

**EVALUATING THE EFFECTIVENESS OF DIFFERENT BIORATIONALS AND
ACARICIDE ON TICKS CONTROL IN CATTLE.**

OYIRWOTH GRACIOUS

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DECLARATION

I Oyirwoth Gracious, do hereby declare that this special project titled “Evaluating the Effectiveness of different Biorationals and Acaricide on Tick control in cattle” is my original work, of my own research and findings; and to the best of my knowledge, has never been published or submitted to any university or institution for the award of any academic award.

Mr. OYIRWOTH GRACIOUS

Faculty of Agriculture

Bachelors of Science in Agriculture

APPROVAL

This special project report has been written by Oyirwoth Gracious and read by Mr. Andrew. E. Briggs, Prof. Peter Odoch, Bro. Aloysius Byaruhanga, Dr. John Byalebeka and Prof. Walter Tita under the supervision of Dr. Nina Pius. I therefore approve and forward to the Department of Agriculture for consideration as a requirement for my final examination.

Dr. Nina Pius

Faculty of Agriculture

DEDICATION

I dedicate this work to the Almighty God who has enabled me achieve success at every stage throughout my academic period. I also dedicate this work to my mother Nagageno M Cheline Family, Uncle Joel Cox Ojuko, FIC Center for Sustainability, Echo community, Uganda Martyrs University and Education for Sustainable Development & Community Engagement for the tireless efforts in supporting me while pursuing my Bachelors of Science in Agriculture program.

ACKNOWLEDGEMENT

The process of writing this report repeatedly was hectic and tedious. Once I was done, I felt great, my posture improved, my eyesight got clearer, and my world seemed great. I imagine this is what giving birth feels like. I was fortunate to have a great team of people who helped me turn this research into its final form. I would like to begin by thanking the Almighty God for the wisdom, knowledge, understanding, strength and health to accomplish this report successfully in time. I would also like to thank my parents, Mrs. Ngageno M Cheline, late Mr. Ngageno J Sanctus and Mr. Joel Cox Ojuko for their financial support and encouragement during my study. Dr. Nina Pius, Dr. John Byarebeka, Br. Aloysius Byaruhanga and Br. Murongo Marius Flarian simply the finest researchers and supervisors, I have ever worked with. They were instrumental in helping me bring this report together through their guidance, tireless effort, time and financial support given during the period of the study and May the almighty God reward them abundantly in all their endeavors. I would also love to thank the animal department assistant farm manager livestock Mr. Gerald kafuuma, Mr. Kato Davis and Dr. John Byalebeka who made it a success for all the Evaluation to be studied well from the laboratory and all the workers at the Equator Valley Farm for making the activities to be a success. They ardently contributed immerse work towards my academics and may the almighty God Sufficiently Bless them. I also thank my colleagues that played a central role as during the research notably, Mr. Kato Davis among others, and I all wish them the very best in their endeavors.

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ABSTRACT

Background

Farmers in Uganda are faced with many diseases that limit the productivity of their animals, many of these are caused by tick infestations. Years of use and overuse of available chemical ectoparasiticides have resulted in the large scale development of resistance in these parasites as well as negative environmental impacts. To reduce these impacts, much focus has been placed on the search for alternative, environmentally friendly parasite control strategies with lower chance of the development of resistance. Many rural farmers have used plants to control ticks. In some cases, the traditional use has been confirmed, in other cases, only the traditional use has been documented. A review of published scientific articles was conducted for medicinal plants with *in vitro* acaricidal or tick-repellent activities against immature and adult stages of ticks.

However, little information has been studied on the organic biorationals and dangers of acaricide in Uganda as a tick control measure in cattle and for its Improvement and Development. The objectives of the study were; To determine the effectiveness of different biorationals as a tick control measure and To determine the effectiveness of different plant extracts on tick mortality

Methodology

Five plant materials were used in the evaluation with 90 – 100% efficacy according to the South African Journal of *Botany* (*Azadirachta Indica*, *Gynandropsis gynandra*, *Lavendula augustifolia*, *cymbopogan spp* and *euphorbia hirta*). The plant materials were washed, shade dried, crushed and maceration method was used for the extraction of the plant phytochemicals which were used for the evaluation.

Results

The results indicated that among the different plant extracts on tick control, *Azadirachta Indica* concoction of ratio 1:1(10ml organic recipe concoction: 10ml water) significantly caused an effect (< .001) on the ticks in respective petri dishes and this was observed after 48 hours and 72 hours of treatment application. At 24 hours of treatment application no single tick was observed dead neither weak because the concoction was not yet very strong. The finding further indicates that 1:1 (10ml organic recipe concoction: 10ml water) of *Azadirachta Indica* at 48 hours, it was observed that from 12 petri dishes having a total of 12 ticks, 3.133 were observed dead whereas at 72 hours many (5.431) ticks continued to die.

CHAPTER ONE: INTRODUCTION

1.0 Introduction

1.1 Background of the Study

The cattle tick, *Rhipicephalus (Boophilus) microplus*, is an economically important ecto-parasite of livestock and creates major problem for milk producers in tropical and subtropical countries including India. It causes severe economic losses by blood loss, reduction in weight gain, direct damage to skin and hides and also by serving as a vector of infectious diseases (Ghosh et al. 2006; Ghosh and Nagar 2014). In order to manage this ecto-parasite, different groups of chemicals viz., synthetic pyrethroids, organophosphates, formamidines and macrocyclic lactones are used widely. Such strategies are aimed to prevent damage and forestall an epidemic by keeping the populations below a critical threshold level. However, widespread use of these chemicals causes serious ecological problems. The use of chemicals to control ticks on cattle usually generates hundreds of gallons of residues (3–4 liters of solution per animal) which are often discarded indiscriminately, leading to soil and water contamination (Gromboni et al. 2007). These chemicals kill non-target organisms and threaten human health due to the toxic residues in milk and meat (Graf et al. 2004). Another most serious problem of intensive use of these chemical acaricides is the development of resistant tick populations which causes failure of the chemical-based tick control program (Rosario-Cruz et al. 2005; Rodriguez-Vivas et al. 2006a, 2006b).

Uganda's agriculture potential is enormous with vast areas of the country suitable for livestock production. The present cattle population of Uganda is estimated at 12 million according to the Uganda Bureau of statistics (UBOS 2017). The report from New Vision of 3rd October 2009 shows that Northern Uganda had 1.6 million cattle as compiled results from National Livestock Census 2008. This livestock sector has been contributing considerable portion to Uganda's economy, and still promising to rally round the economic development of the country. According to previous official estimates, livestock contributed 1.7% to national GDP in 2009 and therefore the revised estimates would now contribute 3.2 of the national total. In Uganda, livestock is a major asset among resource-poor smallholder farmers. It provides cultural pride (traditional purposes), milk, meat, skin, and manure and traction force. The contribution of livestock to the national economy particularly with regard to foreign currency earnings is through export of live animals, milk, meat, skin and hides. The continuous presence of tick infestations and tick-borne diseases is a worry to

the livestock industry. Ticks are nuisance to livestock and have caused economic losses in milk production, quality of hides and productive potential of livestock sector.

According to Wanzala et al. (2005), most animals kept by poor resource farmers are affected by endemic pathogens. Although Uganda's livestock sector has got other factors constraining the production that include, limited access to quality inputs, poor marketing strategies, lack of value addition to livestock products, political rivalry, limited research among many more, the economic control of ticks and tick-borne disease (TBDs) is probably the single most important animal health issue affecting cattle in Uganda. (Mugisha et al., 2005; Ocaido et al., 2009). Despite the potential, the country's livestock has been hardest hit by the presence of ticks and tick-borne diseases (TBD), with over 30% of the calf crop lost to TBDs such as **theileriosis**, **babesiosis**, and **anaplasmosis**. Tick are hematogenous vertebrates' ectoparasites which transmit viral, bacterial and protozoal diseases (De la Fuente et al., 2008). The presence of the ectoparasites cause high economic losses due to effect on the skin and causes anaemia by ingesting the blood of the host (Abbas et al., 2014). The harmful effects of ectoparasites on the productivity of livestock are well documented (Bagavan et al., 2009, Gazim et al., 2011).

