TICK CONTROL IN TSWANA, SIMMENTAL AND BRAHMAN CATTLE BY MEANS OF NEEM SEED EXTRACTS (*AZADIRACHTA INDICA*)

by

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BVM (SUA)

Thesis submitted in partial fulfillment of the requirement for the degree

MSc(AGRIC) ANIMAL SCIENCE

in the

Department of Animal and Wildlife Sciences

Faculty of Natural and Agricultural Sciences

UNIVERSITY OF PRETORIA

PRETORIA

2001

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Statement of originality

The work contained in this Research project was completed by the author at the University of Pretoria between November 1998 and October 2000. It is original work except were due reference is made and neither has been nor will be submitted for the award of a degree at any other university.

Signed ___________________

Students signature

ACKNOWLEDGEMENTS

My sincere gratitude is sent to my supervisor Dr. E C Webb who played an important role in the preparation and caring out of this research project. Without Dr. E C Webb the programme of study would have ended quite prematurely and the study would therefore have been a non-starter.

Special thanks also go to Prof. A J Ngomuo for research funding and guidance. He also provided his expertise on the topic that I was working on.

My special recognition goes to farmers around Serowe Village who allowed me to use their cattle during my study period. I would like to thank all temporary workers who helped me during the tick collection procedures and application of Neem seed extract on the test animals.

I would like to express my gratitude to my typist and my wife Pamela who worked overtime to finish this work. Finally I would like to thank my friends` and family for their support.

Abstract

Matayo David (Dr)

TICK CONTROL IN TSWANA, BRAHMAN AND SIMMENTAL BY MEANS OF NEEM SEED EXTRACTS

Supervisor: Dr. EC Webb

A total of 114 animals (57 bulls and 57 cows) of Tswana, Brahman and Simmental cattle were used for the three trials (Trial 1, 2 and 3). All animals were 15 months old and above. In the first trial a total of 9 animals were spread on the perennial area, ear, dewlap, udder, scrotum, and sternal area with either 5% (W/V) water extract of Neem Seed Kernel NSK at a rate of 5g/kg body weight, some 9 animals were sprayed with tap water (control group).
During the 2nd trial a total of 30 animals were spread with.2% (W/V) ethanolic neem seed extract (NSKE) 2% (W/V) ethanol extract of neem seed oil at a rate of 5g/Kg body weight some 30 animals were similarly sprayed with tap water as control group. During the 1st and the 2nd trial an experimental and control animals were kept in separate partitions of the shelter, but grazed together in the area which was heavily infested with adults and immature stages of ticks. In the 3rd trial a total of 36 animals were artificially infested with tick, 18 cattle were spread with 3% (W/V) ethanolic Neem Seed Kernel (NSKE) at the rate of 5g/kg body weight. Another 18 animals were spread with tap water as control group, there was no contact between animals although were kept in the same environment. The tick index of each animal for all three trials was determined weekly for 14 months consecutively.

Results showed that mean tick population densities were significantly lower in the experimental than control animals. Indigenous Tswana cattle harboured significantly fewer` ticks during periods of peak tick abundance than either Brahman or Simmental cattle. Fewer abscesses due to tick bites were observed in Tswana than the other two breeds, and a large percentage of Tswana cattle showed a high tick resistance. The results suggest a high degree of natural immunity in indigenous Tswana cattle.

It was concluded that NSKE extract was potentially useful in controlling ticks on livestock. Further research is required to study the active ingredients in NSKE as well as the pharmacological action on ticks. It is recommended that NSKE should be considered for inclusion in the management programmes to control ectoparasites on livestock.

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CHAPTER ONE

INTRODUCTION AND MOTIVATION

1.1 Background of the study

Ticks are important ectoparasitic pests and vectors of human and animal diseases in many countries including Botswana. Diseases transmitted by these insects include Heart water, East Coast fever, Babesiosis and Anaplasmosis to name few. Other problems associated with ticks include anaemia, tick worry, toxins, wound, screw worm infections and damage to the hide, ill health and loss of animal productivity, especially in developing countries where control strategies are not carried out regularly (Kilonzo, 1986; Fagbeni, 1983)

Despite the heavy tick infestation of domestic animals, few farmers in Botswana practice regular control strategies. This is attributed mainly to ignorance, negligence and poverty. On most modern farms (Institutional farms) however, animals are grazed within the paddocks separated by fences and hence infestation is generally less than in farms were animal kept with other livestock or wild animals which increases the exposure to tick.

On some modern farms ectoparasite control is mostly by means of regular or occation use of acaricides.

Although most of the commonly available insecticides are effective against livestock ticks in Botswana they have several disadvantages including large costs, contamination of the environment, toxicity to man and animals as well as other non-target organisms. Alternative tick control methods that are effective, safe, economical and socially acceptable and environmentally friendly, are therefore required. Various natural products including plants could be suitable candidates for such an alternative approach.

Azadirachta indica (Neem plant) has been shown to be potentially larvicidal against ticks, fleas, and other insects in other countries like India. It has also been proven to be effective against many insect pests of agricultural products and disease vectors elsewhere (Schmutterer, 1981; Zebitz, 1986; Wilps, 1986).

In the laboratory test the oil-based concentrate at 1% and the cake based concentrate of 0.2% concentration cause 100% and 89% mortality respectively of Nymphs of *idioscopus nitidulus*. (Nigam and Sen, 1989).

Neem, *Azadirachta indica* or *Mauarobaini* in Kiswahili is a tree, which belongs to the family Miliaceae. The tree,which grow up to between 40 to 50 feet high, has a straight trunk and forms a round crown. The tree has been referred to as natures bitter boon, natures gift to human kind, tree that purifies, the wonder tree, the tree of the 21st century and currently a tree for solving global problems (1993), basically due to its plethora of biological activities, that include excellent insect control activity and various medicinal properties in agriculture and medicine (Abdullah *et al*, 1986).

Neem oil at conc. of 1, 2, 3, and 4% was found highly significant in reducing the hopper *nephotettix virescens* population as compared to control (Nigam and Sen, 1989).
Seed extracts of 2.5 to 5% have been used to control helminths, skin infections such as ringworms, rheumatism and muscular pain as a result of its antinflamatory activity (Ketkar and Basu, 1935), stomach ulcer and scabei.

Neem seeds are processed using oil expellers and solvent extraction to yield neem oil and neem cake, which are utilised in the soap Industry (Siddique, 1942) and as manure (Ketkar, 1976), respectively.

In Botswana none of the above characteristics have been developed or exploited for agricultural and livestock production to any significant extent, considering that the number of neem trees has increased considerably over the past several years particularly in Maun, Francistown, Palapye, Lobatse, Hans and some parts of Serowe.

In the Indian subcontinent and the Philippines Islands use of these insecticidal properties has been exploited by scientists and farmers a like for protection of horticulture and cereal crops against pests during growths and production but also during post harvest period (Ketkar, 1976; Jayaraj, 1993. In America and Australia, large neem plantations with several thousand neem trees are being set up by many Scientific Institutions and Agro-industries (Rice, 1993) with the objective of seed production and extraction constituents necessary for production of a variety of industrial goods such as insecticides, soap, shampoo, toothpaste and creams (Rice, 1993).

Neem trees are not only useful in providing seeds and leaves for extraction of pesticidal constituents, but they also provide good shade around homes and high quality building and fencing poles which withstand the attack of termites (Rice, 1993)

Neem tree withstand drought, cyclones, destruction by insects and recover well on exposure to bush fire (Rice, 1993) and is therefore very suitable in arid and semi-arid zones. This property of neem has found application in Kwimba district (Tanzania) in re-forestation projects. Kwimba Association Reforestation Project has grown about 250000 neem trees in Kwimba District in the past several years in this re-forestation programme funded by the government of Australia. About 220000 neem seeds are available to be grown annually (Muyengi, Assistant Project Manager, Kwimba Project Personal Communication).

The total number of neem trees in the country is not yet established but can be estimated to range from 10000 to 50000 at various stages of growth to those that are over 4 years old and producing seeds.

What remains to be done, therefore, is advice and sensitisation/awareness of farmers and livestock keepers on production, preparation, quality control, formulation and dispensing of the neem extracts. This though has to be based on research under local environmental conditions. The field trial studies reveals the effectiveness of neem products like NSKE 5% and Neem oil 3% against insect pests of animals, crops like, rice, pigeon pea and click pea. (Kilonzo, 1986; Fagbeni, 1983)

Not withstanding, Botswana is yet to develop enough data on the number of trees available.

Considering the diversity of properties, it is not surprising that neem is grown as a cash crop in some countries (Anon, 1992). Thus for Tanzanians, besides growing neem to supply locally required extracts for pest control, it could be grown on a large scale as a cash crop to supply oil for the commercial industry. The cake obtained after extraction of oil could be used as a supplemental feed (Rajagopal, 1981) in animals but in addition the residual levels of the active components could be useful if found to inhibit nematode establishment in the gastrointestinal tract; insufficient data is available on the effect of neem on endoparasites (Anon, 1992; Saxena - Neen Awareness Project ICIPE - Personal Commutation.

1.2 Statement of the Problem

Neem is a cheap natural source of insecticides that can play a significant role in reducing the indiscriminate use of the toxic synthetic chemicals which are dangerous to man and the ecosystem (i.e. toxicity to non-target organisms, development of insecticide resistance and consequent pest resurgence, environmental pollution, health hazards to those who cannot afford protective clothing, hazard due to pesticide residues).

Botswana being among the livestock based economy countries stands to benefit from the available trees in insect/pest control to increase livestock production, while at the same time redusing the use of synthetic insecticides/acaricides in the long term. Thus, Botswana, would benefit from home-made crude neem extracts as a source of safe agricultural chemicals, by just pounding leaves, barks or seeds using locally available facilities such as wooden mortars and pestles squeezing out the juice/extract and spraying on crops or animals after mixing with water. However, it is necessary to develop the correct formulations for the different applications, (i.e. horticultural, cereal crops, livestock) so that farmers and livestock keepers can be advised accordingly for home based production which would in turn cause a gradual shift from the use of the more toxic synthetic agriculture chemicals. This would in the long term save the country millions of foreign exchange spent in importing those chemicals (Mbwille et al., 1992).

1.3 Purpose of the study

The purpose of this study was to study the possible uses of neem seed oil extract on controlling ectoparasites of cattle, especially ticks. The study explored the tick control in Tswana, Simmental and Brahman by means of Neem seed extracts (*Azadirachta indica*)

Secondly to make recommendations regarding the cheapest and most effective formulation for use on the cattle. Considering the large amount of literature already available on Neem tree (*Azadirachta indica*) and its medicinal properties, it is rather interesting to note that there is scanty information on its effect against ticks. Tick borne diseases are among the most important diseases affecting livestock in Botswana, causing mortalities and reduction in production.

1.4 Objectives of the Study

The main objective is to test neem tree (*Azadirachta indica)* products as insecticides/pesticides for livestock production.

Specific objectives are as follows:

(1) The effects of NSE on tick counts in different breeds of cattle kept in Botswana.

(2) Quantify seasonal effect (Study effectiveness of NSEK in different seasons).

(3) Study different tick species and anatomical sites that are infested.

(4) Study effect of gender (Male and Female cattle on tick counts)

1.5 Significance of the study/ Importance of the results

In view of its potential pulicidal effects and extensive growth of neem in many parts of Africa, especially dry areas where most animal farming is also a major activity, it is important to study the possible use of Neen seed extract to control ectoparasites on cattle in Botswana.

Farmers who already has neem trees will benefit from this because then they can be advised on how best to feed their animals the neem see cake after oil extraction (Home based or industrial).

The results will be used for making people aware of neem and its variety of uses in livestock production. In areas where neem is not available, programmes will be established through the Governments.

The results of this study will form the basis of starting a neem plantation in order to produce neem extracts for future research.

1.6 Delimitation of the study

The study focused on ticks of cattle, but sheep and goats are also affected by ticks in Botswana. The main priority of livestock farmers is cattle, while a few farmers keep small ruminants (sheep and goats). More over tick control is not generally practiced in small ruminants.

Therefore, the emphasis of the present study is on the possible control of external parasites on cattle.

CHAPTER TWO

LITERATURE REVIEW

2.1.0 Botany of the neem tree

The botanical name of neem is *Azadirachta indica*. The plant is common in India, Java and Burma, either natural or cultivated. *A. indica* is also found in Europe and other warm countries. The tree grows to a height of 40 - 50ft or more depending on the climatic condition of the place (Robert and Henry, 1981).

2.1.1 The neem flowers (Azadirachta flowers)

The calyx of the flower is small, flat with 5 rounded segments, their petals are spathulate, the anthers are 10, its ovary is three celled drupe size of olive, and smooth dark purple when ripe. The flowers are white with short slender pedicaps which have short scattered hairs (Dieltrich, 1974).

2.1.2 Neem leaves *(Azadiractita folia)*

The leaves have an intensively bitter taste. In the India Pharmacopea it is indicated that for medicinal purposes leaves are directly used when fresh. Leaves are imparipinate, 9-15 cm leaflets 9-13 nearly opposite, short petiolated 1-3 cm in length, unequal sided, deep and sharp serrated (Dieltrich 1974).

The bitter substances in the leaves are soluble in water. The leaves have no peculiar alkaloid but have the same properties similar to resin (hydrate) (Robert and Hendry, 1981).

2.1.3 The neem bark

The bark is covered by scaly epidermis and it has a bitter taste How ever, the characteristics of the bark vary according to size and age of the tree. It contains volatile oil, starch, saccharine matter, bitter resin, gum and margnosine, an astringent. The bitter principle of neem back is an amorphous neutral resin, which is readily soluble in alcohol, ether, benzyl and boiling solutions of fixed alkaline, but slightly soluble in water.

2.2 The medicinal value of the tree.

This tree is used as insecticides, pesticides in agriculture and also in human beings as a treatment of some diseases. The following parts of the plant are of medical value; The neem seed, Flower, leaves, bark and fruits

2.2.1 Neem seeds

Neem seed produces oil that is bitter in taste. The oil can be used for the following purposes (Jotwani and Sirca, 1965): as antihelmentic, for skin diseases, insecticide in the control of grain pests and in treatment of sloughing ulcers or chronic disease conditions.

In treatment of ulcers, neem seed extracts stops the sloughing process and prevent production of maggots; it dislodges them if already produced. The oil is also antiseptic. It is further used to treat rheumatism, being taken internally by women during pregnancy. This was practised in India and some parts of Burma (Kirtikar and Basu, 1935)

In veterinary medicine the oil is used to treat mange infection, certain ectoparasites, ringworm's and other skin conditions. The oil is also used as an external stimulant and in the treatment of chronic malaria fever and chronic syphilis (Dieltrich 1974), although presently it is doubtful.

2.2.2. Neem fruits

The fruits may be ripen or unripen. They are produce bitter oil (Margosa) deeply yellow with disagreeable flavour. Unripen fruit oil is used as anthelmintic, in treating skin diseases, tumours, toothache, and piles. It also cures urinary discharges (Kirtikar and Basu, 1935). The Ripe fruit is used to treat eye disease. It has the same characteristic as unripe fruit.

2.2.3 Neem leaves

The young leaves are used as astringent and are good for skin diseases (Kirtikar and Basu 1935). They are bitter and also used as antihelmentic, insecticides and insect repellant, ophthalmic and skin diseases. Other conditions that can be treated with extracts are cough and piles. One can make a bitter tasting medicinal tea by crushing leaves or making it into an infusion with boiling water to form a bitter Vegetable tonic. The later can be used to treat malaria fever (Dieltrich, 1874). The aqueous extract of neem leaves possess an activity, mild diuretic properties. In rats water extracts (4%) produced non-specific inhibition of natural and induced tone in smooth, cardiac and skeletal muscle of isolated muscle preparation (Luscombe et *al.*, 1974)

2.2.4 Neem flowers

The flowers have more or less the same properties as leaves, i.e. bitter tasting, and indications. Some of its indications include cure of snake and scorpion bites, treatment of leucoma, lumbargo, piles, syphilis and wound-like lesions. Neem flowers are also used as anthelmintic and to relieve biliousness (Kirtikar and Basu, 1935).

2.2.5 Neem bark

Like the other parts of the neem, the bark is bitter. Neem bark is often used as a treatment of malaria fever by suing tincture of neem bark stem. Neem bark is also indicated to cure ulcers, coughing, urinary discharges, and antinflamatory processes. The bark relieves burning sensations near the heart, it can cause mutation and is an astringent (Kirtikar and Basu, 1935)

A good paste is prepared from the gum of the bark. The bark is also used to relieve vomiting fatigue, fever and relieves bad taste from mouth (Kirtikar and Basu, 1935)

2.3. Chemical composition of *Azadiracta indica*

Several effective formulations of *Azadiracta indica* have been prepared and their major constituents isolated in a pure state. The chemistry of these constituents is based on classical and modern sophisticated spectroscopic methods. More than 100 new compounds have been isolated and their structures determined.
Azadiracta indica A. Juss (Neem), an indigenous tree belongs to the family Meliaceae and is widely distributed in Asia, Africa and other tropical parts of the world. Almost every part of the tree has found applications in indigenous medicine for the treatment of a variety of human ailments. It has been a practice in the Indo-Pakistan subcontinent to mix dried neem leaves with stored grains and to

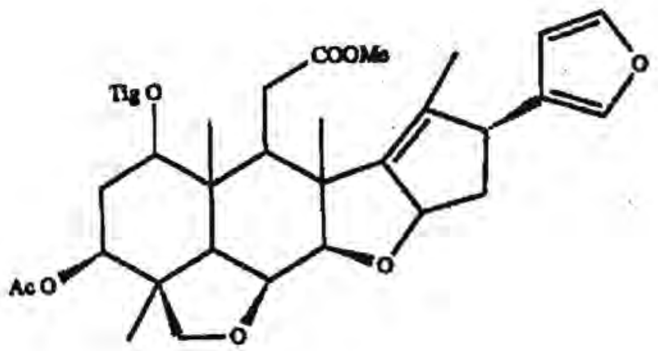
place leaves in folds of clothes and woolen blankets to keep insects away (Chopra et al., 1956; Dymock et al., 1980).

On account of the enormous curative properties of neem, efforts were made by various groups of workers since about the beginning of the current century, to isolate its active constituents, particularly the bitter principles to which the therapeutic virtues of its various parts were attributed. Initially these studies were mainly concerned with the fatty acid components and amorphous bitter substances isolated from neem oil which about 45 per cent of the total weight of seeds.

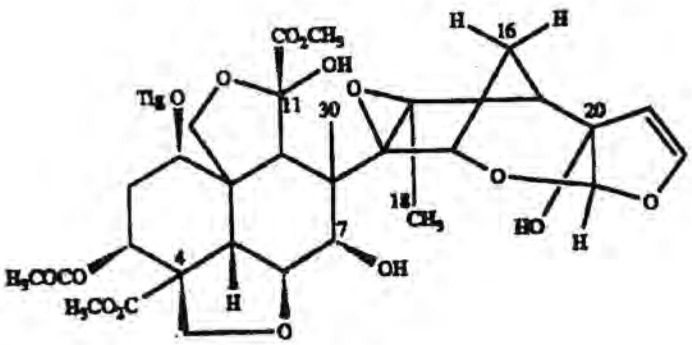
Siddique (1942) undertook a reinvestigation of the oil, employing an analytical procedure based on its partitioning between hexane and slightly diluted alcohol, avoiding its prior saponification. As a result of this study, he reported the isolation and characterization of the first two crystalline bitter constituents nimbin and nimbinin along with an amorphous bitter principle nimbidin from dilute alcoholic extracts of neem oil.

Subsequently, a host of compounds has been isolated from various parts of the tree including meliantriol (Lavie et al., 1967), salannin (Henderson et al., 1968) and azadirachtin (Butterworth and Morgan, 1968) which are active insect feeding deterrents, toxicants and / or disruptants of growth and development against a large variety of insect species and nematodes.

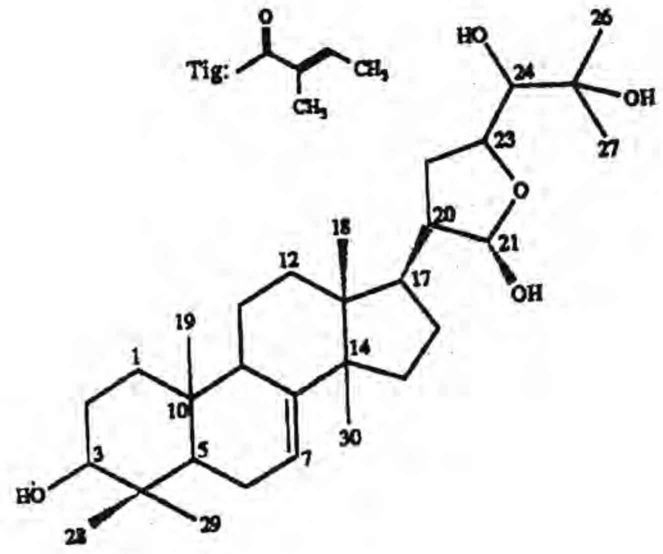
Continuiung research into the various parts of neem is still producing compounds of considerable chemical and biological interest. More recently, two triterpenoids from neem leaves namely nimocinolide and isonimocidolide (Siddiqui et al., 1986) have been reported to behave as growth regulators against *Aedes aegypti*.



