

**ADDIS ABABA UNIVERSITY,
COLLEGE OF HEALTH SCIENCES
SCHOOL OF VETERINARY MEDICINE**

**MAJOR ECTOPARASITES OF SMALL RUMINANTS IN ARSI ZONE OF OROMIA
REGION: PREVALENCE, SPECIES COMPOSITION AND ACARICIDAL EFFICACY OF
MEDICINAL PLANTS AGAINST LICE OF SHEEP**

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DEGREE OF MASTER OF SCIENCE IN TROPICAL VETERINARY PARASITOLOGY

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DEDICATION

This paper is dedicated to the memory of my beloved wife
SEWASEW TADESSE whom I missed due to car accident in
October 2009

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LIST OF ABBREVIATIONS

AAU	Addis Ababa University
CSA	Central Statistics Authority
ELISA	Enzyme-Linked Immunosorbent assays
FAO	Food and Agricultural Organization
Kg. B. wt	Kilo gram per body weight
m.a.s.l	Meters above sea level
MoARD	Ministry of Agriculture and rural development
OIE	Office International des Epizootics
WAAVP	World Association for the Advancement Parasitology guideline
FGD	Focus Group Discussion
PAs	Peasant associations
ANOVA	Analysis of variance
EVM	Ethno-veterinary medicine
TLC	thin Layer chromatography

ABSTRACT

A study on major ectoparasites of sheep in three selected agro-ecological sites of Oromia Region, Ethiopia showed 81.5% overall prevalence of ectoparasites. The ectoparasites recorded were lice (75.5 %) *Damalina ovis* (67.1%), tick infestations (23.8%), *Linognathus ovillus* (14.6%), *Melophagus ovinus* (5.65%) found only in mid and highland, psoroptic mange 1.0% and sarcoptic mange 0.3% were the major ectoparasites. The major ectoparasites identified *Damalina ovis*, ticks and *M. ovinus* were showed statistically significant difference ($P<0.05$) of prevalence in high and midlands and *L.ovillus* in the lowland. The risk of *Damalina ovis* infestation in highland (OR) =2.2, ($P<0.05$) was two times higher than in the lowland. Agro-climate, age and BCs in *Damalina ovis* infestations are found to be the risk factors for the difference of prevalence of ectoparasites: agro-climate (OR=2.22), ($P<0.000$), age (OR=1.5), ($P<0.031$) and BCs (OR=1.09), ($P>0.05$). Favorable climatic conditions, backward level of management, poor level of consciousness and awareness of farmers, and weak animal health extension services are believed to have contributed for widespread distribution and occurrences of ectoparasites. The growing threat of ectoparasites to small ruminant production and tanning industry needs a well-coordinated program. The in-vitro efficacy of 20 mg/ml, 40mg/ml, 80 mg/ml and 160 mg/ml solutions of fixed oil extracts of *Jatropha curcas*, *Melia cafra*, *Ximenia azadrachta*, *pyrethrum cinerariifolium* and *Nicotiana tobacum* and 0.625%, 1.25%, 2.5% and 5% solutions of *Cymbopogon citratus* and *Eucalyptus globulus* essential oils extracts were performed after 10 min, 30 min, 1hr, 2hrs, 3hrs, 6hrs and 12hrs in all tests using usual and strict criteria for mortality. From seven herbal plants' extracts four of them were showed statistically significant difference ($P<0.05$) against *Linognathus ovillus*: *Jatropha curcas* and *Melia azedirachta* at 160, 80, 40 and 20mg/ml, *Cymbopogon citratus* and *Eucalyptus globulus* at 5 % and 2.5% concentrations at 12 hours. Six of the seven herbal plants' extracts were showed statistically significant difference ($P<0.05$) against *Damalina ovis*: *Jatropha curcas*, *pyrethrum* and *Ximenia cafra* at 160mg/ml, *Melia azedirachta* at 160, 80 and 40 mg/ml, *Cymbopogon citratus* and *Eucalyptus globulus* at 5 % and 2.5% concentrations at the end of 12 hours when compared to the commercial acaricide 0.1% diazinone 60 EC.

For therapeutic efficacy study (iv-vivo) in biting/chewing lice /*Damalina ovis* / 40 sites per animal were examined before and after treatment. Lice were counted in five predilection sites /neck, shoulder, wither, flank and rump/ at eight partings in each sites in an area of ten centimeters in length. During in an in-vitro test on *Damalina ovis* with 2.5% *Cymbopogon citratus* and 5% *Eucalyptus globulus*, there were statistical significant differences ($P < 0.05$) in louse count between treated and negative control groups. No apparent signs of any local or general adverse effects were observed during the study after the application of the extracts. The percent louse count reduction obtained was $< 95\%$ efficacy and never greater than 88.11% in an in-vitro test in the current study against *Damalina ovis*. Despite the current in-vivo study's lower results, traditionally claimed herbal plant extracts can be a good substitute for the synthetic compounds if there is a further increase in resistance to the acaricides in use and further studies carried out on synergism of two or more potent plant extracts. The licidal potent of *Melia cafra* fixed and *Cymbopogon citratus* essential oils for instance, suggest that they may find an application in the future. These results demonstrate the possibility of using traditional claimed herbal plant extracts for lice control.

Keywords: Ectoparasites, efficacy, in-vitro, in-vivo, lice, Oromia region, prevalence, Sheep, traditional herbal plants

1. INTRODUCTION

Ethiopia's economy is based on agriculture that accounts for 85% of the total employment and 75% of export (Zewdu, 1995). Livestock is second major source of foreign currency through export of live animals, skins and hides. Skins from goats and sheep are important economic products contributing for the largest share to the total and agricultural export commodities (Ayele *et al.*, 2003, FAO, 2005). Skins from goats and sheep are important economic products contributing for the largest share to the total and agricultural export commodities (Ayele *et al.*, 2003, FAO, 2005) as the largest share of export products followed by live animals (Ayele *et al.*, 2003). However in recent years, this rank has been relegated to fifth level mainly because of rejection and down grading inflicted on hides and skin defects due to infestation by external parasites (Bayou, 2005).

Skin problems caused by ectoparasites such as mange mites, lice, keds and ticks result in serious economic loss to smallholder farmers, the tanning industry and the country as a whole. They can result in mortality, decreased production and reproduction, down grading and rejection of skins (Ayele *et al.*, 2003, Yacob *et al.*, 2008). According to Kassa (1998), skin problems due to ectoparasites cause 35% of sheep skin and 56% of goatskin rejection (Amsalu *et al.*, 2000).

The major observed financial loss due to mange, lice and keds is associated with skin damage. In 1996/97 six tanneries that are found in and around Addis Ababa have rejected 2,037,745 pieces of skins which caused loss of US\$ 6.3 million (Kassa, 1998) and in 1998/99 three tanneries that are found in Amhara region have reported 443,602 pieces of skin rejection per annum which worth US\$1.4 million loss (MoARD, 2005). Even though deterioration in quality of skin is evident from all parts of the country; Tigray was cited among the regions which provide the country's worst skin (Alem, 1998). To combat this problem control program have been designed by MoARD in 2005 to be implemented in Tigray, Amhara and Afar regions. The problem of skin diseases is also evident in Oromia region (Shenkutie, 1987).

To combat this problem control program against ectoparasites and skin diseases have been designed by MoARD in 2005 to be implemented in Tigray, Amhara and Afar regions, which is also necessary to be practiced in Oromia region as the problem is serious in Oromia region, too. Despite the large population of the small ruminants in the region, however, there are no detailed studies that indicate the type of parasites involved their magnitude and relative importance for the farmers and the tanning industry.

Currently, the control of ectoparasites depends on the use of chemical methods. However, affordability for the resource poor farmers, the development resistance of some ectoparasites against the commercially available acaricides and the undesirable side effects of the chemicals require search for alternative treatment and control methods. The use of medicinal plants is one of the best options (Heukelbach.J et al 2006; Jadhav.V et al., 2007). Many farmers and pastoralists in developing countries rely on traditional health care practices to keep their livestock healthy. Indigenous practices, which include use of medicinal plants or ethno-veterinary medicine (EVM), assist farmers /pastoralists to improve the health and welfare of livestock in. (Heukelbach.J et al 2006)

Many of the previous studies on ectoparasites of domestic animals emanate from northern parts of Ethiopia. However, the problem is also serious in eastern, southern, southwest and western parts of Oromia regional state. Despite the large population of the small ruminants in the region, there are no detailed studies that indicate the type of parasites involved their magnitude and relative importance for the farmers and the tanning industry. Various medicinal plants are commonly used for the treatment and control of lice and other ectoparasites of ruminants in different part of the world. However, in vitro and in vivo efficacy evaluation of medicinal plants was never performed in Arsi zone of Oromia Regional State.

Therefore, the present study was designed to achieve the following objectives:

- Determine the prevalence of major ectoparasites of sheep in Arsi zone of Oromia regional state.
- Identify and assess the major risk factors associated with the infestation of these ectoparasites of sheep of the study area.
- Evaluate the in vitro and in vivo acaricidal efficacy of seven medicinal plants against lice in naturally infested sheep.

2. LITERATURE REVIEW

2.1. Ectoparasitism

Ectoparasites are organisms, which inhabit the skin or growth of the skin of the host for various periods (Hopla *et al.*, 1994). The association between arthropod ectoparasite and vertebrate hosts may take on variety of forms. In some cases the parasite may be totally dependent on the host, alternatively, the parasite may feed, or live only occasionally on the host, without being dependent on it (Wall and Shearer, 1997). The effect of skin parasitism usually depends on the size of invading population, on the manner on which the parasite ekes out its existence and the state of nutrition of the host animal when infected. The damage ectoparasites inflict may be mechanical, but the situation is complicated also by host reactions to the presence of the particular parasite, their secretion and excretion (Peter, 1995).

Young animals are generally more susceptible to ectoparasites because of higher region of accessible surface to the body volume and poor grooming behavior (Lehmann, 1993).

2.2. Major ectoparasites that causes skin diseases in small ruminants

2.2.1. Lice infestation

Both biting and sucking lice affect small ruminants. The important species in sheep and goats are found in the genus *Damalina* and *Linognathus*. The important species in sheep being *Linognathus ovillus* (sucking face louse), *Linognathus africanus*, *L. pedalis* (sucking foot louse) and *Bovicola ovis* (biting louse) (Radostits *et al.*, 1994).

Lice usually are unable to survive for more than 1-2 days off their hosts and tend to remain with a single host animal throughout their lives. Most species of lice are highly host specific and many species specialize in infesting only one part of their host body (Wall and Shearer, 1997) and transfer to new hosts is by body contact, particularly under condition of close confinement (Peter, 1995). To allow them survive as permanent

ectoparasites, lice show a number of adaptations which enable them to maintain a life of intimate contact with their hosts. They are small insects, about 0.5-8mm in length, dorsoventrally flattened, wingless and possess stout legs and claws for clinging tightly to fur, hair and feather. They feed on epidermal tissue debris, parts of feathers, sebaceous secretions and blood (Wall and Shearer, 1997).

Life cycles of all lice are similar (Radostits *et al.*, 1994; Peter, 1995). Lice undergo incomplete metamorphosis, which refers Eggs, Nymphs and Adults. In their life cycle, lice hatch from the eggs (nymphs) are tiny replicates of the adults, they change several times but undergo only minor changes in appearance (Bowman *et al.*, 1999). The eggs are laid attached to the coat fiber and there are three nymphal stages before mature lice appear. The life cycle of most species varies from 2-4 weeks under optimum conditions. The rate of development is greatly influenced by temperature and moisture, which are known as Absolute Dynamic Binomic Factors (Kassa, 2007). That is lice breed within narrow temperature range; when temperature is cooler than optimum, eggs do not develop while hotter temperature prevent egg laying and kill the lice (Radostits *et al.*, 1994). Lice have a tightly defined band of humidity and temperature preference and respond to humidity and temperature gradients by showing increased rates of turning in favorable microclimates which tend to keep them in favorable areas (Wall and Shearer, 1997). In the flock, there are often carrier animals that stay heavily infested all year round (Urquhart *et al.*, 1987). All species cause irritation of the skin and stimulate scratching, rubbing, and licking leading to restlessness, damage to the fleece and skin and decrease in milk production. The saliva and faeces of the live contains substances, which are capable of causing allergies, giving rise to severe irritations, followed by the skin thickening and sometimes self trauma (Peter, 1995). Being highly active, *Bovicola* species is usually considered to be most pathogenic in sheep and it can cause great irritation so that the sheep are restless and have their grazing interrupted. Like keds, *B. ovis* is also associated with the development of cockle (Health *et al.*, 1996) which is characterized by formation of brown, or gray discoloration or small nodules of 5-10mm in size which may be found intact or broken to form ulcers on the dermis which result

with scar formation on the leather (Kassa, 2007). In goats the sucking louse *Linognathus stenopsis*, is more pathogenic than the biting lice (Jubb *et al.*, 1993).

While there is disagreement on the effect of lice on the weight gain, animals in poor conditions, improperly fed and exposed to cold and debilitating diseases carry heaviest infestations. Such animals may benefit from removal of the lice while animals in good condition and well fed may not show increased weight gain after treatment (Radostitis *et al.*, 1994). Reduction in the value of the wool clip is economically the most important consequence of ovine pediculosis, but additional hazards in warm countries is that the fleece and skin damage by the rubbing and soiled by louse faeces, is an attractant for blow flies strike (Urquhart *et al.*, 1996).

2.2.2. *Melophagus ovinus*

Keds are brown and hairy wingless biting flies about 5-7 mm in length resembling a tick (Urquhart *et al.*, 1996) with a short head and broad, flattened, brownish thorax and abdomen. Ked live their entire life cycle in the host but able to move from one host to another rapidly within the flock (Sewell *et al.*, 1990). Adult keds are permanent ectoparasites and feed on the blood of sheep and sometimes goats. The female ked live for 4-5 months and lays 10-15 larvae during this time (Radostits *et al.*, 1994) thus build up of infestation is slow. Single larvae are deposited on the host and pupate within a few hours. The larvae are attached to the wool fiber some distance above the skin and many larvae and pupae are removed during shearing. The young keds usually emerges in 20-22 days but this period may be prolonged for up to 35 days in winters. The complete life cycle takes 5-6 weeks in optimum warm conditions (Urquhart *et al.*, 1996).

The parasite is mainly seen in colder and wetter areas and infestations may be lost when sheep are moved to hot dry areas. Keds are most active in cooler months and are common on coarse rather than fine woolen breeds. Sheep in poor conditions suffer most from infestation and goats may also be infested. Resistance is acquired in time and resistant sheep grow better and produce more wool. Keds dislodged from the host can live for up

to 2 weeks in mild moist condition but most die in 3-4 days and probably do not play a part in re-infestation on sheep (Radostits *et al.*, 1994).

The spread of sheep ked is mainly by direct contact and the movement of keds from ewe to lamb is an important route of infestation. The predilection sites of infestation are the neck, around the tail and ventral part of the body (Urquhart *et al.*, 1996). The keds may transmit *Trypanosoma melophagium* and the *Rickettsia melophagi*, which are harmless blood parasites of sheep.

Although the degree of infestations usually encountered cause only irritations (Radostits *et al.*, 1994), the resulting scratching, biting, and rubbing themselves against fences, stones, shrubs, and other things causes severe damage to the skin and wool (Urquhart *et al.*, 1996). Furthermore, both sexes are blood feeders and feed several times each day therefore very heavy infestations may cause severe anaemia (Sewell *et al.*, 1990). Skin punctures made by blood sucking keds causes development of nodules called “cockles” in the finished sheep skins (Health *et al.*, 1995; Kassa, 1998). Cockle is an inflammatory response of the skin to the presence of keds and their saliva which is recognized after the wool or hair has been removed from the skin. Staining of the wool by the faeces of keds reduces its value and gives it a peculiar musty odor (Noble *et al.*, 1982).

2.2.3. Mange mites

The ectoparasitic mites of mammals and birds inhabit the skin, where they feed on blood lymph, skin debris or sebaceous secretions, which they ingest by puncturing the skin, scavenge from the skin surface or imbibe from epidermal lesions. Most ectoparasitic mites spend their entire lives in intimate contact with their host, so that transmission from host to host is primarily by physical contact. The generalized veterinary term for an infestation by mites in an animal is called acariasis and can result in severe dermatitis, known as mange or scabies, which may cause significant welfare problems and economic losses (Wall and Shearer, 1997).

Mange is a widespread and most important ectoparasitic disease of animals. Mange infestation is spread mainly by direct contact between hosts and all the three stages: the larvae, the nymph and the adult are capable of migrating and inert materials such as bedding and grooming tools can act as a carrier. Adult mites do not usually survive more than two weeks away from the host, but in optimum conditions they may remain alive for up to three weeks (Radostits *et al.*, 1994).

Female mites produce relatively large eggs, from which a small, six-legged larva hatches. A few species are ovoviviparous, producing live offsprings. The larva moults to become an eight legged nymph. There may be between one and three nymphal stages, known respectively as the protonymph, deutonymph and tritonymph. At least one of these nymphal stages is usually inactive and development proceeds without feeding. The nymph then moults to become adult (Wall and Shearer, 1997). The number of eggs produced per female is highly variable but lifetime reproductive outputs may be as low as 16 eggs per female. Nevertheless, the life cycle of many parasitic species may be completed in less than 4 weeks and in some species may be, as short as 8 days. Hence, the mites have the potential for explosive increases in their population size (Wall and Shearer, 1997).

High temperature, humidity and sunlight favour mange mite infestations (Pangui, 1994). The disease affects all age groups and runs a more chronic course in adults than younger animals. Animals in poor condition are most susceptible to mange (Radostits *et al.*, 1994). Mange cases due to *Sarcoptes* and *Psoroptes* are often fatal. The mortality rate is higher in younger and poor condition animals (Sewell *et al.*, 1990; Olubunmi, 1995).

Death may be due to dehydration, a direct result of the feeding of huge number of mites, inability to move and feed due to severe lesions on the face, muzzle and on the joints or to secondary causes such as pneumonia or bacterial septicemia introduced through self inflicted bite and scratch wounds (Roberts, 1971). In infestations, which do not end fatally, a marked regression of lesions, with healing of the skin and re-growth of the wool

or hair occurs during dry season. Exposure of lesion and mite to direct sunlight and desiccation may reduce the survival potential of mite populations.