There is resistance of ticks to the different synthetic acaricide compounds used that include Amitraz, Cypermethrine, Dimethrine of common brands like Alfapor, Amitix, Syptertix among many more others in markets in cattle farming communities. Years of use and overuse of available chemical ectoparasites have resulted in the large scale development of resistance in these parasites as well as negative environmental impacts. To reduce the impacts, I have been intrigued to focus on placements that will search for alternative, environmentally friendly parasite control strategies with lower chances of developing resistance. Many rural farmers have used plants to control ticks in many other countries. In some cases, the traditional use has been confirmed, in other cases, only the traditional use has been documented.

Commercial and/or synthetic acaricides are toxic to both livestock and can cause cytotoxic and genotoxic effect on man, and can also be destructive to the environment and the ecosystem if incorrectly handled (Ündeğer and Basaran, 2005). Although ticks and tick borne diseases are a major challenge for many small scale livestock farmers in the developing world, farmers don't have the resources to solve the problem, and generally they cannot afford the high cost of these conventional acaricides (Mwale et al., 2005). Tick-borne diseases are the number one constraint of cattle production in Uganda and not sparing Northern Uganda, Gulu and Omoro . Acaricide failure has been spreading through farms, causing more and more cattle to fall ill with diseases (TBD), and costing farmers' time and money. The development of multi-acaricide resistant ticks could have possibly been delayed or even avoided if farmers had not taken tick control into their own hands.

Therefore, it is important to educate farmers in the sub counties of these Districts and others within the country on how to sustainably manage and control ticks. Acaricide failure places a tremendous financial burden on Ugandan farmers; not only does it lead to a high loss of cattle to TBD, but the costs of the acaricide used themselves account for about 90% of an average farmer's total disease control budget, making non-functional acaricide a major budgetary loss for farmers according to vudriko et al. The frequent occurrence of acaricide failure has raised the possibility that certain ticks are becoming resistant. Farmers have used acaricide, pesticides that target ticks and mites, as a tool to control the vectors. The control of parasitism largely depends on the use of synthetic drugs, however, there is development of resistance in parasites against the drugs (Miller et al., 2007, Saeed et al., 2007). Use of acaricides has also got disadvantages of harmful effects on environment, ecosystem, as well as to humans and livestock (Garcia-Garcia et al., 2000). In addition there is the problem of chemical residue in animal byproducts (Turbin et al., 2006).

The application of acaricides in dips and sprays to control cattle ticks has had a profound influence on livestock productivity through the significant reduction in the prevalence of tick infestations and tick borne diseases (Yilima et al., 2001). However, the progressive evolution of resistance of ticks to almost every available class of acaricide continues to frustrate the efforts of cattle farmers (George et al., 2004). Uganda is attempting to increase cattle production by improving its national herd through the introduction of exotic cattle breeds. The same favorable climatic conditions for cattle also support a large tick population which provides transmission of four important tick-borne

diseases (TBDs): East Coast fever (ECF) (*Theileria parva*), Anaplasmosis (*Anaplasma marginale*), Babesiosis (*Babesia bigemina*) and heart water. In view of the problem associated with use of chemical acaricides, there is need to search for alternatives control strategies that can overcome the difficulties with synthetic product (Christopher et al., 2009, Hamad et al., 2014, Masood et al., 2013). This was a report on the research of the assessment of the efficacy of *Tephrosia vogelii* (*T. vogelii*) plant extracts in tick control and reduction of ticks on selected animals for experiment at Njola veterinary camp in Monze district of Southern province, Zambia.

1.2 Statement of the problem.

To tackle the problem of resistance and other environmental issues linked with chemical control, efforts have been made to develop sustainable immunological means for controlling ticks and tick-borne diseases. As a landmark development, two commercial vaccines against R. (B.) microplus were developed and marketed (de la Fuente et al. 1998, 1999; Willadsen 2004). The vaccine efficacy was reassessed after 10 years of introduction and significant protection was reported with reduced frequency of acaricidal usage (de la Fuente et al. 2007). Encouraging data have also been developed to control other economically important tick species, *Hyalomma anatolicum* (Azhahianambi et al. 2009; Jeyabal et al. 2010; Binod Kumar et al. 2012). However, the efficacy of the vaccine was found highly variable (de La Fuente et al. 2000; Canales et al. 2009) and farmers' expectation has not been met. Moreover, the vaccine is not giving significant protection against multi-species tick infestations, a common problem faced by the livestock owners of Asia and Africa. Other studies involving fungal biopesticides and entomopathogenic nematodes proved unsuccessful (Benjamin et al. 2002; Polar et al. 2005). Thus, there is a keen interest in the development of alternative ecofriendly anti-tick natural products. Amongst the natural products, plant extracts and essential oils have been shown to have significant activity against economically important tick species (Borges et al. 2003; Pereira and Famadas 2006; Fernandes and Freitas 2007; Kamaraj et al. 2010; de Souza Chagas et al. 2012; Juliet et al. 2012; Sunil et al. 2013) including acaricide resistant species (Ghosh et al. 2011, 2013). Moreover, these botanicals are found to contain a mixture of active substances which can delay or prevent the development of resistance to herbal products (Wang et al. 2007).

Although Uganda has got the potential for livestock production due to enormous land, the economic control of ticks and tick borne diseases has still remained the biggest challenge in the

production according to Vudriko. Further still, having experienced and also witnessed the devastating effect of ticks at FIC Center for Sustainability at a Management capacity FIC farm animals, communities surrounding FIC, Gulu, Omoro and Budongo subcounty at NFC, interacting with the farm manager Mr. Bisesira Charles, and also having witnessed the tick invasion, it was concluded on estimation that the cattle are attacked by 150 to 200 plus ticks weekly regardless of whether they are sprayed or not. The same is true with Nkozi community animals – as a student studying at the Equator Valley Farm, Faculty of Agriculture at Uganda Martyrs University, having interacted with the Assistant Farm Manager Livestock, Mr. Kafuuma Gerald who mentioned that on average an estimated 150 to 200 plus ticks attack the farm animals and cases of TBDs have been registered as well.

There is also a deadly effect of the use of acaricides to humans and the environment. Acaricide affect the human health (skin irritations and respiratory system) if used without wearing protective gears (facial and nose mask, overall jacket, hand gloves and gumboots) and when products are mishandled. Knowledge gap in the use of the acaricide among the farmers in Central and Northern Uganda is still a big challenge and besides, most of the farmers lack knowledge on how to make and use organic biorationals in the control of ticks.

Tick-borne diseases (TBDs) are responsible for economic losses in livestock production in the Sub-saharan countries (Makala, 2003). Farmers, lack access to the conventional livestock management skills and financial resources to afford chemical acaricides and curative substances. Njoroge and Bussmann (2006) reported that conventional methods for control of ticks and TBDs are costly for most small scale livestock farmers. Ticks and TBD's are still the major constraints of cattle production in very many countries including Uganda. Southern Province lost 995 (28.9%) cattle out of 34437 due to ECF in 2008 alone (Anon, 2009). It is, therefore, very desirable that alternative methods of tick control that are less costly are sought in order to alleviate the problem of TBD's in resource poor livestock farming sector.

With all the above, there is great need for ecofriendly solutions against this enemy. Since a large portion of the ticks are now resistant to current acaricide, alternative tick control options are needed. (use of organic biorationals). In addition to spreading tick-borne diseases to animals, some of the diseases transmitted by ticks are of concern to humans too. Ticks are capable of transmitting

diseases such as Rocky Mountain spotted fever by just a bite that's caused by bacteria Rickettsia in both humans and pets. They also spread the bacterial diseases tularemia, ehrlichiosis and tick paralysis according to healthy living newsletter (every day Health).

1.3 Research objectives.

The purpose of this research was to evaluate the effectiveness of biorationals in controlling ticks in cattle.

1.3.1 Specific objectives

To determine the effectiveness of different biorationals as a tick control measure.

To determine the effectiveness in different plant extracts on tick mortality

1.4.1 Hypothesis

There is significant difference in different organic biorationals concentration on tick mortality

1.5 Justification for the study

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

Widespread use of chemical acaricide causes serious ecological problems. The use of chemicals to control ticks on cattle usually generates hundreds of gallons of residues (3-4 liters of solution

per animal) which are often discarded discriminately, leading to soil and water contamination (Gromboni et al. 2007). The chemicals kill non-target organisms and threaten human health due to the toxic residues in milk and meat (Graf et al. 2004). Another most serious problem of intensive use of chemical acaricide is the development of resistant ticks' populations which causes failure of the chemical-based tick control program (Rosario-Cruz et al. 2005; Rodriguez-Vivas et al. 2006a, 2006b).