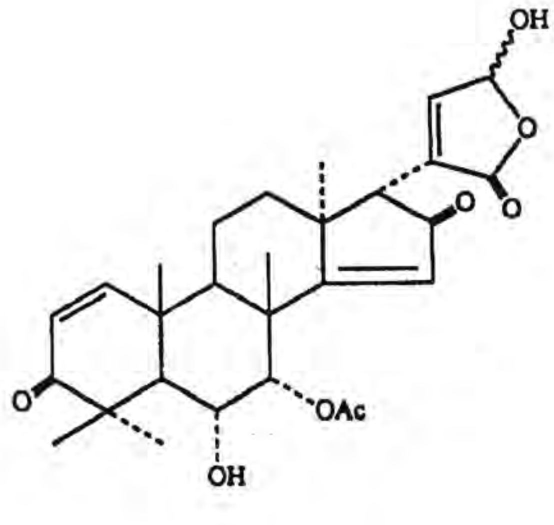
Salannin



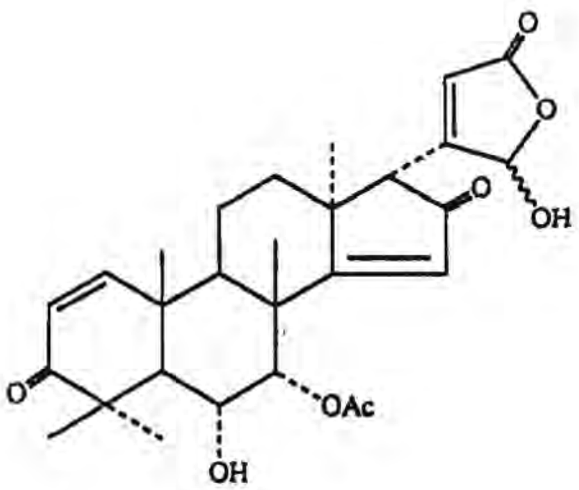
Azadirachtin



Meliantriol



Nimocinolidine



Isonimocinolide

Taking into account the enormous therapeutic and pesticidal properties attributed to neem, and the fact that heavy loss of food and other crops caused by various pest species is a serious problem, particularly in the tropical and sub-tropical regions, studies were started a few years ago on the various parts of the neem tree with the object of:

a) isolation and structure elucidation of the chemical constituents from the various parts of the tree, and

b) obtaining active pesticidal formulations which may prove effective in controlling the target pests with undesirable environmental side effects.

Studies undertaken Siddiqui et al., 1986, in various parts of the tree have resulted in the isolation and structure elucidation of over hundred new compounds on the one hand, and preparation of some simple non-hazardous effective formulations on the other. These formulations can be safely applied against domestic and agricultural pests.

The insects against which the activity has been tested are shown in Table 1 and some interesting and promising results of the biological activity of various fractions and pure compounds obtained from the leaves, and various parts of the fruits are depicted in Table 2.

Table 2.1 Insects which the neem activity has been tested.

Fruit Constituents	Insects	Leave Constitutents
1.	*Musca domestica*	*1. Aedes aegypti*
2.	*Callasobruchus analis*	*2. Musca domestica*
3.	*Alleurobus barodensis*	*3. Blattela germanica*
4.	*Oxyearenus lugubris*	*4. Calasobruchus analis*
5.	*Scintherrista notabilis*	*5. Sitophilus oryzae*
6.	*Sitophilus oryzae*	*6. Tribolium castaneum*
7.	*Amritodus atkinsoni*	
8.	*Culex fatigans*	
9.	*Tribolium castaneum*	

Some of these results need particular attention. For instance, NFD, a fraction of neem leaves and its two pure compounds nimocinolide

(MP1) and isonimocinolide (NP1') have LC_{50}, 0.58, 0.625 and 0.74 ppm against *Aedes aegypti* whereas margosan-O, a neem based pesticide of U.S. origin has LC_{50} 148 ppm. Similarly, NFB, a fraction of neem leaves has LC_{50} 0.15 per cent against *Musca domestica* which is almost of the same order as that of dimilin (LC_{50} 0.14 per cent) and is about three times more effective than malathion (LC_{50} 0.33 percent). A pure compund (NE) from the fruits was a s effective (LC_{50} 0.85 μg/kg) as coopex (LC_{50} 0.80 μg/kg).

The fruit fractions NFC, RBA and RB-a are effective against *Callosobruchus analis, Aleurobus barodensis* and *Amritodus atkinsoni* respectively.

It would be interesting to note that when the activity of neem fruit coats (RB-b) and neem seeds (RB-a) was studied against *M. domestica*, it was noted that RB-b was 200 times more effective (LC_{50} 1.00 ppm) as compared to RB-a (LC_{50} 200 ppm).

A formulation of the same fraction (RB-b) was prepared with piperonyl butoxide and triton X-100 and applied against *Sitophilus oryzae*. It was found that this formulation has LC_{50} 6.28 μg/cm^2 of that of the standard, methoprene.

These observations formed the basis of directing our studies towards the chemical constituents of the fruit coatings (RB-b) in particular and look into their potential biological activity. It may be noted in this connection that azadirachtin has been isolated from the neem seed.

Fresh fruits collected from the Karachi region were manually separated into seeds and coatings, each with some part of the fruit pulp. Fruit coatings were repeatedly extrated with ethyl alcohol at room temperature and the thickish syrupy residue obtained on removal of the solvent from the combined extracts was separated further through solvent fractionation followed by chromatography procedures such as vacuum liquid chromatography (VLC), flash column chromatography (FCC). Thick layer chromatography (TLC) and high pressure liquid chromatography (HPLC). As a result three known triterpenoids – epoxyazadiradione, azadiradione (Lavie et aal., 1971) and kulactone (Chiang and Chang, 1973) and eleven new compounds namely, limocinol, limocinone, limocinin, limocin A, limocin B (Siddiqui et al., 1991a) 17, 20-27 – nonanorapotirucalla (or 17, 20-27 – nonanorapoeupha), 7α-acetoxy, 1, 14-dien, 13 →16-γ-lactone (N-191), 17, 20-27 – nonanorapotirucalla (or 17, 20-27 nonanorapoeupha), 7α-acetoxy, 1, 14-dien, 13-epi, 13→16-γ-lactone (N-192), desfurano-azadiradione (N-193) (Siddiqui et al., 1992), azadirol (Siddiqui et al., 1991b), azadironolide and isoazadironolide (Siddiqui et al., 1990) have so far been isolated and their structures elucidated on the basis of spectro-scopic data and chemical reactions.

Table 2.2. Biological activities of various franction and pure compounds obtained from neem leaves and fruits.

	Leaves			Fruits		
A.aegypti	NFD	NPI	NPI'	RB-b	RBU-9	Margosan-O
LC_{50}	0.58 ppm	0.625 ppm	0.74 ppm	575 ppm	455 ppm	148 ppm

	Leaves			Fruits							
M. domestica	NFA	NFB	NU-1	N-7	RB-a	RB-b	NPE	NE	RBS	RBU	Coopex
LC_{50}	1.4µg per fly						1.4µg per fly	0.85µg per fly		5.6µg per fly	0.8µg per fly
		0.15%	1.06%	3.8%		3.7%			4.8%		0.029%
(Swiss strain) LC_{50}					200 ppm	1.00 ppm					Malathion 0.33% Dimilin 0.14%

	Leaves	Fruits			
C. analis	NFQ	NFC	NPE	NC	Dimilin
LC_{50}	1.12 mg/cm^2	40 µg/ cm^2	1.15 mg/cm^2	0.18	20 µg/ cm^2

A. barodensis	RBA (Fruits)	Malathion
LC_{50}	1.0%	3%

Table 2.2
Contd.

.O lugubris	RBA (Fruits)	Malathion		
LC_{50}	9.2%	0.004%		
S. notabilis	RB-b (Fruits			
LC_{50}	12.5%			
S. oryzae	LPA (Leaves)	RB-b (Fruits+PBO +TX-100	Methoprene	Danitol
LC_{50}	0.50 mg/cm ^{2}	6.28 µg/ cm ^{2}	18.85 µg/ cm ^{2}	0.39 µg/cm ^{2}
A.atkinsoni	RB-a (Fruits)	URN (Unripen fruits)	Dimethoate	Perfekthion
LC_{50}	0.40%	1.40%	0.14%	0.14%
T. castaneum	RB-a (Fruits)+ BPO+ TX-100	Malathion		
LC_{50}	0.05%	0.007%		

Presently the azadirachtin content in various active fractions is being estimated, apart from the characterization of new constituents, it would particularly be interesting to not the azadirachtin content in EB-b a fraction of the neem coatings, since, so far azadirachtin has been reported from the seeds.

2.4 Mode of Action of neem (Azadirachtin)

2.4.1 Antifeedant Action

Through series of experiment conducted at both Reading (UK) and Nyankpala (Ghana), feeding inhibition has been established as a major biological effect of neem extracts on the insects. It can be applied both to the larval/adult of the insect or to the food. Treated insects with at least 1% will experience reduced food conversion and utilization efficiencies resulting in decreased growth rates. The primary effect when neem is applied to the food of insect presumably results from sensory detection and avoidance by the insect, (Schoonhoven and Jermy, 1977).

A secondary anti-feedent effects result when neem extract (above 1%) is applied direct to the insect rather than food, the applied products presumably bypass the taste receptors responsible for this. Neem extract interferes on the centres of the insect that control and regulate feeding and metabolism (Tanzubil, 1989).

Application of neem extracts on the farm animals could protect animals in two ways;

animals would be instantly protected via the primary, gustatory repellent action of extract on the surface of the animals.

larva picking up residues during spray application or through blood sucking can suffer feeding inhibition as a result of decreased activity and metabolic inhibition (Tanzubil, 1989).

2.4.2 Growth regulation effects

Azadiraditin have no knockdown effects. The consistent exert their effects on the insectsi gustatory receptors and neural-endocrine cells. Disruption of corpus cardiacum and corpus allatum cells occurs.

Insects are repelled, pupation is inhibited or pupas develop with severe malformation and inability to moult. Smoke from a mixture of kerosine and neem oil at 2% (v/v) repels mosquitoes (Sharma and Ansari, 1993).

High doses of Azadiractin up to 10g/larva resulted in 100% mortality while lower doses inhibit or disrupt moulting, resulting in the formation of larval-pupal intermediates or abnormal nymph in most of the insect pests of plants and animals (Seiber and Rembold, 1983)

The inability of treated larvae to develop normally could be a consequence of interferences of neem with hormonal events in the insects.

The formation of larvae-pupal (nymph) intermediates can result from interferences of neem with ecolysteriod titres and/or function (Seiber and Rembold, 1983). The activity agaist insects have been demonstrated in more than 250 insect and parasites (Karkit and Basu, 1935; Ketkar, 1976).

2.4.3 Systemic Action

There have been some reports of systemic actions of neem against insects by Gill and Lewis (1977). In Ghana, neem seed powder 3% applied to the soil protected young sorghum seedlings against shoot fly and stemborer attack (Tanzubil and Dekuku, 1991). This implies systemic action of the powder as both insects reside in the stem upon entry.

2.4.4.Diverse mode of Action

Neem extracts usually produces a range of adverse effects on larvae and adult. In principle, it can be argued that it would be unlikely or at least more difficult for insects to develop resistance to neem than to many currently use of synthetic insecticides.

Saxena describes the chances of insects developing resistance to neem seed extract as remote. Working with Plutella xylostella, Vollinger (1987) in fact found no signs of resistance in moths treated with neem seed kernel extracts after 42 generations, while deltamethrin treatment resulted in resistance levels 35 times that of controls.

2.5 Effects of neem on Human and Environment

2.5.1. Safety and selectivity

Neem products are reportedly safe to man, non-target organisms and exhibit a reasonable degree of selectivity. This means they can be used with fewer ecological side effects than current insecticides.

In fact, concentrations of up to 8500mg/kg body wt of neem extract were non-toxic to rats when applied orally (Schmutterer, 1984). Sardeshpande, (1976) noted no carcinogenic effects in rats fed with 25% neem cake protein for nine months. Neem cake is also feed to animals with no side effects in some parts of the world. Neem oil has been shown to cause healing of chronic fungal infections of the skin in children (Chartuverd, V.S ñPersonnal communication 1995)

2.5.2 Toxicity

The neem extracts caused no significant ($P<0.05$) increase in the number of Mononucleated Polychromatic Erythrocytes (PNPCE) after treatment of mice with extracts (Ngomuo et al, 1995). Epidemiological studies conducted in India, where for decades, neem has been consumed for cure and control of various ailments in human; indicate that extracts are free from long term toxicity problems (chaturvedi, V.S. ñ personal communication, 1995).

Qadri et al (1984) reported that a 30% oil formulation fed to rats at doses of up to 600 mg/kg for 5 days per week for 3 weeks caused no toxicity. Furthermore, azadiraclitins showed no mutagenic effects in Salmonella typhinurium (Larson, 1986).

Neem oil is safe to man and is edible (Gaitonde and Seth, 1958) after processing. Neem leaves are eaten as vegetable, and twigs are used as toothbrushes. Neem oil is used as a treatment of various skin diseases and it has anti-biotic properties (Sharma, 1993).

2.5.3. Poor Residue activity

The residue effects of neem products last mostly for five to seven days. Temperature ultraviolet light, rainfall, and other environmental factors may exert negative effects on the active principles (Schmutterer, 1990). The use of neem products for vector protection in animals and plants can help in reducing the atmospheric pollution and preventing food poisoning. The neem-based insecticides are safe, non-toxic and do not keep any residue effects on agricultural produce for more than 7 days.

2.6. Characteristics of neem seed oil and bark

2.6.1 Neem seed

All parts of the plant are used for medicinal purposes. Seed extracts have been used to control helminths, skin infections such as ring worms, rheumatism and muscular pain as a result of its antinflammatory activity (Kirtikar and Basu, 1935), stomach ulcers and scabei. Neem seeds are processed using oil expellers and solvent extraction to yield neem oil and neem cake which are utilised in the soap industry (Siddiquie, 1942) and as manure (Ketkar, 1976), respectively.

The powder from dried seeds is used for the control of insect infestations in homes and cereal grains. It is also used in detergents like shampoo (2.5% to 5.0% azadirachtin) for lice control in animals (Jotwani and Sirca, 1965; Rembold, 1986).

Neem extracts are also used in treating diabetes (Luscombe and Taha, 1974; Ketkar, 1976). Neem is also used to treat malaria fever (Kirtikar and Basu, 1935)

2.6.2 Chemical composition of neem bark

Genudin is an active antimalaria principle of Azadirachtica indica cortex. The bitter components of the bark are resin, magrosine and an astringent substance called catechin.

2.7 The neem plant in Agr iculture industries

2.7.1 Protectant against stored grain:

The repellent properties of the neem tree inhibit locust and other migratory insects. The insecticidal compounds in neem tree are azadirachtin (Butterworth and Morgan, 1968), salannin (Siddique, 1942) and meliantriol (Sankaram, 1986). The three Compounds however are more concentrated in the seeds in comparison with other parts of the plant. Powdered neem seed kernel, when mixed with wheat seed, protects the seeds against the major pests, i.e. beetle and weevils (Jotwani and Sirca, 1965).

Azadirachtin, the active constituent in the seeds, stimulates certain insect hormone and hence the compound repels insects and inhibit pupation or pupa develop with severe malformations and inability to moult (Nagvi 1986)

In India and America the neem insecticide has been shown to be effective against beetles, caterpillars, grasshoppers, locusts white flies, aphids and some bugs. As it is an insect repellant the plant extract tend to repel insects, cockroaches and mosquitoes. It has further been shown that the crushed neem leaves and seeds 2% (v/v) can inhibit the life cycles of mosquitoes in ponds and stagnant water in the breeding places. Soil nematodes can also be killed by neem extract. Neem seed extracts, may be used to protect tobacco plants against Spodoptera litura and insect pest of sorghum under field conditions. Other insect antifeedant components are salannin and meliantriol (Jotwani and Sirca, 1965)

New insect antifeedent and insect growth inhibitors have been isolated from an active fraction of neem seeds. These include nimbidin, isovepaol, and vepaol (Sancaram et al., 1986)

2.7.2 Use of Neem seed cake in the animal industry

About 4.15 tones of neem yield 3.3 tonnes of cake and 0.85 tonnes. The neem cake is a by-product of the neem seed oil industry. The cake is prepared by pressing the neem seed kernel during oil extraction processes (Kerkar; 1976). Neem seed cake contain 12 ñ 17% crude protein. However the cake is not considered as true concentrate due to its higher fibre content (48%). The neem seed cake does not affect both the dry matter intake and digestion coefficient of nutrients (Arora et al., 1975).

However a positive growth response was reported in calves fed various levels of neem seed cake (Arora et al; 1975).

2.7.3 Neem extracts in reproduction.

Neem extracts can be used as safe and effective contraceptives. They are used for birth control and reduce the egg laying capacity in birds (Arora et al., 1975). Neem oil applied intravaginally to rats at 20(l and in humans at 1.0ml prior to sexual intercourse had an efficacy of 100% in inhibiting preginancy and no side effect was observed (G.P. ñPersonal communication, 1995)

2.8 Ticks commonly found in Botswana

Ticks represents one of the major causes of economic losses in animal production. Cattle are hosts to a large number of ectoparasites, but ticks are by far the most important group of cattle ectoparasites.

Ticks are blood feeding external parasites of mammals, birds, and reptiles throughout the world. Approximately 850 species have been described worldwide (Furman and Loomis 1984). There are two families of ticks, the Ixodidae (hard ticks), and Argasidae (soft ticks). Both are important vectors of disease causing agents to humans and animals throughout the world. Ticks transmit the widest variety of pathogens of any blood sucking arthropod, including bacteria, rickettsiae, protozoa, and viruses. Some human diseases of current interest caused by tick-borne pathogens include Lyme disease, ehrlichiosis, babesiosis, rockymountain spotted fever, tularemia, and tick-borne relapsing fever.

Tick, is the common name for members of a group of large mite-like arachnids parasitic on mammals, birds, and reptiles. All ticks are bloodsucking parasites. Ticks are found in most parts of the world but are generally limited to those habitats frequented by their hosts namely, woods, tall grass, and shrubby vegetation where they climb onto plants and wait to jump on a passing host.

Ticks are actually a specialised group of mites and share many features with other mites. In general, they are larger than most mites, ranging from about 0.2 to 0.6 cm (about 0.08 to 0.24 in) in length, although females may be 1 cm (0.4 in) or more in diameter when fully engorged with blood. The adult tick has a mite like body with a tough skin and four pairs of clawed legs; tick larvae have only three pairs of legs. The mouthparts consist of a paired anchoring organ, or rostrum, covered with backward-curving hooks and equivalent to the pedipalps of other arachnids; and a pair of sharp mandibles that move back and forth in two longitudinal channels on the rostrum.

Ticks spend a great deal of time waiting for their hosts. They are particularly sensitive to carbon dioxide and movement signals that a host is nearby. Their grasping forelegs allow them to climb on a host. They quickly find a protected spot on the host's body, sink their mouthparts into the flesh, and begin to feed. When full, they drop off the host. In some species, adult males and some nymph stages do not feed.

Ticks are divided into two families: hard ticks and soft ticks. In hard ticks, the mouthparts are visible from above. Hard ticks are parasites primarily of mammals but are also found on birds and reptiles. The nymphs may feed on a different host species in each developmental stage; in each stage, the nymph feeds only once. The adult female lays a single large batch of eggs after her final meal.

In soft ticks, the mouth parts are hidden underneath the body. In general, soft ticks are parasites of birds, but some feed on other hosts. Usually all the developmental stages feed on a single host species. Each stage may feed many times over a period of at least several days, taking refuge in nearby crevices or under rocks when not feeding. The adult female soft tick lays relatively few eggs over an extended period. The relapsing-fever tick is a soft tick that occasionally bites humans.

Several diseases are transmitted to humans and domestic animals through tick bites or tick excrement. Usually, a particular tick-and-host combination harbours a specific disease organism, and often an infestation is limited to a relatively small geographical area.

2.8.1 Family Argasidae

Ornithodorus spp

Found in and around resting places of their large mammalian hosts (including cattle). However, this tick will readily take blood from almost any warm-blooded mammal in the laboratory (Furman and Loomis 1984). Humans may accidentally encounter this tick when they come in contact with host bedding sites, especially during activities such as hunting and camping. For humans, the bite of this tick is notoriously painful, resulting in a localized inflammatory response due to a toxic substance introduced into the bite site during feeding.

An important pests of livestock and horses. In Botswana, this tick is frequently found in the warm, dry regions of the central valley down to the southern portion of the Country. Rarely, these ticks have been found on humans, dogs, cats, and sheep. Heavy infestations with this tick can result in intense irritation, rubbing, and hair loss in livestock

Argas persicus (poultry ticks)

These ticks are common pests of chickens and turkeys, but generally do not cause serious problems except for small flocks on farms which provide wooden housing that encourages tick establishment. Eggs are laid in crevices in the wood. All stages of these ticks remain around the roosting area of poultry, hiding in crevices during the day and generally feeding at night. Ticks can survive in empty poultry housing for years. Argas persicus infests chickens. (Furman and Loomis 1984).

