
208	1627	Tridecanoic acid, methyl ester	74	0.01
72	580.7	2H-Pyran-3-ol, 6-ethenyltetrahydro-2,2,6-trimethyl-	68	0.01
242	2228	1H-Cyclopenta[c]coumarin, 2,3-dihydro-7-pentyloxy-	115	0.01
106	796.5	Thymol	150	0.01
245	2291	1H-Cyclopenta[c]coumarin, 2,3-dihydro-7-pentyloxy-	230	0.01
244	2240	1H-Cyclopenta[c]coumarin, 2,3-dihydro-7-pentyloxy-	230	0.00*
243	2233	1H-Cyclopenta[c]coumarin, 2,3-dihydro-7-pentyloxy-	173	0.00*
227	1905	2H-1-Benzopyran-2-one, 6,7-dimethoxy-4-methyl-	192	0.00*
241	2221	1H-Cyclopenta[c]coumarin, 2,3-dihydro-7-pentyloxy-	202	0.00*
206	1605	p-Coumaric acid, trans	178	0.00*
290	3177	Dodecanoic acid, 3,7,11-trimethyl-3,4-bis(trimethylsilyloxy)-,trimethylsilyl ester	247	0.00*
53	454.8	Benzene, 1-methyl-4-(1-methylethenyl)-	132	0.00*
62	497.7	Phenylethyl Alcohol	92	0.00*
277	2907	Dodecanoic acid, 3,7,11-trimethyl-3,4-bis(trimethylsilyloxy)-,trimethylsilyl ester	247	0.00*
*Compound present at trace levels				1.38

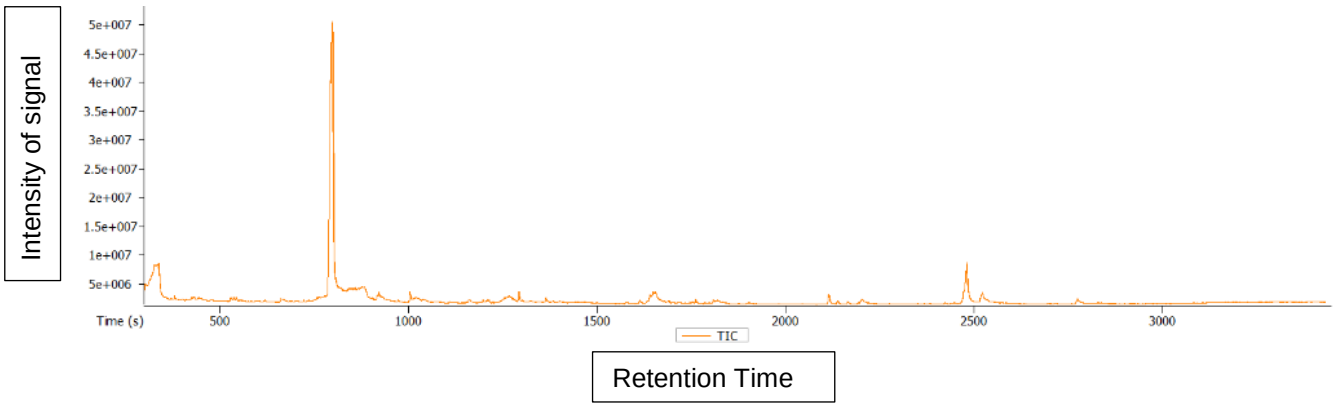

Figure 6.3 A TIC of 20% concentration of acetone extract of *A. ferox*

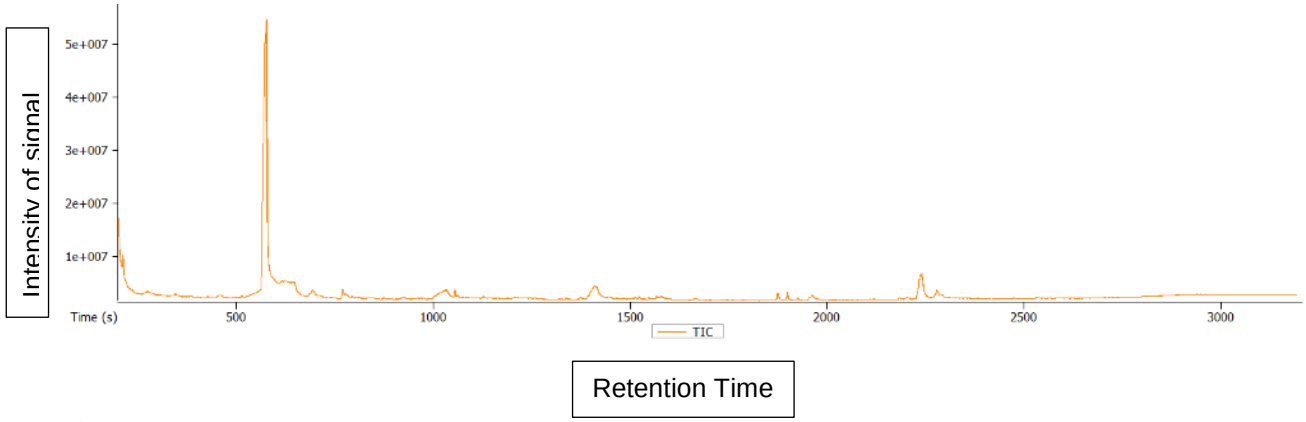

Figure 6.4 A TIC of 20% concentration of ethanol extract of *A. ferox*

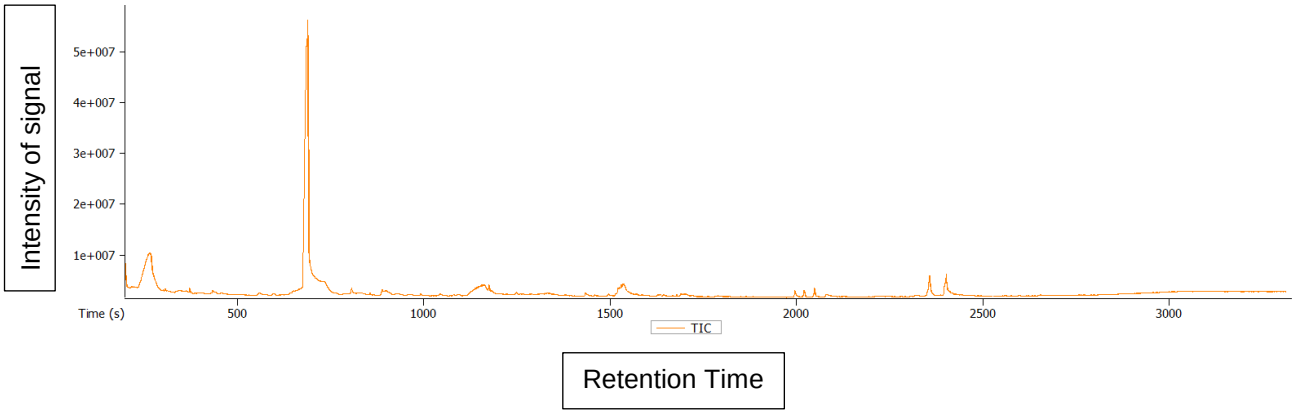

Figure 6.5 A TIC of 20% concentration of methanol extract of *A. ferox*

In Chapter 5 of this study it was demonstrated using the Petri Dish Choice Chamber bioassay that, the acetone extract of *A. ferox* had a high repellency against ticks (with 56.10% repellency), followed by the ethanol extract (with 48.10% repellency) and that of methanol (with 43.75% repellency). Juxtaposing the order of repellency against the order of area of composition and the number of different compounds extracted, it appears that it is the number of different types of compounds that correspond to the repellency strengths of the different extracts. This observation

reinforces the view held by many researchers that the biological activity of plant extracts is largely a product of a number of compounds working synergistically rather than individualistically. However, more research is required to explore this aspect further.

As demonstrated in Table 6.8, five compounds namely, 1H-Cyclopenta[c]coumarin, 2,3-dihydro-7-pentyloxy, 2,7-Octadiene-1,6-diol, 2,6-dimethyl, 2H-Pyran-3-ol, 6-ethenyltetrahydro-2,2,6-trimethyl, p-Coumaric acid, trans and Phenylethyl Alcohol were common in the acetone, ethanol and methanol extracts of *A. ferox*. On the other hand the acetone extract of *A. ferox* presented contained 15 compounds that were not contained in the ethanol and methanol extracts. Although two of these compounds occurred in trace amounts and as a result might be redundant, it would still be interesting to explore the contribution made by these compounds in the tick repellency demonstrated by the acetone extract.