1.6 Significance of the study

The study findings will empower cattle farmers to adapt to the use of organic biorationals in the control of ticks and tick borne diseases (TBD), thus minimizing costs associated with acaricide use. It will also empower the community's realization of the dangers of acaricide when used wrongly and carelessly and their health impacts to humans and the environment and eventually they will learn more technical skills of how to control ticks using locally available organic biorationals from ethno plant materials.

The study will improve on the house hold income of small house hold farmers in a sense that they will not suffer economic loses to TBD and so, more farmers will be motivated, awakened and encouraged to keep cattle. The study will be of relevance to future scholars in the same field of Agriculture and ecological studies as a basis for further research in different areas. The study will help the governments to come up with more stringent sustainable management, realistic policies and interventions on soils, water, human health and the ecosystem as a whole which will be adopted by everyone within the farming and non-farming communities.

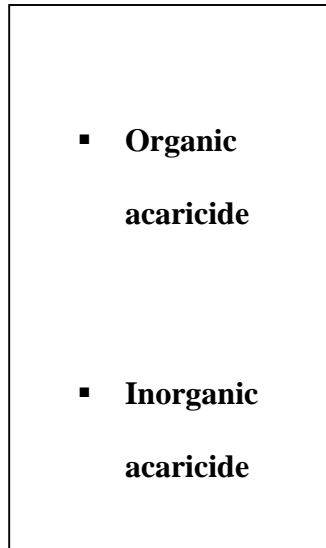
And finally the study will help the researcher to develop organic biorational certified products that will be locally manufactured, packed, branded and distributed to veterinary drug shops and dealers for business and charity. However, in conclusive summary to the significances mentioned above, the research experiment will generate results that will ensure the communities mentioned to adopt to the use of organic recipes. The research experiment will be of importance to farming community and if the problem of ticks in dairy and beef production is not addressed in these farming communities, the farmers will continue to incur high production costs in attempts to control ticks chemically.

If the problem of ticks is not tackled, farmers will continue to lose their animals to TBD and eventually more cattle farmers will be discouraged the more from keeping cattle and so this will eventually affect production in terms of GDP

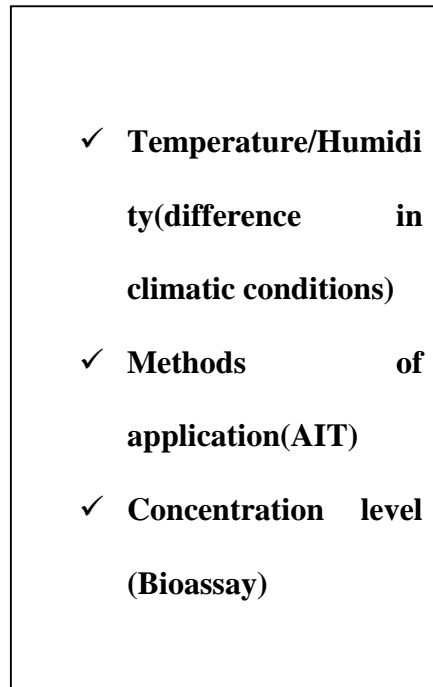
1.9 Conceptual framework

The study experiment is to evaluate the effectiveness of organic biorationals and acaricide on ticks in cattle. The study is guided by three variables, independent, dependent and intervening variables.

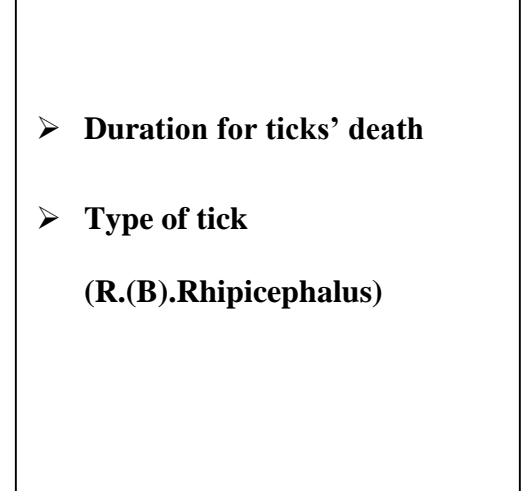
INDEPENDENT VARIABLES



INTERMEDIATE VARIABLE



DEPENDENT VARIABLES



The above diagram shows that the independent variable causes the dependent variable and the intervening variable influences the relationship between the independent and dependent variables. Factors influencing the tick control in cattle that is to say, the use of organic recipe or acaricide has resultant effects on ticks where by the death rate in terms of the duration taken for the ticks mortality after the chemical application (time scope) is basically affected by factors such as the concentration level of either the acaricide or the organic recipe, method of application, specific type of the chemical used, rain, temperature/humidity levels, tick habitat, light effect and rains

1.10 Definition of key important terms concepts.

Investigation. This is the finding more details on a subject (probing more facts on the subject matter)

Organic Biorationals: These are pesticides that have relatively low toxicity and cause relatively little damage to the environment

Acaricide: Acaricides are pesticides that kill members of the arachnid subclass Acari, which include ticks and mites.

TBD: Tick borne disease

Independent variables. These are the causes of the dependent variables and can be manipulated.

Dependent variables. These are the outcomes which has shown either positive or negative as the result of using independent variables

Intervening variables. These are factors which influence both independent and dependent variables.

NFC: Nyabyeya Forestry College

FIC: Freedom in Creation

CHAPTER TWO: LITERATURE REVIEW

2.0 Taxonomy of ticks

Ticks belong to the phylum Arthropoda, Subphylum Chelicerata, Class Arachnida and Subclass Acari. (syn. Acaria, Acarina, Acarida) include ticks. There are two well established families of ticks, the Ixodidae (hard ticks) and the Argasidae (soft ticks), (Sonenshine, 1991). According to (Horak et al., 2002) and (Horak, 2009), there are 867 described species of ticks in the World of which 48 are important species to the health of domestic animals in Africa (Walker et al., 2007). The most important Ixodid tick genera include *Amblyomma*, *Boophilus*, *Dermacentor*, *Hyalomma*, *Haemaphysalis*, *Ixodes* and *Rhipicephalus* (Jongejan and Uilenberg, 2004). Studies by (Murrel et al., 2000) and Baker and Murrel (2002) concluded that the genus *Rhipicephalus* is associated to the genus *Boophilus*.

Also Horak in 2009 reclassified this genera as Subgenera to the genus *Rhipicephalus* in their world list of the valid tick names. Acari consists mostly of mites and ticks, which can be distinguished by their larger size and their exclusive parasitic mode of feeding (Walker et al., 2007). All cattle ticks belong to the family Ixodidae. Thus, the important tick species that infest cattle in Zambia are *Rhipicephalus (Boophilus) decoloratus* (Koch), *Rhipicephalus (Boophilus) microplus* (Canestrini), *Rhipicephalus appendiculatus* Neuman, *Rhipicephalus zambeziensis* Walker, Norval and Corwin, *Amblyomma variegatum* (Fabricius), *Hyalomma truncatum* Koch and *Hyalommama riginatumrufipes* Koch (Speybroeck et al., 2002 and Berkvens et al., 1998).

2.1 Introduction and life cycle

According to Barker (2004) ticks are small arachnids, typically 3 to 5 mm long, part of the order parasitiformes. Along with mites, they constitute the subclass Acari. Ticks are ectoparasites (external parasites), living by feeding on blood of mammals, birds and sometimes reptiles and amphibians. Ticks had evolved by the cretaceous period; the most common form of fossilization being immersed in amber. Ticks are widely distributed around the world, especially in warm, humid climates.