The life stages of soft ticks are not readily distinguishable. The first life stage to come out of the egg, a six legged larva, takes a blood meal from a host, and moults to the first nymphal stage. Unlike hard ticks, many soft ticks go through multiple nymphal stages, gradually increasing in size until the final moult to the adult stage. Some soft ticks pass through up to seven nymphal moults before they become adults. Soft ticks feed several times during each life stage, and females lay multiple small batches of eggs between blood meals during their lives. The time to completion of the entire life cycle is generally much longer than that of hard ticks, lasting over several years. Additionally, many soft ticks have an uncanny resistance to starvation, and can survive for many years without a blood meal (Furman and Loomis 1984).

The mouth parts of soft ticks are not readily visible from above. There are three visible components: the two outside jointed parts are the highly mobile palps; between these are paired chelicerae, which protect the centre rod-shaped structure, the hypostome. The palps move laterally while the tick is feeding and do not enter the skin of the host. The rough hypostome has many beak-like projections on it. This is the structure which plunges into the host's skin while feeding. The backward directed projections prevent easy removal of the attached tick.

Some soft ticks seek hosts by questing on low-lying vegetation, but the vast majority are nest parasites, residing in sheltered environments such as burrows, caves, or nests. Certain biochemical such as carbon dioxide as well as heat and movement serve as stimuli for host seeking behaviour. Soft ticks feed for short periods of time on their hosts, varying from several minutes to days, depending on such factors as life stage, host type, and species of tick. The feeding behaviour of many soft ticks can be compared to that of fleas or bedbugs, as once established, they reside in the nest of the host, feeding rapidly when the host returns and disturbs the contents. The outside surface, or cuticle, of soft ticks expands, but does not grow to accommodate the large volume of blood ingested, which may be anywhere from 5-10 times their unfed body weight

Soft ticks can be readily collected via dry ice traps. Blocks of dry ice emit large amounts of carbon dioxide, a host seeking stimulant. Traps are set in and around nesting areas of animal hosts. Soft ticks can be observed running along the surface of the ground towards the trap and are collected by hand, or inside a collection chamber in the trap. Many types of soft ticks can be artificially fed in the laboratory, thus reducing or eliminating the use of animals for certain studies. Some (ie: Ornithodorus coriaceus) will feed through sausage casings filled with almost any type of mammal blood heated up to 37(C. Others can be fed blood through various types of membranes in the presence of biochemical and environmental feeding stimulants such as ATP, carbon dioxide, heat, and animal fur (Sonenshine 1993).

Family Argasidae comes from the sub order Ixodides. The following are the characteristic feature of this family.

- integument leather-like and frequently mamillated
- no dorsal shield
- capitulum and mouth parts in nymph and adult,

situated anteriorly on ventral surface, not visible from dorsal aspect

- eyes absent or may be situated laterally in the supracoxal folds
- no distinct sexual dimorphism

Argasidae includes from ticks and tampans (Arthur, D.R. 1966)

2.8.2 Genus argas

Argas persicus

Common name: -Fowl tick, Blue bug, Tampan Hosts: -Fowls, Turkeys, Ducks, Geese and Wild birds Geographical distribution: - tropical and subtropical

- Diagnosis:

Finding the tick and/or the dark coloured excrement in hiding places such as cracks and crevices. -

Treatment: seed ticks are resistant to insecticides; can apply Malathion, anthracene (carbolinium), (Oken, 1918).

Morphology and identification:

-Adult is oval in shape 4-10 by 2.5-6 mm; very flat and leathery; dorsal and ventral sides meet on the edges at a pointer suture line; engorged tick has a slaty-blue colour; little sexual dimorphism. Females distinguished from males by a larger genital opening. (Oken, 1918)

Life cycle:

- Eggs laid in cracks and crevices of fowl house and under tree barks
- Larvae hatch in 10 days to 3 months; Larvae attach to chicken and engorge in 3-10 days; Larvae moult in 7 days.
- Two nymph stage each lasting 2 weeks and engorging once during this time; nymphs feed intermittently and at night; They feed rapidly and engorge within 20 minutes to 2 hours; Nymphs can live without food for a year to five years
- Adults are nocturnal; they engorge in a few hours and feed prior to each time they lay eggs usually once a month; can survive 5 years without feeding; mating takes place off the host (Duges, 1987)

Importance: causes worry, anaemia, weakness and unthriftness and death; vector of fowl sprirochaetosis

Classical treatment:

- Birds placed in crates
- Larvae drop off in 10 days
- Meantime the fowl house has been cleaned and treated with insecticide

- Birds returned to house and crates cleaned by scalding with boiling water or spraying with insecticide or by burning. (Oken (1918)

Genus ornithodoros

Species: 0. moubata

Common name: Eyeless tampan; relapsing fever ticks Hosts: Man, pigs and various burrow dwelling wild animals Geographical distribution: Africa, India and Near East Diagnosis:

Finding the tick and/or the dark coloured excrement in hiding places such as cracks and crevices. Treatment: Residual Synthetic pyrethroids, Organophosphates and organochlorides

Morphology: Thick, leathery and pod like; Hypostomes well developed; Head may extend from anterior margin of the body; integument covered with disc and mammillae.

Life cycle: Female lays eggs and then broods over them. Larvae do not feed. Larvae moult into nymphs 1,2,3,4 and 5. Nymphs and adults attack hosts at night (Murray, 1977)

Importance: Annoyance; transmission of Borrelia duttoni, B. Anserna and Aegyptianella pullorum; Reservoir and vector of African Swine Fever.

2.8.4. Family Ixodidae

Hard ticks have three distinct life stages. Larvae which emerges from the eggs have six legs. After obtaining a blood meal from a vertebrate host, they moult to the nymph stage and acquire eight legs. Nymphs feed and moult to the next and final stage - the adult, which also has eight legs. After feeding once more, the adult female hard ticks lay one batch of thousands of eggs and then die. Only one blood meal is taken during each of the three life stages. The time to completion of the entire life cycle may vary from less than a year in tropical regions to over three years in cold climates, where certain stages may enter diapauses until hosts are again available. Many hard ticks can go for several months without feeding if not unduly duressed by environmental conditions.

The mouthparts of hard ticks are readily visible from above. There are three visible components: the two outside jointed parts are the highly mobile palps; between these are paired chelicerae, which protect the centre rod-shaped structure, the hypostome. The palps move laterally while the tick is feeding and do not enter the skin of the host. The rough hypostome has many beak-like projections on it. This is the structure which plunges into the host's skin while feeding. The backward directed projections prevent easy removal of the attached tick. In addition, most hard ticks secrete a cement-like substance produced by the salivary glands which literally glues the feeding tick in place; the substance dissolves after feeding is complete.

Hard ticks seek hosts by an interesting behaviour called "questing." Questing ticks crawl up the stems of grass or perch on the edges of leaves on the ground in a typical posture with the front legs extended, especially in response to a host passing by. Certain biochemicals such as carbon dioxide as well as heat and movement serve as stimuli for questing behaviour. Subsequently, these ticks climb on to a potential host which brushes against their extended front legs. Hard ticks are most commonly collected for research by the use of "flags" or "drags" which are made from 1 m square pieces of roughly textured fabric such as fleece or flannel attached to a rod handle. The flags are slowly dragged across the surface of vegetation to collect questing ticks. Hard ticks feed for extended periods of time on their hosts, varying from several days to weeks, depending on such factors as life stage, host type, and species of tick. The outside surface, or cuticle, of hard ticks actually grows to accommodate the large volume of blood ingested, which, in adult ticks, may be anywhere from 200-600 times their unfed body weight (Sonenshine 1991).

Lifecycles of Hard Ticks

After hatching from the egg, larvae attach to a host, feed and detach, remaining on the animal. Subsequently, they moult to the nymphal stage, resume feeding and detach again. After they develop into adults and feed once again, they drop to the ground and lay their eggs, where the cycle begins once again.

Hard ticks have a variety of life histories with respect to optimizing their chance of contact with an appropriate host to ensure survival. Some ticks feed on only one host throughout all three life stages. These ticks are called one host ticks. This type of tick remains on one host during the larval and nymphal stages, until they become adults, and females drop off the host after feeding to lay their batch of eggs. Other ticks feed on two hosts during their lives and are called two host ticks. This type of tick feeds and remains on the first host during the larval and nymphal life stages, and then drops off and attaches to a different host as an adult for its final blood meal. The adult female then drops off after feeding to lay eggs. Finally, many ticks feed on three hosts, one during each life stage, and are appropriately named three host ticks. These ticks drop off and reattach to a new host during each life stage, until finally the adult females lay their batch of eggs. In each case, the fed adult stage is terminal, that is, after laying one batch of eggs the female dies, and after the male has reproduced, he dies as well.

2.8.5 General morphology of hard ticks:

- possess hard chitinous shield (scutum) extending over whole dorsal surface of male and covering only a small portion behind capitulum in larva, nymph and female
- mouth parts anterior and well visible from dorsal aspect.
- adults and nymphs are octopod while larvae are hexapod
- have festoons (notching) on posterior border of body.
- adult has genital opening - transverse slit in front of the middle
- anus posterior
- has anal groove either anterior of posterior to anus
- scutum ornate (coloured, enamel-like) or inornate (solid colour) (Arthur, 1963).
- Mating takes places on the host (of. soft ticks in which mating takes place off the host).
- Eggs are laid in sheltered sports under stones, in crevices of walls and wood cracks near the ground.
- Eggs are laid in one batch (in thousands) and then the female dies i.e. oviposition is a single uninterrupted process and when completed, the female dies. Number of eggs laid varies; can be 5,000 - 6,000 for Dermacentor spp. and 20,000 for Amblyomma

spp. Egg incubation period varies from 16 - 200 days depending on temperature. (Athur, 1963)

- Newly hatched larvae (seed ticks) climb onto grass and shrubs to await suitable host. Larvae engorges and then moults either on the ground or on the host.

- Nymph seeks a host or reattaches on the same host to engorge and then drop down to moult into adult on the ground or remains to moult into adult on the host.

- Adults seek a new host to attach to or merely reattach on a different site on the same animal. Their feeding activity thereafter is sexually differentiated. Adult male attaches and feeds for 2-3 days and then detaches to seek for females with to mate. After searching or after completion of mating they reattach and feed for a few more days before repeating the process. Females attach and engorge to 10% capacity then remain attached waiting for males to mate with. Upon being mated they engorge to full capacity within 24 hours and ripe off to lay eggs and then die. Unmated females tend to remain in this early stage of engorgement until they die or detach infertile. Males tend to remain on the host for four months or longer and tend to accumulate on the host towards the end of a period of seasonal adult activity e.g. six males to one female. (Soulsby, 1986).

- According to life cycle, hard ticks can be classified into three groups depending on the number of host required to complete the life cycle thus:

One-host tick:

Those in which all three instars engorge on the same animal e.g. Boophilus spp.

Two-host tick:

Those in which larvae engorge and moult into nymph on the same host and then the nymph drops off after having engorged. Moulting into adult takes place on the ground and the adult attaches on a new. Examples: Rhipicephalus evetsi and Rh.bursa.

Three-host ticks:

Are those which require a different host every instar e.g. Ixodes ricinus, Rhipicephalus appendiculatus and Amblyomma variegatum.

Rhipicephalus sanguineus (Brown dog tick)

The brown dog tick, or kennel tick, is a three host tick that is an important pest of dogs in the Botswana especially those housed in sclerotized structure on ventral side lateral to anus in males of some genera of hard ticks; anal, accessory anal and subanal

- present or absent

Basic capitulum

cephalothorax; shape: rectangular, hexagonal etc In the Argasidae family the capitulum is situated anteriorly and ventral, not seen form above. (E.J.L. (Soulsby).

Palp

Use for sensation, the pulp from soft ticks has more hair than that of hard tick.

Hypostome

- size in relation to the palps and number of rows

of teeth

Coxae

first segment of each leg attached to ventral side of body) - bifid or not (armed on unarmed). This segment present in soft and hard tick. They are used in movement.

Eyes;-

presence or absence; position and prominence. In soft tick eyes if present are paired in the supra coxal folds.

Postoons

-Notching on posterior margin on the dorsum; number and colour. Males with a pair of anal and accesory shield and a caudal protrusion when engoge. (present in hard tick). (E.J.L. Soulsby)

Genital opening

- Ventral and anterior to middle of body

- present in adults only and useful for distinguishing adult females from nymphs

Stigimata

-breathing spiracles behind last pair of legs or between 3rd and 4th pair of legs

- present in nymphs and adults only)

- shape (Athur 1963)

Orous area

-present on scutum on female

The family ixodidae is divided into:

Postriate ticks

- anal groove anterior to anus

Metastriate ticks

anal groove posterior to anus

Postriate ticks

Genera

- only one genus Ixodes

Genus Ixodes

Iornate show sexual dimorphism in palps - short in males and long with a characteristic open space between base of pulp and hypostome (seen as a white inverted Y) in female edge of male scutum not coinciding with body margin and therefore leaving an apparent marginal rimventral surface of male covered by a series of plates different from those of metastriates appear festooned but not real festoons have no eyes life cycle involves three hosts species found in East, Central and South Africa are Ixodes lewis, I. pilosus, I. cavipalpus and I.rubicundus.(Soulsby, 1988).

Metastriate ticks

-Are divided into Rhipicephaline group in which males have anterior plates and Amblyommine group in which the male lack ventral plates

Genus Rhipicephalus

- males always have anal and accessory, anal plates may be present in some spp.

- basis capitulum hexagonal
- mouth parts are relatively short and second segment of palps as wide as long
- first coxa bifid
- marginal groove may be well developed by many be absent
- always festooned
- on engorgement, males show one to three caudal protuberances and festoon plates turn form ventral to dorsal
- spiracle always comma shaped
- most species inornate;

Rhipicephalus appendiculatus

Common name, The Brown Ear Tick

Morphology/identification:- basic capitulum hezagonal

-cervical field scapel shaped

-festoons always present

-caudal protuberance on engorged male

-eyes flat.

Geographical distribution:

It is more distributed in East Africa (Kenya, Tanzania and Uganda) plus surrounding countries (Mozambique, Malawi, Zambia, Zaire, Burundi, Rwanda, Sudan) where the rainfall is greater than 600 mm and altitude is 1200 - 2500 meters above sea level.

Seasonal occurrence:

-Adults active just before the rains and through the rainy season. Nymphs are active throughout the dry season.

Hosts:

-Sheep are preferred host but cattle are the most important affected host. The tick may also occur on goats, horses, mules, donkeys and dogs and also on wild antelope

-Preferred sites of attachment: edges and inner surface of the ear, other parts of the head such as base of horns, eyelids, cheeks and other parts of the body such as the tail switch.

Life cycle: Three host tick with periods of survival unfed as follows: -7 month; Nymphs - 6-5 months: Adults - 14 months. Sometimes up to 2 years.

Importance: is the most important tick in Eastern Africa because of its ability to transmit Theileria parva causing East Coast Fever. It also transmits Theileria lawrencei and Th.mutans,

Rhipicephalus evertsi

Common name: Red legged tick

Morphology/identification: Scutum dark, almost black and densely punctated; red margin of body left uncovered by scutum; eyes beady; legs orange in colour

Importance: Transmits tick paralysis in lambs, can transmit Theileria parva experimentally but not in the field since the immature stages feed on small mammals and not cattle usually; also Th. mutans; Th. ovis; Babesia bigeminal; B. equine Spirochaetosis in cattle and horse.

Genus Boophilus

- male very small and have anal and accessory anal plate
- there may be a small caudal protuberance on engorgement of the male in some species
- palps very short; shorter than the hypostome and have trasverse ridges giving them a serracted outline
- first coxa bifid but goove shallow
- inornate
- eyes present
- festoons indistinct
- female scutum heart-shaped

Boophilus decoloratus

- Common name: The Blue Tick

Morphology/Identification:

Basis capitulum hexagonal; eyes frequently difficult to see; tips of anal and accessory anal plates can just be seen from above

Importance:

transmits Babesia bigeminal, B.ovis, Anaplasma marginale, and Babesia trautmanni transovarially. It also transmits spirochaetosis of horses, sheep and goats.

Boophilus microplus

-Common name: The Tropical Cattle Tick.

Importance: transmits Babesia bigeminal B.bovis and Anaplasma marginale, Babesia avian and equi.

The Genus Hylomma (Hyalomma is Greek for glossy eyes)

Common name: Bont-legged ticks

Morphology/Identification:

- Scutum inornate
- Legs ornate - reddish-brown with bright rings
- palps long to intermediate in six and second segment longer than broad
- basis capitulum triangular dorsally
- first coxa bifid
- eyes present and are hemispherical, surrounded by a groove or orbit
- festoons less than 11 and less regular
- male has three pairs of ventral plates with posterior raised margins (NB - this is the only genus with subanal plates)

- Speciation: based on grooves and punctation; several species recongnised but those occurring in Africa south of the Sahara are:-

Hyolomma truncutum

H. ufipes

H. albipermatum

2.6.2.9 Hyalomma truncutum (Soulsby, 1986)

-Common name: The African Bont-legged Tick

Importance:

1) Suspected to cause sweating sickness of cattle, sheep, goats and pigs in South Africa.

2) Causes lameness in sheep (due to attachment in between the hooves)

3) Can cause footrot

4) Can cause tick paralysis and transmit Q-fever in man

The genus Amblyomma (Soulsby, 1986)

Common name: Bont ticks (Bont is an Afrikaans word referring to the presence of brightly coloured patterns on their backs and their white and brown banded legs

Morphology/Identification:

- males without ventral mature (plates) but many species have curious scleritized structures called plaques.

- palps very long and second segment markedly longer than in the other genera and is longer than broad

- eyes present and are of two types: flat, marginal and spherical orbited first coxa usually bifid

Geographical distribution:

- mainly ticks of southern hemisphere; distribution is dependent on rainfall for some species e.g. Amblyomma variegatum occurs in areas with rainfall greater than 100 mm per annum (as for Rhipicephalus appendiculatus) while A.gemma occurs in areas with rainfall less than 100 mm per annum (as for Rh.pravus)

2.9 Methods of tick control in cattle.

The required properties of acaricides are:

1) It must kill the ticks

2) It must be safe to animals and man

3) It must be effective even when fouled by mud, dung, urine and hair and is contaminated by bacteria.

Acaricides most widely used are sold as liquid concentrates or wet table powder diluted with water to form an emulsion and suspension respectively. The acaricides can be grouped into

Arsenicals, Chlorinated Hydrocarbons, Carbamates, Diamidides and Synthetic Pyrethroids.

Arsenicals:

Arsenic was the first chemical used as an ixodicide. It had the advantage of being cheap and stable and also easiness of formulation and assay on account of its complete solubility in water. However it has the disadvantages that it is poisonous causing sever burn when used over strength, has little residual effect (10-12 hours) and easily leads to development of tick resistance (Nicholas,1988).

Benzen hexachlorides exists as alpha, beta, gamma and delta isomers but it is gamma isomer (lindane) which is acaricidal. It is sold as either wettable powder or liquid concentrate. Concentrations used were 0.01-0.05% with Botswana having used 0.02%. It fell into disuse of several disadvantages including the too short residual effect (2 days only) fast degradation in foul dips (due to anaerobic bacteria - hence the practice of using bacteriostatis or and /or regular emptying and refilling of dip (Nicholas, 1988)

Toxaphene (or chlorinated camphene) is very effective against deep feeding Amblyomma, Hyalomma spp., Rhipicephalus spp. and

Boophilus spp. It is formulated as miscible oils containing 75% toxaphene.

This acaricide is notable for several advantages which include having a long residual effect (4 days against adults and much longer against larve) and also it stability in dip washes. It is also comparatively cheap. However, this organochloride has also fallen into disuse in many countries and is no longer being produced on account of general abhorrence of organochlorides due to feared environmental contamination (Nicholas, 1988).

Organophosphates have the advantage of excellent efficiency against Boophilus spp. but there are disadvantages include a lack of residual activity and their higher cost.

Organophasphates are used as contact acaricides, insect repellents, animal systemic topical insecticides and parasiticides; plant systemic insecticides; soil nematocides, fungicide rodenticides etc. The various classes of organaphosphates include, phosphoramidates, pyrophosphates, phosphonates and phosphoramides but not all the compounds are effective (Eto, 1974)

Organphosphates are not very persistent in the environment and dissipate in 2-4 weeks except prophos, trichloronate which may persist in the soil for about 4 weeks. Organophosphates are poorly soluble in water (Nicholas, 1988)

Carbamates: e.g. Cabaryl (SEVIN). These compound have the disadvantage of being ineffective against Boophilus tick.

Diamidines: The most well known Ixodicide in this group is Amitraz. This can safely be used in dip tanks or by spray application and is highly effective against both single and multiple host ticks. It has the added impressive advantage of causing ticks to detach from the host very soon after application.

Synthetic pyrethroid: e.g Decatix. These are now being used extensively for the control of ticks in areas where the tsetse fly abounds since it is also very effective in killing tsetse flies which infest on the animal soon after dipping (Nicholas, 1983)

Principles of Ixodicide Application

The basis of successful tick control is the prevention of the development of the engorged female tick which represent the final stages of the parasitic life cycle. Control of larvae and nymphs of two-and three-host ticks is also important particularly for the prevention of important diseases like theileriosis. The approach to tick control depends on weather the problem is one -host tick or a mixture of one-, two or three-host ticks. (Soulsby, 1986).