Some forms of mange such as demodectic mange, are the result of underlying diseases or immunosuppression (Wall and Shearer, 1997). According to these authors the effects of mite infestations are:

- Direct epidermal damage leading to inflammation; this results in skin erythema, pruritus, scale formation, lichenification (thickening) and crust formation (inflammatory exudates formation);
- The production of cutaneous hypersensitivity (especially type I hypersensitivity);
- Loss of blood or other tissue fluids;
- Mechanical or biological transmission of pathogens.

The clinical signs of erythema, pruritus and scale or crust formation are due to the inflammatory response of the skin and resulting excoriation. This response is stimulated by feeding, burrowing or the production of antigenic material by the mite (Wall and Shearer, 1997).

Some observers suggest that infra-orbital, inguinal pouches, scrotum, under tail, ears, inter digital pouches, perineum, and skin folds are foci for mites and serve as potential dry season hiding places where the mites tend to migrate to the general body surface with the onset of cold season (Sewell *et al.*, 1990). According to the study reports, mange was noticed throughout the year but the incidence was higher during the wet cold months where the moistness and temperature is optimum condition for mite development (Olubunmi, 1995). Mange in sheep and goats is caused by four genera of mites, namely *Sarcoptes*, *Psoroptes*, *Chorioptes* and *Demodex* (Soulsby, 1982).

Sarcoptic mange

Sarcoptic mange occurs in all species of animals and is caused by mite *Sarcoptes scabiei* that has a number of subspecies that affect different hosts but this host specificity is not complete and transference from one host species to another can occur (Radostits *et al.*,

1994). *Sarcoptes* may be transmitted to unusual host in which it might burrow in to the skin and set up a typical mange lesion (Kaufmann, 1996). *Sarcoptes* mites are economically the most important cause of mange in sheep and goats. Sarcoptic mange in sheep and goats is caused by *Sarcoptes scabiei var ovis* and *Sarcoptes scabiei var caprae* respectively (Okoh *et al.*, 1992). Sarcoptic mites are highly specialized for life with in the skin.

Female mites burrow in to the skin and lay eggs in tunnels they made. Mating takes place on the surface of the skin (Sewell and Brockesby, 1990). The life cycle from egg to egg laying female may take 10-14 days (Kaufmann, 1996). The feeding activity of *Sarcoptes* causes intense itching and scratching due to a marked irritation, which causes self inflicted lesions that aggravates the conditions (Jackson, 1991). Sarcoptic mange usually start on relatively hairless part of the skin and may latter generalize (Bowman *et al.*, 1999). The course of Sarcoptic mange is rather more acute than the other forms of mange and may involve the entire body surface in a short time (Blood *et al.*, 1983). It is highly contagious and the spread of *Sarcoptes scabiei* is usually by close physical contact. As a result single cases are rarely seen in groups of animals kept together. Infestation may also occur by indirect transfer, since the mites have been shown to be capable of surviving off the host for short periods. The length of time that *Sarcoptes scabiei* can survive off the host depends on environmental conditions but may be between 2 and 3 weeks (Wall and Shearer, 1997).

Mange caused by *Sarcoptes* was noticed throughout the year but the incidence was higher during the wet cold months where the moistness and temperature is optimum condition for the mites' development (Blood *et al.*, 1983).

Psoroptic mange

Psoroptic mange, also known as sheep scab, is highly contagious disease of sheep and goats. It is caused by the mites, *Psoroptes communis var ovis* in sheep, which affects sides of the body and *Psoroptes communis var cuniculi* in goats, which affects the external ear canal. Psoroptic mange mites are non-burrowing and feed on tissue fluids.

The mite migrates to all parts of the skin and prefers areas covered by wool or hair. The whole life cycle is completed in 3 weeks (Kaufmann, 1996). Infestation by these mites is always superficial on the epidermis, but the piercing of the skin by the mites lead to exudation and exfoliation, causing scabs to form (Sewell *et al.*, 1990). In sheep the cutaneous lesions may occur on any part of the body but characteristically in badly affected sheep, they are most obvious on sides. In goats, lesions can vary from a dry crusty scab on the external ear canal with no clinical signs to severe lesions covering much part of the body and causing death's (Souslby, 1982).

The overall biology of psoroptic mites resembles that of *Sarcoptes*. The only difference is that *Psoroptes* do not burrow in to the skin. Eggs are laid on the skin surface and hatch out in 1-5 days to larval stages. Larvae after feeding moult in to nymphs where finally nymphs moult to adults (Kaufmann, 1996).

Psoroptic mange can affect all ages of sheep but may be particularly severe in young lambs. Mites are usually more active in winter and the oviposition rate is higher at lower temperatures. In summer the disease progress more slowly, lesions are not obvious and can be missed. The disease can become latent in summer, apparently disappearing, with mites taking refuge in protected sites (Wall an Shearer, 1997).

Psoroptic mites pierce the skin and suck the host's tissue fluids causing irritation, inflammation then discharging lymph fluid, which dries to form yellowish crusts and scabs. As a result the affected animals become irritated, rub and bite themselves, resulting in wool drop off (alopecia) and sometimes secondary bacterial infection following severe inflammation (Bowman *et al.*, 1999).

Choriopitc mange

Choriopitic manges (tail, leg, or scrotum mange), which attacks cattle, horse, goats and sheep are now considered to be one species; *Chorioptes bovis* (Radostits *et al.*, 1994). The life cycle of *Chorioptes bovis* is similar to that of *Psoroptes* and is completed in 3 weeks and transmission is by direct contact and contaminated materials. However,

Chorioptes does not survive off the host for more than a few days (Peter, 1995). In goats, lesions of chorioptic mange are usually confined to lower parts of the leg and crusty lesions may be found behind fetlock of all four limbs (Kaufmann, 1996). In sheep it affects the scrotum and may cause decrease in fertility (Radostits *et al.*, 1994).

Demodectic mange

Demodectic mange mites (hair follicle mites) are tiny elongated mites that infest hair follicles of all species of domestic animals. *Demodex species* live as commensals, embedded head down in the hair follicles, sebaceous (oil) glands and meibomian glands of the skin where they spend their entire lives and are unable to survive off their hosts. For the most part they are non pathogenic and form a normal part of the skin fauna (Wall and Shearer 1997). The disease causes little concern but in cattle, there may be significant damage to the hide and rarely deaths due to gross secondary bacterial invasion. The disease may also be severe in goats. The important signs of the disease in goats are the appearance of small nodules and pustules which may develop in to larger abscesses from which large number of *Demodex* mites may be expressed (Jackson, 1991). Lesions induced by *Demodex* mites are of two types (Blood *et al.*, 1983):

- Squamous demodicosis, where hair follicles are distended with mites and cellular debris, follicular epithelium is atrophic and widespread alopecia is evident;
- Pustular (follicular) demodecosis, which results from secondary bacterial complications like staphylococcus species.

The entire lifecycle is spent on the host, where eggs hatch into nymph (protonymph and deutonymph) and then moults to adult stages and the whole life cycle is completed in 18-24 days (Kaufmann, 1996). The disease spreads slowly and transfer of mite is through contact probably early in life (Radostits *et al.*, 1994).

2.2.4. Tick infestations

Ticks are obligate, blood feeding ectoparasites of vertebrates, particularly mammals and birds. They belong to three families *Ixodiadae*, *Argasidae* and *Nutelielidae*. *Ixodidae*, known as hard ticks, contain almost all the species of ticks of veterinary importance. The second family *Argasidae*, known as, soft ticks, contains relatively small number of species of veterinary importance (Wall and Shearer, 1997; Okello-onen *et al.*, 1999). According to Wall and Shearer (1997), ticks are primarily parasites of wild animals and only about 10% of the species feed on domestic animals, primarily sheep and cattle.

Apart from their role as a vector and potential reservoir of infestations can cause direct losses of productivity in domestic animals. Tick bite may be directly debilitating to domestic animals, causing mechanical damage, irritation, inflammation and hypersensitivity and, when present in large numbers, feeding may cause death from severe anemia (Kaufmann, 1996).

According to Wall and Shearer (1997), some species of ticks cause tick paralysis and it is possible that others will elaborate toxins other than those causing paralysis. Heavy tick burden cause sufficient worry to interfere with feeding which may lead to loss of production and weight gain. They also cause greater morbidity and mortality during periods of drought, and delays in fattening so that animals are held longer before they can be sold (Radostits *et al.*, 1994).

Although many ticks favor a particular host, they are usually not completely host specific and may parasitize a wide variety of animals. The life cycle of ticks vary widely. Some species pass their entire life on the host, others pass different stages of the life cycle on successive host, and others are parasitic only at the certain stages. Most ticks spend more time off the host, but are totally dependent on the host for sustenance. They are subjected to microenvironment condition when on the ground and thus tend to be more endemic in specific types of area. Ticks can exist for a long period of time without feeding (Peter, 1995).

Sheep ticks are of great economic importance, but not to the extent that they are in cattle (Kaufmann, 1996). These parasites cause tick-worry, blood loss and damage at feeding sites, which results in skin damage. Damage on sheep skin is primarily found along the belly area or where the fleece is thinner.

Lamb skins are particularly susceptible to skin damage. Secondary bacterial infection of the bite increases the severity of the damage. Wounds left from tick bites are susceptible to additional attack by blowflies and screwworm flies that deposit eggs on the wound and produce a fatal, cutaneous myositis (Bowman *et al.*, 1999). Ticks produce tick marks that look like holes, pinpricks on the grain or scars that mostly occur in the belly (Soulsby, 1982; Asp and Tauni, 1988).

2.3. Diagnosis of ectoparasitic skin diseases

In making a diagnosis of ectoparasitic infestation or an ectoparasite associated dermatosis it is important to have an idea of the parasite involved and its life cycle. Some parasites live in intimate relationship with the host's skin, however, visiting parasites, may be on the skin only for a short period of time and a diagnosis is often made by implication. Hence a working knowledge of the clinical sign of the skin disease is usually also required (Wall and Shearer, 1997). The ideal approach to diagnosis of skin diseases is a logical progression from history to an overall clinical examination, to a detailed examination of the skin, and finally to confirmatory testing or diagnosis by response to treatment (Jackson, 1991; Smith and Sherman, 1994; Kaufmann, 1996).

2.3.1. History

According to Jackson (1991) and Kaufmann (1996), the main points to be noted during history taking includes: Date when symptoms first appeared, symptoms observed by the owner, contact with other flock, spread of the disease within the flock, previous health history of affected animals, other disease problems within the flock at past and present, response to treatment (including traditional and/or home remedies) and detailed management including feeding, de-worming, etc.

2.3.2. Clinical examination

According to Smith and Sherman (1994), simple observation allows identification of most external parasite infestations and clinical signs of skin diseases, thus many conditions can be diagnosed with reasonable certainty. The entire skin surface of the patient should be examined for parasites and lesions and the elasticity of the skin, its temperature, thickness, color and consistency should be noted and response of the animal to palpation of affected area should be observed (Jackson, 1991; Kaufmann, 1996). The appearance and location of lesions are the basis for diagnosis. According to Kaufmann (1996), the primary lesions include papules, vesicles, pustules and nodules. Secondary lesions such as scales, crusts and alopecia are the result of self trauma or super imposed bacterial infections.

During clinical examination unidentified external parasites or their eggs are collected, fixed and stored in 70% alcohol or 10% formalin and identification can be made with the help of identification keys (Soulsby, 1982). Many of the larger ectoparasites, such as blowfly larvae and ticks, may be collected directly from the host using appropriately sized forceps. Small specimens may be picked up with the end of moistened paint brush. Unattached mites and ticks can be removed by combing or brushing of the host animal over a white enamel tray or sheet of paper. Brushing over moistened white blotting paper or paper towel may help to identify flea infestations (Kaufmann, 1996; Wall and Shearer, 1997).

According to Kaufmann (1996), hairs collected by coat brushing and plucking should be mounted in a mineral oil, such as liquid paraffin, and examined microscopically for evidence of ectoparasites. Eggs of some parasites, such as lice may be found attached to the hair shaft and adult ectoparasites, such as lice and various mites, may be also found by this method. The hair bulb and lower third of the shaft should be examined for evidence of follicular mite *Demodex*. In case of alopecia it may be useful to examine the upper portion of the hair for evidence of fracture, which occurs with self-induced alopecia due to pruritus (Wall and Shearer, 1997).

In order to insure that the mouth parts of a tick are not left behind, embedded living ticks may be removed most effectively by dabbing the ticks and the surrounding skin with alcohol. This relaxes the tick, allowing it to be pulled out intact. Alternatively, the tick can be covered with a layer of petroleum jelly, which prevent respiration and, after about 30 minutes, the tick will drop off (Kaufmann, 1996).

2.4. Laboratory diagnosis

2.4.1. Examination of skin scrapings

Examination of skin scrapings is very essential in the diagnosis of mange mites. But in longstanding cases mites are often very few in number and extremely difficult to find and their absence from the skin scraping does not negate a diagnosis (Jackson, 1991). Therefore, multiple sites should be scrapped to increase the likelihood of ectoparasite detection. According to Wall and Shearer (1997), superficial skin scraping (epidermal surface examination) after removing coat hair by gentle clipping can be used to identify surface mites, while deep skin scraping (deep epidermal examination) until capillary ooze occurs is useful in the diagnosis of burrowing and follicular mites such as *Sarcoptes scabiei* and *Demodex species*.

A few drop of 10% potassium hydroxide solution or liquid paraffin are added to the sample, a cover slip applied and cleansing of debris allowed proceeding for 15-30 minutes before microscopic examination. Large samples may be processed by boiling for 10 minutes in 10% potassium hydroxide solution, centrifuging and performing sugar flotation on the sediment (Smith and Sherman, 1994).

2.4.2. Biopsy and histopathology

According to Wall and Shearer (1997), biopsy and histopathology are not as useful as direct identification for the diagnosis of ectoparasitic dermatosis, but they may be valuable in some circumstances, such as insect and arthropod bite lesions. According to Jackson (1991) and Kaufmann (1996), small whole thickness strips of skin 25mm x 5mm are taken from normal and abnormal area and the skin strip should be fixed in formol saline before examining under microscope. According to Wall and Shearer (1997), histological changes often associated with ectoparasites include:

- an eosinophil-rich dermal infiltrate;
- collagen degeneration usually associated with dermal eosinophil infiltration;
- focal dermal necrosis, which occur in tick bite lesion;
- eosinophilic pustule formation, which occurs in cases of fleabite dermatitis and *Sarcoptes scabiei* infestations.

2.4.3. Serology

Enzyme Linked Immunosorbent Assay (ELISA) techniques have been developed to monitor *Psoroptes* infestations of sheep, cattle and non domesticated animals, but none is in routine use. Enzyme Linked Immunosorbent Assays (ELISAs) are used routinely by researchers for detection of *Sarcoptes scabiei* (OIE, 2002).

2.5. The status of small ruminant parasitic skin diseases in Ethiopia

Reports on skin diseases of small ruminants, especially parasitic skin diseases in Ethiopia are scanty and if present are also fragmented. Studies conducted at various localities of the country and tanneries report on magnitude of skin pelts damage due to skin disease, especially ectoparasites indicate that parasitic skin diseases of small ruminants are becoming growing threat for the production of small ruminants and export of skin in Ethiopia. The main parasitic skin diseases reported in Ethiopia are:

2.5.1. Mange

Mange of small ruminants was reported from different areas of the country with different magnitude ranging from 0% to 7.85% in sheep and 0.37% to 11.8% in goats. Higher prevalence rate up to 67.7% in sheep and up to 31.8% in goats were recorded, but these are prevalence on animals with visible skin lesions (Table 1).

Table 1: Overall prevalence of mange in different areas of Ethiopia

Location	Prevalence in %	Source/year
Hararghe	7.85	Gashaw, 1986
Bale Robe	67.7*	Shenkutie, 1987
Addis Ababa	Sheep	Nigatu, 1992
Dire Dawa	0.73	Zelalem, 1994
Mekelle	1.5	Habte, 1994
Wolayta	0	Chalachew, 2001
Central Ethiopia	2.69	Haffeze, 2001
Sidama zone	2.07	Teshome, 2002
Selected zone of Tigray	30.2*	Kedir, 2002
Southern range lands of Oromia	0.15	Molu, 2002
Selected sites of Amhara	0.4	Tefera, 2004

* Study conducted on animals with visible skin lesion

** The study conducted does not include the species.

The variation in the prevalence of mange in different areas of the country is due to lack of consideration seasonal and altitude difference during sample collection. Hence, to avoid this problem consecutive serial sampling should be taken in different seasons of the year and geographical conditions of the country.

According to Amsalu *et al.*, (2000), the disease was reported to occur in lowlands and mid altitude of 59 woredas of Amhara region. It has become an important disease causing a loss of up to 93% of the small ruminants in Yeku water shade development project area of Amhara region (Mekonen *et al.*, 1999). For this reason, lack of small ruminant flock was identified as top ranking problem of the area. The incidence of mange has increased during the past 10 years to a level preventing farmers from keeping sheep and goats in some areas of Amhara region bordering Afar, Tigray and Sudan (Amsalu *et al.*, 2000).

Different species of mange mites were recorded from different parts of the country. Of the mange mite affecting sheep, *Sarcoptes* is the most prevalent species in Ethiopia. *Sarcoptes* was reported to have prevalence of 0.44 in sheep in Sidama zone (Teshome, 2002); 8.5% 0% in sheep from Wolayta (Chalachew, 2001); 0.4% in sheep from selected sites of Amhara region (Tefera, 2004). The prevalence of *Sarcoptes* from clinically diseased animals with visible skin lesion was 2.33% in sheep from Hararghe (Gashaw, 1986); 30.3% in sheep from Tigray (Kedir, 2002) 0% in sheep in Diredawa (Zelalem, 1994). Records of Psoroptic mange were 0.95% in sheep in Sidama zone (Teshome, 2002); 1.8% in sheep in central Ethiopia (Haffeze, 2001). Relatively higher prevalence of 6.15% in goats and 4.67% in sheep from Harrarghe (Gashaw, 1986), 67.6% in sheep from Bale Robe (Shenkutie, 1987), and 32.87% from Addis Ababa (Nigatu, 1994) was recorded. However, these results are from clinically skin disease affected animals. Demodectic mange was reported to occur in central Ethiopia 0.8% in sheep (Haffeze, 2001), in the southern range land of Oromia 0.88% in sheep (Molu, 2002), in Harrarghe 0.84 in sheep (Gashaw, 1986) and in selected sites of Amhara region 0% in sheep (Tefera, 2004).