2.2 Classification of Ticks

Kingdom	Animalia
Phylum	Arthropoda
Subphylum	Chelicerate
Class	Arachnida
Subclass	Acari
Super Order	Parasitiformes
Order	Ixodidae
Phylum	Arthropoda
Super Family	Ixodoidea

There are three families of ticks: The Argasidae, the Ixodidae and the Nuttalliellidae. The Argasidae are known as “soft” ticks and the Ixodidae “hard” ticks, as the Ixodidae have a hard scutum, or shield, on their dorsal surface, whereas the Argasidae have a leathery cuticle. The Nuttalliellidae have features which are intermediate between the other two families. The Ixodidae can be further classified according to the number of hosts required to complete their life cycle from larva to nymph to adult.

Almost all ticks belong to one of two major families (Mans BJ et al 2002), the Ixodidae or hard ticks, which are difficult to crush, and the Argasidae or soft ticks. Adults have ovoid or pear-shaped bodies which become engorged with blood when they feed, and eight legs. As well as having a hard shield on their dorsal surfaces, hard ticks have a beak-like structure at the front containing the mouthparts whereas soft ticks have their mouthparts on the underside of the body. Both families locate a potential host by odor or from changes in the environment. A study conducted by Vudriko et al 2016 set out to uncover the reason behind the increasing reports of acaricide failure in

Uganda. Of the 54 farms tested, 94.4% of them had complaints of acaricide failure, which prompted questions about tick resistance as well as acaricide application methods. To address these questions, Vudriko et al. examined the acaricide application techniques used by farmers and also tested the tick larvae they found on the cattle for acaricide resistance. The results showed that 93.5% of the larvae population they tested was resistant to at least one acaricide. The resistant larvae were identified as *Rhipicephalus* genus ticks. This was the first study in Uganda to report the emergence of multi-acaricide resistant ticks. Ticks' resistance to multiple acaricide increases the danger of cattle being infected with TBD, because these ticks can survive even a combination of tick-attacking techniques.

Vudriko et al. also found that many farmers were guilty of misusing their acaricide. When certain acaricide started to fail, farmers often try to find a quick fix by mixing chemicals, and creating their own acaricide application methods. These farmer-created errors could be partially to blame for the dramatic rise in multi-acaricide resistance. When a mixture of acaricide is applied surviving ticks carry a gene that allow them to resist all of the acaricide in that mixture. After they reproduce, future generations of ticks, harboring the resistance genes, will be resistant to multiple acaricide.

2.2.0 Geographical distribution of ticks

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

2.2.1 Life cycle of ticks

According to Sonenshine (1991), Larvae hatch within days to months of the eggs being laid, depending on the environmental conditions. After finding a suitable host and feeding, the larva drops off and molts into a nymph. The nymph finds the next appropriate host and feeds for several days to a week. After engorging, the nymph drops off and molts to an adult, which must then find a third and final host. After the female tick has fed, she drops off the host and lays up to thousands of eggs in the environment over the following days or weeks, after which she dies. Under ideal conditions, the cycle from egg to egg can be completed in a little over two months.

Ticks find their hosts by ambushing or active hunting. Ticks that employ a hunting strategy actively run or crawl toward their hosts. Ticks that employ an ambush strategy climb onto vegetation (Mans BJ et al 2002) and wait for a host to pass by. Host recognition can be triggered by vibrations, smell, CO heat and visual cues. The dorsal surface of the first pair of legs of ticks contains sensory organs, including the Haller's organ complex of receptors. When a suitable host is detected, the tick adopts a "questing" posture, waving the first pair of legs in the air while it moves itself into a suitable position to crawl onto the host. If hosts are unavailable, unfed Ixodidae adults can survive for up to 14 years, and starvation periods of more than three years are common. Once the tick has found a suitable host, it moves around to find a suitable feeding location. The first stage of feeding is attachment, whereby the tick uses the chelicerae (a component of the mouthparts) to cut through the skin, and then inserts the hypostome into the wound which anchors the tick with recurved teeth. Some species of tick also secrete a cement-like substance from their salivary glands to help anchor them to the skin.

Tick salivary fluid has anticoagulant, vasodilatory, vascular permeability and cytolytic activity to varying degrees depending on the species of tick. The next stage is the slow feeding phase, which takes about four to six days in most species, with minimal blood uptake in the first 12 to 24 hours. During this phase, the female tick can grow to 10 times her unfed weight. The third and final stage is the rapid feeding phase, which occurs within about one to two days and during which the female can grow to 100 times her unfed weight. Male ticks do not ingest as much blood as females. Mating of adult ixodid ticks occurs on the host except in the genus *Ixodes* where mating can occur while ticks are still on vegetation (Klompen et al., 1996 and Walker et al., 2003). Male ticks remain on the host and will mate with as many females as possible resulting in the transfer of spermatheca

(sperm sac) to females. Females mate only once. Fully engorge detach from their host to lay between 2000 and 20000 eggs, in a single batch (Walker et al., 2007). Eggs are never laid on the host. Three distinct life cycles occur among ixodid ticks.

The one-host tick life cycle is less common but occurs in all *Boophilus* subgenera of *Rhipicephalus* genus (Horak et al., 2002). Larvae hatch from eggs and crawl onto vegetation to wait for a host. This behavior of waiting in ambush on vegetation is called questing (Sonenshine, 1991). These ticks are called “one host ticks”. They remain on same host during the larval and nymphal stages until they become adults. Adult females drop off the host after feeding to lay their batch of eggs. The two-host tick life cycle is similar to the one-host tick with larvae and nymphs feeding on the same host while adults attach to another host (Mathysse et al., 1987 and Jongejan and Uilenberg, 2004). Ticks that feed on two hosts during their lives are called “two host ticks”. These ticks feed and remain on the first host during the larval and nymphal life stages, drop off and attach to a different host as an adult for the final blood meal. The engorged adult female drops off from the host after feeding to lay their batch of eggs (Walker et al., 2003).

The commonest life cycle is the three-host tick life cycle (Sonenshine, 1991 and Jongejan and Uilenberg, 2004) in which larvae, nymphs and adults attach to separate hosts. (See figure 3) Upon hatching, larvae will attach and feed on a host, detach from it and hide in the soil or vegetation to moult into a nymph. Nymphs attach to another host, feed and detach to moult into a female or male tick. Females will feed once and lay a huge batch of eggs, after which the depleted females dies. The male will take several small feeds, mate and then die. The three-host life cycle is slow, lasting from six months to several years (Walker et al., 2007). Drawbacks, including the selection of acaricide-resistant ticks, environmental contamination and contamination of milk and meat products with drug residues (Graf et al., 2004). Some reports show that ticks are developing some acaricidal resistance in many African countries where cattle have been treated with conventional acaricides to control tick infestations (Martins et al., 1995; Latif and Jogejan, 2002).

Tick resistance to chemical acaricides has been on the rise (Lane and Crosskey 1996). Chemical acaricides made from arsenic solutions were the first to be used for tick control (Angus, 1996) and in the eradication of *R.(B)microplus* in the United states were dependable products (George et al., 2004). The problem of resistance to arsenic, between effective concentration for tick control

and the toxic concentration to cattle, led to its replacement by synthetic organic compounds (George et al., 2004). Chlorinated hydrocarbon acaricides have been withdrawn from the market (Graham and Hourigan, 1977; Spickett, 1998) because of their high toxicity and long residual effect (lifespan) on cattle products. Carbamates are a little more toxic than the organophosphates for mammals and are much more expensive (Spickett, 1998). There are reports of amitraz resistance to be on the increase with confirmed cases of resistance being reported in Brazil (Furlong, 1999, Miller et al., 2002 and Mendes et al., 2013) and South Africa (Mekonnen, 2002; Ntondini et al., 2008). Organophosphates are generally the most toxic of all pesticides to vertebrates (Ware, 2000)

2.2.2 Non-conventional methods of tick control

Management of traditionally reared animals by small scale livestock farmers depends on the acquisition of indigenous knowledge, skills, methods, practices and beliefs in animal husbandry (McCorkle et al., 1996). Many small scale livestock farmers either do not implement tick control programs at all and/or complement conventional methods with indigenous tick control methods which may include the use of used motor oil, household disinfectants, paraffin, or manually plucking off ticks from the animals (Masika, 1997; Hlatshwayo, 2005). Pasture spelling, pasture burning and use of certain grasses and legumes are also practiced for inhibition or killing of ticks (Branagan, 1973, Suthrest et al., 1982, Chiera et al., 1984). Bush clearing through burning of land annually in Zambia reduces the number of ticks (Baars, 1999). Mbatiet al. (2002) reported that farmers also used oil (12%), Jeyes fluids (24%) and De-ticking (2%). Kaaya and Hassan (2000) reported that the use of entomological fungi to control ticks may reduce the frequency of chemical acaricides applications.