2.10 Cattle breeds commonly found in Botswana.

Cattle are the most important livestock species in Africa and account for approximately 70% of the domestic livestock. In Botswana the major breeds of dairy cattle are the Holstein-Friesian, Ayrshire, Brown Swiss, Guernsey, and Jersey.

Beef cattle have been bred and selected primarily for the production of meat, and many breeds have been developed or adapted for special conditions. The major breeds of beef cattle in Botswana, listed in order of numbers, are Tswana, Afrikander, Tuli, and Brahman.

In recent years, several "exotic" breeds also have been imported, including the Simmental. These later arrivals have been used primarily for crossing with the major Tswana breeds to increase the size and weight of the crossbred offspring for commercial production. These cattle were selected and adapted to the arid region where they originated and have been used successfully in other areas with similar climatic conditions. The various breeds of beef cattle also differ in mature size, growth rate, gestation length, and birth weight. Limited data indicate, however, that strains within the breeds may differ as much as the different breeds in many of these characteristics

CHAPTER 3

3.0 MATERIAL AND METHODS

3.1 Study area

All experimental procedures were conducted on the farms around Serowe Village situated in the central part of Botswana. The study was conducted from November 1998 to December 1999.

The area consists of Acacia mellifera, often a shrub or a small thorn tree of 5 – 8m height. The pods, young twigs, leaves and flowers are highly nutritious and are eagerly eaten by livestock and game. Other types of Acacia include Acacia tortilis.

Rainfall varies between 30mm and 90 mm per annum. Mean monthly rainfall, gleaned from the meteorological service department illustrated in Fig. 3.1. During the experimental period from November 1998 to December 1999, however, rainfall was much reduced because of a prolonged drought (Fig. 3.1)

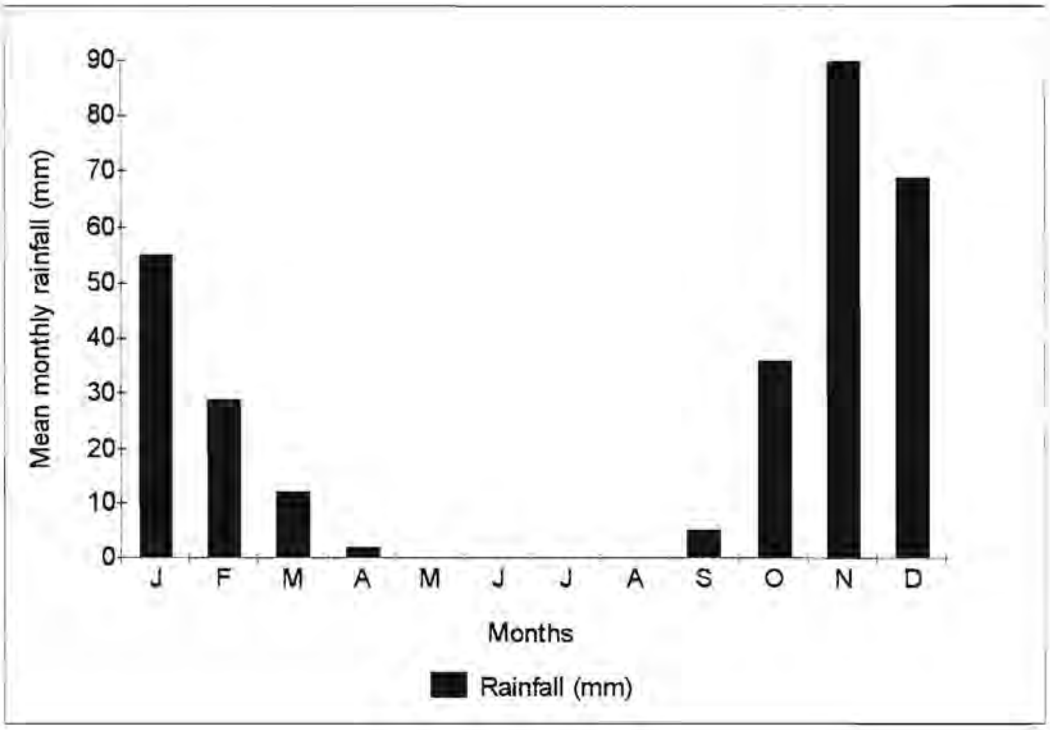


Fig. 3.1 Mean monthly-recorded rainfall (mm)

However this is a fairly normal situation in Botswana due to the fact that a big part of the country is desert. There are two distinct seasons in Botswana namely winter (April to July), and summer (November to March). There is however a short period of spring (August to October). Mean monthly-recorded rainfall (mm) gleaned from Meteorological service department for the experimental area during the study period is given in Figure 3.1.

Mean monthly maximum and minimum temperatures(C°) for the experimental area during the study period was also obtained from the meteorological service department

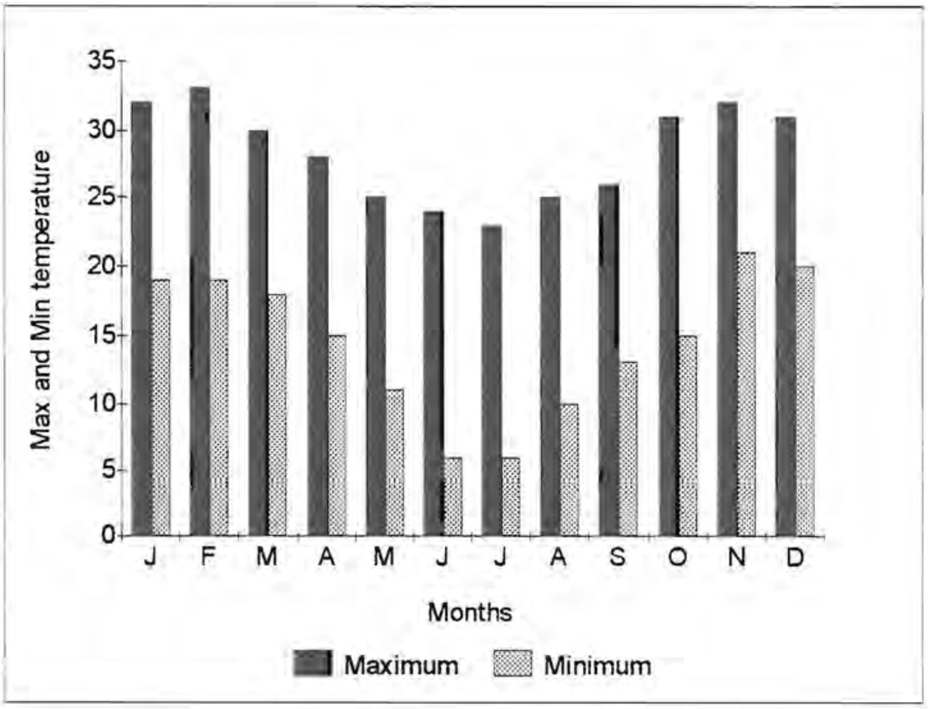


Fig. 3.2 Mean monthly minimum and maximum temperature (°C).

3.2 Animals used.

A total of 114 animals (57 bulls and 57 cows) of Tswana, Brahman and Simmental cattle were used for the three trials (Trial 1, 2 and 3) across all three seasons namely winter, summer and spring. All animals were 15 months old and above.

Animals were identified by means of ear tags. The experimental animals used in the study are listed in Table 3.1.1, 3.1.2 and 3.1.3.

Table 3.1.1

TRIAL No. 1 Natural infestation of ticks (18 animals)

NEEM TREATED GROUP			**CONTROL GROUP**		
Ear tag	**Breed**	**Sex**	**Ear tag**	**Breed**	**Sex**
S1	SIM	F	S4	SIM	F
S2	SIM	F	S5	SIM	M
S3	SIM	M	S6	SIM	M
B1	BRA	F	B4	BRA	F
B2	BRA	M	B5	BRA	F
B3	BRA	M	B6	BRA	M
T1	TSW	F	T4	TSW	F
T2	TSW	M	T5	TSW	F
T3	TSW	M	T6	TSW	M
Total		**9**	**Total**		**9**

Animals in the experimental group (Trial 1 and 2) were naturally infested with ticks without acaricidal intervention. The experimental animals were obtained from separate herds of Tswana, Brahman and Simmental cattle.

In trial three the same animals that were used in trial two were artificially infested with larvae. *A habreum* larvae and *B.decoloratus* were cultured and used in all artificial infestations of the animals (Trial 3) on the farm.

Table 3.1.2
TRIAL NO. 2 Natural Infestation of ticks (60 animals)

NEEM TREATED GROUP			**CONTROL GROUP**		
Ear tag	**Breed**	**Sex**	**Ear tag**	**Breed**	**Sex**
S7	SIM	F	S17	SIM	F
S8	SIM	F	S18	SIM	F
S9	SIM	F	S19	SIM	F
S10	SIM	F	S20	SIM	F
S11	SIM	F	S21	SIM	F
S12	SIM	M	S22	SIM	M
S13	SIM	M	S23	SIM	M
S14	SIM	M	S24	SIM	M
S15	SIM	M	S25	SIM	M
S16	SIM	M	S26	SIM	M
B7	BRA	F	B17	BRA	F
B8	BRA	F	B18	BRA	F
B9	BRA	F	B19	BRA	F
B10	BRA	F	B20	BRA	F
B11	BRA	F	B21	BRA	F
B12	BRA	M	B22	BRA	M
B13	BRA	M	B23	BRA	M
B14	BRA	M	B24	BRA	M
B15	BRA	M	B25	BRA	M
B16	BRA	M	B26	BRA	M
T7	TSW	F	T17	TSW	F
T8	TSW	F	T18	TSW	F
T9	TSW	F	T19	TSW	F
T10	TSW	F	T20	TSW	F
T11	TSW	F	T21	TSW	F
T12	TSW	M	T22	TSW	M
T13	TSW	M	T23	TSW	M
T14	TSW	M	T24	TSW	M
T15	TSW	M	T25	TSW	M
T16	TSW	M	T26	TSW	M
Total		**30**	**Total**		**30**

The animals (Table 3.1.3) were randomly allocated different treatments and were kept in different bomas, the same environment with no direct contact between animals.

Table 3.1.3

TRIAL No.3 Artificial infestation of ticks (36 animals)

NEEM TREATED GROUP			CONTROL GROUP		
Ear tag	**Breed**	**Sex**	**Ear tag**	**Breed**	**Sex**
S27	SIM	F	S33	SIM	F
S28	SIM	F	S34	SIM	F
S29	SIM	F	S35	SIM	F
S30	SIM	M	S36	SIM	M
S31	SIM	M	S37	SIM	M
S32	SIM	M	S38	SIM	M
B27	BRA	F	B33	BRA	F
B28	BRA	F	B34	BRA	F
B29	BRA	F	B35	BRA	F
B30	BRA	M	B36	BRA	M
B31	BRA	M	B37	BRA	M
B32	BRA	M	B38	BRA	M
T27	TSW	F	T33	TSW	F
T28	TSW	F	T34	TSW	F
T29	TSW	F	T35	TSW	F
T210	TSW	M	T36	TSW	M
T31	TSW	M	T37	TSW	M
T32	TSW	M	T38	TSW	M
Total		**18**	**Total**		**.18**

3.3. Tick Collection and identification

Tick counts on different species were made in order to identify tick species at the offset of the trials. During the first week and before any treatment started, ticks were collected from the perennial area, ear, dewlap, udder, scrotum, and sternum.

Table 3.2: Mean number of adult ticks collected for species identification in the Serowe village.

Host	Number of collections	Tick species	Mean No. of adults/collection
Cattle (45)	15	*A. hebraeum*	12
		R. everts	11
		H. truncatum	10
		R. appendiculatus	5.5

All the animals were mustered at 1-weekly intervals and individually neck clamped for examination. Two attendants, one on either side, carefully examined the restrained animals, recording all engorged female ticks according to species, all abscessation sites associated with tick attachments and all ruptured abscesses.
Ticks were fixed in a 70% ethanol solution. These ticks were identified in order to establish the species present and their relative abundance (Table 3.2). Various tick species were identified by stereo microscope and magnifying lens.

The criteria used for identification of various tick species are outlined in section 2.7 of the literature review. A stereomicroscope was used at low magnification of x10 and x40 magnifying lens.

3.4 Experimental procedure

3.4.1 Harvesting and extraction of neem oil from neem fruits

Ripen fruits were harvested from neem trees by shaking the branches. Fruits were collected on a large piece of canvas cloth spread on the ground immediately below the branches. Fruits were transferred into a bucket and soaked in water. The seeds were squeezed out by hand.

A small amount of detergent was added and the contents left over night to allow removal of the sugary coat of the seeds. The peel or 'skin' were removed by soaking for 24 hours in water containing a detergent powder at a low concentration (1g/1), then NSKE several times with clean water and then dried it to open air. Seeds were isolated and NSKE several times with clean water and then dried in open air. The seeds were stored in freezer at -2°C until required.

The local people were trained to collect, and preserve the seed. However most of the neem seed used in the experiment was bought from the National seed, Morogoro, Tanzania

The dried seeds was weighed and introduced into an oil-extracting machine where the seeds were crushed and the oil squeezed out. Therefore, Neem seeds-were used for oil extraction. The extracted oil was weighed to determine the concentration of oil (% w/w) in the seeds. The oil was subjected to extraction with ethanol to obtain the active constituents, or tested as such as described in the following sections.

3.4.2. Extraction of active constituents from the seed oil

Seeds were crushed and pounded thoroughly in a mortar. A 100g of neem seeds were mixed with the following reagents in 1-liter volumetric flask to a total volume of 1000ml of water or 1000 ml of ether or 1000 ml Ethanol alcohol. The reagent was left in a shaker for 12 hours extraction.

The extracts were decanted and sediments discarded after thoroughly filtering. The extracts were allowed to evaporate in a vaporizer to get concentrated extracts.

Ethanol was used for extraction of the active constituents from the oil (Bilton et al., 1987).

A 5% ethanolic neem seed extract (NSKE) for trial No. 1

The ethanolic neem seed extract 5% (NSKE) was prepared by blending 500 g powder of neem depulped seed in ethanol for 4min. The mixture was left to stand for one night, then filtered and condenced by evaporation to a volume of approximately 100ml. When used the extract was diluted with 6 liters of water to obtain a 5% concentation.

A 2% ethanolic neem seed extract (NSKE) for trial No. 2

The second treatment consists of ethanolic neem seed extract 2% (NSKE) prepared by the same procedures as in the second treatment (the extract was evaporated to dryness as paste) plus neem oil. The 50g of paste was dissolved in ethanol and then mixed with 20ml of neem oil and 10ml emulsifier to make 100ml solution. The desired concentration of 2% was prepared by diluting 30ml of the solution in 750ml of water.

A 3% ethanolic neem seed extract (NSKE) for trial No. 3

The third treatment was also ethanolic neem seed extract 3% (NSKE) prepared as in the second treatment but was not mixed with neem oil The 180g of paste was dissolved in ethanol and diluted in 6 liters of water to obtain a 3% concentration.

3.5 Application of neem oil (ethanol) extracts against ticks

A total of nine cattle were randomly selected and sprayed on the perennial area, ear, dewlap, udder, scrotal, and sternal area with 5% (W/V) ethanolic Neem Seed Kernel (NSKE) at the rate of 5g/kg body weight in the first trial. Another nine animals were spread with tap water and considered as a control group.

During the 2nd trial a total of 30 animals were randomly selected and sprayed with.2% (W/V) ethanolic neem seed extract (NSKE) which was mixed with petroleum jelly as the carrier extract. In the same trial some 30 animals were similarly sprayed with tap water and served as control group. During the 1st and the 2nd trial an the experimental and control animals were kept in separate partitions of the shelter, but grazed together in the area which was heavily infested with adults and immature stages of ticks.

In the 3rd trial a total of 18 cattle were randomly selected and sprayed with 3% (W/V) ethanolic Neem Seed Kernel (NSKE) at a rate of 5g/kg body weight. Another 18 animals were spread with tap water as control group. There was no direct contact between these animals, although they were kept in the same environment. All experimental animals were checked for tick burdens prior to the next spraying. Control animals which were not treated with neem extract, were also examined weekly for ticks.

The perennial, external ear, dewlap, udder, scrotum, and sternal area were examined in the weekly basis for the presence of ticks. Ticks, collected were transferred to a bottle containing 70% ethanol for fixing and storage prior to identification. Comparisons of infestation rates with ticks (adults and larvae) of different types were made.

3.6 Statistical analysis for trial No. 1

The data obtained from the tick collections in trial 1 were analysed by means of t-test (self-pairing method, Snedecor and Cochran 1976). The data were tested at a 5% level of significance. The following hypotheses were tested by means of the t-test:

Hypothesis:

Ho = average tick counts on control animals do not differ significantly from treated animals.

Ha: = average tick counts on control animals differ significantly from treated animals

Table 3.3: Tick counts of neem treated and control animals during the study period.

	Control animals	Treated animals	Observed difference	Square of differencere
DATE				
January	59	28	-31	961
February	60	36	-24	676
March	50	35	-15	196
April	18	19	1	1
May	26	18	-8	64
June	11	5	-6	36
July	15	4	-11	121
August	11	2	-9	81
September	30	8	-22	484
October	26	17	-9	81
November	54	30	-24	576
December	90	34	-56	3336
Total	**450**	**237**	**-214**	**6613**

Calculation of group mean *(d)*

$$d = \sum_1 di \,/\, \mathrm{n}$$

$$= -213/12$$

$$= -17.75 \text{ ticks}$$

Standard deviation (sd)

$(sd) = \sqrt{sd^2}$

The hypothesis were treated by calculating the ratio of the observed mean change to its own standard error $sd/\sqrt{n}$ where n is the number of pairs and *sd* is the standard deviation.

$sd^2 = \sum(x^2) - (\sum x)^2 \;\; /n$

$sd^2 = 6313 - (213)^2/12$

$sd^2 = 6313 - 45369/12$

$= 6313 - 3780.75$

$= \underline{2532.25}$

$sd = \sqrt{sd^2/n-1}$

$sd = \sqrt{2532.25}/12-1$

$sd = \sqrt{230.20}$

$sd = 15.17$

Standard Error of the Mean = standard deviations ÷ square root of the number of pairs.

$= sd/\sqrt{n}$

$= 15.17/\sqrt{12}$

$= 4.38$

$tc =$

$= {}^{17.75}\!/_{4.38}$

$= 4.04$

tc it value of ***t*** calculated.

The data obtained from trial 1, 2, and 3 were also analysed by means of multifactor analysis of variance (ANOVA) by employing the GLM-procedure of SAS (SAS, 1996). In most instances it was necessary to employ the GLM-repeated measures analysis procedure since repeated monthly counts were made on the same animals. The assistance of Mrs. Rina Owen and Prof. D. van Zyl from the Statistics department at the University of Pretoria is greatly acknowledged.

CHAPTER 4

RESULTS

4.1 Trial. 1 Natural tick infestation

Tick infestation

Amblyomma hebraeum

Counts of engorged female ticks suggest that the tick species were present throughout the year with marked peaks of abundance during March and November of both years (1998 and 1999). Higher numbers were encountered on all three breeds during the rainy season of each year (Fig. 4.1).

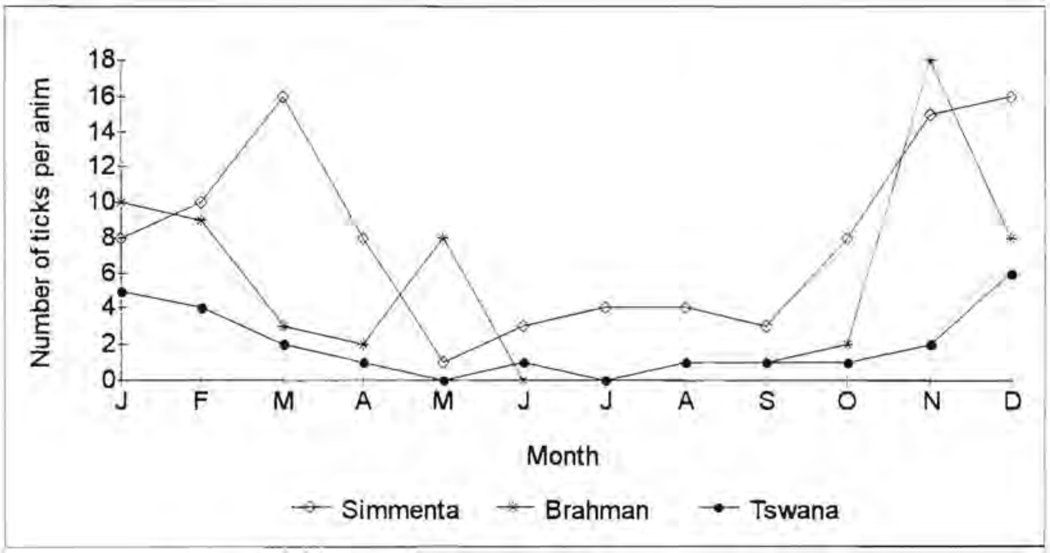


Fig. 4.1 Mean monthly number of engorged *A. hebraeum* females on three breeds of cattle during the trial period.