Table 6.8 A comparative account of compounds with anti-arthropod properties found in the acetone, ethanol and methanol extracts of *A. ferox*

	Acetone	**Ethanol**	**Methanol**
Name	**Area %**	**Area %**	**Area %**
1H-Cyclopenta[c]coumarin, 2,3-dihydro-7-pentyloxy-	0.02	0.02	0.02
2,7-Octadiene-1,6-diol, 2,6-dimethyl-	0.30	0.54	0.14
2H-Pyran-3-ol, 6-ethenyltetrahydro-2,2,6-trimethyl-	0.01	0.13	0.01
p-Coumaric acid, trans	0.70	1.32	0.28
Phenylethyl Alcohol	0.04	0.04	0.00
2-Furanmethanol, 5-ethenyltetrahydro-à,à,5-trimethyl-, cis-	0.15		0.24
trans-Linalool oxide (furanoid)	0.03		0.22
p-Coumaric acid		1.13	0.42
11-Methyldodecanol	0.13		
1-Dodecanol, 3,7,11-trimethyl-	0.02		
1-Undecanol	0.04		
2H-1-Benzopyran-2-one, 5,7-dimethoxy-	0.01		
Aromadendrene oxide-(1)	0.01		
Benzene, 1-methyl-3-(1-methylethyl)-	0.06		
Benzene, 2-methoxy-4-methyl-1-(1-methylethyl)-	0.12		
Cyclopropanetetradecanoic acid, 2-octyl-, methyl ester	0.01		
Furo[3,2-c]coumarin, 2-methyl-3-phenyl-	0.00		
Hexadecanoic acid, 2-hydroxy-, methyl ester, acetate	0.12		
L-à-Terpineol	0.04		
Linalool	0.01		
Oleic Acid	0.02		
p-Cymene-2,5-diol	0.03		
trans-á-Ocimene	0.00		
(3R,6S)-2,2,6-Trimethyl-6-vinyltetrahydro-2H-pyran-3-ol		0.02	

Table continues on the next page

	Acetone	Ethanol	Methanol
Name	**Area %**	**Area %**	**Area %**
2H-1-Benzopyran-2-one, 5,7-dimethoxy-		0.01	
4-Methyl-6,7-methylenedioxycoumarin		1.01	
n-Hexadecanoic acid		0.02	
Octanoic acid, pentafluorobenzyl ester		0.01	
Phenol, 2-methoxy-4-propyl-		0.01	
Phenol, 2-methyl-5-(1-methylethyl)-		0.00	
p-Hydroxycinnamic acid, ethyl ester		1.09	
2H-1-Benzopyran-2-one, 6,7-dimethoxy-4-methyl-			0.00
Benzene, 1-methyl-4-(1-methylethenyl)-			0.00
Dodecanoic acid, 3,7,11-trimethyl-3,4-bis(trimethylsilyloxy)- ,trimethylsilyl ester			0.00
n-Hexadecanoic acid			0.03
Thymol			0.01
Tridecanoic acid, methyl ester			0.01

Compounds identified from *L. leonurus* extracts

Compounds identified to have anti-arthropod properties are listed in Tables 6.9 to 6.11 and illustrated in Figures 6.6 to 6.8. Of the three solvents used in the extraction of *L. leonurus* in this study, ethanol extracted the highest percentage area (6.01%) of compounds reported to have anti-arthropod properties, followed by methanol (4.69%) and acetone (3.81%). However, in terms of the number of compounds identified acetone extracted 36 different compounds followed by ethanol and methanol extracts with 30 compounds each.

Table 6.9 Compounds with arthropod repellent properties identified from the 10% concentration of the acetone extract of *L. leonurus*

Peak #	R.T. (s)	Name	Unique Mass	Area %
114	1309	Dodecanoic acid	191	0.59
115	1310	Caryophyllene oxide	81	0.59
198	1748	Undecanoic acid, methyl ester	74	0.35
19	457.6	Eucalyptol	154	0.20
146	1441	Longiverbenone	175	0.19
142	1434	Caryophyllene oxide	187	0.18
122	1346	(1R,3E,7E,11R)-1,5,5,8-Tetramethyl-12-oxabicyclo[9.1.0]dodeca-3,7-diene	138	0.17
120	1342	(1S,3aS,4S,5S,7aR,8R)-5-Isopropyl-1,7a-dimethyloctahydro-1H-1,4-methanoinden-8-ol	98	0.14
58	806.6	D-Carvone	82	0.13
207	1827	Oleic Acid	69	0.11
181	1619	Cyclopropanecarboxylic acid, 2,2-dimethyl-3-(2-methyl-1-propenyl)-, 2-methyl-4-oxo-3-(2-pentenyl)-2-cyclopenten-1-yl ester, [1R-[1à[S*(Z)],3á]]-	123	0.10
31	537.2	11-Methyldodecanol	70	0.09
53	750.1	Bicyclo[3.1.1]hept-3-en-2-one, 4,6,6-trimethyl-, (1S)-	107	0.09
84	1130	5,9-Undecadien-2-one, 6,10-dimethyl-, (E)-	43	0.08

Table continues on the next page

Peak #	R.T. (s)	Name	Unique Mass	Area %
75	1010	.alfa.-Copaene	161	0.08
47	680.5	endo-Borneol	95	0.08
105	1268	Caryophyllene oxide	106	0.06
38	598.3	Phenylethyl Alcohol	92	0.06
220	1997	n-Hexadecanoic acid	257	0.06
71	931.9	11-Methyldodecanol	84	0.05
83	1125	Humulene	93	0.05
44	654	p-Cymen-7-ol	150	0.05
66	905.5	11-Methyldodecanol	69	0.05
24	481.3	Benzeneacetaldehyde	91	0.04
41	646.8	trans-Verbenol	109	0.03
85	1135	p-Cymene-2,5-diol	151	0.03
81	1061	Benzene, 1,2-dimethoxy-4-(1-propenyl)-	178	0.03
208	1861	Isopropyl palmitate	102	0.03
169	1548	3-Methyl-4-isopropylphenol	152	0.02
201	1767	Caryophyllene oxide	87	0.02
49	716.7	Benzenemethanol, à,à,4-trimethyl-	135	0.02
18	448.6	p-Cymene	119	0.01
67	914	Phenol, 2-methyl-5-(1-methylethyl)-	135	0.01
2	313.5	(1R)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene	93	0.01
150	1447	Apiol	222	0.00*
*Compound present at trace levels				3.81

Table 6.10 Compounds with arthropod repellent properties identified from the 10% concentration of the ethanol extract of *L. leonurus*

Peak #	R.T. (s)	Name	Unique Mass	Area %
198	1577.6	n-Hexadecanoic acid	186	1.01
173	1325.2	Tetradecanoic acid	60	0.69
43	448.6	p-Cymen-7-ol	150	0.64
127	1071.7	Caryophyllene oxide	79	0.60
60	584.4	Carvone	82	0.39
154	1201.3	Longiverbenone	175	0.32
163	1222.3	Dodecanoic acid, 2-(acetyloxy)-1-[(acetyloxy)methyl]ethyl ester	151	0.31
88	839.5	Caryophyllene	175	0.31
234	2403.1	trans-Geranylgeraniol	287	0.27
287	2804.8	à-Amyrin	218	0.22
134	1107.6	(1R,3E,7E,11R)-1,5,5,8-Tetramethyl-12-oxabicyclo[9.1.0]dodeca-3,7-diene	138	0.18
152	1193.7	Caryophyllene oxide	92	0.16
201	1586.4	Hexadecanoic acid, ethyl ester	88	0.14
133	1103.3	(1S,3aS,4S,5S,7aR,8R)-5-Isopropyl-1,7a-dimethyloctahydro-1H-1,4-methanoinden-8-ol	119	0.13
70	670	Thymol	135	0.13
89	853.8	á-copaene	161	0.11
80	776.3	.alfa.-Copaene	161	0.10
93	892.9	5,9-Undecadien-2-one, 6,10-dimethyl-, (E)-	43	0.06
24	310.5	Benzeneacetaldehyde	91	0.05
55	548.6	1-Cyclohexene-1-carboxaldehyde, 2,6,6-trimethyl-	152	0.04
66	629.1	Benzeneacetic acid	91	0.03
22	294.8	Eucalyptol	154	0.03
76	737.9	à-Cubebene	161	0.02

Table continues on the next page

Peak #	R.T. (s)	Name	Unique Mass	Area %
202	1704.5	2(3H)-Furanone, 5-heptyldihydro-	85	0.02
39	401.8	Phenylethyl Alcohol	92	0.01
71	683.2	Phenol, 2-methyl-5-(1-methylethyl)-	135	0.01
19	286.3	p-Cymene	119	0.01
50	519.3	Undecanoic acid, 11-amino-	101	0.01
157	1208	Apiol	222	0.00*
*Compound present at trace levels				6.01

Table 6.11 Compounds with arthropod repellent properties identified from the 10% concentration of the methanol extract of *L. leonurus*

Peak #	R.T. (s)	Name	Unique Mass	Area %
229	1701.4	n-Hexadecanoic acid	111	1.46
162	1191.3	Caryophyllene oxide	79	0.54
227	1692.3	n-Hexadecanoic acid	231	0.35
202	1447.1	Tetradecanoic acid	185	0.32
35	391.3	Benzeneacetaldehyde	91	0.27
222	1628.2	Dodecanoic acid, methyl ester	74	0.25
170	1227.3	(1R,3E,7E,11R)-1,5,5,8-Tetramethyl-12-oxabicyclo[9.1.0]dodeca-3,7-diene	138	0.17
171	1228.8	Phenol, 2,6-dimethoxy-4-(2-propenyl)-	194	0.17
169	1223.4	(1S,3aS,4S,5S,7aR,8R)-5-Isopropyl-1,7a-dimethyloctahydro-1H-1,4-methanoinden-8-ol	167	0.17
234	1852.9	Tridecanoic acid, methyl ester	74	0.16
115	872.5	Eugenol	164	0.16
127	970.8	á-copaene	161	0.11
117	892.2	.alfa.-Copaene	161	0.08
118	893.7	Neric acid	69	0.08
87	694.4	(-)-Carvone	139	0.06
30	370.7	Eucalyptol	154	0.06
131	1006.3	Humulene	93	0.05
132	1010.6	Phenol, 2-methoxy-4-(1-propenyl)-	164	0.03
187	1321.8	Longiverbenone	148	0.03
101	787.2	Thymol	135	0.03
42	432.3	2-Furanmethanol, 5-ethenyltetrahydro-à,à,5-trimethyl-, cis-	94	0.03
80	641	Bicyclo[3.1.1]hept-3-en-2-one, 4,6,6-trimethyl-, (1S)-	107	0.02
125	954.2	Caryophyllene	84	0.02
29	360.4	p-Cymene	119	0.02
56	497.6	Phenylethyl Alcohol	92	0.02
110	853.2	à-Cubebene	161	0.01
248	2248.9	Dodecanoic acid, 10-methyl-, methyl ester	246	0.01
190	1328.5	Apiol	222	0.00*
62	547.4	p-Cymen-7-ol	150	0.00*
*Compound present at trace levels				4.69