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

odours emanating from the leaves of a plant called *Acalypha fruticosa* (Hassan et al., 1994). Certain pasture legumes plants produce sticky secretions which have been reported to immobilize and to kill tick (Elliot et al., 1978; Sutherst, 1982). Some plant products have also been shown to kill ticks and inhibit tick oviposition (Chabra and Saxena 1998). Different reports showed that certain plants and herbs have anti-tick insecticidal properties (Ghosh et al., 2007).

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

2.2.3 Challenges of chemical tick control

Management of ticks and TBDs by small scale farmers has been facing a number of challenges. The cost of conventional acaricides is generally expensive and unsustainable, as such it has resulted in the increase of some of the TBDs which includes Theileriosis, Anaplasmosis, Heart Water and Babesiosis. They are also expensive and unaffordable to resource-limited farmers; as a result the farmers have resorted to ethno-veterinary practices and remedies (Laffont et al., 2001). The conventional control methods include the use of chemical acaricides with partially successful results but this treatment has certain implicit drawbacks, such as the presence of residues in the milk and meat and the development of chemical resistant tick strains (Willadsen and Kemp, 1988; Nolan, 1990). However there is development of resistance in parasite drugs. Tick resistance to acaricide is an increasing problem and real economic threat to the livestock and allied industry (Rajput et al., 2006). The use of acaricides has disadvantages, such as the selection of resistant tick populations and harmful effects on the animals, human beings and the environment (García-García et al., 2000). Increased resistance in target species of ticks to chemical acaricide has

been reported (Currie et al., 2004). Dip tanks are located far away from where cattle are kept and in some cases the dip tanks are non-functional due to the non-availability of water pumps, water and acaricides (Moyo and Masika, 2009).

2.2.4 Economic importance of ticks and Tick borne diseases

According to Simuunza et al. (2011) tick borne diseases are the constraints to livestock production in many developing countries, which has caused high morbidity and mortality, resulting in decreased production of meat, milk and other livestock by-products. Ticks and TBDs continue to be the major constraint to livestock production not only in Zambia, but in many parts of Eastern Southern and Central Africa (Makala et al., 2003). Milk production and weight gain are indirectly affected by tick bites (Hostis and Seeger, 2002; Peter et al., 2005). Increased tick numbers on animals were found to cause proportional bigger live weight losses in tick susceptible Boran cattle than in tick resistant animals of the same breed (De Castro, 1987). Ticks also transmit viral, rickettsial, bacterial and protozoal diseases that affect wild animals, domestic animal, both domesticated and wild animals and indeed human beings (Lane and Crosskey, 1996). Apart from acting as vectors for diseases (TBDs), ticks have been recognized as important ectoparasites of livestock.

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

Ptaeroxylonobliquum for tick control. According to Mwale et al. (2005) the demand for herbal use has increased more than chemical substances due to effectiveness, easy access and low costs

According to Giraldo-Rios and Hurtado (2018), in the economic and health impact of ticks in production animals, the severity of the infection as a result of infestation with ticks will depend on the production animals. Infestation of ticks especially in animals such as cattle for instance, the feeding habits of ticks cause stress in animals affected by bites, blood losses that can lead to anemia and even death. Animals that are infected by ticks or that do not have immunity against them, decline in their capacity to produce quality meat and milk. Ticks present three main dangers to their hosts: the physical skin damage through the bite itself, other systemic effects of the tick's saliva and transmission of infectious diseases. When ticks attach themselves to the host in the first stage of feeding, they cut the skin with their mouthparts and cause damage to tissues and capillaries. Host reactions, such as mast cell degranulation (CDC 2020) leading to histamine release and inflammatory cell infiltration, further contribute to tissue damage. This tissue damage tends to be quite painful and may result in secondary bacterial infections.

Ticks feed on the host's blood, and heavy infestations can also cause anemia (Mans et al., 2002). The systemic effects of a tick bite can be extremely serious. Many species of tick can cause debilitating or even fatal paralysis in their hosts according to Faulde(2008).The engorging adult female tick is usually responsible for paralysis. Ticks inject their saliva into the host while feeding, and paralysis is due to a protein present in the saliva. Tick bites can also cause anaphylactic reactions in humans according to Van et al (1991), that is to say the allergic reactions to ticks. Some of the physical signs and symptoms are pain or swelling at the bite sites, redness on the skin (erythema migrans), rash, burning sensation at the bite site, blisters and difficulty in breathing if severe. These reactions have been reported sporadically in various locations around the world and are thought to be due to reactions to components of the tick's saliva. To the author's knowledge, although anaphylaxis to tick antiserum is reasonably common, anaphylaxis to the tick bite itself has not been reported in companion animals. According to Murray, (1844) ticks also transmit infectious diseases to both companion animals and humans.

Some of these diseases include bacterial such as Tularemia in humans, dermatochalasis a fungal disease transmitted to humans and animals, tick- borne rickettsia infections such as boutonneuse fever, Anaplasmosis, African tick bite fever, spotted fever, ehrlichiosis, and heart water,

spirochetal, tick-borne protozoosis theileriosis known as east coast fever in cattle, etc. The tick's bite causes discomfort and can lead to secondary infections, some species are capable of causing paralysis in animals and small children, and ticks are vectors of a number of diseases affecting both animals and humans (Cooley and Kohls 1942)

Chemical control of ticks (Conventional methods)

Ticks and Tick borne diseases control is mainly by the use of commercial acaricides such as organophosphates, Carbamates, pyrethroids, etc (Ghosh et al., 2007). Acaricides can be applied to cattle using hand sprayers, spray races and in dipping vats (George et al., 2004), in order to control ticks and tick borne diseases. They are often accompanied by serious. Ticks can be treated and/or prevented using both physical and chemical means. Physical means include manual removal of attached ticks and environmental modification to reduce the suitability of the habitat to ticks (Willadsen and Kemp 1988). Chemical treatment and prevention include products for use on companion animals and products for use in the environment. Manual removal of ticks is usually performed as soon as an attached tick is found. It is generally accepted that removing or killing a tick within 24 to 48 hours of attachment will prevent disease transmission (Groocock 1988). There have been few studies comparing the efficacy of different removal strategies, but the most effective means of removal seems to be direct manual removal without twisting.

The few studies examining various removal strategies have shown that fingernail polish, petroleum jelly, 70 percent isopropyl alcohol, a glowing hot match, or gasoline are not effective and that rotation while twisting is more likely to lead to retained mouthparts (Huygelen V et al 2017). To remove a tick, grasp the head of the tick with fine- point tweezers as close to the skin as possible. Avoid squeezing the body and apply gentle traction to pull the tick straight out; this should minimize the chances of leaving mouthparts behind. According to Camus (1993), hundreds of plant species have been identified by traditional practitioners for treating a wide range of livestock (and human) ailments, although the efficacy of plant treatments has often not been tested through formal trials, on which more work is required. Nevertheless, a large body of information on traditional use, over a number of centuries in many cases for indigenous plants, supports their utility for treatment and control.

CHAPTER THREE: MATERIALS AND METHODS

3.1 Geographical scope

The Invitro study was being carried out at the Equator Valley Farm, Faculty of Agriculture Laboratory at Uganda Martyrs University. The Invitro evaluation results was used to further test the efficacy of the recipes in a field study within Nkozi Community, which has not spared by the devastating impacts of the ticks.

3.2 Experimental Materials

In this study, the experiment was carried out using botanically identified ethno-veterinary plant materials of various families and species from a South African Journal of Botany which is a review of the Plant Extracts to Control Ticks of Veterinary and Medical Importance and an Amitraz compound against ticks R (B) Rhipicephalus.

3.3 Experiment design

An In Vitro study was done using the above mentioned plant materials of ethno-veterinary importance against ticks. The amitraz compound chemical was used as a control in the evaluation. Extraction protocol already preceded include collection of plant materials, washing, shade drying. Weighing and aqueous extraction protocol using ethanol 96-97% extractant for the extraction of the phytochemicals for the various phyto-constituents proceeded using Maceration technique.