In general the results showed that mean tick population densities were significantly lower ($P<0.005$) in the experimental than control animals (Table 4.3)

Minor differences in the relative number of ticks on Simmental and Brahman cattle were evident during the first year of study (Figure 4.1). Both breeds were infested with significantly ($P<0.05$) higher numbers of ticks compared to Tswana cattle during the summer months.

During the summer months (February to April) of the experiment (Fig.4.1), tick counts on the Brahman were intermediate between the relatively high numbers found on the Simmental and lower numbers on the Tswana animals. A lower number of ticks were encountered on all three breeds during the dry and winter season of the year.

Rhipicephalus evertsi

Again mean tick population densities observed were lower in the neem treated than control animals ($P<0.05$, Table 4.13). Seasonal peaks of engorged females ticks were evident during the summer months of December 1998 and again in March and December 1999. The trend was similar in all three breeds of cattle (Fig.4.2).

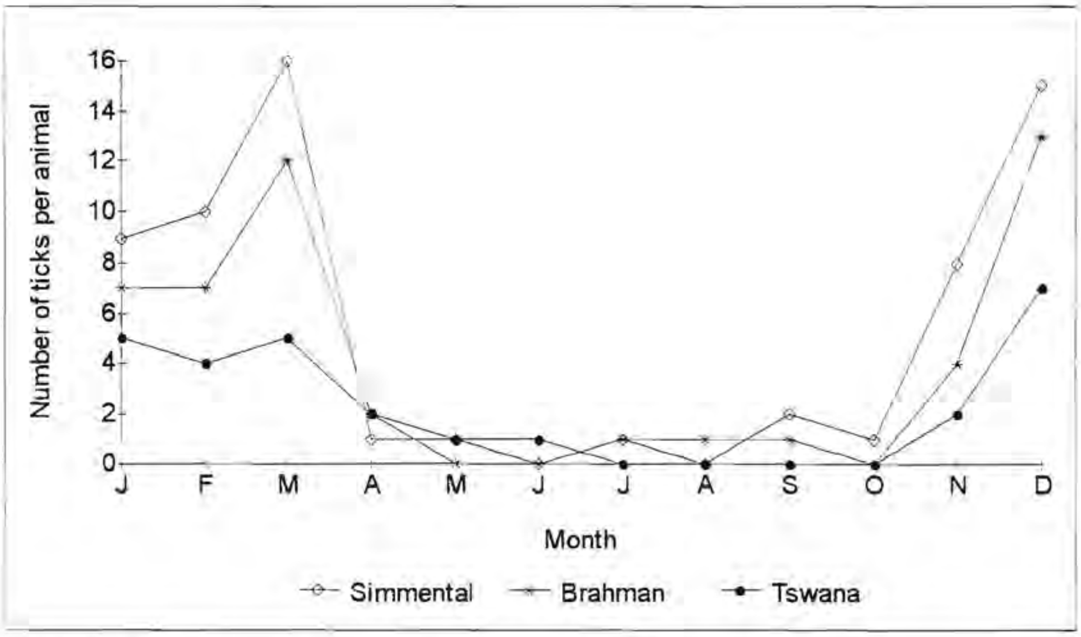


Fig 4.2 Mean monthly number of engorged *R. evetsi*

During both years the counts on Simmental and Brahman cattle were significantly (P = 0,05) higher compared to Tswana cattle in this trial.

Counts of engorged females during winter and spring suggest that *R. evertsi* were present at very low levels through these two seasons of the year. Lower tick counts were obtained during these seasons on all three breeds. (Fig.4.2)

Hyolomma truncutum infestation

Hyolomma truncatum were present although there was some difficulty in distinguishing *Hyolomma* species by means of the stereomicroscope and magnifying lens.

Peak numbers of engorged females ticks occurred during February and October of the 2nd year (1999) of the study (Fig.4.3). Simmental cattle carried significantly more engorged female ticks than Brahman and Tswana cattle during the February peak, but similar numbers were found on all three breeds during winter. There was a sharp increase in tick numbers between September and October, probably due to the abrupt change of weather associated with an increase on temperature and rainfall in the study area.

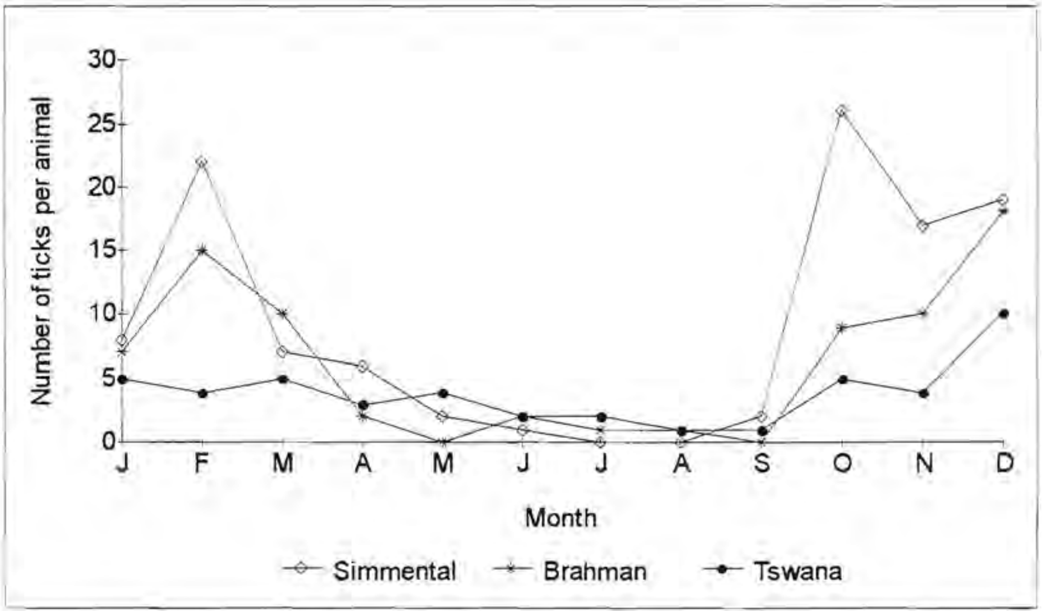


Fig 4.3 Mean monthly number of engorged female *H. truncatum*.

Boophilus decoloratus

Small differences were found between the numbers of engorged females tick on the Simmental and Brahman cattle (Fig.4.4). The ticks occurred throughout the year with peak numbers in March and September. Fewer engorged *B. decoloratus* females were encountered during the study period than any other species of ticks.

During the winter no significant difference was observed in tick counts between neem treated and control animals. This observation was similar for all breeds of cattle, Simmental, Brahman and Tswana.

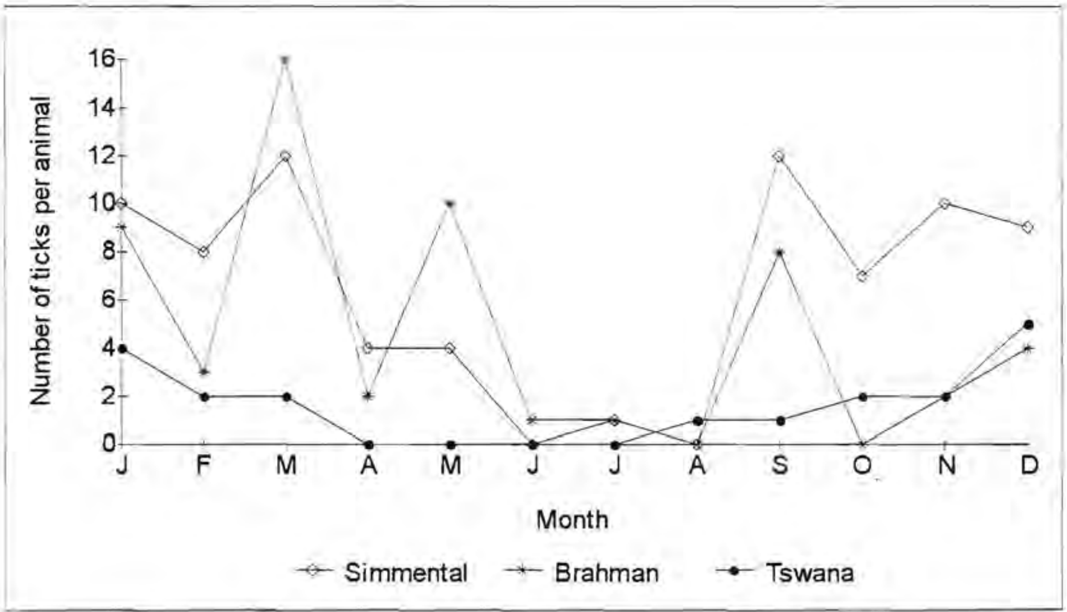


Fig 4.4 Mean monthly number of engorged *Boophilus decoloratus*

Table 4.1 Ticks collected during trial I from neem treated and control animals from all three breeds (pooled results)

	Neem treated Animals		Control Animals	
Date	Number of animals treated	Number of tick collected	Number control Animals	Number of ticks collected
January	9	28	9	59
February	9	36	9	60
March	9	36	9	50
April	9	19	9	18
May	9	18	9	26
June	9	5	9	11
July	9	42	9	15
August	9	24	9	11
September	9	98	9	30
October	9	17	8	26
November	9	30	8	54
December	9	34	7	90

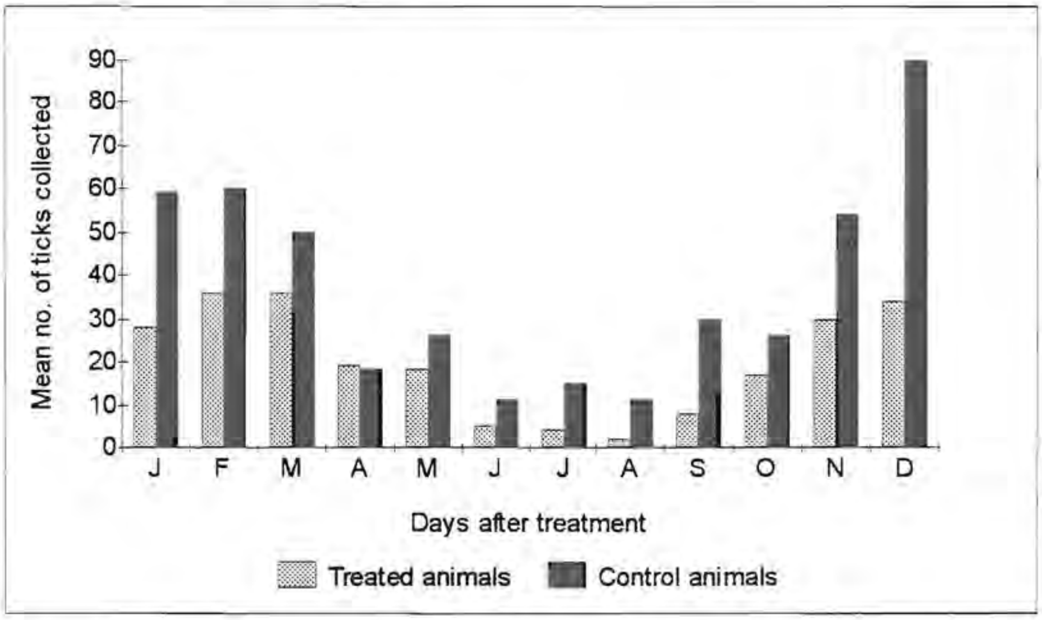


Fig 4.5 Mean monthly number of ticks collected on neem treated and control animals during trial 1.

Table 4.2 Total tick counts from various anatomical sites of neem treated and control animals of all three breeds (Pooled data).

NEEM TREATED GROUP					CONTROL GROUP			
	Ambyomma spp	*Boophilus spp*	*Hyollomma spp*	*R.evertsi*	*Ambyomma spp*	*Boophilus spp*	*Hyollomma spp*	*R.evertsi*
Belly	19	10	25	10	41	21	44	18
Ear	8	8	8	2	17	11	12	10
Perenial	19	14	19	10	35	26	28	11
Scrutum	7	6	5	3	7	10	6	4
Sternum	8	12	16	13	29	24	38	18
Udder	3	2	3	3	13	8	15	7

Incidence of Abscesses

Mean monthly numbers of ruptured abscesses per breed for the trial period are given in Fig 4.6, with most occurring between January, March and December. The Simmental had a significantly (P = 0,05) higher number of abscesses than either the Tswana or Brahman animals. The Brahman was intermediate in this respect while the Tswana had fewer abscesses. Most of the abscesses were encountered in control animal than *neem* treated animals. In other hand there were few number of ruptured abscesses during winter and spring period while most abscesses were present during the rainfall months corresponding with peak tick numbers. Abscesses were attributed mainly to *Hyalomma* and *Amblyomma* infestations, occurring in the regions like udder, root of the tail, vulva lips and on the scrotum in case of males.

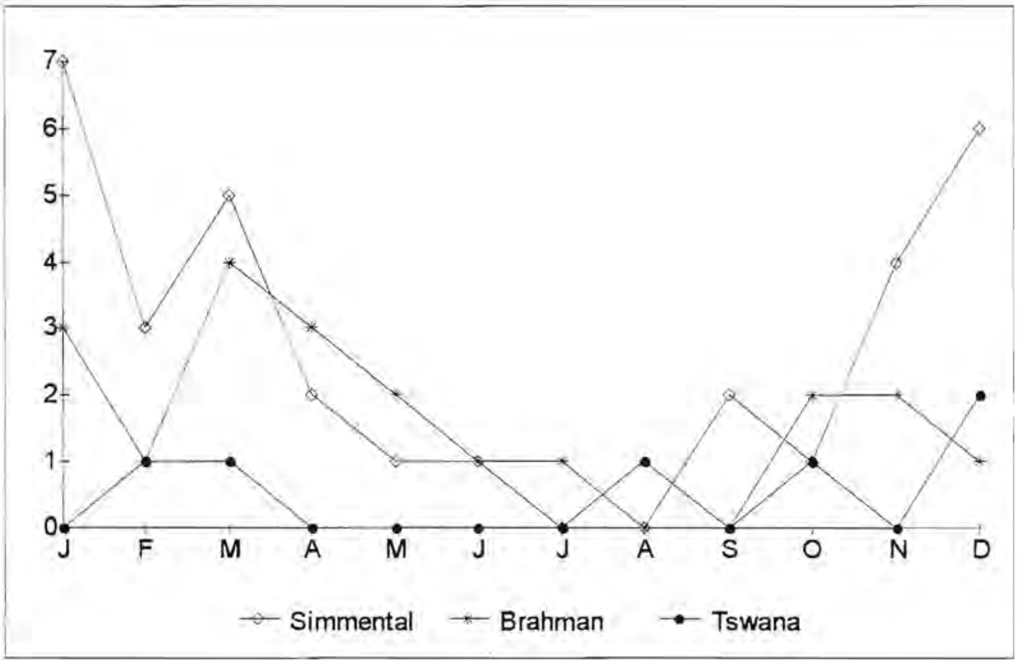


Fig.4.6 Mean Monthly number of ruptured abscesses per breed found on Simmental, Brahman and Tswana animals during the trial 1, 2 and 3.

Table 4.3 Mean total tick counts obtained from treated and control animals on the different anatomical sites.

		CONTROL GROUP		NEEM TREATED GROUP	
Anatomical sites	Tick	Mean	Std. Deviation	Mean	Std. Deviation
Belly (n = 18)	Amblyomma	2.28	1.74	1.06	0.87
	Boophilus	1.11	1.13	0.56	0.70
	Hyollomma	2.44	1.62	1.39	1.20
	R.evertsi	1.00	0.69	0.56	0.51
	Total	1.71	1.49	0.89	0.91
Ear (n = 18)	Amblyomma	0.94	1.00	0.44	0.62
	Boophilus	0.61	0.85	0.44	0.62
	Hyollomma	0.67	1.29	0.44	0.62
	R.evertsi	0.56	1.20	0.11	0.32
	Total	0.69	1.02	0.36	0.56
Perineal (n = 18)	Amblyomma	1.94	1.47	1.06	1.00
	Boophilus	1.44	1.38	0.78	0.88
	Hyollomma	1.56	1.54	1.06	0.73
	R.evertsi	0.61	0.78	0.56	0.62
	Total	1.39	1.39	0.86	0.83
Scrotum (n = 9)	Amblyomma	0.78	0.97	0.67	0.71
	Boophilus	1.11	1.27	0.67	0.87
	Hyollomma	0.67	0.71	0.63	0.74
	R.evertsi	0.44	0.53	0.33	0.50
	Total	0.75	0.91	0.57	0.70
Sternum (n = 18)	Amblyomma	1.44	0.98	0.44	0.62
	Boophilus	1.33	0.69	0.67	0.59
	Hyollomma	2.11	1.53	0.89	0.96
	R.evertsi	1.00	0.77	0.72	0.67
	Total	1.47	1.10	0.68	0.73
Udder (n = 9)	Amblyomma	1.22	1.20	0.33	0.50
	Boophilus	0.89	0.60	0.22	0.44
	Hyollomma	1.67	1.12	0.78	0.67
	R.evertsi	0.78	0.97	0.33	0.71
	Total	1.14	1.02	0.42	0.60
Total (n = 90)	Amblyomma	1.52	1.37	0.70	0.80
	Boophilus	1.10	1.05	0.58	0.70
	Hyollomma	1.59	1.49	0.90	0.91
	R.evertsi	0.76	0.87	0.46	0.58
	Total	1.24	1.26	0.66	0.77

Table 4.4 Mean tick counts obtained from control and treated Simmental cattle

		CONTROL GROUP		NEEM TREATED GROUP	
Anatomical sites	Tick	Mean	Std. Deviation	Mean	Std. Deviation
Belly	Amblyomma	3.33	2.16	1.33	1.03
Ear		1.50	1.38	0.67	0.52
Perineal		2.67	0.82	1.50	0.82
Scortum		1.33	1.53	1.00	1.05
Sternum		2.17	1.17	0.50	1.00
Udder		1.00	0.00	0.67	0.55
Total		2.17	1.51	0.97	0.85
Belly	Boophilus	2.00	1.26	0.83	0.75
(n = 6)	Hyollomma	3.67	1.63	2.00	1.41
	R.evertsi	1.33	0.82	0.50	0.55
	Total	2.33	1.57	1.11	1.13
Ear	Boophilus	1.17	0.98	0.67	0.82
(n = 6)	Hyollomma	1.33	1.37	0.67	0.82
	R.evertsi	1.00	2.00	0.00	0.00
	Total	1.17	1.42	0.44	0.70
Perineal	Boophilus	2.33	1.21	1.33	1.03
(n = 6)	Hyollomma	1.83	1.94	1.00	0.89
	R.evertsi	1.00	0.89	0.83	0.75
	Total	1.72	1.45	1.06	0.87
Scrotum	Boophilus	2.00	2.00	0.67	1.15
(n = 3)	Hyollomma	1.00	1.00	0.67	1.15
	R.evertsi	1.00	0.00	0.67	0.58
	Total	1.33	1.22	0.67	0.87
Sternum	Boophilus	1.50	0.55	0.83	0.75
(n = 6)	Hyollomma	3.50	1.38	1.33	1.03
	R.evertsi	1.17	0.98	0.83	0.41
	Total	2.06	1.43	1.00	0.77
Udder	Boophilus	1.00	0.00	0.67	0.58
(n = 3)	Hyollomma	2.67	1.53	1.33	0.58
	R.evertsi	1.33	1.53	0.67	1.15
	Total	1.67	1.32	0.89	0.78
Total	Boophilus	1.70	1.12	0.87	0.82
	Hyollomma	2.43	1.76	1.20	1.06
	R.evertsi	1.13	1.14	0.57	0.63
	Total	1.76	1.46	0.88	0.88

Table 4.5 Mean tick counts obtained from control and treated Tswana cattle

		CONTROL GROUP		NEEM TREATED GROUP	
Anatomica sites	Tick	Mean	Std. Deviation	Mean	Std. Deviation
Belly (n = 6)	Amblyomma	1.00	0.63	0.33	0.52
	Boophilus	0.33	0.52	0.17	0.41
	Hyollomma	1.67	1.03	0.50	0.55
	R.evertsi	0.83	0.41	0.33	0.52
	Total	0.96	0.81	0.33	0.48
Ear (n = 6)	Amblyomma	0.33	0.52	0.00	0.00
	Boophilus	0.17	0.41	0.00	0.00
	Hyollomma	0.00	0.00	0.17	0.41
	R.evertsi	0.60	0.55	0.17	0.41
	Total	0.25	0.44	0.28	0.28
Perineal (n = 6)	Amblyomma	1.17	0.98	0.67	0.82
	Boophilus	0.50	0.55	0.17	0.41
	Hyollomma	0.67	1.03	1.00	0.63
	R.evertsi	0.17	0.41	0.33	0.52
	Total	0.63	0.82	0.33	0.66
Scrotum (n = 3)	Amblyomma	0.67	0.58	0.00	0.00
	Boophilus	0.67	0.58	0.00	0.00
	Hyollomma	0.33	0.58	0.50	0.71
	R.evertsi	0.00	0.00	0.00	0.00
	Total	0.42	0.51	0.09	0.30
Sternum (n = 6)	Amblyomma	0.83	0.75	0.00	0.00
	Boophilus	0.67	0.52	0.67	0.41
	Hyollomma	0.83	0.75	0.33	'0.52
	R.evertsi	0.67	0.52	0.17	0.41
	Total	0.75	0.61	0.17	0.38
Udder (n = 3)	Amblyomma	0.67	1.15	0.00	0.00
	Boophilus	0.67	0.58	0.00	0.00
	Hyollomma	1.00	0.00	0.33	0.58
	R.evertsi	0.67	0.58	0.33	0.58
	Total	0.75	0.62	0.17	0.39
Total	Amblyomma	0.80	0.76	0.20	0.48
	Boophilus	0.47	0.51	0.10	0.31
	Hyollomma	0.77	0.90	0.48	0.57
	R.evertsi	0.50	0.51	0.23	0.43
	Total	0.63	0.70	0.25	0.47