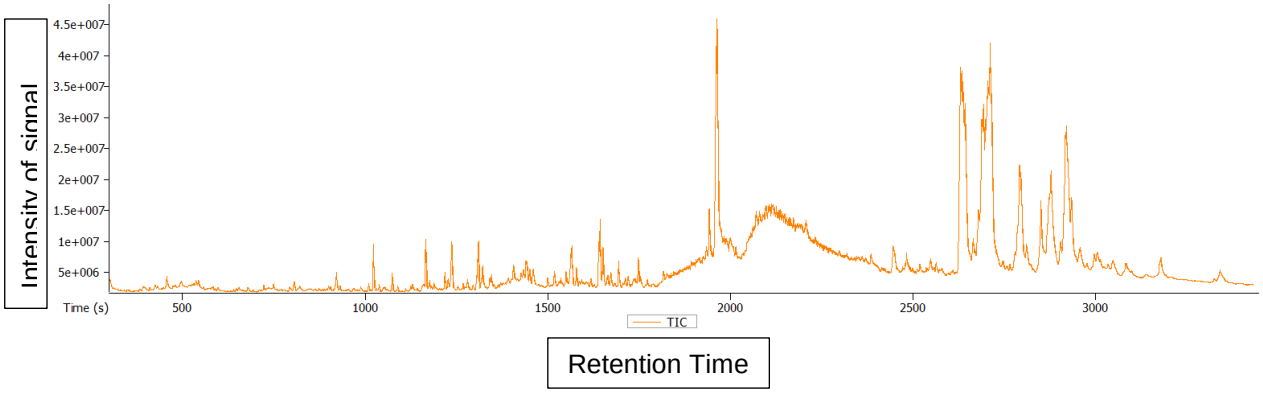

Figure 6.6 A TIC of 10% concentration of acetone extract of *L. leonurus*

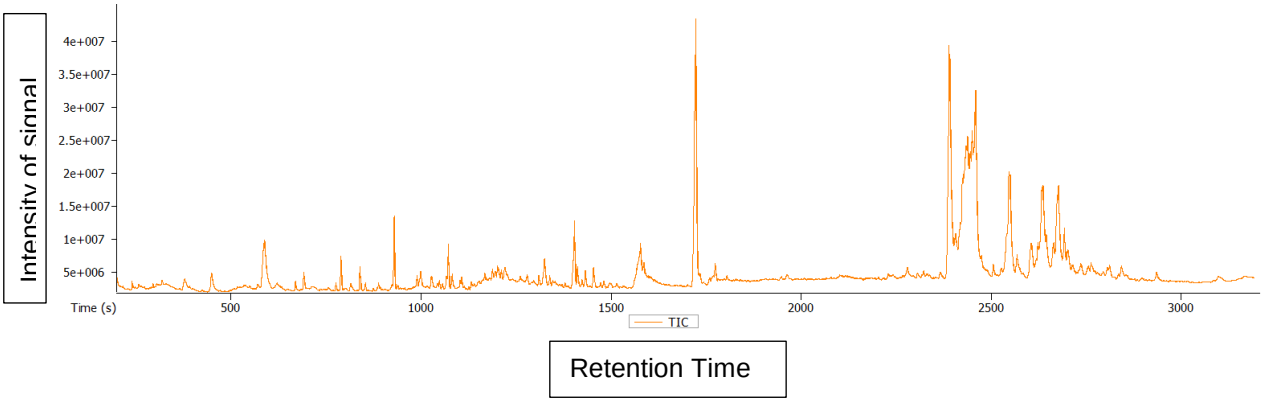

Figure 6.7 A TIC of 10% concentration of ethanol extract of *L. leonurus*

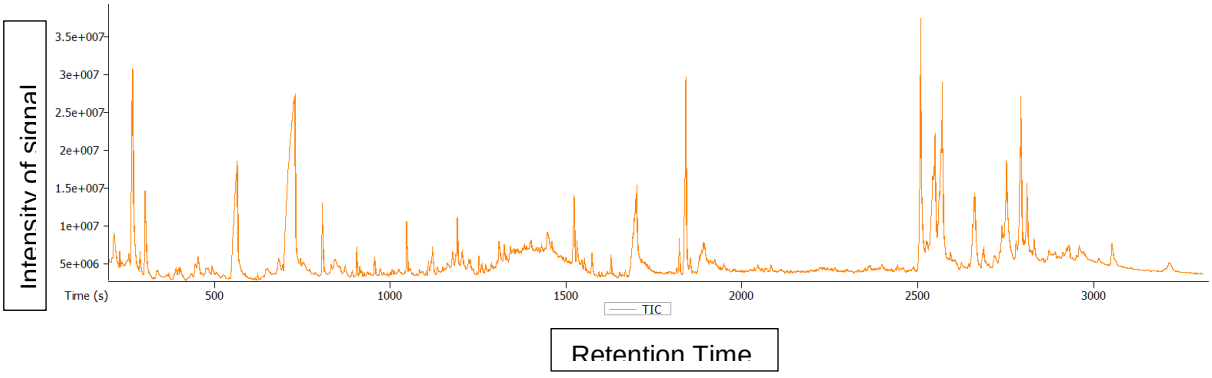

Figure 6.8 A TIC of 10% concentration of methanol extract of *L. leonurus*

As noted in Chapter 5 of this study it was demonstrated that the ethanol extract of *L. leonurus* in the Petri Dish Choice Chamber bioassay, had stronger tick repellent effects (with 81.58% repellency), followed by those of the methanol extract (with 73.94% repellency) and lastly the acetone extract (with 53.89% repellency). In the Tick Climbing bioassay the repellent effects of the acetone (with 48.10% to 56.00% repellency) and ethanol (with 58.27% to 65.17% repellency) extracts were relatively similar with those of methanol (with 46.15% to 60.00% repellency) extract being

slightly lower. However, statistical comparison among the repellencies of the three extracts revealed no significant differences. It is interesting to observe that the three extracts had approximately the same number (36 in acetone extract, 29 in each of ethanol and methanol extracts) of compounds known in literature to have anti-arthropod properties. This observation may also reinforce the view that it is that number of compounds in an extract that act synergistically in effecting a biological effect.

As demonstrated in Table 6.12, the three extracts of *L. leonurus* contained 12 similar compounds known to have anti-arthropod properties. This level of commonality among the extracts might have contributed towards the observed similarities in tick repellency strength of the three extracts.

Table 6.12 A comparative account of compounds with anti-arthropod properties found in the acetone, ethanol and methanol extracts of *L. leonurus*

	Acetone	**Ethanol**	**Methanol**
Name	**Area %**	**Area %**	**Area %**
(1R,3E,7E,11R)-1,5,5,8-Tetramethyl-12-oxabicyclo[9.1.0]dodeca-3,7-diene	0.17	0.18	0.17
(1S,3aS,4S,5S,7aR,8R)-5-Isopropyl-1,7a-dimethyloctahydro-1H-1,4-methanoinden-8-ol	0.14	0.13	0.17
.alfa.-Copaene	0.08	0.21	0.19
Apiol	0.00	0.00	0.00
Benzeneacetaldehyde	0.04	0.05	0.27
Caryophyllene oxide	0.85	0.76	0.54
Eucalyptol	0.20	0.03	0.06
Longiverbenone	0.19	0.32	0.03
n-Hexadecanoic acid	0.06	1.01	1.81
p-Cymen-7-ol	0.05	0.64	0.00
p-Cymene	0.01	0.01	0.02
Phenylethyl Alcohol	0.06	0.01	0.02
Bicyclo[3.1.1]hept-3-en-2-one, 4,6,6-trimethyl-, (1S)-	0.09		0.02
Humulene	0.05		0.05
Phenol, 2-methyl-5-(1-methylethyl)-	0.01	0.01	
Caryophyllene		0.31	0.02
Tetradecanoic acid		0.69	0.32
Thymol		0.13	0.03
à-Cubebene		0.02	0.01
(1R)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene	0.01		
11-Methyldodecanol	0.19		
3-Methyl-4-isopropylphenol	0.02		
5,9-Undecadien-2-one, 6,10-dimethyl-, (E)-	0.08		
Benzene, 1,2-dimethoxy-4-(1-propenyl)-	0.03		
Benzenemethanol, à,à,4-trimethyl-	0.02		
Cyclopropanecarboxylic acid, 2,2-dimethyl-3-(2-methyl-1-propenyl)-, 2-methyl-4-oxo-3-(2-pentenyl)-2-cyclopenten-1-yl ester, [1R-[1à[S*(Z)],3á]]-	0.10		

Table continues on the next page

	Acetone	Ethanol	Methanol
Name	**Area %**	**Area %**	**Area %**
D-Carvone	0.13		
Dodecanoic acid	0.59		
endo-Borneol	0.08		
Isopropyl palmitate	0.03		
Oleic Acid	0.11		
p-Cymene-2,5-diol	0.03		
trans-Verbenol	0.03		
Undecanoic acid, methyl ester	0.35		
1-Cyclohexene-1-carboxaldehyde, 2,6,6-trimethyl-		0.04	
2(3H)-Furanone, 5-heptyldihydro-		0.02	
5,9-Undecadien-2-one, 6,10-dimethyl-, (E)-		0.06	
à-Amyrin		0.22	
Benzeneacetic acid		0.03	
Carvone		0.39	
Dodecanoic acid, 2-(acetyloxy)-1-[(acetyloxy)methyl]ethyl ester		0.31	
Hexadecanoic acid, ethyl ester		0.14	
trans-Geranylgeraniol		0.27	
Undecanoic acid, 11-amino-		0.01	
(-)-Carvone			0.06
2-Furanmethanol, 5-ethenyltetrahydro-à,à,5-trimethyl-, cis-			0.03
Dodecanoic acid, 10-methyl-, methyl ester			0.01
Dodecanoic acid, methyl ester			0.25
Eugenol			0.16
Neric acid			0.08
Phenol, 2,6-dimethoxy-4-(2-propenyl)-			0.17
Phenol, 2-methoxy-4-(1-propenyl)-			0.03
Tridecanoic acid, methyl ester			0.16

Compounds identified from *T. vulgaris* extracts

Compounds identified to have anti-arthropod properties are listed in Tables 6.13 to 6.15 and illustrated in Figures 6.9 to 6.11. Of the three solvents used in the extraction of *T. vulgaris* in this study, the percentage areas of compounds known to have anti-arthropod properties constituted approximately 30% each for acetone and ethanol, whereas those of methanol constituted 21.33%. However, in terms of the number of identified compounds with known anti-arthropod properties the three extracts contained approximately the same number with acetone extract containing 39, ethanol extract 42 and the methanol extract containing 39 of these compounds.