2.5.2. Lice

Lice infestations in small ruminants were reported with overall prevalence of 2% in sheep (Haffeze, 2001) from central Ethiopia; 0% in sheep (Molu, 2002) from southern range land; and 2.4% in sheep (Tefera, 2004). The louse species identified were 0.8% *B. ovis* and 1.2% *Linognathus species* in sheep in central Ethiopia (Haffeze, 2001). However,

results obtained from examination of fresh sheep pelts showed a much higher infestation rate of 89.55% (Ermias, 2000).

Both lice and keds are considered as cause of “ekek” in Ethiopian sheep pelts (Kassa, 1998). However, information on prevalence and distribution of keds are scarce but examination of fresh sheep pelts indicates 32.7% infection rates (Ermias, 2000), and “ekek” was responsible for 70.8% of the pickled sheep pelt downgrading and rejection (Tefera, 2004).

2.5.3. Ticks

Reports from different areas of Ethiopia indicate that ticks are also among the skin parasites affecting small ruminants. The overall prevalence of ticks infestation reported was 0.96% in sheep (Haffeze, 2001) from central Ethiopia; 5.27% in sheep (Molu, 2002) from southern range lands; 23.8% in sheep (Teshome, 2002) from Sidama zone; 66.5% in sheep (Zelalem, 1994) from Diredawa; and 0.1% in sheep (Tefera, 2004) from selected sites of Amhara region.

The species identified were *Amblyomma spp.* (11.4%), *Hyaloma spp.* (5.3%), *Boophilus spp.* (7.1%) from sheep in Sidama zone (Teshome, 2002); *Amblyomma spp.* (4.58%) from goats in Kombolcha (Numery, 2001); and *Boophilus spp.* (0.1%), *Amblyomma spp.* (2.1%) and *Rhipicephalus spp.* (2.3%) from sheep in selected sites of Amhara region (Tefera, 2004).

3. MATERIALS AND METHODS

3.1. Study area

This study was conducted in three agro ecological districts of Arsi Zone of Oromia Region: Adaba (highland), Asasa (midland) and Ziwaydugda (lowland) with ranging from 500 to 4130 m.a.s.l, experiencing binomial rainfall occurring from July to October and April to May, with an average annual temperature of 20-25° C and rainfall of 200 mm in the lowland, where 10-15 ° C with a rainfall of 400 mm in the highlands. The production system of the area is mixed crop-livestock in all three agro ecological zones. The three climatic zones are characterized by an arid lowland zone (Ziwaydugda-Arsi zone Rift valley area 201kms), tropical highlands (Adaba- interface area of Bale Mountain ranges 325kms) and tropical form of midland (Asasa 283kms) away from Addis Ababa respectively. Vegetation of the area changes with altitude and rainfall ranging from scattered acacia trees and bushes to dense bushes and forest in different altitudes and from thorny and fibrous grass of dry season to bushy and soft grass of rainy season. The livestock reared in the area consists of cattle, sheep, goats, horses, donkeys, mules and poultry. Arsi-bale indigenous sheep are one of the known highest populations of sheep species in the country (CSA, 2003).

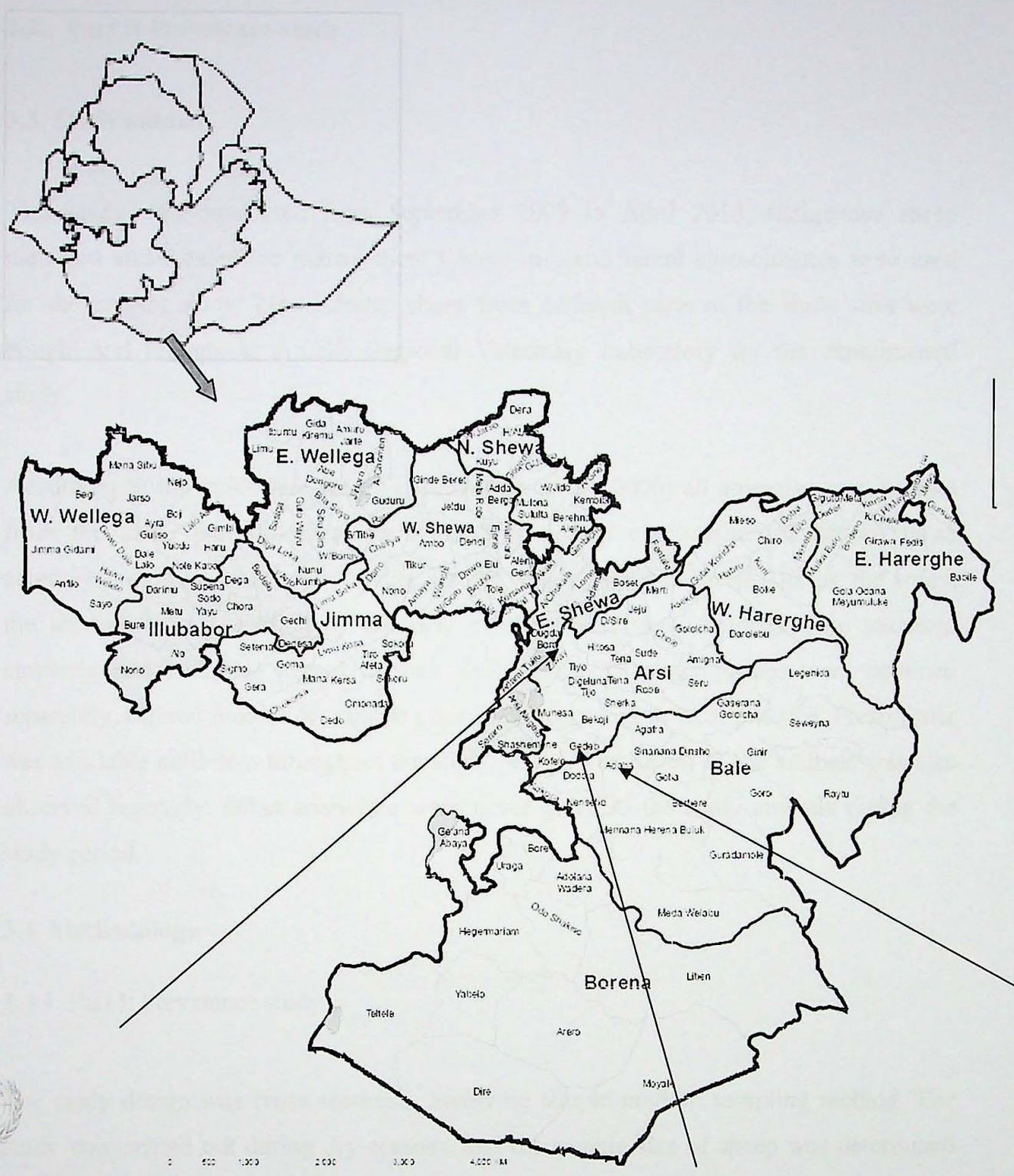


Figure 1: Map showing the study district in Oromia Regional state.

3.2. Part I: Prevalence study

3.3. Study animal

This study was conducted from September 2009 to April 2010. Indigenous sheep managed under extensive management system in the different agro-climates were used for the present study. Lice infested sheep from different parts of the study sites were bought and brought to Assella Regional Veterinary Laboratory for the experimental study.

According to the guide line set by (Holdsworth *et al.*, 2006) all animals were selected from the same parasitological background (exposure of parasite infestation) those acquired the disease naturally, having similar weight, age and breed. During the study, the animals were identified by uniquely numbered ear tags. Animals were managed similarly and with due regard to their well being. All study groups were confined separately, offered food under similar conditions according to local practice. Fresh water was available adlibitem throughout the study period. The health of the animals was also observed regularly. Other acaricides were never given to the study animals during the study period.

3.4. Methodology

3.4.1. Part I: Prevalence study

The study design was cross sectional, involving simple random sampling method. The study was carried out during dry seasons, and the sample size of sheep was determined according to Thrusfield, (2005). The age of animals was determined primarily based on the information obtained from the framers and also by looking to the dentition pattern of the animals Gatenby (1991) for sheep. Animals were grouped (as young up to one year of age and adults older than one year) and body condition score of the animal was made as poor and good, by modifying the system as described by Gatenby (1991) for sheep.

3.4.2. Questionnaire survey

In an attempt to obtain overall information, a pre-structured FGD(Focus Group Discussion) system was designed. The system was set up to reduce the time required to be spent on an individual interviewing and broaden the out come and come up with the community perception rather than the individual's concept about the overall information on livestock ownership pattern, importance of small ruminant keeping, awareness the community have on ectoparasites and effect ectoparasites have on production and market value of skins, control practices and the traditional herbal medicine usage in livestock disease.

8 to 10 selected people: animal owners, skin traders, elders, religious leaders, shepherds and respective animal health personnel of the respective districts will constitute one FGD group.

The main livestock diseases identified were, Anthrax, Blackleg, pasteurellosis, external and internal parasites, sheep-pox, African horse sickness, fowl diseases and mastitis accordingly as prioritized by FGD. After thoroughly discussion, the main livestock skin diseases prioritized was tick and lice infestation, sterpthothricosis, and mange mites. Earlier, lice infestation is known and pronounced in calves during dry period according to FGD and lice infestation in small ruminants is the recently (four-five years) problem. Lice infestation problem is encountering in equines and cattle nowadays, particularly during the prolonged dry period.

When discussed on the use of traditional medicine in treating livestock dermal diseases, at present, the community is practicing treating lice infestation only in calves by traditional means. The intervention methods used to tackle veterinary diseases are mainly, modern medicine and there are also a lot of traditional plants to treat Blackleg and Anthrax. The awareness the animal owners, skin traders, elders, religious leaders, shepherds and in general different community members have on ectoparasites effect (lice) is very great. According to FGD members in all nine PAs in the three districts, the

low price of skin is known to be due to lice infestation. The lice on slaughtered or dead sheep skins are clearly observed and such skins are rejected by the hide and skin traders. The price of un-rejected sheep skin is in the range of three to five birr, so this concern brought awareness to the community that the skins infested with lice are rejected.

The knowledge of traditional herbal drug handling in skin disease control practices in the community is unsatisfactory, unless for the calves lice treatment. In one of the study district, Gedeb, the FGD members discussed and agreed that traditional herbal plant utilization is not practiced in their area nowadays.

The preparations of traditional medicine are carried out either at home or seek assistance from knowledgeable community members, with minimum payment or for free. Traditional medicine preparations are cheaper and mostly used in areas where there is no accessibility. They prefer modern medicine than traditional one, as traditional medicine shows variable results.

The local name for lice is 'chini' and it is extremely prevalent, the clinical manifestation of lice is easily observable. When livestock are affected by lice, the measure usually taken to tackle this problem is intervening with modern medicine. There is no practice to treat lice in sheep, however, 'chekata' is a plant used in treating calves infested by lice.

When asked, whether they encourage medicinal plants to undergo scientific investigation to develop herbal based livestock medicine for lice, they show great interest towards this idea and they hope traditional medicine can be prior to modern acaricide to treat lice in calves. They clarified that, traditional medicine often will cure or kill, when used, because of lack of knowledge to determine the amount required to use.

3.4.3. Clinical and laboratory examination

All 1200 sheep clinically examined for presences of ectoparasites and/or lesions. Prior to clinical examination, the sex, age and body condition of the animals was recorded. Body condition score of the animals was considered as poor and good; by modifying the system described in Gatenby (1991). The clinical examination was performed by multiple fleeces parting in the direction opposite that in which hair or wool normally rests and visual inspection and palpation of the skin for parasites and/or lesion on all parts of the animals including the ears and digits. Those sheep found infested by single parasite was considered positive Kumsa, B. and Bekele, M. 2008. The sites of infection on the animal body were recorded and the parasites were identified on the basis of their morphological structure as described in Wall and Shearer (1997).

3.4.4. Specimen collection and examination

Those ectoparasites and their larvae unidentified during clinical examination and scrapings of mange like lesions from clinically suspected animals were collected in a clean container, for detailed laboratory examination.

Samples of mange like lesions were collected by cutting the hair around affected area, scrapping the edges of the lesion with scalpel as described by Soulsby (1982) until capillary bleeding is seen in such a way that the bled is being held at an angle that the material scraped falls on Petri dish held underneath. The lesions were then dressed and the scrapped, material transferred to a clean container and unreserved carried for laboratory examination. Ectoparasites such as ticks, lice were collected by forceps/ hand picking, respectively from their attachment site, put into container and preserved with 70% alcohol as described by Urquhart et al. (1996).

Collected ectoparasites were examined under stereomicroscope and identification was performed according to the identification key described by Okello-onen *et al.* (1999) for tick; and wall and shearer (1997) for lice. Skin scrapings were processed according to the

method described by Coles (1986). According to this method, a few drops of 10% potassium hydroxide was added to the sample, allowed to stand for 30 minutes and the sediment was examined under different microscopic magnifications for mites, their eggs and fragments. Mites were identified according to the identification key described by Wall and Shearer (1997).

3.5. Sample size

The study design was cross sectional, involving simple random sampling method to determine prevalence of major ectoparasites in indigenous sheep and to identify major species of ectoparasites in the study areas were applied according to Thrusfield (2005). With the assumption of possible prevalence rate of the disease 50% (as there are no data record for these particular study areas) absolute desired precision of 5% and confidence level of 95% for estimation prevalence in the simple random sampling according to Thrusfield (2005).

The sample size was determined as follow:

$$n = \frac{1.962 P_{exp} (1-P_{exp})}{d^2}$$

Where:

n = required sample size,

P_{exp} = expected prevalence

d= desired absolute precision (Thrusfield, 2005).

Therefore based on the above formula the sample size was 384 sheep by considering 50% prevalence of ectoparasites in small ruminants from each agro climatic zones. This makes a total of 1152 sheep but the sample size is inflicted to 1200 in three agro-ecological zones.

Part II: Experimental study

3.6. In vitro and in vivo acaricidal efficacy of medicinal plants

3.6.1. Plant collection and identification

Plant collection, identification, extract preparation was performed by National Herbarium, AAU and Herbarium of the Directorate of Traditional and Modern Medicine Research of the Ethiopian Health and Nutrition Research Institute, Addis Ababa.

The plants (*C.citratus*, *E.globulus*, *Nicotiana tobacum*, *Ximenia caffra*, *Melia azedirachta*, *pyrethrum cinerariifolium*, and *Jatropha curcas*) investigated in this study were collected from their natural habitat and identified by a taxonomist using standard Flora, and voucher specimens were deposited in the National Herbarium, AAU and Herbarium of the Directorate of Traditional and Modern Medicine Research of the Ethiopian Health and Nutrition Research Institute, Addis Ababa, which was used in an in-vitro and in-vivo studies.

3.6.2. Plant extract preparation

Air dried and powdered plant materials (300-550 g) were extracted exhaustively with a hydroalcoholic solvent by percolation at room temperature. The menstruum filtered and concentrated in rotary evaporator or lyophilizer to give the crude extracts. The concentrated extract was kept in tightly closed bottle in refrigerator until used for efficacy evaluation. Small portion of the extract/fraction was used for identification of the constituents (secondary metabolites) of the extract by examining the developed TLC chromatogram with appropriate chromogenic reagents. The plant extracts (*C.citratus* and *E.globulus* extracts) that showed pronounced activity against the test parasites during in-vitro tests were fractionated (partitioned) among solvents of varying polarities, concentrated and utilized for assay guided identification and characterization of active constituent(s) by employing different chromatographic and spectroscopic techniques.

Furthermore, the crude active extract and active fractions were used for toxicological studies in order to ensure their safety.

Extract concentration: 20mg/ml, 40 mg/ml, 80mg/ml and 160 mg/ml solutions of fixed oils extracts and 0.625%, 1.25%, 2.5% and 5% solutions of essential oils with their respective vehicles (2% Tween 80 for *C.citratus* and *E.globulus*, 3% ethanol for *Nicotiana tobacum*, 4% Tween 20 for *Ximenia cafra* and *Melia azadrachta*, kerosene: 2% Tween 80: 3% ethanol in 1:1:1 ratio for *pyrethrum cinerariifolium* , and 4% Tween 80 for *Jatropha curcas*) were tested for licidal activity. Distilled water was used for dilution preparation. Distilled water and 0.1% diazinone 60 EC was used for negative and positive control.

3.6.3. Collection and identification of lice

The lice used for the study, *Damalinia ovis* and *Linognathus ovillus* species were obtained from naturally infested local Arsi-Bale sheep breed. The animals were restrained and the body surface of the animal was visually examined for presence of lice. All isolated from clinical cases were characterized and identified before the assay according to Urquhart (1996) and Soulsby (1982). The lice were collected by using painting brush and by hand picking. After collection, the lice were pooled and placed in the Petri dish. The *in vitro* tests were started within one hour after lice collection (Heukelbach. J et al, 2006). Ten active alive lice were put into the Petri dish and within one hour of collection immersed completely in the test solution for one minute according to (Jadhav.V et al, 2007). The test solutions, positive (0.1% diazinone 60 EC) and negative (distilled water) controls were removed just after one minute contact time, using whatman filter paper No 1. Each louse in each Petri dish was closely observed for death under dissecting microscope at 10 min, 30 min, 1 hr, 2hrs, 3hrs, 6hrs and 12 hrs time intervals. The criteria used for death of lice were extremely strict. If any minor signs of live such as movement of antennae, gut cells or minimal legs movements were observed (with or without stimulation touching it by forceps) the lice were categorized as alive. The lice were judged as dead, if there were no vital signs at all. The test was done in six duplicates

/replica/ and average is considered, Percentage mortality was calculated and graphs of the percentage mortality versus time were plotted (Jadhav.V et al, 2007) Fig.2-15

3.7. Susceptibility test against lice

3.7.1. In vitro efficacy

Prior to in vivo efficacy study, the medicinal plants were evaluated for their good efficacy against *Damalinia ovis* and *Linognathus ovillus* in vitro in the area according to Urquhart (1996) and Soulsby (1982). The in vitro and in vivo efficacy of plant extracts was assessed and compared with the commercially known products such as 0.1% diazinon 60% E.C.

3.7.2. In-vivo /therapeutic/efficacy/ test

Application of the herbal extracts on randomly selected clinically positive animals according to the schematic design of the experimental study (Table 2) was conducted.