Table 4.6 Mean tick counts obtained from control and treated Brahman cattle

		CONTROL GROUP		NEEM TREATED GROUP	
Anatomical sites	Tick	Mean	Std. Deviation	Mean	Std. Deviation
Belly (n = 6)	Amblyomma	2.50	1.38	1.50	0.55
	Boophilus	1.00	0.89	0.67	0.82
	Hyollomma	2.00	1.55	1.67	1.03
	R.evertsi	0.83	0.75	0.83	0.41
	Total	1.58	1.32	1.17	0.82
Ear (n = 6)	Amblyomma	1.00	0.63	0.67	0.82
	Boophilus	0.50	0.84	0.67	0.52
	Hyollomma	0.67	0.82	0.50	0.41
	R.evertsi	0.17	0.41	0.17	0.41
	Total	0.58	0.72	0.50	0.59
Perineal (n = 6)	Amblyomma	2.00	2.10	1.00	1.10
	Boophilus	1.50	1.64	0.83	0.75
	Hyollomma	2.17	1.33	1.17	0.75
	R.evertsi	0.67	0.82	0.50	0.55
	Total	1.58	1.56	0.88	0.80
Scrotum (n = 3)	Amblyomma	0.33	0.58	1.00	0.00
	Boophilus	0.67	0.58	1.33	0.58
	Hyollomma	0.67	0.58	0.67	0.58
	R.evertsi	0.33	0.58	0.33	0.58
	Total	0.50	0.52	0.83	0.58
Sternum (n = 6)	Amblyomma	1.33	0.52	0.83	0.75
	Boophilus	1.83	0.41	1.00	0.00
	Hyollomma	2.00	1.10	1.00	1.10
	R.evertsi	1.17	0.75	1.17	0.75
	Total	1.58	0.78	1.00	0.72
Udder (n = 3)	Amblyomma	2.00	1.73	0.33	0.58
	Boophilus	1.00	1.00	0.00	0.00
	Hyollomma	1.33	0.58	0.67	0.58
	R.evertsi	0.33	0.58	0.00	0.00
	Total	1.17	1.11	0.25	0.45
Total (n = 30)	Amblyomma	1.60	1.38	0.93	0.78
	Boophilus	1.13	1.04	0.77	0.63
	Hyollomma	1.57	1.22	1.00	0.87
	R.evertsi	0.63	0.72	0.57	0.63
	Total	1.23	1.17	0.82	0.74

Table 4.7 Tick counts at different anatomical location on the various cattle breeds of during the trial 1 (Pooled data)

Anatomic site	Tick	Mean	Std.Deviation
Belly	Amblyomma	1.67	1.49
(n = 36)	Boophilus	0.83	0.97
	Hyollomma	1.92	1.50
	R.evertsi	0.78	0.64
	Total	1.30	1.30
Ear	Amblyomma	0.69	0.86
(n = 36)	Boophilus	0.53	0.74
	Hyollomma	0.56	0.84
	R.evertsi	0.33	0.89
	Total	0.53	0.84
Perineal	Amblyomma	1.50	1.32
(n = 36)	Boophilus	1.11	1.19
	Hyollomma	1.31	1.21
	R.evertsi	0.58	0.69
	Total	1.13	1.17
Scrotum	Amblyomma	0.72	0.83
(n = 18)	Boophilus	0.89	1.08
	Hyollomma	0.65	0.70
	R.evertsi	0.39	0.50
	Total	0.66	0.81
Sternum	Amblyomma	0.94	0.95
(n = 36)	Boophilus	1.00	0.72
	Hyollomma	1.50	1.40
	R.evertsi	0.86	0.72
	Total	1.08	1.01
Udder	Amblyomma	0.78	1.00
(n = 18)	Boophilus	0.56	0.62
	Hyollomma	1.22	1.00
	R.evertsi	0.56	0.86
	Total	0.78	0.91
Total	Amblyomma	1.11	1.19
	Boophilus	0.84	0.93
	Hyollomma	1.24	1.28
	R.evertsi	0.61	0.75
	Total	0.95	1.08

Table 4.8 Factors included in multifactorial analysis of variance (ANOVA) analysis"with type III sum of squares (Trial 1)

		N
Treatment	C	360
	T	359
Breed	BRAHMAN	240
	SIM	240
	TSWANA	239
Tick	Ambyomm	180
	Boophilus	180
	Hyolomma	179
	R.evertsi	180
Anatomical sites	Belly	144
	Ear	144
	Perineal	144
	Scortum	71
	Sternum	144
	Udder	72
Sex	Female	360
	Male	359

Table 4.9 Effects of treatment, breed and season on tick counts based on GLM-repeated measures analysis (Pooled data)

	PR>F
Treatment	< 0.0001
Breed	< 0.0001
Trt*breed	0.4856
Season	< 0.0001
Season*trt	0.8256
Season*breed	0.9258
Season*trt*breed	0.6198

The results of the GLM-repeated measures procedure on the pooled data suggest that treatment (P<0.0001), breed (P<0.0001) and season (P<0.0001) significantly influenced the tick counts on cattle in trial 1. No statistically significant interactions were observed. These results will be discussed in more detail by referring to the GLM-repeated measures procedures for the various tick species in the following sections (Table 4.9)

Table 4.10 Effect of treatment, breed and season on the number of Amblyomma ticks based on GLM repeated measures analysis.

	PR>F
Treatment	< 0.0001
Breed	< 0.0001
Trt*breed	0.4553
Season	< 0.0001
Season*trt	0.0846
Season*breed	0.1529
Season*trt*breed	0.9296
Difference between seasons	
Spring	
Trt	0.0347
Breed	0.0320
Trt*breed	0.8445
Summer	
Trt	0.1265
Breed	0.7397
Trt*breed	0.7785

Treatment (P<0.0001), breed (P<0.0001) and season (P<0.0001) significantly affect the number of engoged female *Amblyomma* ticks on the cattle. It is important to note that the differences in the *Amblyomma* ticks treatment (P<0.034 and breeds (P<0.032) were only significantin spring. In summer most of these differences ere not significant (Table 4.10). This means that the effect of the 5% NSK treatment in reducing the number of *Amblyomma* tick was was particularly significant in spring while the effect was less obvious in summer when the number of ticks started to decline. A similar trend was observed for the effect of breed on tick counts. In spring it was quite clear that Tswana cattle harboured significantly less (P<0.0320 ticks compared to Brahman and Simmental cattle. This typically coincides with times of peak tick abundance. When the tick count start to decline (e.g. in winter), the differences between the various breeds of cattle tend to be less important. No significantly interaction were observed between these factors for *Amblyomma* ticks (Table 4.10).

Table 4.11 Effect of treatment, breed and season on the number of Boophilus ticks based on GLM repeated measures analysis.

	PR>F
Treatment	< 0.0001
Breed	< 0.0001
Trt*breed	0.4825
Season	< 0.0001
Season*trt	0.0796
Season*breed	0.4896
Season*trt*breed	0.4975
Difference between seasons	
Spring	
Trt	0.7795
Breed	0.4226
Trt*breed	0.4009
Summer	
Trt	0.0541
Breed	0.6463
Trt*breed	0.6055

Treatment ($P<0.0001$), breed ($P<0.0001$) and season ($P<0.0001$) significantly influenced the number of engorged female *Boophilus* ticks. No statistically significant interactions were observed. Treatment significantly reduced ($P<0.0001$) the number of *Boophilus* ticks on all breeds. Tswana cattle carried significantly less ($P<0.0001$) *Boophilus* ticks compared to Brahman and Simmental cattle. Minor differences were found between the numbers of engorged female *Boophilus* ticks on Simmental and Brahman cattle (Table 4.11).

Table 4.12 Effect of treatment, breed and season on the number of Hyolomma ticks based on GLM repeated measures analysis.

	PR>F
Treatment	< 0.0082
Breed	< 0.0002
Trt*breed	0.7031
Season	< 0.0001
Season*trt	0.9032
Season*breed	0.0099
Season*trt*breed	0.2982
Difference between seasons	
Spring	
Trt	0.6748
Breed	0.0632
Trt*breed	0.4006
Summer	
Trt	0.7490
Breed	0.0143
Trt*breed	0.3861

Treatment (P<0.001), breed (P<0.001) and season (P<0.0001) significantly influence the tick number of engorged female *Hyolomma* ticks (Table 4.12). The interactions between season and breeds tended toward significance (P<0.05). Simmental cattle carried significantly (P<0.05) more engorged *Hyolomma* female ticks than Brahman and Tswana cattle during the February peak, but similar numbers were found on all three breeds during winter months. The effect of breed of cattle on the number of *Hyolomma* ticks was particularly evident in summer (P<0.0143), while a tendency toward significance was also noted in spring (P<0.0632). This suggest that the differences between breeds in terms of the numbers of ticks harboured are more significant during times of higher tick infestations. This agrees with the observations of the number of *Amblyomma* ticks in the trial.

Table 4.13 Effect of treatment, breed and season on the number of R. evertsi ticks based on GLM repeated measures analysis.

	PR>F
Treatment	< 0.0249
Breed	0.0056
Trt*breed	0.3320
Season	< 0.0001
Season*trt	0.7271
Season*breed	0.2145
Season*trt*breed	0.2116
Difference in season	
Spring	
Trt	0.4680
Breed	0.1621
Trt*breed	0.4788
Summer	
Trt	0.5775
Breed	0.1478
Trt*breed	0.1440

Treatment ($P<0.05$), breed ($P<0.05$) and season ($P<0.0001$) significantly affected the number of engorged female *R. evertsi* ticks on the cattle. These results are similar to the main effects reported in section 4.1.1 for *R. evertsi* ticks. No significant interaction was observed between these factors for *Amblyomma* ticks (Table 4.13).

4.2 TRIAL 2 RESULTS (Naturally tick infestation)

4.2.1 Tick counts

The effect of 2% NSKS treatment on the number of ticks on cattle was not significant (P<0.701; F=0.148). It is evident that at least 5% NSKS should be used for effective tick treatment in Simmental, Brahman and Tswana cattle like in trial 1 (Table 4.20). Peak numbers of engorged female ticks were evident during the summer months of March and December 1999. The situation was similar in all three breeds of cattle. Tick counts on Simmental and Brahman cattle were significantly (P = 0.05, Table 4.19. and 4.21) higher than on Tswana cattle. After 12 months of treatment it was found that the numbers of ticks infesting animals were less as compared to the initial number at the first treatment. It is evident from these observations that the nymphs that hatched later were also affected by the treatment.

Results from trial 1 and 2 with 5% and 2% concentrations of NSKE respectively resulted in very similar reductions in tick counts on the treated animals. Counts of engorged female ticks during dry seasons suggest that ticks were present at very low levels during winter and spring. During dry seasons no significant difference was observed in tick counts between neem treated and control animals. This observation was nearly similar for all breeds of cattle, Simmental, Brahman and Tswana. Small differences were found between the numbers of engorged female ticks on the Simmental and Brahman cattle.

4.2.2.Effects of season on tick counts.

The highest number of engorged female ticks was observed during the summer (Table 4.22). There were only minor differences between tick counts in the winter and spring.

Table 4.18 Number of various treatment groups naturally infested with ticks during trial 2.

Between-Subjects Factors		
		N
Treatment	**C**	30
	NT	30
Breed	**BRA**	20
	SIM	20
	TSW	20
Sex	**F**	30
	M	30

Table 4.19 Mean tick counts obtained from natural infested animals during trial 2

			Control group			Neem treated group	
	Breed	Sex	Mean	Std. Dev.	N	Mean	Std. Dev.
Belly	BRA	F	23.4000	7.1624	5	6.0000	4.3589
		M	24.6000	6.2690	5	2.4000	2.0736
		Total	24.0000	6.3770	10	4.2000	3.7357
	SIM	F	24.8000	5.8907	5	12.4000	3.9115
		M	24.8000	8.7579	5	11.4000	3.7815
		Total	24.8000	7.0364	10	11.9000	3.6652
	TSW	F	12.0000	6.2048	5	4.6000	1.5166
		M	8.4000	4.3359	5	5.0000	1.5811
		Total	10.2000	5.3914	10	4.8000	1.4757
	Total	F	20.0667	8.4131	15	7.6667	4.7759
		M	19.2667	10.0887	15	6.2667	4.6209
		Total	19.6667	9.1363	30	6.9667	4.6719
Ear	BRA	F	6.0000	2.9155	5	4.6000	3.3615
		M	2.6000	3.7815	5	2.4000	2.0736
		Total	4.3000	3.6530	10	3.5000	2.8771
	SIM	F	5.8000	2.2804	5	4.8000	1.7889
		M	5.6000	3.2863	5	4.4000	2.9665
		Total	5.7000	2.6687	10	4.6000	2.3190
	TSW	F	2.4000	2.3022	5	1.6000	1.5166
		M	4.0000	1.5811	5	1.0000	1.2247
		Total	3.2000	2.0440	10	1.3000	1.3375
	Total	F	4.7333	2.8900	15	3.6667	2.6637
		M	4.0667	3.0814	15	2.6000	2.5014
		Total	4.4000	2.9548	30	3.1333	2.5962

Perineal	**BRA**	**F**	17.4000	3.4351	5	7.6000	3.6469
		M	17.8000	3.7683	5	11.0000	4.8477
		Total	17.6000	3.4059	10	9.3000	4.4234
	SIM	**F**	17.0000	4.5826	5	9.0000	2.7386
		M	17.4000	4.2190	5	8.6000	4.0373
		Total	17.2000	4.1580	10	8.8000	3.2592
	TSW	**F**	9.4000	2.4083	5	4.4000	1.5166
		M	9.4000	5.4129	5	3.8000	1.0954
		Total	9.4000	3.9497	10	4.1000	1.2867
	Total	**F**	14.6000	5.0540	15	7.0000	3.2514
		M	14.8667	5.7924	15	7.8000	4.6167
		Total	14.7333	5.3430	30	7.4000	3.9444
Scrotum	**BRA**	**F**	.0000	.0000	5	.0000	.0000
		M	4.0000	3.6742	5	1.4000	1.1402
		Total	2.0000	3.2318	10	.7000	1.0593
	SIM	**F**	.0000	.0000	5	.0000	.0000
		M	3.4000	3.0496	5	3.2000	.8367
		Total	1.7000	2.7101	10	1.6000	1.7764
	TSW	**F**	.0000	.0000	5	.0000	.0000
		M	1.0000	1.2247	5	2.0000	.7071
		Total	.5000	.9718	10	1.0000	1.1547
	Total	**F**	.0000	.0000	15	.0000	.0000
		M	2.8000	2.9568	15	2.2000	1.1464
		Total	1.4000	2.4997	30	1.1000	1.3734
Sternum	**BRA**	**F**	10.8000	5.3572	5	3.4000	1.3416
		M	4.8000	1.9235	5	4.0000	2.3452
		Total	7.8000	4.9396	10	3.7000	1.8288
	SIM	**F**	15.2000	6.1400	5	4.6000	1.5166
		M	18.0000	3.9370	5	3.2000	1.4832

		Total	16.6000	5.0816	10	3.9000	1.5951
	TSW	**F**	4.4000	1.8166	5	2.6000	.5477
		M	6.6000	2.1909	5	3.0000	2.5495
		Total	5.5000	2.2236	10	2.8000	1.7512
	Total	**F**	10.1333	6.4016	15	3.5333	1.4075
		M	9.8000	6.5922	15	3.4000	2.0633
		Total	9.9667	6.3869	30	3.4667	1.7367
Udder	**BRA**	**F**	5.4000	3.7148	5	3.6000	2.5100
		M	.0000	.0000	5	.0000	.0000
		Total	2.7000	3.7727	10	1.8000	2.5298
	SIM	**F**	5.2000	3.1145	5	3.0000	1.8708
		M	.0000	.0000	5	.0000	.0000
		Total	2.6000	3.4383	10	1.5000	2.0138
	TSW	**F**	3.8000	3.4205	5	2.0000	1.4142
		M	.0000	.0000	5	.0000	.0000
		Total	1.9000	3.0350	10	1.0000	1.4142
	Total	**F**	4.8000	3.2558	15	2.8667	1.9591
		M	.0000	.0000	15	.0000	.0000
		Total	2.4000	3.3280	30	1.4333	1.9945

Table 4.20. GLM-repeated measures analysis results on the effect of treatment, breed, sex and season on tick counts obtained from different breeds of cattle during trial 2.

Source	**PR<F.**
TREATMEN	0.701
BREED	0.000
SEX_	0.181
SEASON	0.000
BREED * SEASON	0.058

In general treatment did not significantly affect the tick counts on cattle in trial 2 (Table 4.20). This result is in contrast with the findings in the first trial. This could be partly due to the fact that the cattle were older and or better adapted to the tick infestation; or that the tick burden was reduced by the previous treatment (carry-over effects). It is also possible that the concentration of NSKE in trial 2 was too low to effectively reduce the tick numbers. However, significant differences in tick counts were observed between breeds and between seasons, which agrees with the findings in trial 1.

Table 4.21. GLM-repeated measures analysis results of the effect of treatment, breed and sex on tick counts on cattle.

Source	Dependent Variable	PR<F.
TREATMEN	**Belly**	.000
	Ear	.060
	Perineal	.000
	Scrotum	.441
	Sternum	.000
	Udder	.065
BREED	**Belly**	.000
	Ear	.003
	Perineal	.000
	Scrotum	.164
	Sternum	.000
	Udder	.419
SEX_	**Belly**	.411
	Ear	.194
	Perineal	.579
	Scrotum	.000
	Sternum	.769
	Udder	.000
	Sternum	.769
	Udder	.000

Table 4.22. Mean tick counts obtained in different seasons from Control and neem treated animals during trial 2.

			Control group			Neem treated group	
Breed	**Sex**	**Season**	**Mean**	**Std. Dev**	**N**	**Mean**	**Std. Dev**
BRA	F	spring	17.0000	7.4833	5	17.0000	7.4833
		summer	30.6000	6.6933	5	30.6000	6.6933
		winter	15.4000	6.6182	5	15.4000	6.6182
		Total	21.0000	9.5469	15	21.0000	9.5469
	M	spring	15.6000	4.3932	5	15.6000	4.3932
		summer	27.2000	4.4385	5	27.2000	4.4385
		winter	11.0000	3.4641	5	11.0000	3.4641
		Total	17.9333	8.0220	15	17.9333	8.0220
	Total	spring	16.3000	5.8319	10	16.3000	5.8319
		summer	28.9000	5.6460	10	28.9000	5.6460
		winter	13.2000	5.4934	10	13.2000	5.4934
		Total	19.4667	8.8033	30	19.4667	8.8033
SIM	F	spring	18.0000	4.7434	5	14.4000	8.3247
		summer	32.2000	4.0249	5	32.2000	4.0249
		winter	17.8000	5.4955	5	17.8000	5.4955
		Total	22.6667	8.2693	15	21.4667	9.8406
	M	spring	20.0000	4.4159	5	20.0000	4.4159
		summer	32.6000	6.3087	5	32.6000	6.3087
		winter	16.6000	9.0443	5	14.6000	5.8992
		Total	23.0667	9.5429	15	22.4000	9.3717
	Total	spring	19.0000	4.4472	10	17.2000	6.9410
		summer	32.4000	4.9933	10	32.4000	4.9933
		winter	17.2000	7.0836	10	16.2000	5.6332
		Total	22.8667	8.7759	30	21.9333	9.4538

TSW	F	**spring**	6.8000	2.8636	5	6.8000	2.8636
		summer	19.4000	6.6558	5	19.4000	6.6558
		winter	5.8000	3.9623	5	5.8000	3.9623
		Total	10.6667	7.7797	15	10.6667	7.7797
	M	**spring**	6.4000	2.6077	5	6.4000	2.6077
		summer	14.2000	6.0581	5	14.2000	6.0581
		winter	8.8000	3.6332	5	8.8000	3.6332
		Total	9.8000	5.2536	15	9.8000	5.2536
	Total	**spring**	6.6000	2.5906	10	6.6000	2.5906
		summer	16.8000	6.5963	10	16.8000	6.5963
		winter	7.3000	3.9172	10	7.3000	3.9172
		Total	10.2333	6.5373	30	10.2333	6.5373
Total	F	**spring**	13.9333	7.2256	15	12.7333	7.6295
		summer	27.4000	8.0516	15	27.4000	8.0516
		winter	13.0000	7.3776	15	13.0000	7.3776
		Total	18.1111	9.9412	45	17.7111	10.2217
	M	**spring**	14.0000	6.8868	15	14.0000	6.8868
		summer	24.6667	9.5593	15	24.6667	9.5593
		winter	12.1333	6.4903	15	11.4667	4.8236
		Total	16.9333	9.4205	45	16.7111	9.2211
	Total	**spring**	13.9667	6.9356	30	13.3667	7.1703
		summer	26.0333	8.7945	30	26.0333	8.7945
		winter	12.5667	6.8415	30	12.2333	6.1738
		Total	17.5222	9.6480	90	17.2111	9.6925

TRIAL 3 RESULTS (Artificial tick infestation)

Amblyomma hebraeum

The results suggest that the mean tick population densities were significantly lower in the neem treated than control animals (Table 4.25 and Table 4.27). The tick counts indicate that the various tick species were present throughout the year with marked peaks of abundance during the summer of both years (1998 and 1999).