Table 6.13 Compounds with arthropod repellent properties identified from the 10% concentration of the acetone extract of *T. vulgaris*

Peak #	R.T. (s)	Name	Unique Mass	Area %
71	912.5	Phenol, 2-methyl-5-(1-methylethyl)-	118	10.76
76	920.9	Phenol, 2-methyl-5-(1-methylethyl)-	134	3.24
77	921.4	Phenol, 2-methyl-5-(1-methylethyl)-	51	3.24
152	1313	Caryophyllene oxide	147	2.25
69	900.3	Thymol	133	1.77
39	681.1	endo-Borneol	95	1.70
30	576.8	Linalool	71	1.46
17	448.7	Benzene, 1-methyl-3-(1-methylethyl)-	134	1.12
55	793.4	Benzene, 2-methoxy-4-methyl-1-(1-methylethyl)-	164	0.86
108	1076	Caryophyllene	133	0.73
42	698.2	(3R,6S)-2,2,6-Trimethyl-6-vinyltetrahydro-2H-pyran-3-ol	59	0.52
43	698.7	Terpinen-4-ol	71	0.52
56	807.5	Benzene, 2-methoxy-1-methyl-4-(1-methylethyl)-	149	0.44
169	1432	Caryophylla-4(12),8(13)-dien-5à-ol	92	0.42
149	1295	p-Cymene-2,5-diol	151	0.33
19	457.3	Eucalyptol	154	0.22
14	435.5	1,3-Cyclohexadiene, 1-methyl-4-(1-methylethyl)-	121	0.21
65	872.1	Bornyl acetate	95	0.17
28	553.3	Benzene, 1-methyl-4-(1-methylethenyl)-	132	0.14
21	503	ç-Terpinene	93	0.13
58	817.2	Thymoquinone	164	0.11
196	1747	Undecanoic acid, methyl ester	74	0.10
100	1014	Isobornyl propionate	95	0.08
156	1347	(1R,3E,7E,11R)-1,5,5,8-Tetramethyl-12-oxabicyclo[9.1.0]dodeca-3,7-diene	138	0.08
99	1011	.alfa.-Copaene	161	0.07
62	825.1	Thymoquinone	164	0.06
63	845.5	Geraniol	123	0.06
95	984.1	Phenol, 5-methyl-2-(1-methylethyl)-, acetate	135	0.05
24	537.2	11-Methyldodecanol	70	0.04
23	527.3	trans-Linalool oxide (furanoid)	59	0.04
197	1764	4-Terpinenyl acetate	136	0.03
112	1125	Humulene	93	0.02
54	766.5	8,9-Dehydrothymol	148	0.01
27	550.2	1,3-Cyclohexadiene, 1-methyl-4-(1-methylethyl)-	121	0.01
247	2081	Phenol, 2-methyl-5-(1-methylethyl)-, acetate	135	0.01
106	1067	Benzene, 1,3,5-trimethoxy-	168	0.01
61	823.1	Carvone	54	0.01
81	928	Dodecanoic acid, methyl ester	87	0.01
182	1599	Caryophyllene oxide	120	0.00*
*Compound present at trace levels				31.05

Table 6.14 Compounds with arthropod repellent properties identified from the 10% concentration of the ethanol extract of *T. vulgaris*

Peak #	R.T. (s)	Name	Unique Mass	Area %
134	903.9	Bicyclo[3.1.1]hept-2-ene-2-ethanol, 6,6-dimethyl-	64	5.83
88	682.9	Phenol, 2-methyl-5-(1-methylethyl)-	117	5.62
93	692.6	Phenol, 2-methyl-5-(1-methylethyl)-	107	2.55
164	1076	Caryophyllene oxide	79	2.05
17	286.4	Benzene, 1-methyl-3-(1-methylethyl)-	117	1.91
18	286.5	Benzene, 1-methyl-3-(1-methylethyl)-	63	1.88
53	473.7	endo-Borneol	95	1.15
34	382	Linalool	93	0.94
268	2042	Phenol, 2-methyl-5-(1-methylethyl)-, acetate	136	0.77
66	570.2	Benzene, 2-methoxy-4-methyl-1-(1-methylethyl)-	149	0.76
216	1575	n-Hexadecanoic acid	43	0.63
161	1063	p-Cymene-2,5-diol	151	0.62
128	840.4	Caryophyllene	133	0.60
163	1074	3-(4-Isopropylphenyl)-2-methylpropionaldehyde	190	0.58
183	1195	Caryophylla-4(12),8(13)-dien-5à-ol	92	0.55
16	284.5	Dodecane, 1,1-dimethoxy-	47	0.38
182	1177	Caryophylla-4(12),8(13)-dien-5à-ol	187	0.38
215	1570	n-Hexadecanoic acid	229	0.32
56	488.6	Terpinen-4-ol	71	0.32
90	684.6	3-Methyl-4-isopropylphenol	149	0.30
69	583.5	Benzene, 2-methoxy-1-methyl-4-(1-methylethyl)-	149	0.26
217	1586	Undecanoic acid, ethyl ester	88	0.25
32	365.3	trans-Linalool oxide (furanoid)	59	0.25
31	364.7	Benzene, 1-methyl-4-(1-methylethenyl)-	132	0.25
58	502.8	Benzenemethanol, à,à,4-trimethyl-	135	0.18
82	645.4	Bornyl acetate	154	0.18
22	325.5	ç-Terpinene	93	0.17
214	1567	n-Hexadecanoic acid	150	0.13
194	1325	Tetradecanoic acid	60	0.13
19	294.5	Eucalyptol	154	0.12
213	1563	n-Hexadecanoic acid	241	0.11
251	1804	Hexadecanoic acid, ethyl ester	88	0.10
48	451.8	p-Cymene	119	0.08
168	1110	(1R,3E,7E,11R)-1,5,5,8-Tetramethyl-12-oxabicyclo[9.1.0]dodeca-3,7-diene	96	0.08
119	781.2	Isobornyl propionate	136	0.07
114	751.9	Phenol, 5-methyl-2-(1-methylethyl)-, acetate	135	0.07
274	2115	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	239	0.07
208	1508	Undecanoic acid, methyl ester	74	0.06
14	276.9	1,3-Cyclohexadiene, 1-methyl-4-(1-methylethyl)-	136	0.06
118	777.4	.alfa.-Copaene	161	0.05
158	1047	Butanoic acid, 3,7-dimethyl-2,6-octadienyl ester, (E)-	68	0.04
156	1036	Homovanillyl alcohol	137	0.03
192	1291	4-(1-Hydroxyallyl)-2-methoxyphenol	180	0.02
80	642.8	Benzeneacetic acid	91	0.01
63	542	Terpinen-4-ol	86	0.01
132	889	Humulene	93	0.01
				30.95

Table 6.15 Compounds with arthropod repellent properties identified from the 10% concentration of the methanol extract of *T. vulgaris*

Peak #	R.T. (s)	Name	Unique Mass	Area %
80	576.3	Isoborneol	95	5.59
117	795.8	Phenol, 2-methyl-5-(1-methylethyl)-	39	2.77
122	806.7	Phenol, 2-methyl-5-(1-methylethyl)-	91	1.74
37	362	Benzene, 1-methyl-3-(1-methylethyl)-	134	1.20
58	456.4	trans-Linalool oxide (furanoid)	94	0.95
56	454.7	Benzene, 1-methyl-4-(1-methylethenyl)-	132	0.95
178	1193.1	Caryophyllene oxide	106	0.94
60	476.8	Linalool	71	0.77
225	1693.8	n-Hexadecanoic acid	257	0.74
226	1694.5	Pentadecanoic acid	199	0.73
84	592.9	Terpinen-4-ol	71	0.58
175	1184.6	p-Cymene-2,5-diol	151	0.54
101	680	Benzene, 2-methoxy-4-methyl-1-(1-methylethyl)-	149	0.50
147	956.9	Caryophyllene	133	0.45
191	1314.8	Caryophylla-4(12),8(13)-dien-5à-ol	92	0.32
105	694.7	Carvone	82	0.27
43	408.9	ç-Terpinene	93	0.25
190	1296.1	Caryophyllene oxide	187	0.24
104	693.8	Benzene, 2-methoxy-1-methyl-4-(1-methylethyl)-	149	0.21
219	1627.8	Dodecanoic acid, methyl ester	74	0.19
88	610.9	Benzene, 2-methoxy-4-methyl-1-(1-methylethyl)-	149	0.17
224	1683.5	n-Hexadecanoic acid	241	0.16
21	305.4	Decanal dimethyl acetal	75	0.14
38	372.1	Eucalyptol	154	0.13
35	350.3	1,3-Cyclohexadiene, 1-methyl-4-(1-methylethyl)-	136	0.13
52	433.7	Methyl 3-hydroxytetradecanoate	97	0.12
51	433.2	trans-Linalool oxide (furanoid)	94	0.12
182	1228.2	(1R,3E,7E,11R)-1,5,5,8-Tetramethyl-12-oxabicyclo[9.1.0]dodeca-3,7-diene	96	0.09
97	656.3	8,9-Dehydrothymol	148	0.07
196	1358.2	Phenol, 2,6-dimethoxy-4-(2-propenyl)-	194	0.06
202	1445	Tetradecanoic acid	185	0.05
72	531.7	2(3H)-Furanone, 5-hexyldihydro-	85	0.04
255	1897.1	Cyclopropaneoctanoic acid, 2-[[2-[(2-ethylcyclopropyl)methyl]cyclopropyl]methyl]-, methyl ester	242	0.04
140	897.6	Isobornyl propionate	136	0.04
139	892.8	.alfa.-Copaene	161	0.03
148	1006.5	Humulene	93	0.01
142	908	4-Terpinenyl acetate	92	0.01
113	746.1	Benzene, 2-chloro-1-methyl-4-(1-methylethyl)-	153	0.00*
149	1010.3	trans-Isoeugenol	164	0.00*
*Compound present at trace levels				21.33