Table 2: Group of experimental animals and description of the treatment administration

Groups	No of animals	Treatment groups		Control groups	
		C.citratus	E.globulus	Diazinone	NC
I	6	2.5%	-	-	-
II	6	-	5%	-	-
III	6	-	-	0.1%	-
IV	6	-	-		Untreated

After through clinical examination, sheep naturally infested with lice were randomly selected and purchased. At arrival, live body weight, body condition scores, sex, age, animal species and status of lesion were recorded and randomly allocated in to four experimental groups of six animals after two weeks of adaptation period. All animals were randomly assigned into four groups. Group I was randomly assigned to be treated with 2.5% *C.citratus* and Group II with 5 % *E.globulus* herbal extracts respectively,

Group III as a positive control treated with 0.1% Diazinone 60 EC and the negative control Group IV was left untreated, according to method adapted by Holdsworth et al (2005). Animals in all groups, at their arrival were treated with broad spectrum anthelmintic to make them free of any possible natural infestation by endo and ectoparasites and vaccinated against anthrax, sheep-pox and ovine pasteurellosis.

All groups were kept in separate boxes supplied with straw and concentrate feed and water *ad-labitum*. All experimental animals were with comparable body condition, size, same age and sex. Body weight, BCS, sex and age were recorded at the start of the study (Holdsworth et al, 2006). Group I and II sheep were treated with medicinal plant extracts treated; group III positive control 0.1% diazinone treated and group IV negative control remain untreated. Dosage of the test herbal plant extracts were determined according to the efficacy showed during the *in vitro* study and application of the commercial acaricide was according to the manufacturers recommendations (Table 2). For efficacy study in *Damalinia ovis* 40 sites per animal were examined. Lice were counted in five predilection sites including the neck, shoulder, wither, flank and rump and eight partings in each sites in an area of ten centimeters in length. Only alive lice were counted (Heukelbach.J et al., 2006).

3.8. Data management and analysis

Collected raw data was carefully recorded and stored in Microsoft Excel database system used for data management. Statistical software package called SPSS for windows version 15.0 was used for data analysis. Descriptive statistics, percentages and 95% confidence intervals were used to summarize the proportion of infected and non-infected animals. The effects of different environmental and host risk factors were analyzed by regression. Statistical significance was set at $P < 0.05$ and Analysis of variance (one- way ANOVA-LSD test) was also used to compare the means of different treatments (concentrations) of the extracts and controls in different time used for *in vitro* and *in vivo* efficacy studies of medicinal plants.

In vitro and in vivo efficacies of different plants were determined as per the standard guidelines set by Abbott (1925) and Heukelbach.J et al., (2006)

3.9. Study period.

The total project period was 10 months from September 2009 to April 2010.

4. RESULTS

4.1. Questionnaire survey result

In an attempt to obtain overall information, a pre-structured FGD (Focus Group Discussion) system was designed. FGD is different from other Questionnaire in analyzing that the forwarded questions or ideas were discussed by FGD members in the floor and through their own justifications practice; they come up to the only one answer. The system was set up to reduce the time required to be spent on an individual interviewing and broaden the out come and come up with the community perception rather than the individual's concept about the overall information on livestock ownership pattern, importance of small ruminant keeping, awareness the community have on ectoparasites and effect ectoparasites have on production and market value of skins, control practices and the traditional herbal medicine usage in livestock disease.

There were 9 FGD 8 to 10 selected people: animal owners, skin traders, elders, religious leaders, shepherds and respective animal health personnel of the respective districts will constitute one FGD group.

The main livestock diseases identified were, Anthrax 100% (9), Blackleg 100 % (9), ovine pasteurellosis100% (9), external and internal parasites100% 8, sheep-pox, African horse sickness, fowl diseases and mastitis accordingly as prioritized by FGD. After thoroughly discussion, the main livestock skin diseases prioritized was tick and lice infestation, sterpthothricosis, and mange mites. Earlier, lice infestation is known and pronounced in calves during dry period according to FGD and Lice infestation in small ruminants is the recently (four-five years) occurred problem. Lice infestation problem is encountering in equines and cattle nowadays, particularly during the prolonged dry period.

When discussed on the use of traditional medicine to treat livestock dermal diseases, at present, the community is practicing treating lice infestation only in calves by traditional means. The intervention methods used to tackle animal diseases are mainly, modern medicine and there are also a lot of traditional plants to treat Blackleg and Anthrax, table:

3. The awareness the animal owners, skin traders, elders, religious leaders, shepherds and in general different community members have on ectoparasites effect (lice) is very great. According to FGD members, of all nine PAs in three districts, the lowered price of skin was due to lice infestation. The lice on the infested skins are clearly observed and such skin is rejected by the hide and skin traders. The price of un-rejected sheep skin is in the range of three to five birr, so this concern brought about the awareness to the community.

The knowledge of traditional herbal drug handling in skin disease control practices in the community is unsatisfactory, unless for the calves lice treatment. In one of the study district, Gedeb, the FGD members discussed and agreed that traditional herbal plant utilization is not practiced in their area nowadays.

The preparations of traditional medicine are carried out either at home or seek assistance from knowledgeable community members, with minimum payment or for free. Traditional medicine preparations are cheaper and mostly used in areas where there is no accessibility. They thrust modern medicine than traditional one, which a traditional medicine shows variable results, because of the unknown amount used /dosage/.

The local name for lice is 'chini' and it is extremely prevalent, the clinical manifestation of lice is easily observable. When livestock are affected by lice, the measures usually taken to tackle this problem are modern medicine. There is no practice to treat lice in sheep, however, 'chekata' is a plant used in treating calves infested by lice.

When asked, whether they encourage medicinal plants to undergo scientific investigation to develop herbal based livestock medicine for lice, they show great interest towards this idea and they hope traditional medicine is prior to modern acaricide to treat lice, if its dosage is determined. They clarified that, traditional medicine often will cure or kill, when used, because of lack of knowledge to determine the amount required to use.

Table 3: Herbs used for the treatment of skin diseases and parasites in the study area

	<i>Botanical name of the plant</i>	<i>Parts used</i>	Parasites
1. Bissana(Am)	Caraton macristachys	Leaf	Lice
2.cheketa (OR)		Steam	Lice
3.Endod (Am)	Pytolacca dodecandra	Leaf	Lice
4. Korch (Am)		Leaf	Lice
5. Enkoye (Am)		Seed	Mange
6.wofkollo(Am)		Leaf	Lice
7. yeahya shito(Am)		Leaf and fruit	Lice

4.2. Prevalence of ectoparasites

Table 4: Overall prevalence of Ectoparasites

	<i>Sheep</i>	<i>95% CI</i>	X² (P-Value)
	<i>N=1200</i>		
	<i>Prevalence%</i>		
Lice infestation by the either spp	906(75.5)	73.07-77.93	X ² =146.155 (P< 0.000)
Mixed Lice infestation	63(6.3)	60.27-65.73	X ² = 21.209(P<0.000)
D.ovis	805(67.1)	64.44-69.76	X ² = 295.027(P < 0.000)
L.ovillus	175(14.6)	12.61-16.59	X ² =83.262(P<0.000)
M.ovinus	67(5.65)	4.72-6.58	X ² =36.074(P<0.000)
Sarcoptic mange	3(0.3)	-0.01-0.61	X ² =6.015(P<0.049)
Psoroptic mange	12(1)	-0.97-2.97	X ² =16.606(P<0.005)
Ticks	285 (23.8)	21.39-26.21	X ² =223.669P<0.000)
Overall	978 (81.5)	73.87-83.63	

4.2.1. Overall prevalence of ectoparasites

Out of a total of 1200 sheep examined for ectoparasite (81.5%) 978 sheep were infested with one or more ectoparasites (table 4).The major ectoparasites identified were *Damalinia ovis*, *Linognathus ovillus*, tick infestation, *melophagus ovinus* and psoroptic and sarcoptic mange in order of their prevalence.

The common sites of *D.ovis* and *Linognathus ovillus* infestation were the skin of wither, flank, shoulder, neck and rump, however infestation of *Linognathus ovillus* is pronounced on the neck, flank, thigh, and ribs regions. For *M. ovinus* infested sheep, the common site commonly parasitized were the rump, shoulder, tail and neck but the load of the parasites can vary in the degree of their fleece length over the body. The main attachment sites were ear/head, sternum under the tail, anus region and interdigital space in both sexes, scrotum in males and udder in females.

The tick genera (*Amblyomma*, *Rephiccephalus* and *Boophilus*) were identified. *Am. varigatum*, *Rh. pulcalus*, *Rh. evertsi evertsi*, *Bo. decoloratus* were the tick species identified.

Table 5: Prevalence of ectoparasites by sex

	Female (n= 916)	Male (n= 284)		
Lice infestation	679(74.1)	227(79.9)	3.947	0.047
Damalinia ovis	601(65.6)	204(71.8)	3.798	0.51
Linognathus spp	131(14.3)	44(15.5)	0.247	0.631
Mixed lice	46(5%)	17(6)	0.405	0.543
infestation				
Melophagus ovinus	52(5.7)	15(5.3)	0.604	0.883
Sarcoptic mange	3(0.30)	0(0)	0.932	1.000
Psoroptic mange	9(1)	3(1.1)	0.12	1.000
Ticks	221(24.1)	64(22.8)	0.303	0.632
Overall	916(76.33)	284(23.67)		

4.2.2. Prevalence of ectoparasites by sex

The overall prevalence of ectoparasites in female and male sheep was 916(76.33%) and 284 (23.67%) respectively (Table: 5). There were statistically significant differences between the prevalence of lice infestation in female and male sheep, ($P<0.049$).

Table 6: Prevalence of ectoparasites by agro-climate

<i>Sheep n=(1200)</i>				
Parasite species	Highland	Midland	Lowland	P-Value
Lice infestation by either of the spp	359(89.8)	328(82)	219(54.8)	$X^2=146.155$ ($P<0.000$)
Mixed Lice infestation	7(1.8)	36(9)	20 (5.0)	$X^2=21.209$ ($P<0.000$)
<i>Damalinia ovis</i>	355(88.8)	311(77.8)	139(34.8)	$X^2 = 295.027$ ($P<0.002$)
<i>L. ovillus</i>	14(3.5)	56(14)	105(26.3)	$X^2=83.262$ ($P<0.000$)
<i>M. ovinus</i>	36(9)	31(7.8)	0	$X^2=36.074$ ($P<0.000$)
Sarcoptic Mange	0	3(0.8)	0	$X^2=6.015$ ($P<0.000$)
Psoroptic mange	9(2.3)	0.8	0	$X^2 =10.606$ ($P<0.000$)
Ticks	84(21)	190(47.5)	11(2.8)	$X^2=223.669$ ($p<0.000$)
Overall	(94.5)378	(94.5)378	(55.5) 222	

4.2.3. Prevalence of ectoparasites by agro-climate

The prevalence of ectoparasites in highlands, midland and lowlands were (94.5 %) 378, (94.5 %) 378 and (55.6%) 222 respectively. Table:6 shows agro-climate specific prevalence of ectoparasites identified in sheep.

The prevalence of *Damalinia ovis* in sheep was significantly higher in highlands and midlands (OR = 2.22) ($P<0.000$) than lowland, *Melophagus ovinus* was found statistically significantly in high and midlands ($P<0.000$), but not in lowlands. *Linognathus ovillus* was significantly higher in the lowlands than highlands ($P<0.000$). The prevalence of tick was significantly higher in midlands than lowlands ($P<0.000$). The prevalence of sarcoptic and psoroptic mange was significant in midlands ($P<0.000$), but not found in both low and highlands.

Table 7: The prevalence of ectoparasites by age group

<i>Parasites</i>	<i>Young n=437</i>	<i>Adult n = 763</i>	<i>X²</i>	<i>P-Value</i>
Lice infestation	352(80.5)	554(72.6)	9.473	0.002
Damalinia ovis	320(73.2)	485(63.3)	11.7.46	0.001
Mixed lice infestation	20(4.6)	43(5.6)	0.626	0.502
Linognathus spp	57(13)	188(15.5)	1.308	0.270
Melophagus ovinus	35(8)	32(4.2)	7.672	0.008
Sarcoptic mange	1(0.1)	2(0.4)	0.11	1.000
psoroptic mange	4(0.9)	8(1)	0.50	1.000
Ticks	81(18.5)	204(26.7)	10.320	0.001
Overall	437(36.41)	763(63.59)		

4.2.4. The prevalence of ectoparasites by age group

The overall prevalence of ectoparasites in young and adult sheep was (36.41) 437 and (763(63.59) respectively (Table-8). There were statistically significant differences observed in prevalence of lice infestation ($P < 0.002$), *Damalinia ovis* ($P < 0.001$), *Melophagus ovinus* ($P < 0.008$) and tick infestations ($P < 0.001$) between adult and young, while there were no statistically significant differences ($P > 0.05$) for the remaining ectoparasites species.

Table 8: Prevalence of ectoparasites by body conditions

Parasites	Poor n=636	Good n=564	X2	P
Lice infestation	493(77.5%)	413(73.2)	2.972	0.093
Damalinia ovis	418(65.7)	387(68.6)	1.134	0.296
Linognathus spp	123(19.3)	52(9.2)	24.575	0.000
Mixed lice infestation	38(6)	25(4.4)	1.429	0.246
Melophagus ovinus	39(6.1)	28(5)	0,773	0.450
Sarcoptic mange	19.2)	2(0.4)	0.467	0.604
psoroptic mange	10(1.6)	2(0.4)	4.477	0.042
Ticks	143(22.5)	142(25.2)	1.197	0.278
Overall	636(53)	564(47)		

4.2.5. Prevalence of ectoparasites by body conditions

The overall prevalence of ectoparasites in poor and good BSc sheep was (53) 636 and 564(47) respectively (Table: 9). There were statistically significant differences observed in prevalence of Linognathus ovillus ($P < 0.000$), and psoroptic mange ($P < 0.042$), while there were no statistically significant differences ($P > 0.05$) for the remaining ectoparasites species.

Table 9: The binary logistic regression result showing the association of lice and *Damalinia ovis* prevalence to the risk factors (agro-ecology, sex, age and BSc)

Factors	Lice			D.ovis		
	P	OR	CI	P	OR	CI
Lowland	0.000	0.247	0.178-0.343	0.000	0.145	0.105-0.199
Highland	0.001	1.977	1.301-3.004	0.000	2.223	1.449-3.298
Midland	0.000	0.125	0.085-0.184	0.000	0.065	0.045-0.095
Sex	0.139	0.72	0.467-1.112	0.289	0.797	0.524-1.212
Age	0.160	1.311	0.899-1.912	0.031	1.502	1.037-2.175
BSC	0.002	1.579	1.182-2.111	0.527	1.096	0.825-1.456

Table 10: The binary logistic regression indicating association of *L.ovis* and *M.ovinus* infestations to the risk factors (agro-ecology, sex, age and BSc).

Factors	L.ovis			M.ovinus		
	P	OR	CI	P	OR	CI
Lowland	0.000	0.217	0.178-0.343	0.992	0.000	0.000
Highland	0.000	1.977	1.301-3.004	0.730	1.095	0.654-1.832
Midland	0.000	8.807	4.923-15.758	0.992	0.000	0.000
Sex	1.139	0.720	0.467-1.112	0.109	1.751	0.883-3.473
Age	0.160	1.311	0.899-1.912	0.003	2.446	1.366-4.381
BSC	0.002	1.579	1.182-2.111	0.341	1.287	0.765-2.165

Table 11: The binary logistic regression indicating association of Mange suspected lesion and tick infestations the risk factors (agro-ecology, sex, age and BSc).

Factors	Mange suspected lesion			Tick infestations		
	P	OR	CI	P	OR	CI
Lowland	0.000	0.096	0.029-0.321	0.000	0.031	0.016-0.058
Highland	0.063	0.512	0.253-1.036	0.000	0.302	0.220-0.415
Midland	0.011	0.188	0.052-0.678	0.000	0.102	0.053-0.196
Sex	0.005	0.268	0.107-0.670	0.089	0.680	0.436-1.060
Age	0.004	0.247	0.095-0.641	0.001	0.513	0.343-0.767
BSC	0.002	3.168	1.549-6.480	0.487	0.898	0.663-1.216

4.5. In vitro acaricidal efficacy

In the current in-vitro licidal activity study of seven plants extracts at different concentrations against *Damalinia ovis* and *Linognathus ovillus* different results were obtained. 20 mg/ml, 40mg/ml, 80 mg/ml and 160 mg/ml solutions of fixed oil of *Jatropha curcas*, *Melia cafra*, *Ximenia azadrachta*, *pyrethrum* and *Nicotiana tobacum* and 0.625%, 1.25%, 2.5% and 5% solutions of *Cybopogon citratus* and *Eucalyptus globulus* essential oils were tested. Examinations for the death of lice was performed by checking the vital signs after 10 min, 30 min, 1hr, 2hrs, 3hrs, 6hrs and 12hrs in all tests. The vehicle used for dilution in *Jatropha curcas* test, was 4% Tween 80. As a positive control 0.1% diazinone and negative control vehicle and distilled water were used.

In the test against *Linognathus ovillus*, mortality of 1.7 % was recorded at 10 min treated with *Jatropha curcas* 160 mg/ml concentration. After one and two hrs time, mortality of 5% and 6% were recorded in *Jatropha curcas* 80mg/ml and 40 mg/ml concentrations respectively. Mortality of 73.3%, 66.6%, 61% and 48.8 % were noted at 12 hrs time (Fig. 2). When the mortality of lice was compared to the positive control, there were no statistically significant difference ($P<0.05$) at 12hrs against. While *Linognathus ovillus* treated with *Jatropha curcas* 160mg/ml 80mg/ml and 40mg/ml concentrations, compared to the negative controls (the vehicle and the distilled water), there were statistically significant difference ($P<0.05$) (Fig. 2).