Higher numbers of *Ambyomma hebraeum* were encountered on all three breeds during the rainy season of each year. Counts of *Ambyomma hebraeum* on the Brahman were intermediate between the relatively high numbers found on the Simmental and lower numbers on the Tswana animals. During winter months (April, May and June) there were minor differences in tick counts on Simmental and Brahman cattle. On the other hand Brahman and Simmental breeds were infested with significantly (P=0.05) higher numbers of ticks compared to Tswana cattle during the summer months of the experiment. Similar results were obtained in the first trial, where the tick counts were lower on all three breeds during the dry season of the year.

Boophilus decoloratus

The effect of treatment on tick counts tended toward significance (P=0.06; Table 4.25 and 4.27). A large number of ticks was observed on Simmental cattle followed by Brahman and Tswana cattle.

The ticks occurred throughout the year with peak numbers in February and November 1999. The general observation was that *B. decoloratus* tick counts from neem treated and control animals during the trial were low compared to that of *Amblyomma* spp. During the dry season no significant difference was observed in tick counts between animals.

Table 4.23 Number of various treatment groups artificially infested with ticks.

Between-Subjects Factors		
		N
Treatment	C	18
	NT	18
Breed	BRA	12
	SIM	12
	TSW	12
Sex	F	18
	M	18

Table 4.24. Effect of anatomical location on the tick counts of artificial infested animals

			Control group			Neem treated group	
Anatomic site	**Breed**	**Sex**	**Mean**	Std.Dev	**N**	**Mean**	**Std.Dev**
Belly	BRA	F	10.3333	6.1101	3	6.6667	2.3094
		M	11.0000	7.9373	3	4.6667	1.5275
		Total	10.6667	6.3456	6	5.6667	2.0656
	SIM	F	15.0000	8.0000	3	7.6667	1.1547
		M	14.6667	12.4231	3	5.0000	1.0000
		Total	14.8333	9.3470	6	6.3333	1.7512
	TSW	F	6.0000	1.0000	3	2.3333	.5774
		M	5.3333	1.5275	3	3.6667	1.5275
		Total	5.6667	1.2111	6	3.0000	1.2649
	Total	F	10.4444	6.3857	9	5.5556	2.7889
		M	10.3333	8.4558	9	4.4444	1.3333
		Total	10.3889	7.2691	18	5.0000	2.1963
Ear	BRA	F	2.6667	2.0817	3	.6667	1.1547
		M	2.0000	3.4641	3	1.3333	.5774
		Total	2.3333	2.5820	6	1.0000	.8944
	SIM	F	2.6667	1.5275	3	1.3333	1.1547
		M	2.0000	1.0000	3	1.0000	.0000
		Total	2.3333	1.2111	6	1.1667	.7528
	TSW	F	2.3333	.5774	3	.6667	.5774
		M	2.3333	1.5275	3	.6667	.5774
		Total	2.3333	1.0328	6	.6667	.5164
	Total	F	2.5556	1.3333	9	.8889	.9280
		M	2.1111	1.9650	9	1.0000	.5000
		Total	2.3333	1.6450	18	.9444	.7254

Perineal	BRA	F	9.0000	5.1962	3	6.6667	3.7859
		M	4.3333	1.5275	3	4.3333	2.5166
		Total	6.6667	4.2740	6	5.5000	3.1464
	SIM	F	9.6667	2.5166	3	5.0000	1.0000
		M	13.6667	4.0415	3	7.6667	2.5166
		Total	11.6667	3.7238	6	6.3333	2.2509
	TSW	F	1.3333	.5774	3	1.3333	1.5275
		M	2.0000	1.0000	3	.6667	.5774
		Total	1.6667	.8165	6	1.0000	1.0954
	Total	F	6.6667	4.9497	9	4.3333	3.1623
		M	6.6667	5.7879	9	4.2222	3.5277
		Total	6.6667	5.2244	18	4.2778	3.2504
Scrotum	BRA	F	.0000	.0000	3	.0000	.0000
		M	1.6667	2.0817	3	1.6667	1.5275
		Total	.8333	1.6021	6	.8333	1.3292
	SIM	F	.0000	.0000	3	.0000	.0000
		M	1.6667	1.5275	3	2.0000	1.7321
		Total	.8333	1.3292	6	1.0000	1.5492
	TSW	F	.0000	.0000	3	.0000	.0000
		M	1.3333	1.5275	3	1.0000	1.0000
		Total	.6667	1.2111	6	.5000	.8367
	Total	F	.0000	.0000	9	.0000	.0000
		M	1.5556	1.5092	9	1.5556	1.3333
		Total	.7778	1.3086	18	.7778	1.2154
Sternum	BRA	F	4.0000	2.0000	3	5.0000	2.6458
		M	5.6667	4.1633	3	3.6667	4.6188
		Total	4.8333	3.0605	6	4.3333	3.4448
	SIM	F	10.0000	5.1962	3	3.0000	1.0000
		M	8.3333	1.5275	3	5.0000	2.6458

		Total	9.1667	3.5449	6	4.0000	2.0976
	TSW	**F**	2.6667	1.5275	3	1.6667	1.1547
		M	2.0000	.0000	3	2.0000	1.0000
		Total	2.3333	1.0328	6	1.8333	.9832
	Total	**F**	5.5556	4.4472	9	3.2222	2.1082
		M	5.3333	3.5355	9	3.5556	3.0046
		Total	5.4444	3.8991	18	3.3889	2.5237
Udder	**BRA**	**F**	2.0000	.0000	3	1.0000	1.0000
		M	.0000	.0000	3	.0000	.0000
		Total	1.0000	1.0954	6	.5000	.8367
	SIM	**F**	3.3333	2.5166	3	1.6667	.5774
		M	.0000	.0000	3	.0000	.0000
		Total	1.6667	2.4221	6	.8333	.9832
	TSW	**F**	1.3333	1.1547	3	1.0000	1.0000
		M	.0000	.0000	3	.3333	.5774
		Total	.6667	1.0328	6	.6667	.8165
	Total	**F**	2.2222	1.6415	9	1.2222	.8333
		M	.0000	.0000	9	.1111	.3333
		Total	1.1111	1.6047	18	.6667	.8402

Table 4.25 GLM-repeated measures analysis results of the effect of treatment, breed and sex on tick counts on artificially infested cattle.

Source	Dependent variable	PR<F
TREATMEN	Belly	.005
	Ear	.009
	Perineal	.012
	Scrotum	1.000
	Sternum	.035
	Udder	.164
BREED	Belly	.026
	Ear	.914
	Perineal	.000
	Scrotum	.756
	Sternum	.002
	Udder	.269
SEX_	Belly	.731
	Ear	.737
	Perineal	.950
	Scrotum	.000
	Sternum	.952
	Udder	.000

Table 4.26. Effect of treatment on tick counts of artificial infested animals with *Amblyomma* and *Boophilus spp.*

CONTROL GROUP					NEEM TREATED		
	Breed	**Sex**	**Mean**	**Std. Dev.**	**N**	**Mean**	**Std. Dev.**
Amb	**BRA**	**F**	13.3333	2.8868	3	12.0000	2.6458
		M	14.6667	8.0208	3	7.6667	6.0277
		Total	14.0000	5.4406	6	9.8333	4.7924
	SIM	**F**	24.0000	6.0828	3	9.0000	4.5826
		M	22.3333	15.5027	3	11.6667	3.0551
		Total	23.1667	10.5720	6	10.3333	3.7771
	TSW	**F**	7.6667	1.1547	3	2.3333	1.1547
		M	7.3333	2.5166	3	4.0000	2.0000
		Total	7.5000	1.7607	6	3.1667	1.7224
	Total	**F**	15.0000	7.9530	9	7.7778	5.0690
		M	14.7778	10.9519	9	7.7778	4.8419
		Total	14.8889	9.2856	18	7.7778	4.8088
Boop	**BRA**	**F**	9.3333	3.7859	3	9.6667	3.2146
		M	10.0000	6.0828	3	7.6667	4.6188
		Total	9.6667	4.5461	6	8.6667	3.7238
	SIM	**F**	16.6667	4.6188	3	9.6667	1.1547
		M	18.0000	2.6458	3	9.0000	3.4641
		Total	17.3333	3.4448	6	9.3333	2.3381
	TSW	**F**	5.6667	1.5275	3	4.6667	1.5275
		M	5.6667	1.5275	3	4.3333	1.5275
		Total	5.6667	1.3663	6	4.5000	1.3784
	Total	**F**	10.5556	5.7470	9	8.0000	3.1225

		M	11.2222	6.3988	9	7.0000	3.6401
		Total	10.8889	5.9100	18	7.5000	3.3299

Table 4.27. GLM-repeated measures analysis results of the effect of treatment, and breed on the tick counts of artificial infested animals with *Ambyomma* and *Boophilus spp*

Source	Dependent Variable	PR<F
TREATMEN	**Amblyomma spp**	.002
	Boophilus spp	.006
BREED	**Amblyomma spp**	.000
	Boophilus spp	.000

In the cattle artificially infested with *Amblyomma* and *Boophilus* larvae the treatment significantly affected the number of engoged female *Boophilus* (P<0.002) and *Ambyomma* (P<0.006) ticks (Table 4.27). Significant differences in the number of *Amblyomma* and *Boophilus* ticks were also observed on Simmental, Brahman and Twana cattle regardless of the artificial infestations of cattle with similar amounts of larvae.

CHAPTER FIVE

5.0 DISCUSION

5.1 TRIAL 1

5.1.1 Effects of treatment on tick counts.

Neem treatment (5% NSKE) significantly reduced the tick counts ($P<0.0001$, Table 4.9) of cattle in trial 1. Average tick counts were 37.5±12.75 for control animals and 19.75±12.75 for treated animals (Table 3.3). The fact that all cattle grazed together and were equally exposed to a highly infested shelter suggests that all breeds of cattle had equal chances of being infested by ectoparasites. The observation was that the population densities of ticks were significantly lower on neem treated (5% NSKE) than control animals during the experimental period (summer, winter and spring) in trial1. This can be attributed, partly, to the lethal or repellent effects of the Neem Seed Kernels Extracts, (NSKE) on the various tick species. These results agree with that of Jotwarni and Sirca, (1965) and Butterworth et al., (1971). It appears that, Neem extracts have insect repelling properties, reduce infestation and digestion of feed and cause poor development and growth of insects. These results are also in agreement with that of Rembold et al., (1986) and Saxena, (1993). The extracts cause a disruption in mating, oviposition and inhibit eggs hatchability and exposed insects fail to moult.

Incidence of Abscesses and neem treatment

The heavy tick infestations of control animals were at least partly responsible for the higher incidence of abscesses during the three trial I, II and III. Fewer abscesses due to tick bites were observed in the Tswana cattle as compared to Brahman and Simmental (Figure 4.6). Most abscesses were present during the summer months (December and March) corresponding with peak tick numbers. More abscesses were encountered in control animals (72%) than Neem treated (28%) animals. The incidence of abscesses was attributed particularly to Hyalomma and Amblyomma infestations, occurring in specific anatomical areas like the udder, root of the tail, vulva lips and on the scrotum. These results are in agreement with report from the Department of Animal Health and Production, Serowe that during the rainy season animals some animals are subjected to screw worm due to abscesses occurring in the region of clumping of the former species and at the isolated attachment sites of the latter species, especially on the udder, scrotum axillae and at the tail root.

5.1.2 Effect of Breeds on tick counts

There was a large variation in tick counts among breeds ($P<0.0001$; Table 4.9). Counts of engorged female ticks on naturally infested cattle over a one-year period, showed that indigenous Tswana cattle harboured significantly fewer ticks during periods of peak abundance than either Brahman or Simmental cattle. The results suggest that Tswana cattle were infested with fewer ticks as compared to Brahman and Simmental. These results are in agreement with that of Norval et al, 1998a and Scholtz, 1989) that the use of indigenous breeds that possess a very high resistance to tick infestation may be a solution to the dilemma of tick infestation and frequent dipping in the undeveloped parts of Africa.

5.1.3 Effect of the interaction between treatment and anatomical sites on tick counts

Neem treatment significantly reduced the number of ticks on different anatomical sites of treated animals ($P < 0.05$). In all treated and control animals, ticks were found at nearly all anatomical sites (Table 4.2). Most of the Amblyomma species were found on the Belly area followed by the perineal area. On the other hand most of the other tick species were observed on the anatomical sites with longer hair. Simmental cattle had the highest tick counts and therefore the assumption that longer hair is conducive to tick infestations is true as far as tick selection of cattle for resistance is concerned. No significant correlation between hair length of different anatomical sites and tick counts could be demonstrated between the sexes within each of the three breeds.

5.1.4 Interaction between treatment and sex on tick counts

There was no significant difference among different sexes. Treatment did not affect the tick counts in either sex, although some Tswana bulls had lower tick count compared to the females counterparts.

5.1.5 Effect of breed on tick counts.

Breed significantly influenced the tick counts (P<0.001; Table 4.9). The average values obtained from tick counts among the different breeds were as follows; Tswana cattle (0.4435±0.6251); Brahman cattle (1.0250±1.0018); Simmental cattle (1.3167±1.2793). The consistently large percentage of Tswana cattle showing low tick counts indicates a higher level of natural resistance in this breed. Tick counts of individuals, which could indicate a high or low resistance, suggest that there are differences among breeds and even individual animals. These results suggest that there is a potential for the selection for tick resistance within all three breeds.

5.1.6 Effect of the interactions between breed and anatomical sites on tick counts

There was no significant interaction between anatomical sites of different breeds on tick counts. No significant interaction was observed between breeds and anatomical sites for the different tick species.

5.1.7 Effect of the interaction between breed and sex on tick counts

The effect of the interaction between breed and sex on tick counts tended toward significance (P=0.088). Males of some breeds exhibited lower tick counts compared to those of other breeds. The interaction effects for specific tick species were not significant namely: Ambyomma P=0.1795, Boophilus P=0.0781; Hyolomma P=0.149; R. evertsi P=0.6163.

5.1.8 Effects of season on tick counts.

Season significantly affected the tick counts on cattle (P<0.01; Table 4.9). The results show that the highest number of engorged female ticks was observed during the summer season. Higher tick counts were obtained during summer probably because ticks breed during the rainy season when the ambient temperature is also high. There was no significant difference between tick counts in the winter and spring. This is in agreement with that of Chavunduka et al, (1990), that, during summer tick populations build up more rapidly resulting in an upsurge of tick infestations on cattle. It is also in agreement with the information I got from the Department of Animal Health, Serowe that tick burden depend on the weather condition, the eggs hatch out more rapidly if the weather is warm and moist than cold dry winter months.

5.1.9 The effect of the interactions between season, breed and sex on tick counts

No significant interaction between breed, seasons and sex on tick counts was observed (P>0.05; Table 4.9)

5.1.10 Effect of anatomical sites on tick counts.

The anatomical sites significantly affected the number of ticks counted on the experimental animals ($P < 0.01$). Ticks were found at all anatomical sites (Table 4.3 to 4.7) regardless of the breed of cattle or sex. More ticks were found on the belly, perineal and sternum while fewer tick were found on the udder and scrotum. On the other hand more ticks were observed at the anatomical areas covered with longer hair because the longer hair provides an ideal site for tick breeding. This result is in agreement with Chavunduka at al., (1990) that different species have different predilection sites and that the length of the animalsí coat tends to affect the degree of tick infestation. He further noted that the level of tick infestation might differ markedly in two animals of the same breed and on the same pastures.

5.1.11 Effect of the interaction between anatomical sites and treatment on tick counts.

Generally there was a significant interaction between treatment and anatomical sites on the numbers of ticks (P= 0.031).

The treatment generally affected the tick counts at different anatomical sites of experimental animals of both sexes. The belly had the highest tick counts while the lowest tick counts were observed on the udder and scrotum. The various anatomical sites and predominant tick species are as follows; Belly (Hyolomma spp), Ear (Amblyomma spp), Perineal (Ambyomma spp), Scrotum (Boophilus spp), Sternum (Hyolomma spp) and udder (Hyolomma spp), (Table 4.2).

5.1.12 Effect of the interaction between anatomical sites and sex on tick counts

Significant interaction effects between anatomical sites and sex were observed for specific tick species. There were more ticks on the udder (1.139±1.02) of the control animals compared to the number of ticks collected from the scrotum (0.75±0.906) of the same group of animals. Therefore sex affected the tick counts at specific anatomical sites. The opposite is true in the case of treated animals, where more ticks were collected from the

5.2 DISCUSSION OF TRIAL 2

5.2.1 Effects of treatment on tick counts.

The effect of 2% NSKE treatment on the number of ticks on cattle was not significant (P<0.701; F=0.148). It is evident that at least 5% NSKS should be used for effective tick treatment in Simmental, Brahman and Tswana cattle like in trial 1 (Table 4.20). Peaks of engorged female ticks were evident during the summer months of March and December 1999.

The situation was similar in all three breeds of cattle. Tick counts on Simmental and Brahman cattle were significantly (P = 0.05, Table 4.19. and 4.21) higher than on the Tswana breed. After 12 months of treatment it was found that the numbers of ticks infesting animals were less as compared to the initial number at the first treatment. It is evident from these observations that the nymphs that hatched later were also affected by the treatment.

Results from trial 1 and 2 with 5% and 2% concentrations of NSKE respectively resulted in very similar reductions in tick counts on the treated animals. These results are in agreement with that of Schmutterer, (1990) that, temperature, ultraviolet light, rainfall, and other environmental factors may exert a negative effect on the active principles of *Azadirachta indica* when low dosage is used.

The neem-based insecticides are safe, non-toxic but due to its short LC_{50}, insect pests can develop resistance when low dosage is used at short interval.

5.2.2 Effect of Breeds on tick counts

There was a large variation in tick counts among breeds (P<0.005; Table 4.21). Indigenous Tswana cattle harboured significantly fewer` ticks during periods of peak abundance than Brahman and Simmental cattle. These results are the same as trial 1 as discussed in section 5.1.2.

5.2.3 Effect of the interaction between treatment and anatomical sites on tick counts

Neem treatment significantly reduced the number of ticks on different anatomical sites of treated animals (P < 0.05, Table 4.21). In all treated and control animals, ticks were found nearly at all anatomical sites. Except in scrotum the neem treatment did not reduce the tick counts (P=0.441; Table 4.21).

5.2.4 Effect of sex on tick counts

There was no significant difference among different sexes (P>0.05)

Table 4.21) except on the other anatomical locations named scrotum (P<0.01; F=41.978; Table 4.21) and udder (P<0.01;F=56.277, Table 4.21). Treatment did not affect the tick counts in either sex.

5.2.5 Effects of season on tick counts.

Season significantly affected the tick counts on cattle (P<0.001; Table 4.20). The highest number of engorged female ticks was observed during the summer. There were little differences between tick counts in the winter and spring. Higher tick counts were obtained during summer probably because ticks breed during the rainy season when the ambient temperature is also high. The effect of season in trial 2 is similar to that of trial 1 discussed in section 5.1.8 of this chapter.

5.2.6 The effect of the interactions between breed and season

The effect of the interaction between breed and season on tick counts tended toward significance (P=0.058; F=2.339; Table 4.20). High tick counts was observed on Simmental cattle followed by Brahman and Tswana cattle the least.

5.3 DISCUSSION OF TRIAL 3 (ARTIFICIAL TICK INFESTATION)

5.3.1 Effects of treatment on tick counts.

Neem treatment with 3% NSKE on artificially infested animals significantly reduced the tick counts of *Amblyomma spp* ($P<0.01$; $F=12.507$, Table 4.27) and *Boophilus spp* ($P<0.01$; $F=9.256$, Table 4.27). Animals on trial were all infested with *Amblyomma* and *Boophilus spp* ticks artificially and grazed together, and therefore ticks had equal chances of infesting all the animals equally, however the population densities of ticks were significantly lower on neem treated than control animals during the trial period. This can be attributed, partly, to the lethal or repellent effects of the Neem Seed Kennels Extracts, (SKE) on the various tick species. These results agree with that of Rembold et al., (1986) and Saxena, (1993). The extracts cause a disruption in mating, oviposition and inhibit egg hatchebility and exposed insects fail to moult.

5.3.2 Effect of Breeds on tick counts

As it was the case for the first trial a large variation in tick counts among breeds were observed in this trial for *Amblyomma spp* ($P<0.01$; $F=10.830$, Table 4.27) and *Boophilus spp* ($P<0.01$; $F=18.286$, Table 4.27). Tick counts on artificially infested cattle over a year, indicate that Tswana cattle harboured significantly fewer ticks than either Brahman or Simmental cattle. These results obtained in trial 1 suggest that the use of indigenous breeds that possess a very high resistance to tick infestation may be a solution to the dilemma of tick infestation in the some parts of Africa (Norval et al., 1998a).