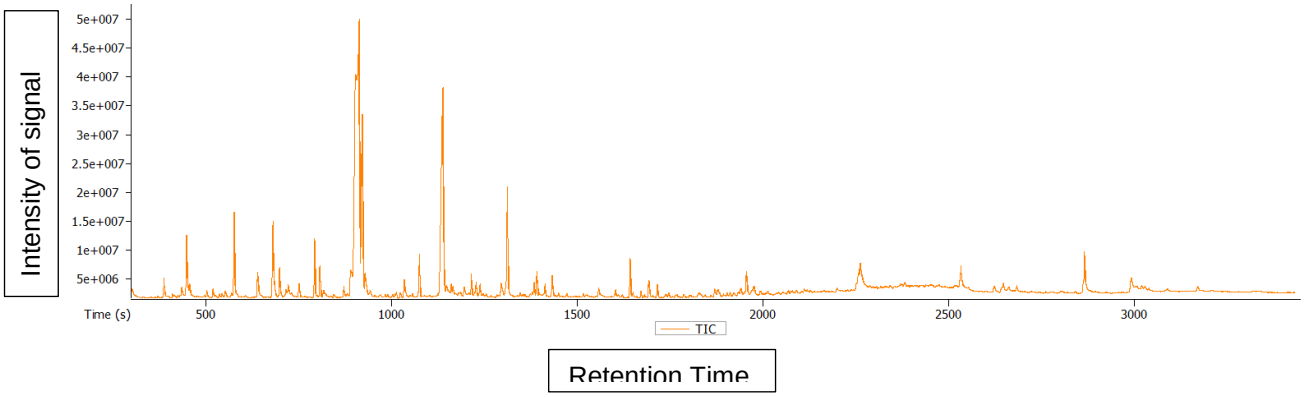

Figure 6.9 A TIC of 10% concentration of acetone extract of *T. vulgaris*

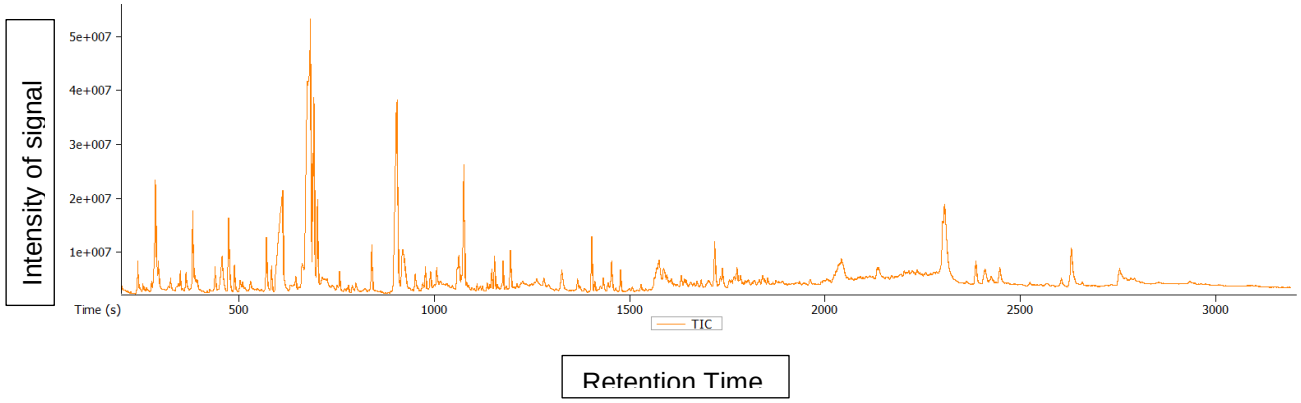

Figure 6.10 A TIC of 10% concentration of ethanol extract of *T. vulgaris*

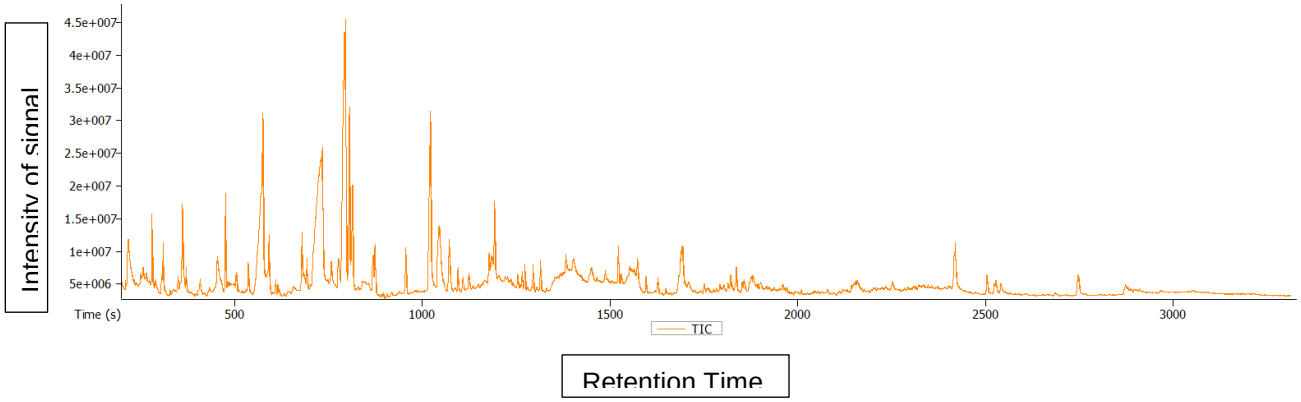

Figure 6.11 A TIC of 10% concentration of methanol extract of *T. vulgaris*

As noted in Chapter 5 of this study it was demonstrated that the acetone, ethanol and methanol extracts of *T. vulgaris* had significantly high repellency effects (with percentage repellencies for acetone ranging from 82.35% to 96.29%, ethanol 75.00% to 94.22% and methanol 80.00% to 88.28%) against ticks in both the Petri Dish Choice Chamber and the Tick Climbing bioassays. The tick repellency values recorded using these two bioassays were significantly higher compared to those of extracts from the other two plant species. Acetone extracts registered the highest

repellency followed by that of ethanol and lastly that of methanol. It is interesting to note that the acetone, ethanol and methanol extracts of *T. vulgaris* contained 18 common compounds known to contain anti-arthropod compounds among them. Based on this observation it is reasonable to suggest that this high number of commonalities might be the basis for the generally high levels of tick repellency observed in this study.

In studies conducted by Pavela (2007) and Pavela *et al.,* (2009) the following active compounds of *T. vulgaris* essential oils with insecticidal and repellent effects include, thymol, p-Cymene, linalool, carvacrol, γ-terpinene, (-)-Borneol, terpineol, α-pinene, α-terpineol, carvone, 4-Allylanisole and trans-Sabinene hydrate. Results from this study, as listed in Tables 6.13 to 6.15, however did not yield α-pinene, α-terpineol, 4-Allylanisole and trans-Sabinene hydrate from the extracts tested. This highlights that sample preparation, essential oil or solvent-specific extraction may have an effect on the compounds obtained in an extract.

As demonstrated in Table 6.16, it is interesting to note that some of the compounds common to the three extracts varied greatly in terms of percentage area. For example, Phenol, 2-methyl-5-(1-methylethyl)- yielded 17.24% from the acetone extract, 8.17% from ethanol and 4.51 from methanol. The observed difference in yield amounts was probably a result of the different solvents used (Azmir *et al.,* 2013).

Table 6.16 A comparative account of compounds with anti-arthropod properties found in the acetone, ethanol and methanol extracts of *T. vulgaris*

	Acetone	**Ethanol**	**Methanol**
Name	**Area %**	**Area %**	**Area %**
(1R,3E,7E,11R)-1,5,5,8-Tetramethyl-12-oxabicyclo[9.1.0]dodeca-3,7-diene	0.08	0.08	0.09
.alfa.-Copaene	0.07	0.05	0.03
1,3-Cyclohexadiene, 1-methyl-4-(1-methylethyl)-	0.22	0.06	0.13
Benzene, 1-methyl-3-(1-methylethyl)-	1.12	3.79	1.20
Benzene, 1-methyl-4-(1-methylethenyl)-	0.14	0.25	0.95
Benzene, 2-methoxy-1-methyl-4-(1-methylethyl)-	1.30	1.02	0.88
Caryophylla-4(12),8(13)-dien-5à-ol	0.42	0.93	0.32
Caryophyllene	0.73	0.60	0.45
Caryophyllene oxide	2.25	2.05	1.18