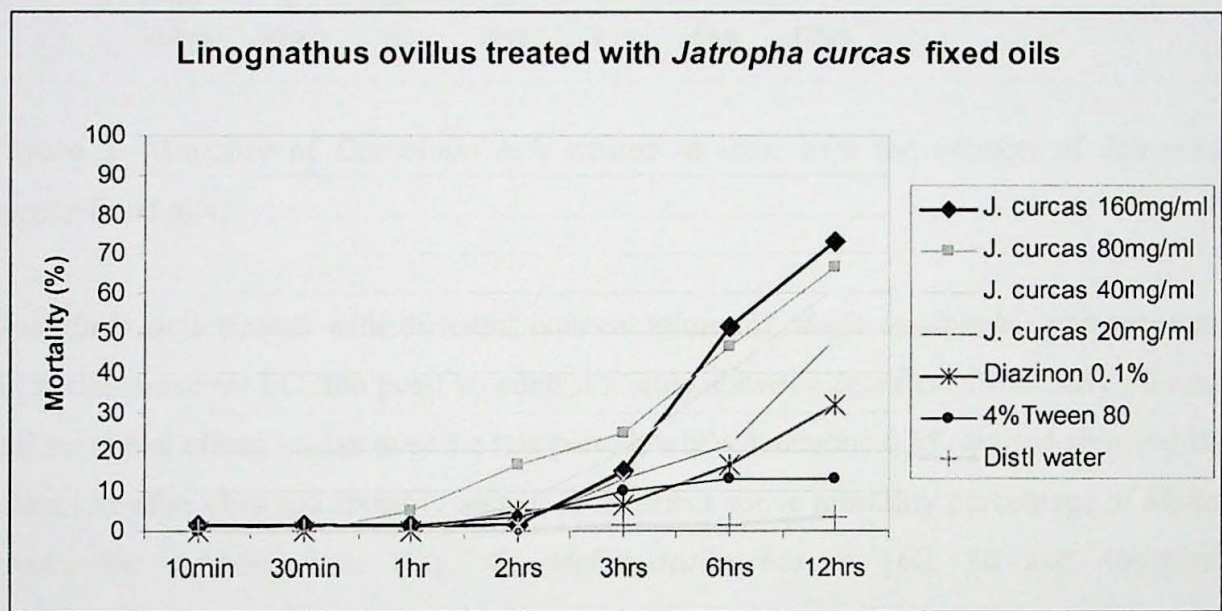


Figure 2: Mortality of *Linognathus ovillus* treated in vitro with the extracts of *Jatropha curcas* fixed oils

Damalinia ovis treated with *Jatropha curcas* at different concentrations a mortality of 5% were observed at 10 min by both *Jatropha* 160 and 80 mg/ml concentrations. The effect of the extracts observed was lower and at 12hrs mortality of 75%, 58% and 45% were recorded for *Jatropha curcas* at 160, 80, and 40 mg/ml respectively (Fig. 3). When compared to positive control there was statistically significant difference ($P<0.05$) for *Jatropha curcas* 160 mg/ml, while there were no statistically significant difference ($P>0.05$) between *Jatropha curcas* 80, 40 and 20 mg/ml concentrations, vehicle and distilled water.

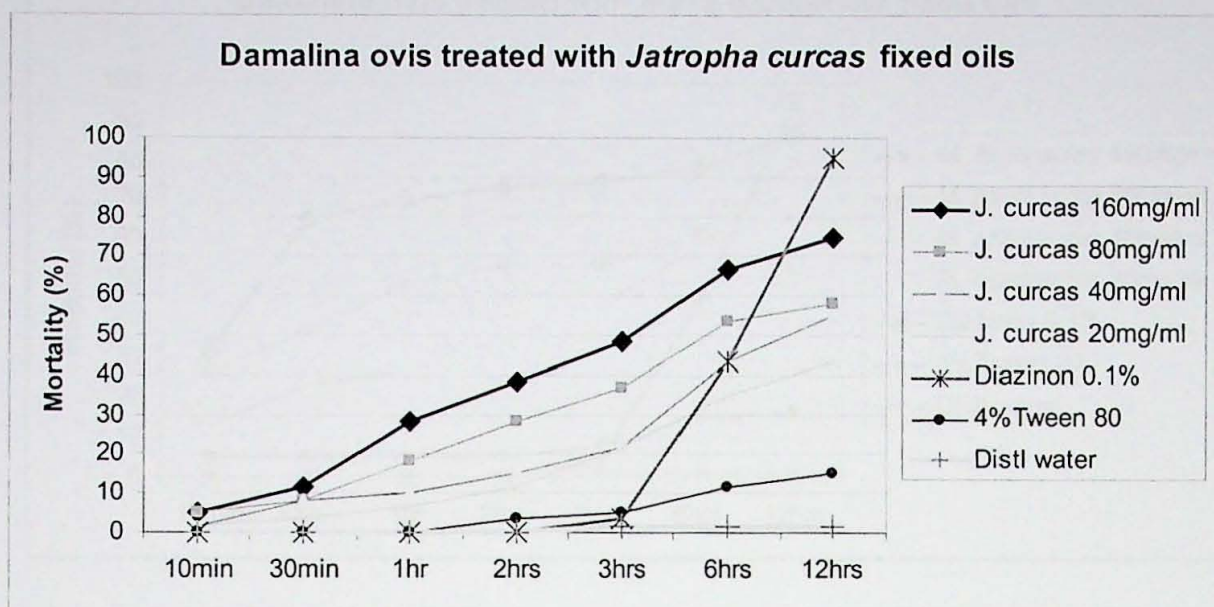


Figure 3: Mortality of *Damalina ovis* treated in *vitro* with the extracts of *Jatropha curcas* fixed oils.

Damalina ovis treated with different concentrations of *Melia azadrachta* compared to 0.1% diazinone 60 EC, the positive control started showing its effect from early 30 min and sustained effectiveness over the test period, while diazinone 0.1% started showing its effect late after 6hrs and speedily increased its effect above mortality percentage of *Melia azadrachta* concentrations (Fig. 4). *Melia azadrachta* at 160, 80 and 40mg/ml concentrations never showed statistically ($P>0.05$) significant difference compared to the positive control, while the vehicle and distilled water showed statistically significant difference ($P<0.05$) compared to the positive control.

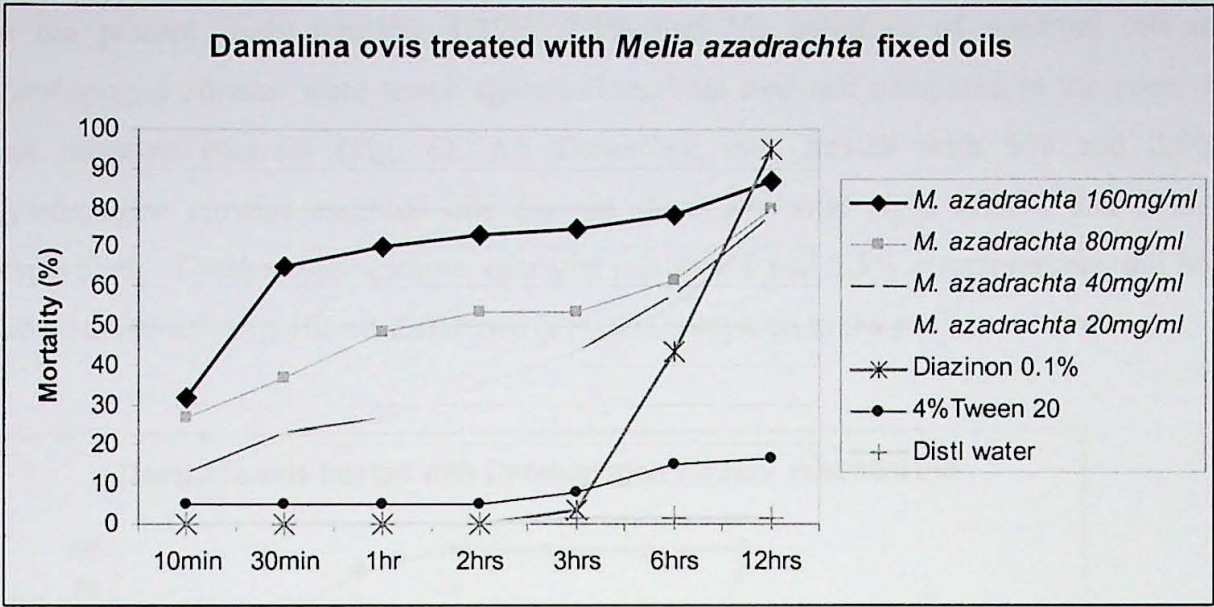


Figure 4: Mortality of *Damalina ovis* treated in *vitro* with the extracts of *Melia azadrachta* fixed oils.

Melia azadrachta extracts with different concentration compared to diazinone 0.1%, the positive control drug. All concentrations were effective against *Linognathus ovillus* from early 10 min to 12hrs (Fig. 5). There were statistically significant difference ($P>0.05$) between different concentration of *Melia azadrachta* solutions compared to the positive control drug 0.1%.diazinone 60EC.

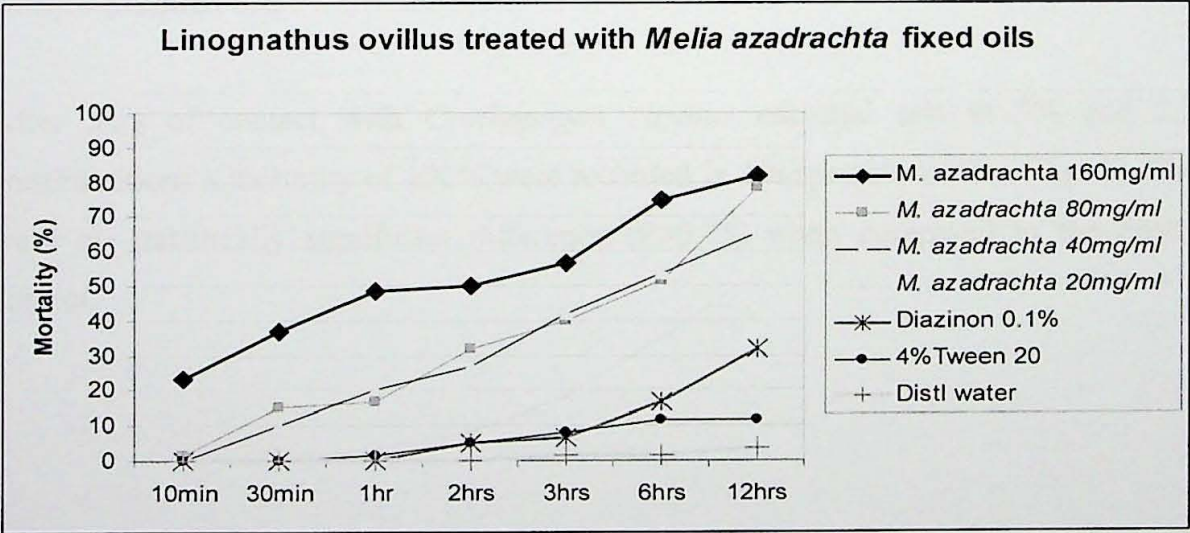


Figure 5: Mortality of *Linognathus ovillus* treated in-vitro with the extracts of *Melia azadrachta* fixed oils.

In the present study 0.625%, 1.25%, 2.5% and 5% solutions of essential oils of *Cymbopogon citratus* were tested against *Damalinea ovis* and compared to the positive and negative controls (Fig. 6). All *Damalinea ovis* treated with 5% and 2.5% *Cymbopogon citratus* essential oils did not show any vital signs after 2 and 3 hrs, respectively. *Cymbopogon citratus* essential oils at 5% and 2.5% concentrations did not show statistically significant difference ($P>0.05$) compared to the positive control.

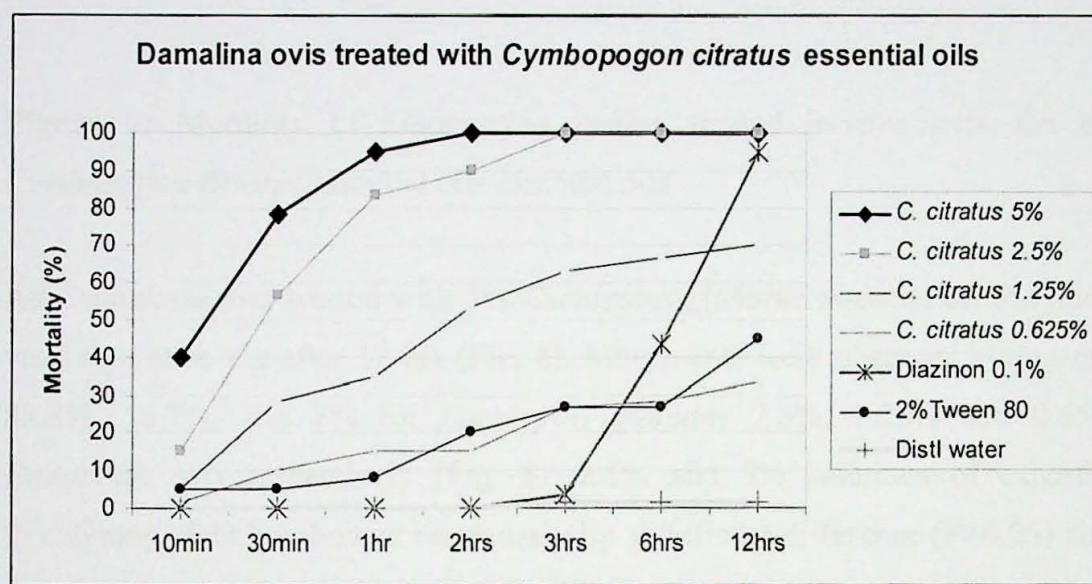


Figure 6: Mortality of *Damalinea ovis* treated in-vitro with the extracts of *Cymbopogon citratus* essential oils

After 3hrs of contact with *Cymbopogon citratus* essential oils at 5% and 2.5% concentrations a mortality of 100% were recorded in *Linognathus ovillus* (Fig. 7). There were no statistically significant difference ($P>0.05$) when compared to the positive control.

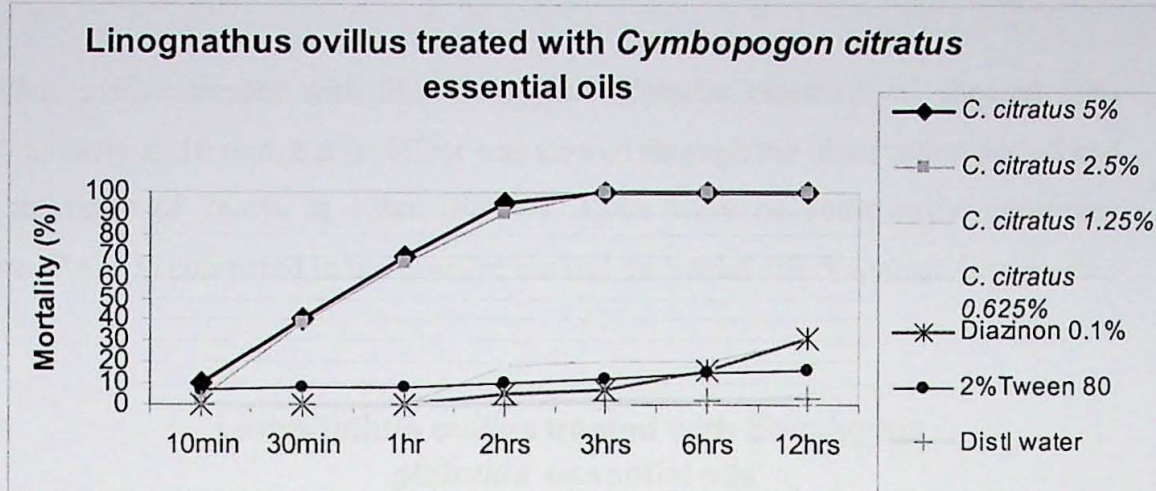


Figure 7: Mortality of *Linognathus ovillus* treated in-vitro with the extracts of *Cymbopogon citratus* essential oils

All *Damalinea ovis* treated with 5% *Eucalyptus globulus* essential oil did not show any vital sign after 1hr after 12 hrs (Fig. 8). Movements were observed giving mortality of 88.8%, 56.7%, and 5% for *Eucalyptus globulus* 2.5%, 1.25% and 0.65% against *Damalinea ovis* respectively (Fig. 8). 2.5% and 5% solutions of essential oils of *Eucalyptus globulus* showed no statistically significant difference ($P>0.05$) compared to the positive control on licidal effect against *D. ovis*.

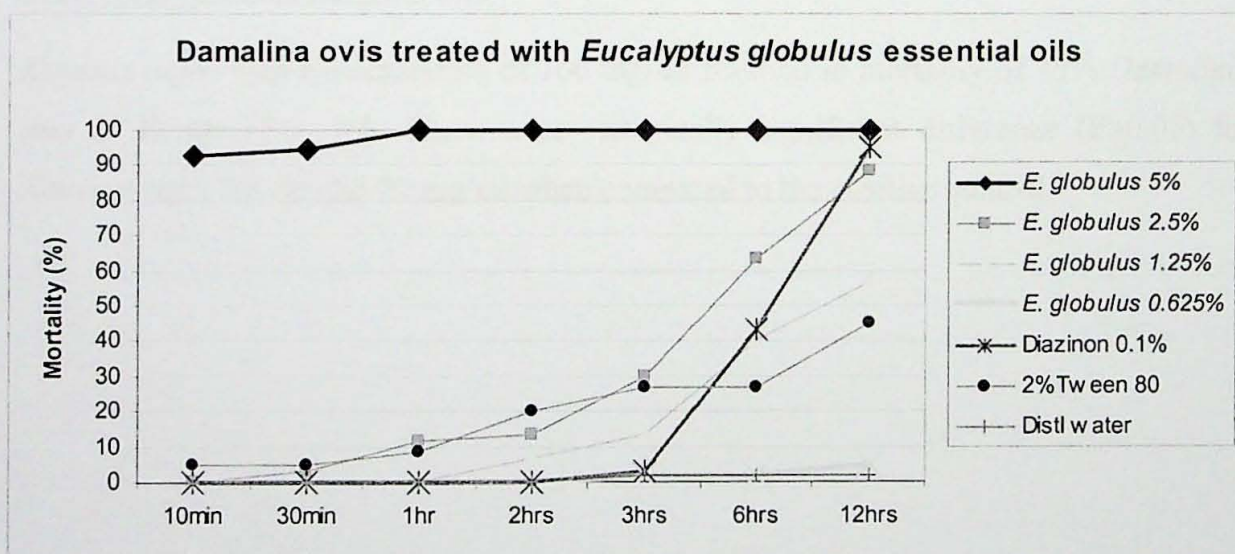


Figure: 8. Mortality of *Damalinea ovis* treated in-vitro with the extracts of *Eucalyptus globulus* essential oils.

Linognathus ovillus treated with 5% *Eucalyptus globulus* essential oil showed 30% mortality as early as 10 min, but its effect was slowed through the observation period and showed mortality of 76.6% at 12hrs (Fig. 9). There were no statistically significant difference ($P>0.05$) compared to the positive control on licidal effect against *Linognathus ovillus*.