5.3.3 Effects of treatment and anatomical location on tick counts.

Neem treatment significantly reduced the tick counts (P<0.05, Table 4.25) on different anatomical sites with the exception of the udder, belly (P=0.005; F=9.372), ear (P=0.009; F=013), perineal area (P=0.012; F=7.337), scrotum (P=0.001; F=0.00), sternum (P=0.035; F=4.978), and udder (P=0.164; F=2.065). Average total tick counts were as follows; belly (5.000±2.1963) for neem treated and (10.3889±7.2692) for control, ear (0.9444±0.7264) for neem treated and (2.3333±1.6450) for control, perineal (4.2778±3.2504) for neem treated and (6.6667±5.2244) for control, scrotum (0.7778±1.2154) for neem treated and 37.5±12.75 for control, sternum (3.3889±2.5237) for neem treated and (5.4444±3.8991) for control, and udder (0.6667±0.8402) for neem treated and (1.111±1.6047) for control.

5.3.4 Interaction between breed, anatomical sites on tick counts

In some anatomical locations there was a variation in tick counts among breeds for example belly, perineal and sternum ($P<0.05$, Table 4.25). But it was not the case for the other anatomical sites like ear, udder and scrotum where ($P>0.05$).

5.3.5 Effect of the interaction between treatment and breed on tick counts..

Neem treatment significantly reduced the number of *Boophilus* ticks on of treated animals ($P < 0.05$; $F=4.286$; Table 4.27). There was no significant difference between treatment and breeds in *Amblyomma* species.

5.4 Effect of the geographical locations on neem seed oil concentration

It has already been shown that the concentrations of the azadirachtin vary with geographical location and the time when the seeds was harvested i.e. seeds harvested a considerable time ago tend to be more concentrated than newly harvested seeds. It follows that the preparation of the formulations/dosage forms in the different parts of the country will depend on the chemical content of these constituents.

The terms inhibition, suppression and anti-feeding activity however provide a good description of the phenomenon (Butterworth and Morgan, 1971). Both the water extract and ether extract of the kernels contain active feeding inhibitors. The studies conducted on filter paper impregnated with Neem seed fraction and sucrose has shown that locust was not able to feed on filter papers (Butterworth and Morgan, 1971). These results were obtained from laboratory-based studies, which agree with the field observations in the present study.

In general there was a marked difference in tick counts between those animals treated and the control. Some live ticks were collected from treated animals. The presence of live ticks on animals treated with neem extracts can be explained as follows;

- Firstly the simple spray machine, which was used, was not efficient due to the low pressure and leakage of the chemicals.

- Secondly the small sized nozzle of the sprayer effected the application of the extracts, so that neem extract probably did not penetrate well through the hair to the skin.

- Thirdly the concentration of the neem extract use could have been lower than that required, so it failed to maximize its efficacy on ticks.

- Absence of a crush makes the restraining of animals during application so difficult, that a large proportion of the chemical was lost.

As neem is an insect repellent the plant extract tends to repel insect, cockroaches and mosquitoes. It has further been shown that the crushed neem leaves and seeds can inhibit the life cycles of mosquitoes in ponds and stagnant water in the breeding places.

Although the cake is not considered as a true concentrate due to its higher fibre content, Neem seed cake may be used as anthelmintic for animal production. Further investigation is required to determine the level at which neem seed cake can be incorporated in the feed without causing toxicity to animals.

The use of the Modern hand sprayer proved to be very efficient due to the fact that the drug penetrates well to the skin due to the large sized nozzle hence high pressure. The drug also showed high efficacy because even those animals sprayed by simple hand killed a large number of ticks.

Further investigations however should be pursued to determine the actual nature of the effects of NSKE on ticks, the active ingredients affecting ticks, concentration in the carrier or any solvent with subsequent effects on the efficacy and optimum treatment period and interval in beef cattle.

CHAPTER SIX

CONCLUSSION AND RECOMMENDATIONS

Neem is a cheap natural source of insecticide that can play a significant role in reducing the indiscriminate use of synthetic chemicals which are potentially dangerous to man and the ecosystem i.e. toxicity to non-target organisms, development of insecticide resistance and consequent pest resurgence, environmental pollution, health hazards to those who cannot afford protective clothing and hazard due to pesticide residues.

Neem trees also provide a good shade around homes and high quality building and fencing poles, which withstand the attack of termites.

Neem is used as a pesticide and it disrupts metamorphosis in many insect larvae. Moulting is inhibited and hence larvae die. Neem repels insects, a property which is important in crop protection during pre- and post-harvest periods.

It was concluded that, the Tswana breed is more tick resistant than Simmental or Brahman cattle. Simmental cattle appeared to be the most susceptible to tick infestation. The use of local breeds (i e Tswana) that possess a high resistance to tick infestation may be a solution to the dilemma of infestation and frequent dipping in the undeveloped parts of Africa, as pointed out by Norval et al, (1988a). This may be combined with strategic dipping e.g. dipping every three weeks during the wet season and only when necessary in the dry season. This strategy will to a large extent prevent the loss of endemic stability.

Frequent dipping is essential to avoid drastic losses in productivity when exotic breeds, with a high susceptibility to tick infestation, are utilised in farming areas with a high tick incidence.

It is recommended that the choice of acaricides and the dosage form should be based on epidemiological studies. It is further recommended that acaricides control programs be initiated in villages.

Improvement in the health of animals should result in enhanced meat and milk production for domestic use as well as for sale. In the commercial sector appropriate management practices in conjunction with strategic tick spraying and dipping should be adopted to reduce the prevalence of ticks and tick borne diseases.

Further studies are needed to determine the efficacy of Neem seed, leaf and bark extracts/powders against gastrointestinal parasites of cattle and other animals when administered in feed or water at various concentrations.

From the prevalence study it was established that Hyolomma truncutus is the most prevalent species of ticks on Serowe village animals, followed by Ambyomma hebraeum. In the study it was also possible to determine which season has the highest tick burden. The prevalence of ticks was highest in the summer, thus it is recommended that further studies be carried out to establish the following: -

The species of ticks that is mostly affected after neem treatment and the species of ticks that shows neem extract resistance.

To establish the persistent effect (residual effect) of neem seed extracts. The findings from this study will be of importance in epidemiological based strategic dipping and spraying with the aim to reduce the frequency of treatment and therefore slow the evolution of acaricides resistance.

However, as far as animal production is concerned I also recommend the following: -

- The minimum use of effective acaricide,
- Integration of chemotherapy and pasture management,
- Avoid under dosing during spray or dipping of the animals,
- Adequate nutrition,
- Monitoring of the tick burden and insecticide efficacy,

Introduction of a proper insecticide treatment regime and recommended dosage rates by the manufacturer. These will reduce the rate of development of insecticide resistance and maintain the efficacy of the drugs (chemicals).

Farmers and livestock keepers should be;

Sensitised on production, preparation, quality control, formulation and dispensing of the neem extracts as ticks, fleas, lice and mites etc transmit some of the most important diseases of livestock in the country.

Advised to use an efficient spraying machine for effective tick control when acaricides are used.

Provided with a modern hand sprayer (perhaps on loan bases) for effective tick control.

Educated through extension officers about the economic losses attributed to tick borne diseases.

In view of the present observations and easy availability and preparation of neem products, consideration of its inclusion in the programmes for tick management on livestock in developing areas of southern Africa (Botswana) is recommended.

Botswana should develop enough data on the number of Neem trees available, the content of active constituents and efficacy of these constituents in different formulations against the huge variety of pests in agriculture and livestock production.

On the other hand Neem needs to be grown on large scale in Botswana as a cash crop to supply oil for the commercial industry. The cake obtained after extraction of oil could be used as a supplemental feed in animals.

Botswana being among the livestock based economies, stands to benefit from the available trees in insect/pest control to increase livestock production, while at the same time reducing the use of synthetic insecticides/acaricides in the long term. The country will save millions of Pula spent on importing acaricides. Botswana, should benefit from home-made crude neem extracts as a source of safe agricultural chemicals, by just preparing extracts using locally available facilities such as wooden mortars and pestles squeezing out the juice/extract and spraying on animals after mixing with water. Brooms can be used in small-scale production systems. This would be the most practical way to prepare dosage forms of extracts from seeds, leaves and bark of neem tree for use against ectoparasites and pests of livestock.

It is justifiable that the plant could be effectively used for controlling ectoparasites on cattle in many parts of Africa especially dry areas where most animal farming is also a major activity.

Farmers who already have neem trees will benefit from this because they can be advised on how to best feed their animals on neem seed cake after oil extraction (Home based or industrial).

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APPENDIX 1

Ticks collected on various anatomical locations of control and treated group during the trial 1.

		NEEM TREATED GROUP										CONTROL GROUP									
	BREED	SIMMENTAL			BRAHMAN			TSWANA				SIMMENTAL			BRAHMAN			TSWANA			
		S1	S2	S3	B1	B2	B3	T1	T2	T3		S4	S5	S6	B4	B5	B6	T4	T5	T6	
	SEX	FEMALE	FEMALE	MALE	FEMALE	MALE	MALE	FEMALE	MALE	MALE	Total	FEMALE	MALE	MALE	FEMALE	FEMALE	MALE	FEMALE	FEMALE	MALE	Total
Amblyomma	Perineal	5	1	3	1	4	1	3	0	1	19	6	6	4	3	6	3	4	2	1	35
	Belly	3	3	2	2	3	4	0	2	0	19	9	9	2	5	5	5	3	1	2	41
	Sternum	1	1	1	1	3	1	0	0	0	8	3	3	7	3	2	3	2	3	0	26
	Udder	2	0	0	1	0	0	0	0	0	3	2	1	0	5	1	0	0	4	0	13
	Ear	2	1	1	0	1	3	0	0	0	8	3	5	1	3	1	2	0	1	1	17
	Scrotum	0	2	1	0	2	2	0	0	0	7	0	1	3	0	0	1	0	1	1	7
Hyolomma	Perineal	2	0	4	4	2	1	3	2	1	19	4	2	5	5	4	4	2	2	0	28
	Belly	3	7	2	3	5	2	2	1	0	25	7	8	7	4	6	2	4	4	2	44
	Sternum	2	4	2	1	2	3	0	2	0	16	7	9	5	4	3	5	3	2	0	38
	Udder	3	1	0	1	1	0	0	1	0	7	7	1	0	3	1	0	2	1	0	15
	Ear	1	2	1	1	1	1	0	0	1	8	1	3	4	2	2	0	0	0	0	12
	Scrotum	0	2	0	0	1	1	0	0	1	5	0	2	1	0	0	2	0	0	1	6
R. evertsi	Perineal	3	1	1	2	0	1	0	2	0	10	1	2	3	1	1	2	0	1	0	11
	Belly	1	0	2	1	2	2	1	0	1	10	4	2	2	1	3	1	2	1	2	18
	Sternum	2	2	1	3	1	3	1	0	0	13	3	2	2	2	3	2	2	1	1	18
	Udder	2	0	0	0	0	0	1	0	0	3	3	1	0	1	0	0	2	0	0	7
	Ear	0	0	0	0	1	0	1	0	0	2	5	1	0	1	0	0	2	1	0	10
	Scrotum	0	1	1	0	1	0	0	0	0	3	0	1	2	0	1	0	0	0	0	4
Boophilus	Perineal	4	2	2	2	3	0	0	0	1	14	7	3	4	5	1	3	1	1	1	26
	Belly	1	2	2	3	1	0	1	0	0	10	5	2	5	2	1	3	0	1	2	21
	Sternum	1	1	3	2	2	2	0	0	1	12	3	2	4	4	3	4	2	1	1	24
	Udder	1	1	0	0	0	0	0	0	0	2	2	1	0	1	2	0	1	1	0	8
	Ear	1	3	0	0	2	2	0	0	0	8	2	1	4	1	2	0	0	1	0	11
	Scrotum	0	0	2	0	1	3	0	0	0	6	0	0	6	0	1	1	0	1	1	10
	TOTAL	40	37	31	28	39	32	13	10	7	237	84	68	71	56	49	43	32	31	16	450

APPENDIX 2

Ticks collected on various anatomical sites of control and treated animals during the trial 2.

NEEM TREATED

Ear tag				Season of the year			Tick counts on anatomical locations						
No.	Treatment	Breed	Sex	Spring	Summer	Winter	Belly	Ear	Perineal	Scotum	Sternum	Udder	Total
S7	NT	SIM	F	12	20	8	16	3	11	0	7	3	**40**
S8	NT	SIM	F	9	13	7	10	6	5	0	5	3	**29**
S9	NT	SIM	F	10	18	8	11	7	8	0	4	6	**36**
S10	NT	SIM	F	7	10	13	8	5	12	0	4	1	**30**
S11	NT	SIM	F	11	17	6	17	3	9	0	3	2	**34**
S12	NT	SIM	M	10	10	7	11	9	3	3	1	0	**27**
S13	NT	SIM	M	7	22	11	17	3	13	2	5	0	**40**
S14	NT	SIM	M	6	9	14	8	4	10	3	4	0	**29**
S15	NT	SIM	M	11	14	7	13	1	11	4	3	0	**32**
S16	NT	SIM	M	12	7	7	8	5	6	4	3	0	**26**
B7	NT	BRA	F	11	13	9	11	4	8	0	4	6	**33**
B8	NT	BRA	F	10	15	3	7	7	12	0	1	1	**28**
B9	NT	BRA	F	7	10	7	4	2	10	0	4	4	**24**
B10	NT	BRA	F	8	14	9	7	9	5	0	4	6	**31**
B11	NT	BRA	F	5	9	4	9	1	3	0	4	1	**18**
B12	NT	BRA	M	10	17	11	14	5	15	1	3	0	**38**
B13	NT	BRA	M	10	11	4	9	0	12	0	4	0	**25**
B14	NT	BRA	M	5	9	9	8	4	7	2	2	0	**23**
B15	NT	BRA	M	11	4	3	6	1	5	3	3	0	**18**
B16	NT	BRA	M	13	15	8	9	2	16	1	8	0	**36**
T7	NT	TSW	F	2	6	6	3	2	6	0	2	1	**14**
T8	NT	TSW	F	3	8	3	5	1	3	0	2	3	**14**
T9	NT	TSW	F	4	7	6	6	4	3	0	3	1	**17**
T10	NT	TSW	F	3	10	2	3	1	4	0	3	4	**15**
T11	NT	TSW	F	5	8	3	6	0	6	0	3	1	**16**
T12	NT	TSW	M	3	6	0	3	0	4	1	1	0	**9**
T13	NT	TSW	M	0	8	6	4	3	2	3	2	0	**14**
T14	NT	TSW	M	4	3	5	5	0	4	2	1	0	**12**
T15	NT	TSW	M	4	10	7	6	1	5	2	7	0	**21**
T16	NT	TSW	M	4	9	5	7	1	4	2	4	0	**18**

KEY:

C=Treated animals ***NT=Neem treated anaimals***

SIM = Simmental cattle
BRA = Brahman cattle
TSW = Tswana cattle

... Appendix 2 continue.........

CONTROL

Animal N	Treatment	Breed	Sex	Season of the year			Tick count of anatomical locations						
				Spring	Summer	Winter	Belly	Ear	Perineal	Scotum	Sternum	Udder	
S17	C	SIM	F	14	30	20	30	8	12	0	9	5	**64**
S18	C	SIM	F	20	36	20	28	8	14	0	20	6	**76**
S19	C	SIM	F	12	28	8	17	4	17	0	8	2	**48**
S20	C	SIM	F	23	37	20	20	6	24	0	20	10	**80**
S21	C	SIM	F	21	30	21	29	3	18	0	19	3	**72**
S22	C	SIM	M	27	34	11	23	10	12	7	20	0	**72**
S23	C	SIM	M	20	38	10	18	4	20	6	20	0	**68**
S24	C	SIM	M	18	30	10	23	2	22	0	11	0	**58**
S25	C	SIM	M	15	23	30	20	8	19	1	20	0	**68**
S26	C	SIM	M	20	38	22	40	4	14	3	19	0	**80**
B17	C	BRA	F	20	24	16	20	4	20	0	10	6	**60**
B18	C	BRA	F	23	26	22	33	6	22	0	6	4	**71**
B19	C	BRA	F	8	28	12	18	5	16	0	9	0	**48**
B20	C	BRA	F	24	40	21	29	11	15	0	20	10	**85**
B21	C	BRA	F	10	35	6	17	4	14	0	9	7	**51**
B22	C	BRA	M	16	24	5	20	3	18	0	4	0	**45**
B23	C	BRA	M	20	22	13	33	0	12	4	6	0	**55**
B24	C	BRA	M	14	33	13	23	9	17	9	2	0	**60**
B25	C	BRA	M	19	27	13	29	0	22	1	7	0	**59**
B26	C	BRA	M	9	30	11	18	1	20	6	5	0	**50**
T17	C	TSW	F	6	14	9	4	1	11	0	4	9	**29**
T18	C	TSW	F	3	23	4	18	0	8	0	2	2	**30**
T19	C	TSW	F	11	22	2	7	6	12	0	5	5	**35**
T20	C	TSW	F	7	11	11	14	2	6	0	4	3	**29**
T21	C	TSW	F	7	27	3	17	3	10	0	7	0	**37**
T22	C	TSW	M	10	21	9	8	5	18	3	6	0	**40**
T23	C	TSW	M	7	12	9	7	3	11	1	6	0	**28**
T24	C	TSW	M	5	5	10	3	2	5	0	10	0	**20**
T25	C	TSW	M	7	17	13	15	6	8	1	7	0	**37**
T26	C	TSW	M	3	16	3	9	4	5	0	4	0	**22**

APPENDIX 3

Ticks collected on various anatomical sites and seasons of control and treated animals during the trial 3.

CONTROL GROUP

Ear tag				Season of the year			Tick counts on anatomical locations						Tick specie	
No.	Trt	Breed	Sex	Spring	Summer	Winter	Belly	Ear	Perineal	Scotum	Sternum	Udder	Amb	Boop
S27	NT	SIM	F	4	10	8	9	2	5	0	4	2	13	9
S28	NT	SIM	F	2	7	6	7	0	4	0	3	1	4	11
S29	NT	SIM	F	6	11	2	7	2	6	0	2	2	10	9
S30	NT	SIM	M	4	9	3	6	1	5	1	3	0	9	7
S31	NT	SIM	M	7	7	8	5	1	8	4	4	0	15	7
S32	NT	SIM	M	7	9	8	4	1	10	1	8	0	11	13
B27	NT	BRA	F	5	11	1	4	0	5	0	6	2	11	6
B28	NT	BRA	F	3	8	5	8	2	4	0	2	0	10	11
B29	NT	BRA	F	7	13	7	8	0	11	0	7	1	15	12
B30	NT	BRA	M	3	7	2	5	1	4	1	1	0	7	5
B31	NT	BRA	M	1	4	2	3	1	2	0	1	0	2	5
B32	NT	BRA	M	4	12	11	6	2	7	3	9	0	14	13
T27	NT	TSW	F	3	5	1	3	1	3	0	1	1	3	6
T28	NT	TSW	F	0	4	2	2	0	1	0	1	2	3	3
T29	NT	TSW	F	1	3	2	2	1	0	0	3	0	1	5
T30	NT	TSW	M	1	6	1	5	0	1	1	1	0	2	6
T31	NT	TSW	M	2	5	1	2	1	1	0	3	1	4	4
T32	NT	TSW	M	2	5	2	4	1	0	2	2	0	6	3

CONTROL GROUP

Ear tag				Season of the year			Tick counts on anatomical locations						Tick specie	
No.	Trt	Breed	Sex	Spring	Summer	Winter	Belly	Ear	Perineal	Scotum	Sternum	Udder	Amb	Boop
S33	C	SIM	F	10	22	9	7	3	12	0	13	6	27	14
S34	C	SIM	F	5	20	6	15	1	10	0	4	1	17	14
S35	C	SIM	F	10	22	18	23	4	7	0	13	3	28	22
S36	C	SIM	M	9	21	10	8	2	10	3	7	0	11	19
S37	C	SIM	M	8	20	3	7	1	13	0	10	0	16	15
S38	C	SIM	M	13	27	20	29	3	18	2	8	0	40	20
B33	C	BRA	F	6	14	0	5	1	6	0	6	2	15	5
B34	C	BRA	F	7	9	8	9	5	6	0	2	2	10	11
B35	C	BRA	F	9	20	11	17	2	15	0	4	2	15	12
B36	C	BRA	M	5	14	1	8	0	3	0	9	0	14	6
B37	C	BRA	M	4	7	3	5	0	4	4	1	0	7	7
B38	C	BRA	M	2	18	20	20	6	6	1	7	0	23	17
T33	C	TSW	F	6	7	2	6	2	2	0	3	2	9	6
T34	C	TSW	F	3	7	4	7	3	1	0	4	0	7	7
T35	C	TSW	F	2	4	5	5	2	1	0	1	2	7	4
T36	C	TSW	M	2	8	1	7	1	1	0	2	0	5	6
T37	C	TSW	M	5	6	6	5	4	3	3	2	0	10	7
T38	C	TSW	M	4	6	1	4	2	2	1	2	0	7	4