Table continues on the next page

	Acetone	Ethanol	Methanol
Name	**Area %**	**Area %**	**Area %**
ç-Terpinene	0.13	0.17	0.25
Eucalyptol	0.22	0.12	0.13
Humulene	0.02	0.01	0.01
Linalool	1.46	0.94	0.77
p-Cymene-2,5-diol	0.33	0.62	0.54
Phenol, 2-methyl-5-(1-methylethyl)-	17.24	8.17	4.51
Terpinen-4-ol	0.52	0.33	0.58
trans-Linalool oxide (furanoid)	0.04	0.25	1.07
Isobornyl propionate	0.08	0.07	0.04
4-Terpinenyl acetate	0.03		0.01
8,9-Dehydrothymol	0.01		0.07
Carvone	0.01		0.27
Dodecanoic acid, methyl ester	0.01		0.19
Bornyl acetate	0.17	0.18	
endo-Borneol	1.70	1.15	
Phenol, 2-methyl-5-(1-methylethyl)-, acetate	0.01	0.77	
Phenol, 5-methyl-2-(1-methylethyl)-, acetate	0.05	0.07	
Undecanoic acid, methyl ester	0.10	0.06	
n-Hexadecanoic acid		1.19	0.90
(3R,6S)-2,2,6-Trimethyl-6-vinyltetrahydro-2H-pyran-3-ol	0.52		
11-Methyldodecanol	0.04		
Benzene, 1,3,5-trimethoxy-	0.01		
Geraniol	0.06		
Thymol	1.94		
Thymoquinone	0.17		
3-(4-Isopropylphenyl)-2-methylpropionaldehyde		0.58	
3-Methyl-4-isopropylphenol		0.30	
4-(1-Hydroxyallyl)-2-methoxyphenol		0.02	
Benzeneacetic acid		0.01	
Benzenemethanol, à,à,4-trimethyl-		0.18	
Bicyclo[3.1.1]hept-2-ene-2-ethanol, 6,6-dimethyl-		5.83	
Butanoic acid, 3,7-dimethyl-2,6-octadienyl ester, (E)-		0.04	
Dodecane, 1,1-dimethoxy-		0.38	
Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester		0.07	
Hexadecanoic acid, ethyl ester		0.10	
Homovanillyl alcohol		0.03	
p-Cymene		0.08	
Tetradecanoic acid		0.13	
Undecanoic acid, ethyl ester		0.25	
2(3H)-Furanone, 5-hexyldihydro-			0.04
Benzene, 2-chloro-1-methyl-4-(1-methylethyl)-			0.00
Cyclopropaneoctanoic acid, 2-[[2-[(2-ethylcyclopropyl)methyl]cyclopropyl]methyl]-, methyl ester			0.04
Decanal dimethyl acetal			0.14
Isoborneol			5.59
Methyl 3-hydroxytetradecanoate			0.12
Pentadecanoic acid			0.73
Phenol, 2,6-dimethoxy-4-(2-propenyl)-			0.06
Tetradecanoic acid			0.05
trans-Isoeugenol			0.00

Section C – Discussion

6.5 Summary

Data presented in this chapter, demonstrated that *A. ferox, L. leonurus* and *T. vulgaris* contain compounds that have anti-arthropod properties. These compounds appear to vary in type and amount in different plant species. In addition, data obtained from this study suggest that the higher the number of the compounds with anti-arthropod properties in an extract, the stronger the repellent effect it showed against ticks. This observation reaffirms the view held by many researchers that biological activities shown by plants are products of synergistic effects of different compounds. Among the three plant species, namely *A. ferox, L. leonurus* and *T. vulgaris*, it is the latter plant species *T. vulgaris* that appears to contain more of the compounds that are known in literature to have anti-arthropod properties. *A. ferox* which contained the least number of compounds known to have anti-arthropod properties, demonstrated a relatively weaker repellent effects against ticks. These observations may have far reaching implications since the yellow powder of *A. ferox* is widely used by small scale farmers in the belief that it reduces tick loads on their animals. However, more studies have to explore the effects of quality or quantity of compounds with anti-arthropod properties on the repellent strength of extracts.

CHAPTER 7
CONCLUSION AND RECOMMENDATIONS

7.1 Conclusions

Introduction

As seen in the literature review chapters, ticks have a significant impact as parasites of animals and are associated with numerous veterinary, medical and economic conditions. Currently, synthetic chemicals are the mainstay in tick control strategies. However, in spite of most chemicals being effective as agents of tick control, the impact of ticks continues to be felt across the world and much more in developing countries. This reality is heightened also by problems such as emergence of tick resistant strains and environmental pollution which accompany the use use and over use of synthetic chemicals for tick control. Undoubtedly, this state of affairs necessitates more research on alternative tick control measures that have less problems.

Literature review and the results obtained in this study have demonstrated that some plant species have compounds that have anti-tick properties. The development of such compounds as agents of tick control and their inclusion as part of the integrated tick control strategy might reduce over-reliance on synthetic chemicals in tick controlled. The benefits of using plants as agents of tick control is that they are biodegradable and as a result pose less threat to the environment.

Evaluation of selected plants as potential repellents

As set out in the first chapter, the second and third study objectives were to test whether the extracts of *A. ferox*, *L. leonurus* and *T. vulgaris* have tick repellent properties or not and also to identify the biological active compounds associated with anti-tick properties.

Using the modified petri dish and tick climbing bioassays described in this study, it was demonstrated that all concentrations (2.5%, 7.5% and 10%) of the acetone, ethanol and methanol extracts of *T. vulgaris* used in this study showed a stronger repellency against both *Am. hebraeum* and *Rh. appendiculatus* ticks compared to commercialised repellents and similar extracts from *L. leonurus*.and *A. ferox*. For *L. leonurus* only the 10 % concentration extracts showed a stronger repellent

potency against *Am. hebraeum* and *Rh. appendiculatus*. Whereas *A ferox* demonstrated a relatively weaker repellent strength compared to the other two bioassays.

It is also important to note results indicated that *T. vulgaris* expressed a strong repelling effect in both species, compared to the *L. leonurus* and *A. ferox* extracts that had a stronger effect on *Am. hebraeum* compared to *Rh. appendiculatus*, highlighting (1) the strength of *T. vulgaris* as repellent, and (2) the importance of testing potential repellents using multiple species, as different tick species might respond variedly to treatment products.

Evaluation on modified *in vitro* repelling bioassays

As seen in Chapter Five, various bioassays, each with its own advantages and disadvantages, can be considered as part of a research model to investigate the potential effect of plant-based repellents on ticks. It is important that both the advantages and disadvantages of all bioassays be evaluated to determine the most cost-effective and minimally invasive tests are conducted to test the effect, if any, of a tested product on the target organism prior to introduction to live hosts.

Results from this study have shown that modifications can be successfully implemented to traditionally used repellent bioassays, such as the petri dish and tick climbing bioassays. The traditional petri dish bioassay used a standard 90mm petri dish to observe the behaviour of ticks. The modification to use a choice chamber allows for a bigger surface area for treated and control filter papers and to have a narrow neutral area to which ticks can be moved during time intervals from where tick movement can be observed. The modified petri dish bioassay using a choice chamber can therefore be considered a viable alternative in the place of the traditional petri dish bioassay. However, as the bioassay is conducted within a closed unit, there is still a concern that the bioassay could report an increased potential effect of an extract, when in the open as in the natural environment it might not be so.

The modified tick climbing bioassays used in this study allowed for simultaneous observation and recording of results on the impact and effect of extracts from the three plant species and a control. As a petri dish bioassay is limited to a horizontal

base, when in the natural plants are vertically positioned, it cannot be entirely relied upon particularly when studying the behaviour of ticks such as *Rh. appendiculatus* which use the climbing questing behaviour in the natural. It is therefore important that a tick climbing bioassay should also form part of an *in vitro* repellent study design.

In addition, the modified tick climbing bioassays used to test for repellency of different plant extracts in this study allowed for simultaneous observation and recording of results on the impact and effect of extracts from the three plant species and a control. In addition, the partitions placed between the individual units may have contributed in limiting the effect of neighbouring units on the behaviour of ticks, and thereby improving the results obtained from the traditional model used. By performing a tick climbing bioassay with the inclusion of attractants has shown a slight decrease in the efficacy of repellent plant extracts, especially when the results are compared with results from the tick climbing bioassay without attractants. This bioassay can assist in distinguishing stronger and weaker repellents from the stronger ones. Nonetheless, *T. vulgaris* demonstrated stronger repellent properties even in the presence of attractants. across all bioassays conducted, supporting the view that this plant species is endowed with compounds that have anti-tick properties.

The efficacy of the preference tick climbing bioassay indicated the potential use of the bioassay to determine the preference in rod selection for questing by *Rh. appendiculatus* ticks. This bioassay is much closer to what is happening in the natural and may be used to determine if ticks would approach a treated rod over the untreated one when questing for hosts.

It is therefore evident in all the bioassays used, that the repellency observed was dose dependent.

Chemical analysis

The repellency effects observed from the plant species tested in this study are supported by the plant compounds identified through GC-MS analysis. *A. ferox* which demonstrated the least repellency across the bioassay, had a lower number (22) of compounds known to have anti-arthropod properties compared to

L. leonurus which had 30 and *T. vulgaris* with 39 compounds. Acetone appears to have extracted more of these compounds than other solvents. Also, based on the evidence provided in this study it appears that it is the number of these compounds in an extract that contributes towards the potency of the repellency compared to the amount of each individual compound.

In summary, this study demonstrated that *T. vulgaris* had stronger repellent tick properties compared to *L. leonurus* and *A. ferox*.

7.2 Recommendations

Based on the evidence reported in this study and that elsewhere in literature, it is increasingly becoming evident that *A. ferox* does may not have the anti-arthropod properties as it is reported to have based on the frequent use by small scale farmers. It is therefore important to further explore the anti-tick properties of *A. ferox* in order to clarify its use for tick control.

The two modified bioassays (Petri Dish Choice Chamber and Tick Climbing bioassays) developed for use in this study demonstrated that they may be considered for use in testing for anti-arthropod properties of plant extracts. Although, these bioassays retained the substantive design of the traditional bioassays, the motivation for their design was to address some of the disadvantages that accompany their use.

Also, future studies should incorporate attractants which play an important role in influencing tick behaviour in the natural as demonstrated in this study. The extent to which a repellent overcomes the stimulus by an attractant may provide a good measure of the repellent strength of an extract.

In addition, this study has demonstrated that *T. vulgaris* was strongly repellent against ticks suggesting that it contains compounds that can be effective agents for tick control. Future studies should further examine the extracts of this plant species particularly their effect against immature tick stages which did not form part of this study.

For the purpose of this study only extracts prepared from acetone, ethanol and methanol were evaluated. Additional analysis can be done for these plant species using alternative solvents. In addition, as highlighted in Chapter Six, sample preparation, such as extract or essential oil, may have an effect on the compounds obtained, therefore the preparation and analysis on the effect of essential oils of these plant species as alternative to extracts tested could also be explored.

As suggested from data obtained in the chemical analysis done in this study, the number of compounds with known anti-arthropod properties appear to be important in determining the strength of the extract repellency. This aspect should be further investigated since it appears to support the synergistic effect theory compared to the individualistic theory.