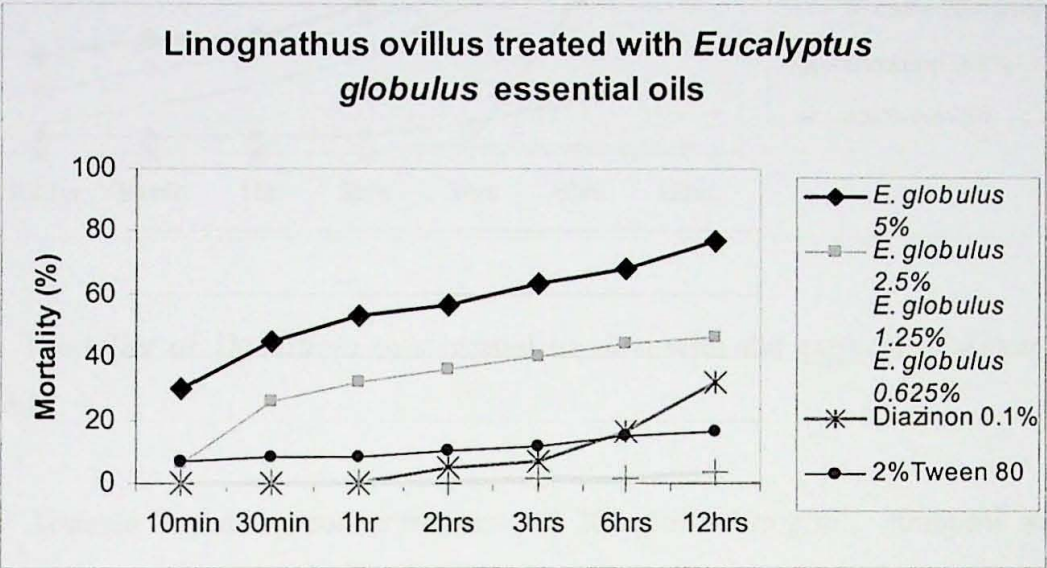


Figure 9: Mortality of *Linognathus ovillus* treated in-vitro with the extracts of *Eucalyptus globulus* essential oils.

Ximenia cafra with concentration of 160 mg/ml resulted in mortality of 75% *Damalinia ovis* at 12 hrs (Fig. 10). There were statistically significant difference ($P>0.05$) for *Ximenia cafra* 80, 40 and 20 mg/ml when compared to the positive control.

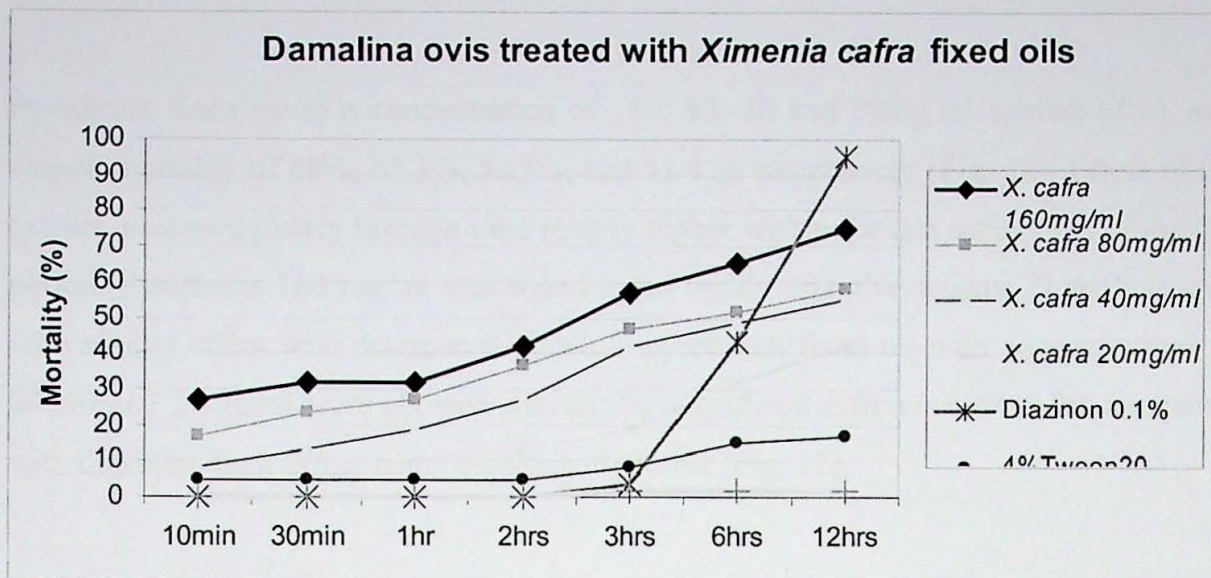


Figure 10: Mortality of *Damalania ovis* treated in-vitro with the extracts of *Ximenia cafra* fixed oils.

Efficacy of *Ximenia cafra* in concentrations of 20mg/ml, 40mg/ml, 80mg/ml and 160mg/ml against *Linognathus ovillus* was significantly higher ($P < 0.05$) compared with diazinon 0.1% (Fig. 11).

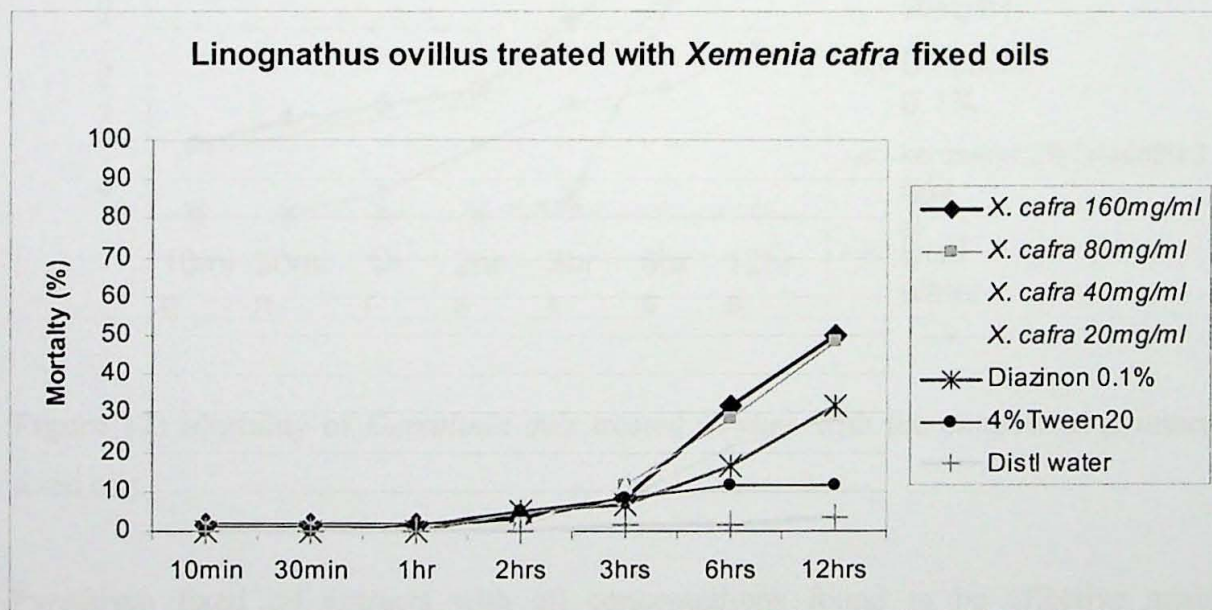


Figure 11: Mortality of *Linognathus ovillus* treated in-vitro with the extracts of *Ximenia cafra* fixed oils.

Pyrethrum fixed oil in a concentration of 160, 80, 40 and 20mg/ml against of *D. ovis* caused mortality of 88%, 63.3%, 58.3%, and 51.6 % respectively (Fig. 12). Effect of the extract increased slowly through time mainly higher within the last nine hours of the test period. Pyrethrum 160 mg/ml was found to be highly effective against *Damalinia ovis* with similar effect with diazinon 01% while pyrethrum fixed oil with concentrations of 80, 40 and 20mg/ml were showed statistically significant difference ($P<0.05$) compared with diazinon, their effect being significantly lower (Fig. 12).

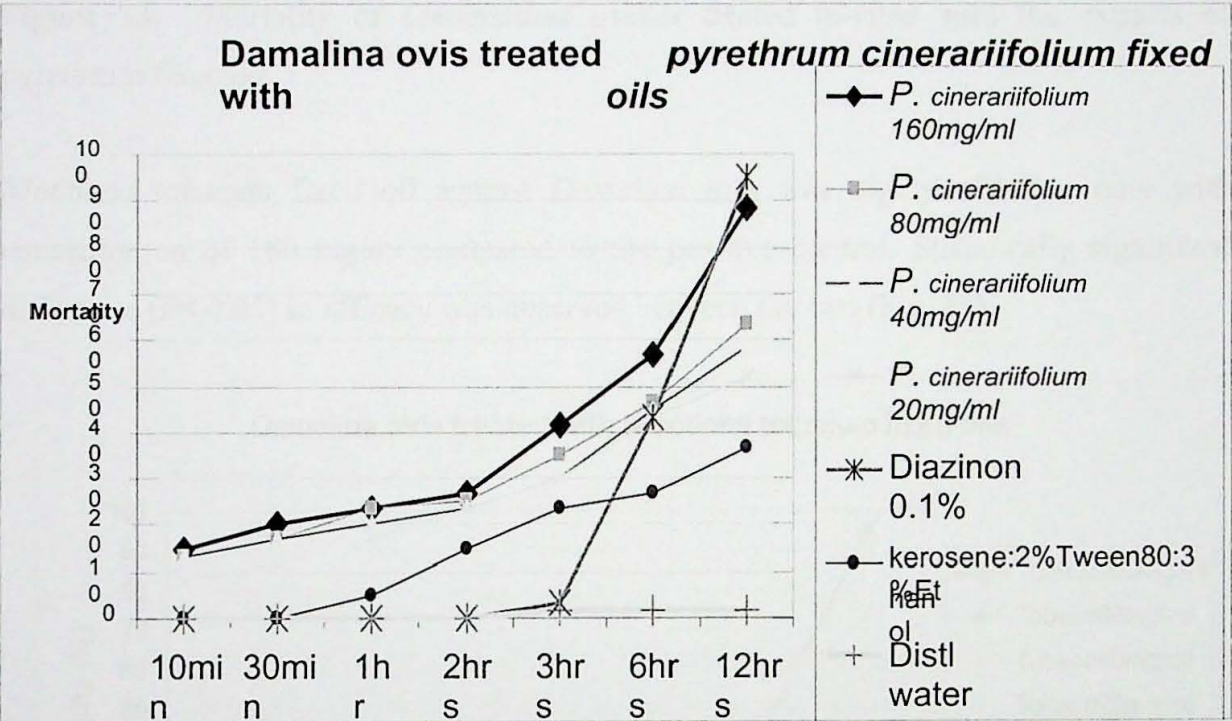


Figure 12: Mortality of *Damalinia ovis* treated in-vitro with the extracts of pyrethrum fixed oils.

Pyrethrum fixed oil extracts with all concentrations found to be effective against *Linognathus ovillus* with comparable effect with diazinon (positive control). There were no statistical difference ($P>0.05$) in effect between the two (Fig. 13).

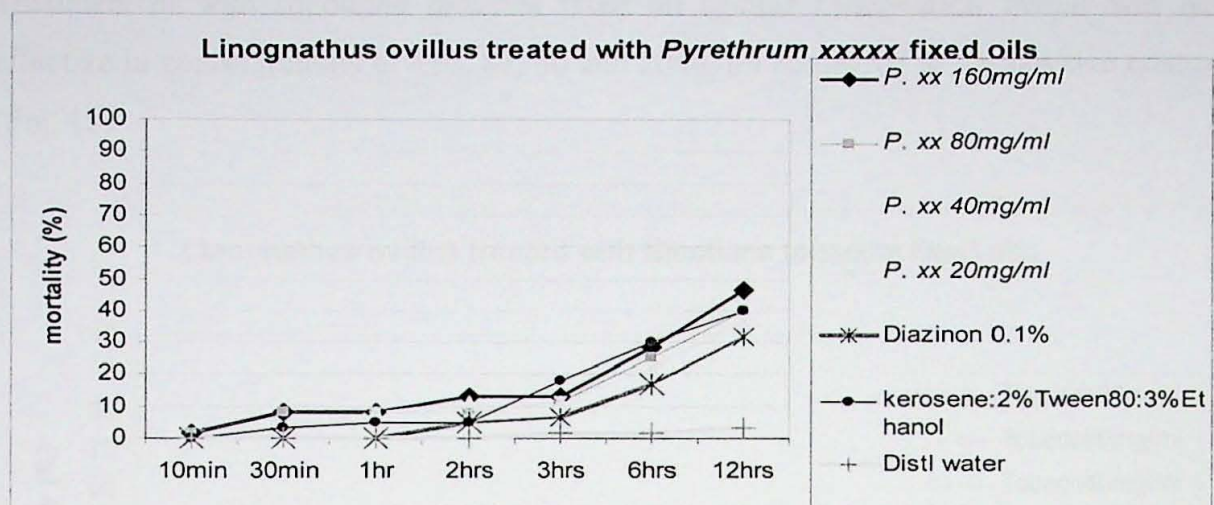


Figure 13: Mortality of *Linognathus ovillus* treated in-vitro with the extracts of pyrethrum fixed oil.

Nicotiana tobacum fixed oil against *Damalina ovis* was highly effective only with concentration of 160 mg/ml compared to the positive control. Statistically significant difference ($P < 0.05$) in efficacy was observed between the two (Fig. 13).

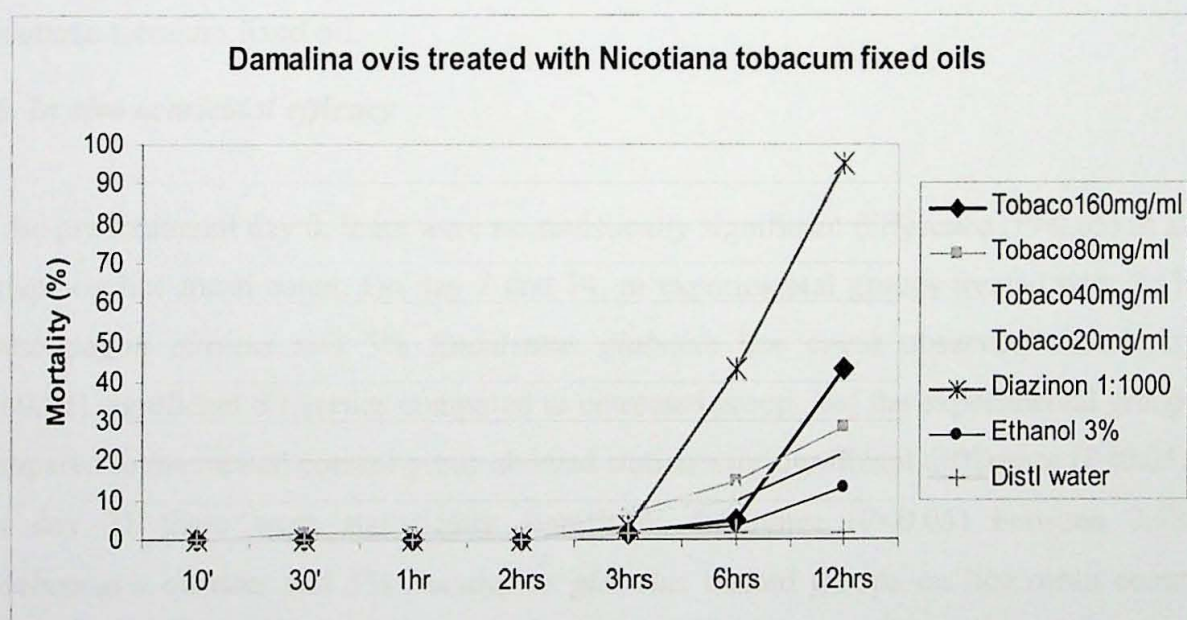


Figure 14: Mortality of *Damalinea ovis* treated in-vitro with the extracts of *Nicotiana tobacum* fixed oil.

Treatment of with *Nicotiana tobacum* fixed oil against *Linognathus ovillus* was not effective in concentrations of 160, 80, 40 and 20mg/ml compared to the positive control (Fig. 15).

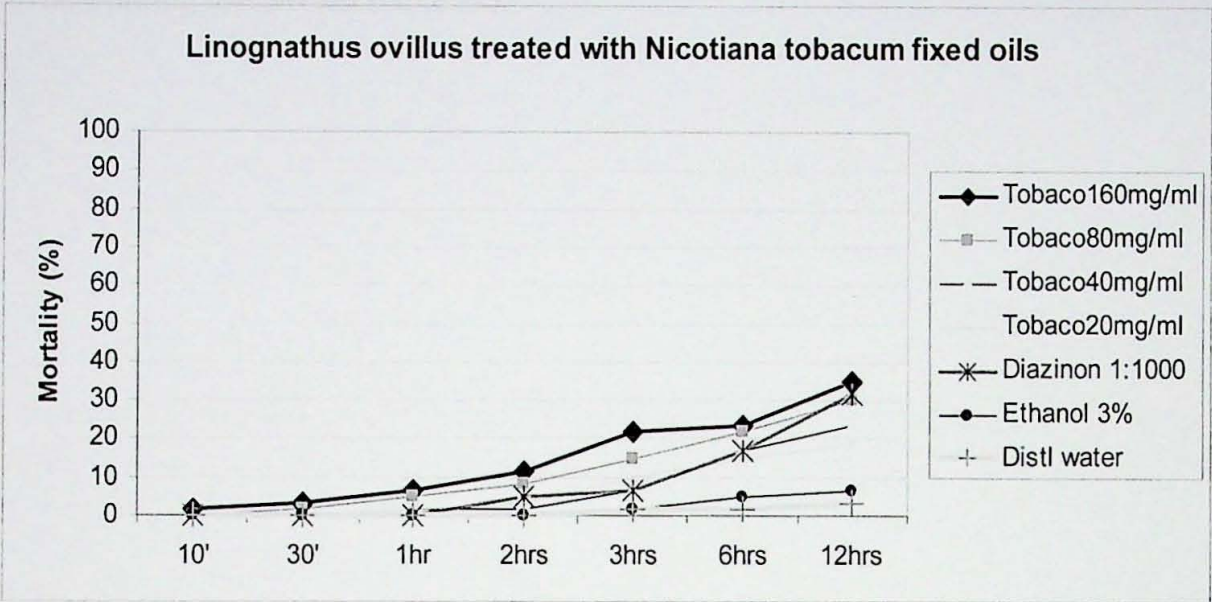


Figure 15: Mortality of *Linognathus ovillus* treated in-vitro with the extracts of *Nicotiana tobacum* fixed oil.