Bioassay method used is Petri dish method of Adult Immersion Test.

3.4 Collection of plant materials

Five plant with possible efficacy of 95-100% from the above mentioned plant materials were used in the evaluation against ticks on the basis of available literatures and ethno-veterinary usage. The plant materials used were *gynandropsis gynandria*, *azadirachta indica*, *Lavendula augustifolia*, *Ocimum gratissimum*, and *Targetes erecta*.

3.5 Extraction procedure

The Biomass was crushed to make a powdery consistency using traditionally local grinding stone. Then 15g of each biomass crushed material was positioned separately, and 10ml quantity of

ethanol was provided for extraction. Ethanol was added to the plant and soaked for 3 days while covered using paraffin film wax to prevent evaporation due to volatility. The extraction process, filtration was repeated using cotton/cheese cloth up to 12cycle.

The different concentrations 1:1 and 1:2, Three replicates with four tick's samples in each concentration. Two dilutions in each, meaning 1:1 and 1:2 of each. This therefore means, 2 dilutions X 5 concentrations = 10 concentrations

Each of the 10 repeated 3 times with equal treatments making 30 parts for the case of organic recipe, meaning same quantity of concentration, same number of ticks, equal monitoring of the experiment daily.

Amitraz Compound 3 replicates, 4 ticks, 2 dilutions meaning ratio of 1:1 and 1:2 of each concentration.

Where $2 \times 3 = 6$ Concentrations. Equal treatment to eliminate biasness meaning, Same quantity of concentration/chemical, same number of ticks, monitoring the experiment every day.

The test was run twice to repeat for second test and more confirmation for results.

3.5 Data collection

Five plant materials were used against the ticks for the case of organic recipe and one acaricide type of synthetic components in the evaluation process.

Duration for ticks' death

The period for the ticks' mortality was timed in terms of number of days and recorded for the organic recipe versus the synthetic acaricide.

Type of tick

The specific species of tick whose mortality was recorded in terms of number was mainly R.B. Rhipicephalus. Four ticks were placed in each petri dish and specific number of drops of the different dilutions was applied.

3.7 Data analysis

Analysis of variance was performed using GenStat analytical software version 14th Edition (VSN International, 2012) to evaluate the significance difference within the treatments. The least

significant difference (LSD) test at 5% probability level was used for mean comparison from the ANOVA results obtained.

CHAPTER FOUR: RESULTS AND DISCUSSION

4.0 Introduction.

This chapter presents the results and Discussion of findings. The data presentation was done in accordance with the objectives of the study; To determine the effectiveness of different biorationals as a tick control measure and To determine the effectiveness of different plant extracts on tick mortality were discussed in relation to the literature reviewed.

4.1 Objective one: To determine the effectiveness of Organic Biorationals as a tick control measure

Table 1: Effectiveness of Organic Biorationals as a Tick Control

Treatment		Time Of Data Collection		
Plant Specie	Conc	24Hrs(1 day)	48Hrs(2 days)	72Hrs(3 days)
<i>Azadirachta Indica</i>	1:1	1.000	3.133	5.431
<i>Azadirachta Indica</i>	1:2	0.000	1.010	1.101
<i>Gynandropsis gynandria</i>	1:1	0.000	1.201	2.610
<i>Gynandropsis gynandria</i>	1:2	0.000	0.000	0.002
<i>Lavendula augustifolia</i>	1:1	0.000	0.213	2.221
<i>Lavendula augustifolia</i>	1:2	0.000	0.000	0.000
<i>Targets erecta</i>	1:1	0.000	0.000	1.210
<i>Targets erecta</i>	1:2	0.000	0.000	0.000
<i>Ocimum gratissimum</i>	1:1	0.000	0.000	1.152
<i>Ocimum gratissimum</i>	1:2	0.000	0.000	0.000
Fpr		0.234	<. 001	<.001
E.S.E		0.0560	0.0962	0.1716
S.E.D		0.2351	0.1361	0.2427
L.S.D		0.1430	0.0434	0.5034
CV%		19.4	5.6	15.7

LSD (0.05) = Least Significant Difference at 5% level; S.E.D= Standard Error of Difference; F-pr = Fisher's Probability; CV%= Percentage Coefficient of Variations, *Conc: 1:1 (10ml organic*

recipe concoction: 10ml water) and Conc 1:2 (10ml organic recipe concoction: 20ml water),

The findings presented in Table above indicates that among the different plant extracts on tick control *Azadirachta Indica* concoction of ratio 1:1 (10ml organic biorational concoction: 10ml water) significantly caused an effect ($<. 001$) on the ticks in respective petri dishes and the this was observed after 48 hours and 72 hours of treatment application. At 24 hours of treatment application, no single tick was observed dead neither weak because the concoction was not yet very strong. The finding further indicates that 1:1 (10ml organic biorational concoction: 10ml water) of *Azadirachta Indica* at 48 hours, it was observed that from 12 petri dishes having a total of 12 ticks, 3.133 were observed dead whereas at 72 hours many (5.431) ticks continued to die. This ratio of *Azadirachta Indica* showed a strong reaction on the ticks indicating that if farmers can adopt the use of this readily available plant extract can offer relief to the farmers from high risks of using chemical acaricide. It was further observed that ration 1:2 or organic recipe did not show any effect on the control of ticks because it was over diluted if the ticks can develop resistance over chemical acaricide. This was followed by ticks which were subjected to *Gynandropsis gynandria* recipe (1:1 ratio) at 72 hours of treatment application some a small percentage of ticks (2.410) were observed dead which was not the case at 24 hours and at 48 hours (1.601) tick was observed dead out of 12 ticks, *Lavendula augustifolia* recipe of 1:1 ratio (2.221) and *Targets erecta* 1:1 ratio (1.210) and lastly the least number ticks dead were observed from *ocimum gratissimum* in 1:1 ratio (1.152). These findings are support with to tackle the problems many methods have been recommended (Ghosh et al. 2007) and phyto-acaricide has been considered as a viable option. Plants provide a number of natural compounds which can intervene on all biological processes of insects interrupting their life cycle and are considered as an important part of ethno-veterinary practices (Habeeb 2010; Zaman et al. 2012). In comparison to synthetic acaricides, the botanicals are usually less toxic to mammals, have no residual effects and have less chance of development of resistant tick populations (Chungsamarnyart et al. 1991). The possibility of using botanicals for the control of arthropods of veterinary importance has recently been reviewed by Ghosh and Ravindran (2014) and a few plants were identified as most promising against ticks. In the present study, the anti-tick potentiality of two plant extracts was identified to explore further the possibility of development of plant-based herbal acaricides for the control of resistant tick species.

4.2 Objective two: To determine the effectiveness of different plant extracts on tick mortality

This study examined the **effect of plant extracts on tick mortality**. This was determined by looking at dead ticks, number of ticks which completed the cycle and lastly the number of tick larvae produced. The data collected was analyzed and the results were established or presented in Table 2 below.

4.2.1 Effect of plant extracts on tick mortality rate

Table 2: Effect of plant extracts on tick mortality.