A number of plant species has already been tested and has been identified to have repelling effects on various other arthopods not specifically ticks. Therefore, further research should be done to investigatre these plants for anti-tick properties. For example, *Artemisia afra* and *Cymbopogan* spp. are among some of the plant species that could be investigated. Zoubiri and Baaliouamer (2011), for example, indicated the repelling effect of *Artemisa herba-alba* and *Artemisa monsperma* against *Chrysomya albiceps* (blow flies) and various *Cymbopogan* spp. against mosquitoes. Both *Artemisia afra* and *Cymbopogan* spp. can be found locally in South Africa and it would therefore be very interesting to know if these plant species may be effective repellents against ticks.

From the results obtained in the study it also highlighted the importance that standardised methods for extractions preparation be developed to ensure that the optimum procedure is followed to yield the highest concentration of biologically active compounds and thereby the strongest acaricides or repellents, and to ensure uniformity in procedures followed in tick research which will contribute to the comparability of results.

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APPENDIX A

ETHICS AND SECTION 20 APPROVALS

UNISA university of south africa

CAES ANIMAL RESEARCH ETHICS REVIEW COMMITTEE

Date: 12/11/2015

Ref #: **2015/CAES/100**
Name of applicant: **Ms EMC Theron**
Student #: **33793298**

Dear Ms Theron,

Decision: Ethics Approval

Proposal: In vitro studies on the anti-tick properties of plant-based compounds against veterinary and economical important ticks in South Africa

Supervisor: Prof SR Magano

Qualification: Postgraduate degree

Thank you for the application for research ethics clearance by the CAES Animal Research Ethics Review Committee for the above mentioned research. Final approval is granted for the duration of the project.

Please note point 4 below for further action.

The application was reviewed in compliance with the Unisa Policy on Research Ethics by the CAES Animal Research Ethics Review Committee on 11 November 2015.

The proposed research may now commence with the proviso that:

1) *The researcher/s will ensure that the research project adheres to the values and principles expressed in the UNISA Policy on Research Ethics.*
2) *Any adverse circumstance arising in the undertaking of the research project that is relevant to the ethicality of the study, as well as changes in the methodology, should be communicated in writing to the CAES Animal Ethics Review Committee. An amended application could be requested if there are substantial changes from the existing proposal, especially if those changes affect any of the study-related risks for the research participants.*
3) *The researcher will ensure that the research project adheres to any applicable*

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national legislation, professional codes of conduct, institutional guidelines and scientific standards relevant to the specific field of study.

4) *The application states that should any compound be found that is effective, further trials will be undertaken to test the compound on animal subjects. The researcher is reminded that a new application will have to be submitted if this further stage of the research becomes a reality.*

Note:

The reference number [top right corner of this communiqué] should be clearly indicated on all forms of communication [e.g. Webmail, E-mail messages, letters] with the intended research participants, as well as with the CAES Animal RERC.

Kind regards,

Signature
CAES Animal RERC Chair: Prof EL Kempen

Signature
CAES Executive Dean: Prof MJ Linington

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agriculture,
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REPUBLIC OF SOUTH AFRICA

Directorate Animal Health, Department of Agriculture, Forestry and Fisheries
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Enquiries: Mr Herry Gololo • Tel: +27 12 319 7532 • Fax: +27 12 319 7470 • E-mail: HerryG@daff.gov.za
Reference: 12/11/1/1/23

Ms EMC Theron
UNISA
Florida (Science) Campus
c/o Pioneer and Christiaan de Wet Avenue
Florida
Johannesburg

RE: Permission to do research in terms of Section 20 of the ANIMAL DISEASES ACT, 1984 (ACT NO. 35 of 1984)

Dear Ms Theron,

Your application received via email 2017-08-30, requesting permission under Section 20 of the Animal Disease Act, 1984 (Act No. 35 of 1984) to perform a research project or study, refers.

I am pleased to inform you that permission is hereby granted to perform the following research/study, with the following conditions :

Conditions:

1. This permission does not relieve the researcher of any responsibility which may be placed on him by any other act of the Republic of South Africa;
2. All potentially infectious material utilised or collected during the study is to be destroyed at the completion of the study. Records must be kept for five years for audit purposes. A dispensation application may be made to the Director Animal Health in the event that any of the above is to be stored or distributed;
3. The study is approved as per the application form dated 28 August 2017 and the correspondence thereafter. Written permission from the Director: Animal Health must be obtained prior to any deviation from the conditions approved for this study under this Section 20 permit. Please apply in writing to HerryG@daff.gov.za;
4. Only disease-free *Rhipicephalus appendiculatus, Hyalomma (marginatum) rufipes and Amblyomma hebraeum*, obtained from the laboratory reared tick colonies from the ARC-OVI Parasites, Vector and Vector-Borne Diseases Laboratory, may be utilised for this study;
5. The above-mentioned ticks must be accompanied by an official letter from the head of the laboratory, confirming that the ticks come from pre-established

colonies already maintained at the OVI and that they are maintained on animals that are disease-free;

6. Collection of blood for tick feeding must be in compliance with the Meat Safety Act, 2000 (Act 40 of 2000) and my only be obtained from CMP in Chamdor abattoir;
7. Permission for the removal of blood from the abattoir must be obtained from the abattoir owner;
8. The blood collected at the abattoir must be accompanied by a letter from the meat inspection official that the blood was collected from animals that passed ante- and post-mortem inspection and did not show any signs of disease;
9. Samples must be packaged and transported in accordance with International Air Transport Association (IATA) requirements and/or the National *Road Traffic Act*, 1996 (*Act* No. 93 of 1996);
10. Ethical approval for the study must be obtained from the relevant authority before the study may start;
11. If required, an application for an extension must be made by the responsible researcher at least one month prior to the expiry of this Section 20 approval

Title of research/study: In Vitro studies on the anti-tick properties of plant-based compounds against veterinary and economically important ticks in South Africa

Researcher (s): Ms EMC Theron

Institution: UNISA

Your Ref./ Project Number: 2015/CAES/100

Our ref Number: 12/11/1/1/23

Expiry date: 2018-12

Kind regards,

DR. MPHO MAJA
DIRECTOR OF ANIMAL HEALTH
Date: 2017 -09- 28

agriculture,
forestry & fisheries

Department:
Agriculture, Forestry and Fisheries
REPUBLIC OF SOUTH AFRICA

Directorate Animal Health, Department of Agriculture, Forestry and Fisheries
Private Bag X138, Pretoria 0001

Enquiries: Mr Henry Gololo • Tel: +27 12 319 7532 • Fax: +27 12 319 7470 • E-mail: HenryG@daff.gov.za
Reference: 12/11/1/1/23

Ms EMC Theron
UNISA
Florida (Science) Campus
c/o Pioneer and Christiaan de Wet Avenue
Florida
Johannesburg

Dear Ms Theron,

RE: Amendment to permission to do research in terms of Section 20 of the ANIMAL DISEASES ACT, 1984 (ACT NO. 35 of 1984)

Your request received via email 2017-11-14, requesting an amendment to the permission given under Section 20 of the Animal Disease Act, 1984 (Act No. 35 of 1984) to perform a research project or study, refers. I am pleased to inform you that the following amenments are hereby granted, with the following conditions :

Conditions:

1. This permission does not relieve the researcher of any responsibility which may be placed on him by any other act of the Republic of South Africa;
2. All potentially infectious material utilised or collected during the study is to be destroyed at the completion of the study. Records must be kept for five years for audit purposes. A dispensation application may be made to the Director Animal Health in the event that any of the above is to be stored or distributed;
3. Disease-free *Rhipicephalus appendiculatus* ticks, obtained from the laboratory reared tick colonies from Clinvet, may also be utilised for this study;

4. The above-mentioned ticks must be accompanied by an official letter from the head of the laboratory, confirming that the ticks come from pre-established colonies already maintained at Clinvet and that they are maintained on animals that are disease-free;

Title of research/study: In Vitro studies on the anti-tick properties of plant-based compounds against veterinary and economically important ticks in South Africa
Researcher (s): Ms EMC Theron
Institution: UNISA
Your Ref./ Project Number: 2015/CAES/100
Our ref Number: 12/11/1/1/23
Expiry date: 2018-12

Kind regards,

MMaja.