4.6. In vivo acaricidal efficacy

In the pre treatment day 0, there were no statistically significant difference ($P>0.05$) in all groups on lice mean count. On day 7 and 14, in experimental groups treated with 2.5% *Cymbopogon citratus* and 5% *Eucalyptus globulus* lice count observed statistically ($P<0.05$) significant difference compared to untreated group. But the experimental groups compared to the treated control group showed statistically significant difference ($P<0.05$). On day 21 there were statistically significant difference ($P<0.05$) between 2.5% *Cymbopogon citratus* and 5% *Eucalyptus globulus* treated groups on lice mean count, whereas, no statistically significant difference ($P>0.05$) between treated and negative control groups (Table 12).

On day 28 there was no significant difference ($P<0.05$) between 2.5% *Cymbopogon citratus* and 0.1% diazinone 60 EC on the mean lice count while 2.5% *Cymbopogon citratus* significantly reduced the lice to the lower number compared to 0.1% diazinone 60 EC. Mean while, *Eucalyptus globules* 5% was not found effective against *Damalina ovinus* during in-vivo test (table 12).

Table 12: *In vitro* pre and post treatment lice mean count in experimental animals

<i>Sampling day</i>	<i>Group</i>	<i>N</i>	<i>Mean Count (SE)</i>	<i>95% CI</i>	<i>P-value</i>
Day 0	Cymbopogon	30	40.97 (4.60)	31.57-50.37	0.449
	Eucalyptus	30	30.23 (5.36)	19.27-41.20	
	Diazinone	30	38.37 (4.68)	28.80-47.93	
	Negative control	30	34.03 (5.41)	22.96-45.11	
	Total	120	35.90 (2.51)	30.93-40.87	
Day 7	Cymbopogon	30	9.43 (1.42)a	6.53-12.34	0.000
	Eucalyptus	30	13.87 (2.20)ab	9.36-18.38	
	Diazinone	30	0.00 (0.00)c	0.00-0.00	
	Negative control	30	31.73 (4.32)d	22.90-40.57	
	Total	120	13.76 (1.64)	10.52-17.00	
Day 14	Cymbopogon	30	13.93 (1.62)a	10.61-17.25	0.000
	Eucalyptus	30	23.33 (2.64)b	17.93-28.84	
	Diazinone	30	0.00 (0.00)c	0.00-0.00	
	Negative control	30	39.00 (5.84)d	27.06-50.94	
	Total	120	19.07 (2.09)	14.93-23.20	
Day 21	Cymbopogon	30	7.23 (1.04)a	5.10-9.37	0.000
	Eucalyptus	30	12.83 (1.76)ab	9.24-16.42	
	Diazinone	30	0.00 (0.00)ac	0.00-0.00	
	Negative control	30	46.27 (8.20)d	29.51-63.03	
	Total	120	16.58 (2.64)	11.35-21.82	
Day 28	Cymbopogon	30	4.87 (0.73)a	3.38-6.35	0.000
	Eucalyptus	30	20.90 (2.42)b	15.96-25.84)	
	Diazinone	30	0.00 (0.00)ac	0.00-0.00	
	Negative control	30	43.93 (6.40)d	30.84-57.02	
	Total	120	17.43 (2.31)	12.84-22.01	
Day 35	Cymbopogon	30	8.47 (1.35)a	5.71-11.22	0.000
	Eucalyptus	30	26.50(3.62)b	19.10-33.90	
	Diazinone	30	0.00(0.00)c	0.00-0.00	
	Negative control	30	29.43(5.5)d	18.19-40.68	
	Total	120	16.10(2.00)	12.13-20.07	
Day 42	Cymbopogon	30	8.63(1.79)a	4.97-12.30	0.000
	Eucalyptus	30	28.00(4.19)b	19.44-36.56	
	Diazinone	30	0.00(0.00)c	0.00-0.00	
	Negative control	30	38.13(5.05)d	27.80-48.47	
	Total	120	18.69(2.18)	14.38- 23.00	
Day 49	Cymbopogon	30	11.57(1.90)a	7.68-15.45	0.000
	Eucalyptus	25	46.60(7.6)b	30.96-62.24	
	Diazinone	30	0.00(0.00)c	0.00-0.00	
	Negative control	20	27.35(7.44)d	11.79-42.91	
	Total	105	19.61(2.9)	13.87-25.35	
Day56	Cymbopogon	30	16.67(2.9)a	5.71-22.54	0.000
	Eucalyptus	25	39.72(5.15) b	29.10-50.34	
	Diazinone	30	0.00(0.00)c	0.00-0.00	
	Negative control	20	15.40(4.07) d	6.89-23.91	
	Total	101	17.15(2.16)	12.87-21.44	

During the in vivo study after one week time, after the first treatment, the lice percent reduction calculated according to Abbott (1925) was 76.98%, 54.11%, 100% and 6.75% in group I, II, III and IV respectively (Table 13). However, best result (complete cure) was recorded in positive control group treated with (0.1% diazinone 60 EC) followed by group I treated with 2.5% *C.citratus* and group II treated with *E. globulus* 5% ($P<0.05$) compared to the positive control group.

After two weeks of treatment time, (on day 14) the lice were counted on all individual animals in every group, then after the second time treatment was applied in group I, II and III with respective extracts/acaricide of the same volume and concentrations used on day 0. The negative control group was left untreated. The result of the second week treatment was 65.99%, 22.82%, 100% and -14.6% in group I, II, III and IV respectively. However, the percent reduction in group II was poor 22.82% ($P>0.05$) and the percent reduction in group I was reduced compared to the day 7 result and still statistically no significant difference ($P>0.05$) compared to the positive control group. The lice count in negative control group increased by 14.6%.

After three weeks of treatment time, (on day 21) the lice were counted as it was done on day 14. The result of the third week (day 21) was 82.36%, 57.56%, 100% and (-35.97%) in group I, II, III and IV respectively. After application of the second treatment on day 14, and evaluating the lice count, after a week on day 21, the result of percent reduction obtained in experimental groups I and II were showed better result (percent lice reduction of group I improved from 65% to 82.35% and of group II improved from 22.33% to 57.56 %, percent reduction) and yet, 2.5% *C.citratus* is prior to 5% *E. globulus*. The result of 0.1% diazinone 60EC was consistent 100 %, while the lice count in group IV (untreated) was increasing by 35.97%.

After four weeks of treatment time, (on day 28) the lice were counted. The result of the fourth week lice percent reduction was 88.11% ($P>0.05$), 30.86% ($P<0.05$), 100% and (-29.1%) in group I, II, III and IV respectively, when compared to day 0 lice count. The treatment efficacy of 5% *E. globulus* observed decreasing in the second weeks of

treatment in the two occasions and lice count is increasing. 0.1% diazinone 60EC treated group was cleared from the lice up to day 28. In untreated control group, the lice count trend is in increasing fashion though; the lice count in one occasion was found decreased.

After five weeks of treatment time, (on day 35) the lice were counted. The result of the fifth week lice percent reduction was 79.33% ($P>0.05$), 32.65% ($P<0.05$), 100% and (-29.43%) ($P<0.05$), in group I, II, III and IV respectively, when compared to day 0 lice count. The treatment efficacy of 5% *E. globulus* was poor 0.1% diazinone 60EC treated group was cleared from the lice. In untreated control group, the percent lice reduction was 29.43% ($P<0.05$).

After five weeks of treatment time, (on day 42) the lice were counted. There was slight change in percent lice reduction in 2.5% *C.citratus* treated group but the percent lice reduction in 5% *E. globulus* treated group was minimized to 7.38% and then after the lice mean count raised above the initial time (day 0), $P>0.05$.

After sixth to seventh week of treatment time, (on day 49 and 56) there was slight decrease in percent lice reduction in 2.5% *C.citratus* treated group but the percent lice reduction in 5% *E. globulus* treated group was minimized to 7.38% and then after day 49, the lice mean count raised above the initial time (day 0), $P<0.05$. The percent lice reduction in 0.1% diazinone 60EC treated group was consistent (100%), there were no statistically significant difference ($P>0.00$) from day 7 to day 56.

Mean lice count in untreated group, decreased by 5%, *E. globulus* treated group by 27.35 and 15.40% in the last two observations time on Day 49 and 56 respectively.

Table 13: In vivo efficacy of 2.5% *Cymbopogon citratus* and 5% *Eucalyptus globulus* against lice in naturally infested sheep

Group	No. of sheep	Used materials	Average number of lice per animal per treatment (% lice reduction)				
			Day 0	Day 7	Day 14	Day 21	Day 28
1	6	C. citratus 2.5%	40.97	9.43 (76.98%)	13.93 (65.99%)	7.23 (82.35%)	4.87 (88.11%)
2	6	E. globulus5%	30.23	13.87 (54.11%)	23.33 (22.82%)	12.83 (57.56%)	20.9 (30.86%)
3	6	0.1% Diazinone 60EC	38.37	0 (100%)	0 (100%)	0 (100%)	0 (100%)
4	6	Un treated	34.03	31.73 (6.75%)	39.0 (-14.6%)	46.27 (135.97%)	43.93 (-29.09%)
			Day 0	Day 35	Day 42	Day 49	Day 56
1	6	C. citratus 2.5%	40.97	8.47 (79.33%)	8.63 (78.94%)	11.57 (71.76%)	16.67 (59.31%)
2	6/5*	E. globulus5%	30.23	20.50 (32.65%)	28.0 (7.38%)	46.6□ (-35.13%)	39.72□ (-31.39 %)
3	6	0.1% Diazinone 60EC	38.37	0 (100%)	0 (100%)	0 (100%)	0 (100%)
4	6/4**	Un treated	34.03	29.43 (13.52 %)	38.13 (-12.05%)	27.35 (19.63%)	15.40 (54.75%)

* =One animal was dead and 5 were used in the experiment.

** = Two animals were dead and 4 were used in the experiment.

5. DISCUSSION

The result of Questionnaire survey revealed that ectoparasites and related skin disorders are the most important problem in the midlands area of Adeba districts. The disease caused lice in the district referred as “chini”. It became more severe and spread intensively in the last four-five years. The problem of ectoparasites in small ruminants in the study area seems to be very serious as they are distributed in all agro-ecologies affecting all age and sex groups. Poor management and poor level of awareness of farmers regarding the effect of ectoparasites are believed to have contributed to widespread occurrence of infestation. These conditions were noted during the questionnaire study where only a few respondents understood the effect of ectoparasites as either causing health problem or reducing the quality of skin.

The present study showed that the overall prevalence of the ectoparasites is (81.5%) 978 and the prevalence of lice infestation is (75.6%) 907. The prevalence of ectoparasites recorded in the present study was higher than the observations made in other parts of Ethiopia. This might be due to the environmental and climatic condition of the area which is known by its highest temperature and humid due to large area of wet land used for communal grazing (OZARDO, 2006). High temperature, humidity and sunlight favor lice infestation (Pangui, 1994). The current study result is also different from pervious works: 2% (Haffeze, 2001) from central Ethiopia; 0% (Molu, 2002) from southern range land; 2.4% (Tefera, 2004). The louse species identified 0.8% *B.ovis* and 1.2% *Linognathus species* in central Ethiopia (Haffeze, 2001). This might be associated with season of study, difference in agro-climatic condition and animal management system.

Poor management, nutrition and hygienic conditions, in addition less awareness of the owners on the effect of ectoparasites are believed to be contributed to wide spread occurrence of infestation.

The results of the current study demonstrated that among the different ectoparasites, lice infestation (*Damalinia ovis*), tick infestation and *Linognathus ovillus* are 67.7%, 23.8%

and 14.6% respectively. This implies that *Damalinia ovis* is the most prevalent ectoparasites in the study area. *Damalineia ovis* in sheep was highly prevalent indifferent agroecologies with the prevalence of 88.8% in the highlands followed by 77.8% in midland and 67.7 % lowlands of infestation. According to Radostitis *et al* (1994) when the temperature is cooler than optimum, egg do not develop while hotter temperature prevent egg lying and kill the lice. In this study lice infestation of 34.8% was recorded in lowlands (OR=2.55, $P<0.000$); this is may be due to hot climate that the prevalence of lice in the lowland was significantly lower than the higher altitudes of the area.

There was significant difference in age groups, young animals were more affected than adults (OR=1.16, $P < 0.002$). This may be explained the transfer of lice to new host is by contact, from dam to lambs. Lice infestation may indicate some other underlying problem such as malnutrition and chronic diseases according to Wall and Shearer, (1997). The present study confirmed that there was also significant difference between, poor and good body conditioned animals (OR=2.09, $P < 0.05$).

In this study *Melophagus ovinus* was not found in the lowland. The finding is in agreement with Radostits *et al* (1994) and Kettel (1984) who reported that in the hot humid climate, the parasite is restricted to cooler highlands and infestation may be lost when sheep are moved to hot dry areas. All animals infested by *M.ovinus* were also found to be infested by *D. ovis*. The present study indicates that young animals were significantly infested than adults (OR=3.82, $P<0.005$). According Radostitis, et al., (1994) young animals are generally more susceptible to ectoparasites because of higher ratio of accessible surface to body volume and grooming behavior. This high prevalence of lice is most probably attributed to the nutritional stress following the expansion of farm lands as a result of which feed scarcity is the problem of sheep of the study area. And also the common watering and grazing sites favoring the establishment and spread of lice infestation..

In this study an overall prevalenve of 23.8% of ticks recorded in sheep. The three genera of ticks observed were Amblyomma, Rhipicephalus and Boophilus. All sheep positive for tick infestation were also positive for lice.

Among all tick infested animals 15.6 % of them were with heavy infestation. *Rhiphicephalus* spp were the dominant genera found in the high and midlands, while *Boophilus* spp found in all agro-ecological climates. People in the midlands claim about the heavy ticks infestation at the beginning of the rainy season but this needs further investigation and confirmation. High temperature, humidity and prolonged sunlight favor the survival and reproduction of ticks (Pangue, 1994). According to the present study the risk of tick infestations was two 17 times higher in midland than the high and lowlands respectively.

Most ticks spend more time off the host, but are totally dependant on the host for sustenance (Peter, 1995). In lowlands tick infestations were found less, this is may be due to low moisture and poor vegetation cover of the study area, which abstain animals contact with ticks longer months. An odds ratio of 0.89 and $P < 0.278$ describes that tick infestation in sheep were significant in poor body conditioned animals.

In the current in-vitro licidal activity study of seven plants extracts were tested against *Damalinia ovis* and *Linognathus ovillus* using different concentrations fixed oils of well as *Jatropha curcas*, *Melia cafra*, *Ximenia azadrachta*, *pyrethrum* and *Nicotiana tobacum* a volatile essential oils of *Cymbopogon citratus* and *Eucalyptus globules*. Examinations for the death of lice was performed by checking the vital signs of tested lice after 10 min, 30 min, 1hr, 2hrs, 3hrs, 6hrs and 12hrs in all tests.

Out of all seven plant extracts tested in the present study only *Cymbopogon citratus* and *Eucalyptus globulus* were found to be highly effective against both sucking and biting lice. Essential oils of *Cymbopogon citratus* in concentrations of 0.625%, 1.25%, 2.5% and 5% were tested against *Damalinia ovis* and compared to the positive and negative controls (Fig. 6). All *Damalinia ovis* treated with 5% and 2.5% *Cymbopogon citratus* essential oils did not show any vital signs after 2 and 3 hrs, respectively. Its licidal effect was quite comparable with commercially available products such as diazinon. Mean while, all *Damalinia ovis* treated with 5% *Eucalyptus globulus* essential oil did not show any vital sign after 1hr post treatment. After 3hrs of contact with *Cymbopogon citratus* essential oils at 5% and 2.5% concentrations a mortality of 100% were recorded

in *Linognathus ovillus*. Comparable results were observed in the test conducted against *Linognathus ovillus* which was treated with 5% *Eucalyptus globulus* essential oil showing mortality of 76.6% within 12hrs.

In the current study results of the *in vitro* efficacy test showed that *Cymbopogon citratus* and *Eucalyptus globulus* had 100% efficacy against *Damalinina ovis*. The findings were in agreement with the work of Heukelbach et al. (2006) and confirmed the *in-vitro* effectiveness of these extracts against tested lice. The mortality of lice treated with these two extracts was similar with commercially available products like diazinon. This is also in agreement with the results of Heukelbach et al. (2006) who report the high *in vitro* efficacy of extract concentration of dimeticon and 1% of malathion and their similar licide effect as of diazinon. In this study results of *in-vivo* tests using extracts of *Cymbopogon citratus* and *Eucalyptus globulus* against *Damalinina ovis* was conducted according to Holdsworth et al (2006) and the lice percent reduction calculated, examination was conducted at weekly intervals for 56 days covering at least two complete life cycles. *In vivo* test result demonstrated 88.11% lice count reduction which was lower efficacy but promising compared with result obtained by Holdsworth et al. (2005) which was 95%. Concordant data with our result was also observed in works of Kaufman et al 1996 conducted against *Linognathus vituli* on cattle where the percent lice reduction never reached even 90%. This difference might be due to technical errors which might be encountered during extraction and concentration of the plant extracts.

Our results obtained both during *in vitro* and *in vivo* tests using these extracts reflects the potency of their major constituents and confirms that, as expected can be used to predict activity with some success. These results suggest that further investigations into the use of essential oils as acaricides and pediculocides are warranted, especially in view of the public dislike of synthetic insecticides, and resistance and toxicity problems associated with their use.

6. CONCLUSION AND RECOMMENDATIONS

This study clearly showed that ectoparasites are among highly prevalent and the major constraints of sheep production in the study area. The major ectoparasites affecting sheep were lice, ticks, ked and mites. Ectoparasites of sheep are among one of the most serious productivity and health problems of the study area and are rapidly spreading. Moreover, the impact of the disease in causing skin damage may severely affect the performance of the tanning industry, which in turn affects the country's foreign currency. Among risk factors attributing to the prevalence and distribution of ectoparasites and parasitic skin disease in the study area were favorable climate and humidity for parasites development, sex as well as age of animals. Meanwhile, poor veterinary service and absence of strategic treatment of animals were also among highly contributed factors for the widespread of these ectoparasites and skin diseases.

From seven herbal plants using different concentrations during in vitro and in vivo tests four of them were found to be effective against sucking and biting sheep lice their effect was comparable with commercially available products such as diazinon.