Treatment		Time Of Data Collection		
Plant Specie	Conc	24hrs(1 day)	48hrs(2 days)	72hrs(3 days)
<i>Azadirachta Indica</i>	1:1	1.000	3.133	5.431
<i>Azadirachta Indica</i>	1:2	0.000	1.010	1.101
<i>Gynandropsis gynandria</i>	1:1	0.000	2.201	4.410
<i>Gynandropsis gynandria</i>	1:2	0.000	0.000	1.002
<i>Lavendula augustifolia</i>	1:1	0.000	0.213	3.421
<i>Lavendula augustifolia</i>	1:2	0.000	0.000	0.000
<i>Targets erecta</i>	1:1	0.000	0.000	2.000
<i>Targets erecta</i>	1:2	0.000	0.000	0.000
<i>Ocimum gratissimum</i>	1:1	0.000	0.000	1.952
<i>Ocimum gratissimum</i>	1:2	0.000	0.000	0.000
<i>Amitix (Chemical accaricide)</i>	1:1	0.000	3.411	6.200
<i>Amitix (Chemical accaricide)</i>	1:2	0.000	0.000	0.000
Fpr		<.001	<.001	<.001
E.S.E		0.2643	0.0962	0.5430
S.E.D		0.7752	0.1361	0.1234
L.S.D		0.1254	0.2822	0.5210
CV%		9.6	17.6	14.6

LSD (0.05) = Least Significant Difference at 5% level; S.E.D= Standard Error of Difference; F-pr = Fisher's Probability; CV%= Percentage Coefficient of Variations, *Conc: 1:1 (10ml organic*

recipe concoction: 10ml water) and Conc 1:2 (10ml organic recipe concoction: 20ml water),

Based on the results presented in (Table) at 72 hours of treatment application, of the five plant extracts tested against ticks mortality, at a ratio of 1:1 (10ml organic biorational concoction: 10ml water) of *Azadirachta Indica*, *Gynandropsis gynandria* and *Lavendula augustifolia* ethanolic extracts were identified for further study as they caused a significant (<.001) mortality of 5 ticks (41.6%) out of 12 ticks, 4 ticks (33.33%) and *Gynandropsis gynandria* 3.421 (25%) respectively (Table) and this effectiveness was observed at 72 hours because the plant extracts presented toxicity which never allowed the ticks to survive and the rest of the plant extract, *Targets erecta* 2.000 (16.6%) and *ocimum gratissimum* 1.952 (16.26%) did not show strong acaricidal activity in them hence presenting the least percentage of tick mortality. Previously, ethanolic leaf extracts of *Azadirachta Indica* were reported for acaricidal, repellent, and reproductive inhibitory properties against two-spotted spider mites, *Tetranychusurticae* and *Panonychusulmi* (Kumral et al. 2010, 2013), flat mite, *Brevipalpusphoenicis* and the coconut eriophyid mite, *Aceriaguerreronis* (Acari: Eri- opyhidae) (Guirado et al. 2001; Thevan et al. 2005). Shyma et al. (2014) reported significant acaricidal activities of *D. stramonium* extracts against *R. (B.) microplus*. Kuganathan and Ganeshalingam (2011) reported toxic effect of the leaf extracts of *D. metel* plants at various concentrations on grasshoppers and red ants. The anti-feedant activity of aqueous leaf extract of *D. metel* exhibited significant larval mortality against *Helicoverpaarmigera* after 24 h of exposure (Ramya and Jayakumararaj 2009). However, none of the studies have addressed the activity of the solvent guided extracts of *D. metel* or *D. stramonium* against the arthropods resistant to synthetic acaricides.

4.2.2 Number of ticks, which completed life cycle at 72hour treatment application

Table: 3 Number of live ticks, which completed 72 hour treatment application

Treatment		After treatment application
Plant Specie	Conc	Number of ticks completed life cycle
<i>AzadirachtaIndica</i>	1:1	7
<i>AzadirachtaIndica</i>	1:2	11
<i>Gynandropsisgynandria</i>	1:1	8
<i>Gynandropsisgynandria</i>	1:2	11
<i>Lavendulaaugustifolia</i>	1:1	9
<i>Lavendulaaugustifolia</i>	1:2	12
<i>Targets erecta</i>	1:1	10
<i>Targets erecta</i>	1:2	12
<i>ocimumgratissimum</i>	1:1	11
<i>ocimumgratissimum</i>	1:2	12
<i>Amitix (Chemical accaricide)</i>	1:1	6
<i>Amitix (Chemical accaricide)</i>	1:2	12

On the basis of tick mortality pattern within 72hrs of treatment application, fewer number of (7) ticks were observed from the petri dishes of *Azadirachta Indica* in the ratio of 1:1(10ml organic biorational concoction: 10ml water) whereas higher number of ticks were able to complete their cycle in the ratio of 1:2 (10ml organic biorational concoction: 20ml water) because the accaricidal activity in the recipe was weak to kill the ticks because it was diluted too much. Another recipe which recorded some small number of (8) ticks completing their cycle was recorded from *Gynandropsis gynandria* ration 1:1 (10ml organic recipe concoction: 10ml water) which was not the same case with ratio 1:2 (10ml organic biorationals concoction: 20ml water). Whereas the rest of the biorationals reported higher numbers of ticks which completed their cycles. In ticks, the moulting hormone (ecdysteroids) plays a role in the regulation of salivary gland function, production of pheromones, and oogenesis and oviposition (Rees 2004). In fully engorged-adult female ticks, the level of ecdysteroids rises in the hemolymph which in turn causes salivary gland

to degenerate and as a result the ticks lay more eggs. This hormone also triggers vitellogenesis (Sankhon et al. 1999) and inhibits reattachment to the host (Weiss and Kaufman 2001). The neuronal involvement in the control of salivary gland degeneration in the ixodid tick, *Amblyomma haebraeum*, has been proved (Harris and Kaufman 1984). The neurotransmitter dopamine regulates the synthesis of ecdysteroids. Dopamine was identified in the salivary gland and salivary gland nerves of *R. (B.) microplus* and *A. haebraeum* (Kaufman and Harris 1983). The probable effect of the plant extracts on these hormones and neurotransmitters may be the reason for the former observations and needs further validation by detailed studies.

4.2.3 Number of tick larvae produced at 72 hours of treatment application

Table 4: Number of tick larvae produced at 72 hours of treatment application

Treatment		After treatment application
Plant Specie	Conc	Number of tick larvae produced
<i>Azadirachta Indica</i>	1:1	100
<i>Azadirachta Indica</i>	1:2	150
<i>Gynandropsis gynandria</i>	1:1	120
<i>Gynandropsis gynandria</i>	1:2	164
<i>Lavendula augustifolia</i>	1:1	110
<i>Lavendula augustifolia</i>	1:2	120
<i>Targets erecta</i>	1:1	100
<i>Targets erecta</i>	1:2	125
<i>Ocimum gratissimum</i>	1:1	100
<i>Ocimum gratissimum</i>	1:2	120
<i>Amitix(Chemical accaricide)</i>	1:1	102
<i>Amitix(Chemical accaricide)</i>	1:2	129

Findings on the mean number of tick larvae produced at 72 hours of treatment application presented above indicates that more tick larvae were observed from all the recipe which was diluted in the ratio of 1:2 (10ml organic biorational concoction: 20ml water) of all the recipe and

less was observed from the ratio of 1:1 (10ml organic recipe concoction: 10ml water) of all the recipe which strong prove that though some recipe are weak but the ratio have high active ingredients. Of all the recipe in the study, fewer number of (100) tick larva produced at 72 hours was observed from *Azadirachta Indica* 1:1 ratio recipe and amitraz chemical acaricide recorded lower numbers of tick larvae. This was so because the activity ingredient damaged the egg system of most ticks hence leading to lower number of tick larvae. Among the various ixodid ticks *R. (B.) microplus* is one of the important ticks infesting dairy animals of India (Ghosh et al. 2007) particularly Punjab state (Haque et al. 2011; Singh and Rath 2013). The most widely used method for the control of ticks is the direct application of acaricides to host animals, and thus, the consumption of insecticides has been increased manifold during last decades. SPs are the most widely and frequently used acaricides to control tick populations globally thus resulting in development of resistance. Presence of wide spread resistance to cypermethrin and deltamethrin in *R. (B.) microplus* has been recently reported from different places of India including Punjab state (Singh et al. 2010, 2012; Sharma et al. 2012).

CHAPTER FIVE: SUMMARY FINDINGS, CONCLUSIONS AND RECOMMENDATIONS

5.0 Introduction

This chapter in particular presents a summary of findings, conclusion and recommendation of the study. The study drew conclusion from the findings based on the objectives of the study. The recommendation from the findings are two fold that is recommendation in accordance to the conclusions made on the study objectives and lastly further studies.