DR. MPHO MAJA
DIRECTOR OF ANIMAL HEALTH
Date: 2017 -11- 30

APPENDIX B

RESULTS RECORD SHEETS

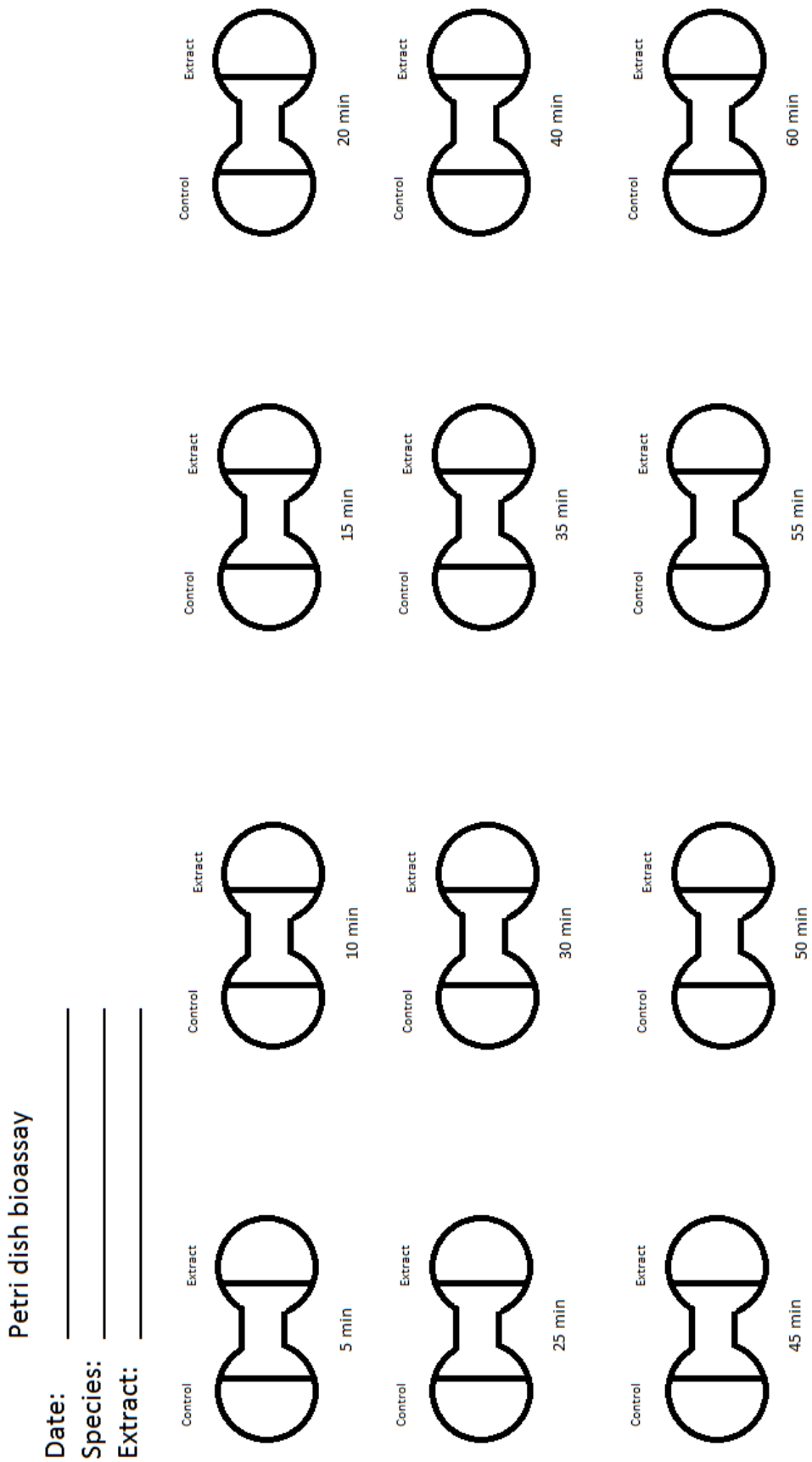


Figure B.1 Choice Chamber Bioassay results sheet

Tick climbing assay

Date: ____________________

Species: ____________________

Solvent: ____________________

Control

Time	# on Treated Filter paper			# on Neutral filter paper			# below filter paper		
15 Min									
30 Min									
45 Min									
60 Min									
90 Min									
120 Min									

Plant: ____________________

Time	# on Treated Filter paper			# on Neutral filter paper			# below filter paper		
15 Min									
30 Min									
45 Min									
60 Min									
90 Min									
120 Min									

Plant: ____________________

Time	# on Treated Filter paper			# on Neutral filter paper			# below filter paper		
15 Min									
30 Min									
45 Min									
60 Min									
90 Min									
120 Min									

Plant: ____________________

Time	# on Treated Filter paper			# on Neutral filter paper			# below filter paper		
15 Min									
30 Min									
45 Min									
60 Min									
90 Min									
120 Min									

Figure B.2 Tick Climbing Bioassay results sheet

Preference Tick climbing assay

Date: _____

Species: _____

Rod 1: _____

Rod 2: _____

Run 1

Time	Rod 1	Rod 2	Not Climbing
15 Min			
30 Min			
45 Min			
60 Min			

Run 2

Time	Rod 1	Rod 2	Not Climbing
15 Min			
30 Min			
45 Min			
60 Min			

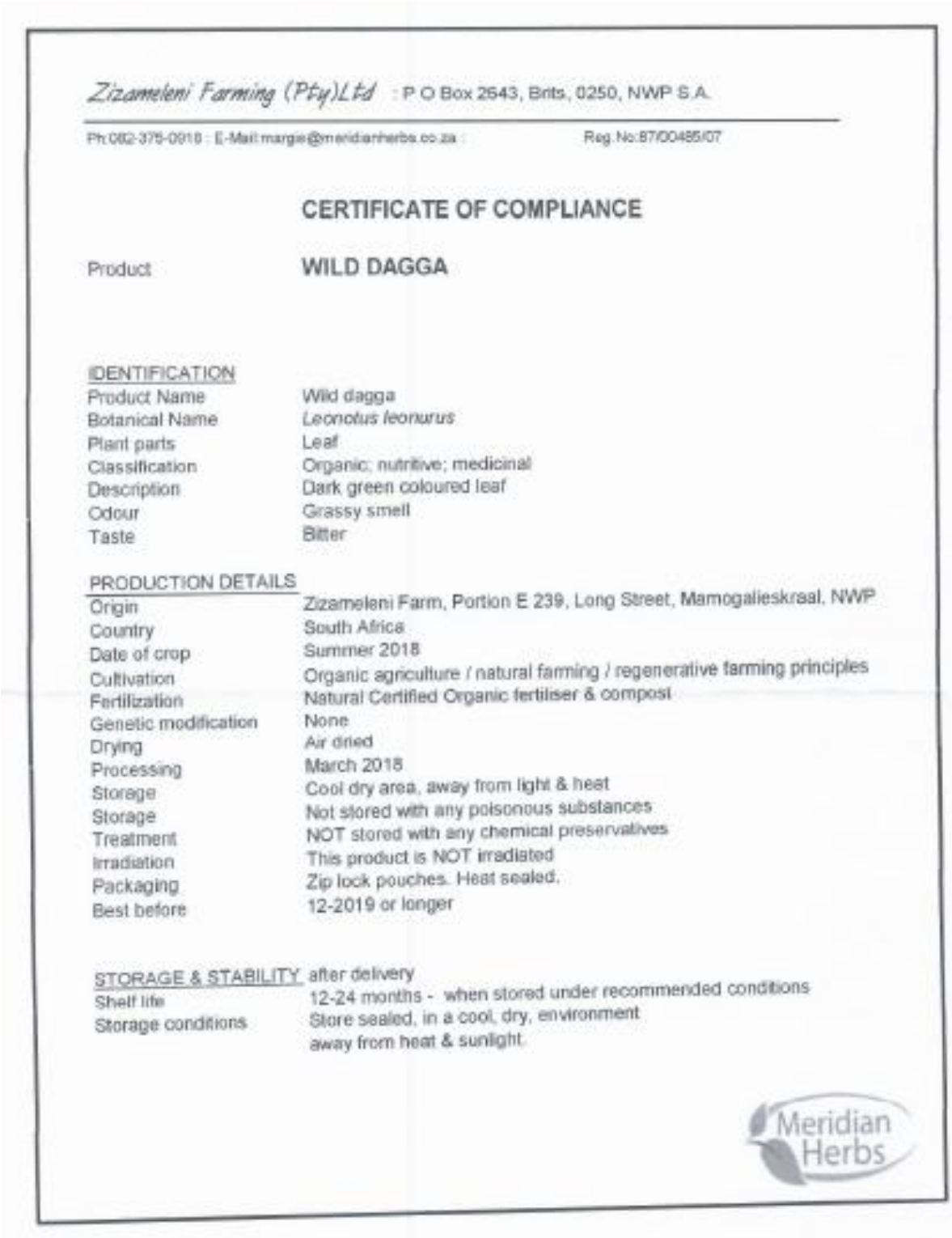
Run 3

Time	Rod 1	Rod 2	Not Climbing
15 Min			
30 Min			
45 Min			
60 Min			

Figure B.3 Preference Tick Climbing Bioassay results sheet

APPENDIX C

PLANT CERTIFICATES



Zizameleni Farming (Pty)Ltd : P O Box 2643, Brits, 0250, NWP S.A.

Ph:082-375-0918 : E-Mail:margie@meridianherbs.co.za : Reg.No:87/00485/07

CERTIFICATE OF COMPLIANCE

Product **WILD DAGGA**

IDENTIFICATION

Product Name	Wild dagga
Botanical Name	*Leonotus leonurus*
Plant parts	Leaf
Classification	Organic; nutritive; medicinal
Description	Dark green coloured leaf
Odour	Grassy smell
Taste	Bitter

PRODUCTION DETAILS

Origin	Zizameleni Farm, Portion E 239, Long Street, Mamogalieskraal, NWP
Country	South Africa
Date of crop	Summer 2018
Cultivation	Organic agriculture / natural farming / regenerative farming principles
Fertilization	Natural Certified Organic fertiliser & compost
Genetic modification	None
Drying	Air dried
Processing	March 2018
Storage	Cool dry area, away from light & heat
Storage	Not stored with any poisonous substances
Treatment	NOT stored with any chemical preservatives
Irradiation	This product is NOT irradiated
Packaging	Zip lock pouches. Heat sealed.
Best before	12-2019 or longer

STORAGE & STABILITY after delivery

Shelf life	12-24 months - when stored under recommended conditions
Storage conditions	Store sealed, in a cool, dry, environment away from heat & sunlight.

Meridian Herbs

Zizameleni Farming (2018a): Certificate of Compliance – Wild Dagga.

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CERTIFICATE OF COMPLIANCE

Product **THYME**

IDENTIFICATION	
Product Name	Thyme
Botanical Name	*Thymus vulgaris*
Plant parts	Leaf with some fine stalk
Classification	Organic; nutritive; medicinal
Description	Dark green coloured leaf
Odour	Slightly spicy smell
Taste	Slightly savoury

PRODUCTION DETAILS	
Origin	Zizameleni Farm, Portion E 239, Long Street, Mamogalleskraal, NWP
Country	South Africa
Date of crop	Summer 2017
Cultivation	Organic agriculture / natural farming / regenerative farming principles
Fertilization	Natural Certified Organic fertiliser & compost
Genetic modification	None
Drying	Air dried
Processing	March 2018
Storage	Cool dry area, away from light & heat
Storage	Not stored with any poisonous substances
Treatment	NOT stored with any chemical preservatives
Irradiation	This product is NOT irradiated
Packaging	Zip lock pouches. Heat sealed.
Best before	12-2019 or longer

STORAGE & STABILITY	after delivery
Shelf life	12-24 months - when stored under recommended conditions
Storage conditions	Store sealed, in a cool, dry, environment away from heat & sunlight.

Meridian Herbs

Zizameleni Farming (2018b): Certificate of Compliance – Thyme.