5.1 Summary of findings

To determine the most effective biorational as a tick control measure

The findings presented in Table above indicates that among the different plant extracts on tick control *Azadirachta Indica* concoction of ratio 1:1 (10ml organic biorational concoction: 10ml water) significantly caused an effect ($<. 001$) on the ticks in respective petri dishes and the this was observed after 48 hours and 72 hours of treatment application. At 24 hours of treatment application no single tick was observed dead neither weak because the concoction was not yet very strong. The finding further indicates that 1:1 (10ml organic biorational concoction: 10ml water) of *Azadirachta Indica* at 48 hours, it was observed that from 12 petri dishes each having a total of 12 ticks, 3.133 were observed dead whereas at 72 hours many (5.431) ticks continued to die. This ratio of *Azadirachta Indica* showed a strong reaction on the ticks indicating that if farmers can adopt the use of this readily available plant extract can offer relief to the farmers from high risks of using chemical accaricide. It was further observed that ration 1:2 or organic biorational did not show any effect on the control of ticks because it was over diluted, if the ticks can develop resistance over chemical accaricide. This was followed by ticks which were subjected to *Gynandropsis gynandria* biorational (1:1 ratio) at 72 hours of treatment application some a small percentage of ticks (2.410) were observed dead which was not the case at 24 hours and at 48 hours (1.601) tick was observed dead out of 12 ticks, *Lavendula augustifolia* biorational of 1:1 ratio (2.221) and *Targets erecta* 1:1 ratio (1.210) and lastly the least number of ticks dead were observed from *ocimum gratissimum* in 1:1 ratio (1.152)

To determine the effectiveness of different plant extracts on tick molarity

Effect of plant extracts on tick mortality rate

Based on the results presented in (Table) at 72 hours of treatment application, of the five plant extracts tested against ticks mortality, at a ratio of 1:1 (10ml organic biorational concoction: 10ml water) of *Azadirachta Indica*, *Gynandropsis gynandria* and *Lavendula augustifolia* ethanolic extracts were identified for further study as they caused a significant (<.001) mortality of 5 ticks (41.6%) out of 12 ticks, 4 ticks (33.33%) and *Gynandropsis gynandria* 3.421 (25%) respectively (Table) and this effectiveness was observed at 72 hours because the plant extracts presented toxicity which never allowed the ticks to survive and the rest of the plant extract, *Targets erecta* 2.000 (16.6%) and *ocimum gratissimum* 1.952 (16.26%) did not show strong acaricidal activity in them hence presenting the least percentage of tick mortality

Number of ticks, which completed life cycle at 72hour treatment application

On the basis of tick mortality pattern within 72hrs of treatment application, fewer number of (7) ticks were observed from the petri dishes of *Azadirachta Indica* in the ratio of 1:1(10ml organic recipe concoction: 10ml water) whereas higher number of ticks were able to complete their cycle in the ratio of 1:2 (10ml organic recipe concoction: 20ml water) because the accaricidal activity in the recipe was weak to kill the ticks because it was diluted too much. Another recipe which recorded some small number of (8) ticks completing their cycle was recorded from *Gynandropsis gynandria* ration 1:1 (10ml organic recipe concoction: 10ml water) which was not the same case with ratio 1:2 (10ml organic recipe concoction: 20ml water).

Number of tick larvae produced at 72 hours of treatment application

Findings on the mean number of tick larvae produced at 72 hours of treatment application presented above indicates that more tick larvae were observed from all the recipe which was diluted in the ratio of 1:2 (10ml organic recipe concoction: 20ml water) of all the recipe and less was observed from the ratio of 1:1 (10ml organic biorational concoction: 10ml water) of all the recipe which strong prove that though some recipe are weak but the ratio have high active ingredients. Of all the recipe in the study, fewer number of (100) tick larva produced at 72 hours was observed from *Azadirachta Indica* 1:1 ratio recipe and amitraz chemical accaricide recorded

lower numbers of tick larvae. This was so because the activity ingredient damaged the egg system of most ticks hence leading to lower number of tick larvae.

5.2 Conclusion of the study

According to the findings, it was concluded that the most effective ratio of the selected organic recipe is 1:1 (10ml organic biorational concoction: 10ml water) because the concentration in the recipe acaricidal activity is higher as compared to the other ratio 1:2 (10ml organic biorational concoction: 20ml water). The study further more concluded out of the five organic biorationals from different plant materials, *Azadirachta Indica* in the ratio of 1:1(10ml organic biorational concoction: 10ml water) was the most effective organic biorational on the control of ticks. Ticks which were treated with *Azadirachta Indica* in the ratio of 1:1(10ml organic biorational concoction: 10ml water) majority died as compared to other plant materials. Few ticks were able to complete their life cycle in the *Azadirachta Indica* in the ratio of 1:1(10ml organic biorational concoction: 10ml water) as compared to other plant materials The results of the present study showed that various plant extracts are toxic to R. (B.) microplus adults. Also, there was a significant reduction in the egg production in ticks treated with *Azadirachta Indica* in the ratio of 1:1(10ml organic biorational concoction: 10ml water). The reductions in the egg mass production in ticks reflect the direct effect of the extracts, because the surviving ticks were weak enough to produce optimum egg mass and thus reflect the population

5.3 Recommendation

The beneficial medicinal effects of plant materials can be due to effects of a single compound or combination of one or more active compounds present in the plant. The chemical constituents of the plants may exert acaricidal effects in different ways; therefore, the development of resistance seems difficult against botanical acaricides in combination. Further, studies are needed to identify the active ingredients present in these plants that caused the mortality of tick, decrease in egg production and inhibition of hatching of the eggs.

The study recommends that in order to develop an eco-friendly tick control method, farmers should adopt the use of *Azadirachta Indica* in the ratio of 1:1(10ml organic biorational concoction: 10ml water) plant extracts.

Suggestion for further study

The preliminary studies indicated that both the plants have potential for developing acaricides to counter-act problems associated with the chemical acaricides. However, studies on the possibilities of large scale economical harvesting of the plants, stability pattern of active components at different environmental conditions and safety aspects of the extracts containing alkaloid, terpenoids, flavonoids and phenolics need to be under- taken in phased manner.

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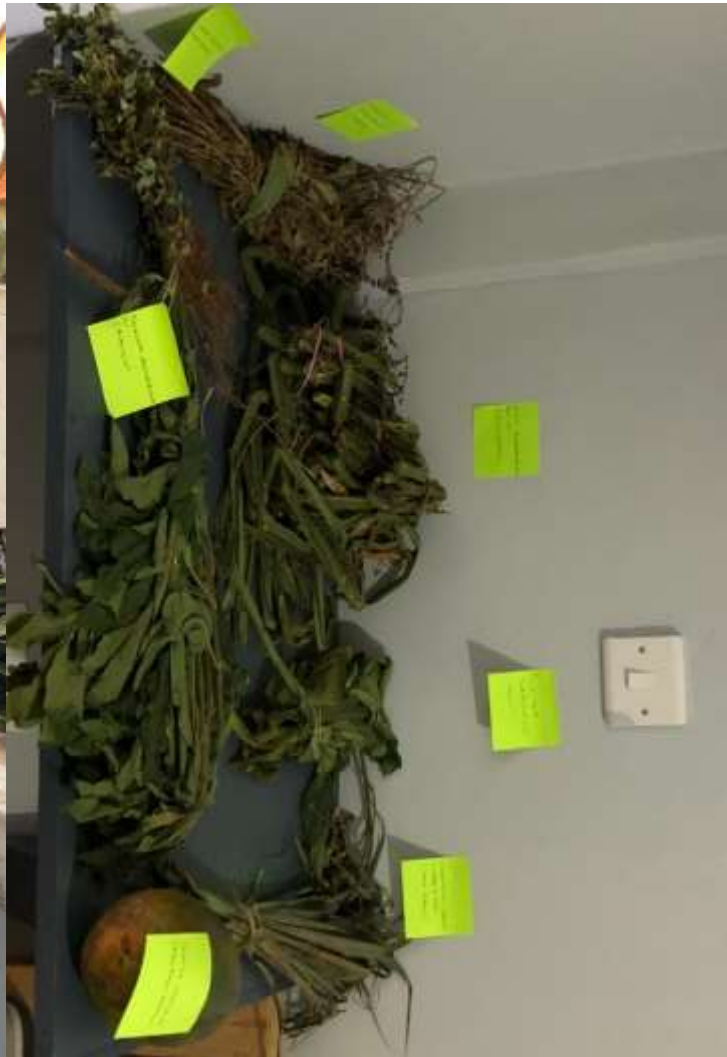
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APPENDICES

Appendix 1



Immersion of the ticks in the different plant concoctions



Washing and shade drying of the plant materials as extraction protocol