Based on the above conclusive remarks, the following recommendations are forwarded:

- Animals should be Strategically treated by against ectoparasites
- The extension services should focus on training of farmers to create awareness about the correct dosing to minimize resistance ectoparasites and skin diseases as well as their impact on the productivity of animals
- Further epidemiological studies should be conducted in the area in order to determine seasonality of the ectoparasites
- Further study on claimed herbal plants should be continued and such study should also be conducted against other ectoparasites of domestic animals.

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8. ANNEXES

Annex 1: FGD Points on KAP of Ethnoveterinary Medicine

Ethnobotanical study on Ethnoveterinary Medicine in ----- and ----- woredas of Oromiya (Dr Hailu)/Amhara (Dr Ahmed) Regional State: Points to be raised during FGD about the use of medicinal plants used against livestock ectoparasites. (Indicate the parasites)

1. Date _____

2. Study wereda _____

3.

P.A/Kebele _____

4. Altitude where the FGD is conducted _____

5. Participants of the FGD

Name of participant	Age	Gender	Religion	Education al status	Occupati on	Represented from

- What are the main livestock diseases in the area? List in terms of priority.
- What are the main livestock skin diseases in the area? List in terms of priority.
- What are the intervention methods used to tackle veterinary diseases? Seeking modern medicine and/or traditional medicine (explain the method(s)).
- Do you use traditional medicine to treat livestock dermal diseases? If yes, explain the method(s).
- Do you use medicinal plants to treat livestock dermal diseases? If yes, which main diseases?
- If you used medicinal plants to treat livestock dermal diseases, do you prepare the preparations at home or seek assistance from knowledgeable community members?

12. If you seek assistance from knowledgeable community member, do they charge money (in kind) for their assistance?
13. If yes to Q 12., how do you compare the cost with the modern medicine? Cheaper/expensive/depends on the disease?
14. If there is modern medicine for a particular livestock dermal disease, why using traditional medical preparations prepared at home or seek assistance from knowledgeable community members? Cost is cheaper/accessible/more effective than modern medicine/other _____
15. Is there any secrecy associated with the knowledge of traditional medicine used to treat livestock diseases by the community?
16. How do you evaluate prevalence of lice? (Use local name for the respective disease if it explicitly describes the disease. If the term denotes more than one, show affected animal to FGD participants during interview) in this area? Very low/Low/high/very high/extremely prevalent?
17. What are the clinical manifestations when livestock are affected by lice, describe.
18. What are the measures usually taken to tackle lice ?Describe
19. Do you use medicinal plants to treat lice?
20. If yes, to Q 18, do you prepare at home or seek assistance from knowledgeable person?
21. If yes, to Q 18, do you prefer modern or traditional medicine to treat livestock affected by lice ? Why?
22. Do you encourage medicinal plants to undergo scientific investigation to develop herbal based livestock medicine for lice? If yes, why?
23. Any _____ other _____ valuable information: _____

I thank you for sharing your valuable knowledge and sparing precious time!!!!

Annex 2: Definition and synonyms

Alopecia: spontaneous hair loss occurs more often in crossbreeds, mainly at the end of winter. Shaving at inappropriate time is thought to increase the risk of hair loss. Nutritional deficiencies or imbalances (such as high calcium with low zinc) have also been incriminated. Partial alopecia (hypotrichosis) is a non specific secondary lesion.

Body condition: Poor body condition score will be given to sheep and goats which are extremely thin to those with smooth and less prominent spinous process, transverse process in which finger can be pushed and moderate depth loin muscle. Good body condition score will be given for sheep and goats in which the spinous process only stick up very slightly; smooth, rounded and well covered transverse processes and those having full loin muscle and very fat. Age categorization in to young (lamb/kid) and adult will be performed as described by Gatenby (1991) for sheep and steele (1996) for goats. Accordingly those sheep and goats under 1 year will be categorized as young and the rest as adult.

Cockle/ "Ekek": is allergic dermatitis which causes itching, observable only after removal of the hair or wool and is characterized by multiple small circular lesions on the grain side of the skin.

Erythema (reddening): it occurs in many acute disease conditions and is not diagnostic.

Hyperkeratosis: is an increased thickness of stratum corneum. During clinical examinations hyperkeratosis is used to refer to accumulation of adherent keratinized material.

Scratches: are elongated scars of different sizes. They are the results of the healing of any wound in the skin with that elongated shape.

Skin: is the outer covering of mature fully grown animals of the smaller kinds such as sheep, goats, pigs, reptiles, birds and fishes, or of the immature animals of the larger species such as calves and colts in raw and/or untanned form.

Annex 3: Death 10 min, *Damalina ovis*, Test of Between- Subject Effects

Test materials	Mean	Std. Error	95% Confidence Interval for Mean		P=Value
			Lower Bound	Upper Bound	
Jatropha curcas 160mg/ml	,00	,000	,00	,00	0.000
Jatropha curcas 80mg/ml	,50	,342	-,38	1,38	
Jatropha curcas 40mg/ml	,50	,342	-,38	1,38	
Jatropha curcas 20mg/ml	,17	,167	-,26	,60	
4%Tween80	,17	,167	-,26	,60	
Melia160mg/ml	,00	,000	,00	,00	
Melia80mg/ml	3,17	,307	2,38	3,96	
Melia40mg/ml	2,67	,211	2,12	3,21	
Melia20mg/ml	1,33	,494	,06	2,60	
4%Tween20	1,00	,365	,06	1,94	
Cymbopogon citratus 5%	,50	,500	-,79	1,79	
Cymbopogon citratus 2. 5%	4,00	,775	2,01	5,99	
Cymbopogon citratus 1.25%	1,50	,806	-,57	3,57	
Cymbopogon citratus 0.625%	,50	,342	-,38	1,38	
2%Tween80	,17	,167	-,26	,60	
Eucalyptus globulus 5%	,50	,500	-,79	1,79	
Eucalyptus globulus2.5%	9,17	,477	7,94	10,39	
Eucalyptus globulus 1.25%	,00	,000	,00	,00	
Eucalyptus globulus 0.625%	,00	,000	,00	,00	
Ximenia160mg/ml	,00	,000	,00	,00	
ximenia80mg/ml	2,67	,333	1,81	3,52	
ximenia40mg/ml	1,67	,667	-,05	3,38	
ximenia20mg/ml	,83	,307	,04	1,62	
Pyrethrum160mg/ml	,33	,333	-,52	1,19	
pyrethrum80mg/ml	1,50	,500	,21	2,79	
pyrethrum40mg/ml	1,33	,422	,25	2,42	
pyrethrum20mg/ml	1,33	,558	-,10	2,77	
kerosene:2%Tween80:3%Ethanol	,83	,307	,04	1,62	
Tobaco160mg/ml	,00	,000	,00	,00	
Tobaco80mg/ml	,00	,000	,00	,00	
Tobaco40mg/ml	,00	,000	,00	,00	
Tobaco20mg/ml	,00	,000	,00	,00	
Ethanol 3%	,00	,000	,00	,00	
Diazinon 0.1%	,00	,000	,00	,00	
Distl water	,00	,000	,00	,00	
Total	1,04	,131	,78	1,30	

Annex 4: Death 30 min, *Damalina ovis*, Test of Between- Subject Effects

Test materials	Mean	Std. Error	95% Confidence Interval for Mean		P=Value
			Lower Bound	Upper Bound	
Jatropha curcas 160mg/ml	1,17	,477	-,06	2,39	0.000
Jatropha curcas 80mg/ml	,83	,401	-,20	1,87	
Jatropha curcas 40mg/ml	,83	,401	-,20	1,87	
Jatropha curcas 20mg/ml	,33	,333	-,52	1,19	
4%Tween80	,00	,000	,00	,00	
Melia160mg/ml	6,50	,563	5,05	7,95	
Melia80mg/ml	3,67	,667	1,95	5,38	
Melia40mg/ml	2,33	,422	1,25	3,42	
Melia20mg/ml	2,17	,307	1,38	2,96	
4%Tween20	,50	,500	-,79	1,79	
Cymbopogon citratus 5%	7,83	,401	6,80	8,87	
Cymbopogon citratus 2. 5%	5,67	,919	3,30	8,03	
Cymbopogon citratus 1.25%	2,83	,601	1,29	4,38	
Cymbopogon citratus 0.625%	1,00	,516	-,33	2,33	
2%Tween80	,50	,500	-,79	1,79	
Eucalyptus globulus 5%	9,33	,333	8,48	10,19	
Eucalyptus globulus2.5%	,33	,211	-,21	,88	
Eucalyptus globulus 1.25%	,00	,000	,00	,00	
Eucalyptus globulus 0.625%	,00	,000	,00	,00	
Ximenia160mg/ml	3,17	,307	2,38	3,96	
ximenia80mg/ml	2,33	,760	,38	4,29	
ximenia40mg/ml	1,33	,333	,48	2,19	
ximenia20mg/ml	,50	,342	-,38	1,38	
Pyrethrum160mg/ml	2,00	,516	,67	3,33	
pyrethrum80mg/ml	1,67	,615	,09	3,25	
pyrethrum40mg/ml	1,67	,494	,40	2,94	
pyrethrum20mg/ml	1,17	,477	-,06	2,39	
kerosene:2%Tween80:3%Ethanol	,00	,000	,00	,00	
Tobaco160mg/ml	,00	,000	,00	,00	
Tobaco80mg/ml	,17	,167	-,26	,60	
Tobaco40mg/ml	,00	,000	,00	,00	
Tobaco20mg/ml	,00	,000	,00	,00	
Ethanol 3%	,00	,000	,00	,00	
Diazinon 0.1%	,00	,000	,00	,00	
Distl water	,00	,000	,00	,00	
Total	1,71	,172	1,37	2,05	

Annex 5: Death 1hr, *Damalina ovis*, Test of Between- Subject Effects

Test materials	Mean	Std. Error	95% Confidence Interval for Mean		P=Value
	Upper Bound	Upper Bound	Lower Bound	Upper Bound	
Jatropha curcas 160mg/ml	2,83	,477	1,61	4,06	0.000
Jatropha curcas 80mg/ml	1,83	,477	,61	3,06	
Jatropha curcas 40mg/ml	1,00	,447	-,15	2,15	
Jatropha curcas 20mg/ml	,67	,333	-,19	1,52	
4%Tween80	,00	,000	,00	,00	
Melia160mg/ml	7,00	,447	5,85	8,15	
Melia80mg/ml	4,83	,792	2,80	6,87	
Melia40mg/ml	2,67	,333	1,81	3,52	
Melia20mg/ml	2,67	,422	1,58	3,75	
4%Tween20	,50	,500	-,79	1,79	
Cymbopogon citratus 5%	9,50	,342	8,62	10,38	
Cymbopogon citratus 2. 5%	8,33	,422	7,25	9,42	
Cymbopogon citratus 1.25%	3,50	,719	1,65	5,35	
Cymbopogon citratus 0.625%	1,50	,671	-,22	3,22	
2%Tween80	,83	,477	-,39	2,06	
Eucalyptus globulus 5%	10,00	,000	10,00	10,00	
Eucalyptus globulus2.5%	1,17	,401	,13	2,20	
Eucalyptus globulus 1.25%	,00	,000	,00	,00	
Eucalyptus globulus 0.625%	,00	,000	,00	,00	
Ximenia160mg/ml	3,17	,307	2,38	3,96	
ximenia80mg/ml	2,67	,803	,60	4,73	
ximenia40mg/ml	1,83	,477	,61	3,06	
ximenia20mg/ml	1,00	,516	-,33	2,33	
Pyrethrum160mg/ml	2,33	,494	1,06	3,60	
pyrethrum80mg/ml	2,33	,494	1,06	3,60	
pyrethrum40mg/ml	2,00	,516	,67	3,33	
pyrethrum20mg/ml	1,67	,494	,40	2,94	
kerosene:2%Tween80:3%Ethanol	,50	,224	-,07	1,07	
Tobaco160mg/ml	,00	,000	,00	,00	
Tobaco80mg/ml	,17	,167	-,26	,60	
Tobaco40mg/ml	,17	,167	-,26	,60	
Tobaco20mg/ml	,17	,167	-,26	,60	
Ethanol 3%	,00	,000	,00	,00	
Diazinon 0.1%	,00	,000	,00	,00	
Distl water	,00	,000	,00	,00	
Total	2,20	,195	1,81	2,58	

Annex 6: Death 2hrs, *Damalina ovis*, Test of Between- Subject Effects

Test materials	Mean	Std. Error	95% Confidence Interval for Mean		P=Value
			Lower Bound	Upper Bound	
Jatropha curcas 160mg/ml	3,83	,703	2,03	5,64	0.000
Jatropha curcas 80mg/ml	2,83	,477	1,61	4,06	
Jatropha curcas 40mg/ml	1,50	,806	-,57	3,57	
Jatropha curcas 20mg/ml	1,17	,167	,74	1,60	
4%Tween80	,33	,333	-,52	1,19	
Melia160mg/ml	7,33	,422	6,25	8,42	
Melia80mg/ml	5,33	,919	2,97	7,70	
Melia40mg/ml	3,50	,428	2,40	4,60	
Melia20mg/ml	3,50	,563	2,05	4,95	
4%Tween20	,50	,500	-,79	1,79	
Cymbopogon citratus 5%	10,00	,000	10,00	10,00	
Cymbopogon citratus 2. 5%	9,00	,365	8,06	9,94	
Cymbopogon citratus 1.25%	5,33	,422	4,25	6,42	
Cymbopogon citratus 0.625%	1,50	,671	-,22	3,22	
2%Tween80	2,00	,577	,52	3,48	
Eucalyptus globulus 5%	10,00	,000	10,00	10,00	
Eucalyptus globulus2.5%	1,33	,422	,25	2,42	
Eucalyptus globulus 1.25%	,67	,333	-,19	1,52	
Eucalyptus globulus 0.625%	,33	,211	-,21	,88	
Ximenia160mg/ml	4,17	,401	3,13	5,20	
ximenia80mg/ml	3,67	,803	1,60	5,73	
ximenia40mg/ml	2,67	,760	,71	4,62	
ximenia20mg/ml	1,33	,494	,06	2,60	
Pyrethrum160mg/ml	2,67	,422	1,58	3,75	
pyrethrum80mg/ml	2,50	,428	1,40	3,60	
pyrethrum40mg/ml	2,33	,615	,75	3,91	
pyrethrum20mg/ml	2,33	,422	1,25	3,42	
kerosene:2%Tween80:3%Ethanol	1,50	,224	,93	2,07	
Tobaco160mg/ml	,00	,000	,00	,00	
Tobaco80mg/ml	,17	,167	-,26	,60	
Tobaco40mg/ml	,33	,211	-,21	,88	
Tobaco20mg/ml	,17	,167	-,26	,60	
Ethanol 3%	,00	,000	,00	,00	
Diazinon 0.1%	,00	,000	,00	,00	
Distl water	,00	,000	,00	,00	
Total	2,68	,204	2,28	3,08	

Annex 7: Death 3hrs, *Damalinia ovis*, Test of Between- Subject Effects

	Mean	Std. Error	95% Confidence Interval for Mean		P=Value
			Lower Bound	Upper Bound	
Jatropha curcas 160mg/ml	4,83	,477	3,61	6,06	0.000
Jatropha curcas 80mg/ml	3,67	,558	2,23	5,10	
Jatropha curcas 40mg/ml	2,17	1,222	-,98	5,31	
Jatropha curcas 20mg/ml	2,17	,307	1,38	2,96	
4%Tween80	,50	,342	-,38	1,38	
Melia160mg/ml	7,50	,428	6,40	8,60	
Melia80mg/ml	5,33	,919	2,97	7,70	
Melia40mg/ml	4,33	,667	2,62	6,05	
Melia20mg/ml	4,33	,494	3,06	5,60	
4%Tween20	,83	,477	-,39	2,06	
Cymbopogon citratus 5%	10,00	,000	10,00	10,00	
Cymbopogon citratus 2. 5%	10,00	,000	10,00	10,00	
Cymbopogon citratus 1.25%	6,33	,667	4,62	8,05	
Cymbopogon citratus 0.625%	2,67	,333	1,81	3,52	
2%Tween80	2,67	,558	1,23	4,10	
Eucalyptus globulus 5%	10,00	,000	10,00	10,00	
Eucalyptus globulus2.5%	3,00	1,317	-,38	6,38	
Eucalyptus globulus 1.25%	1,33	,715	-,50	3,17	
Eucalyptus globulus 0.625%	,50	,224	-,07	1,07	
Ximenia160mg/ml	5,67	,667	3,95	7,38	
ximenia80mg/ml	4,67	,615	3,09	6,25	
ximenia40mg/ml	4,17	1,014	1,56	6,77	
ximenia20mg/ml	1,83	,703	,03	3,64	
Pyrethrum160mg/ml	4,17	,980	1,65	6,69	
pyrethrum80mg/ml	3,50	,619	1,91	5,09	
pyrethrum40mg/ml	3,00	,447	1,85	4,15	
pyrethrum20mg/ml	3,00	,447	1,85	4,15	
kerosene:2%Tween80:3%Ethanol	2,33	,558	,90	3,77	
Tobaco160mg/ml	,17	,167	-,26	,60	
Tobaco80mg/ml	,67	,494	-,60	1,94	
Tobaco40mg/ml	,83	,307	,04	1,62	
Tobaco20mg/ml	,67	,333	-,19	1,52	
Ethanol 3%	,17	,167	-,26	,60	
Diazinon 0.1%	,33	,211	-,21	,88	
Distl water	,17	,167	-,26	,60	
Total	3,36	,213	2,94	3,78	

Annex 8: Death 6hrs, *Damaliina ovis*, Test of Between- Subject Effects

Test materials	Mean	Std. Error	95% Confidence Interval for Mean		P=Value
			Lower Bound	Upper Bound	
Jatropha curcas 160mg/ml	6,67	,760	4,71	8,62	0.000
Jatropha curcas 80mg/ml	5,33	,667	3,62	7,05	
Jatropha curcas 40mg/ml	4,33	1,520	,43	8,24	
Jatropha curcas 20mg/ml	3,50	,563	2,05	4,95	
4%Tween80	1,17	,477	-,06	2,39	
Melia160mg/ml	7,83	,543	6,44	9,23	
Melia80mg/ml	6,17	,749	4,24	8,09	
Melia40mg/ml	5,83	,980	3,31	8,35	
Melia20mg/ml	5,50	,847	3,32	7,68	
4%Tween20	1,50	,428	,40	2,60	
Cymbopogon citratus 5%	10,00	,000	10,00	10,00	
Cymbopogon citratus 2. 5%	10,00	,000	10,00	10,00	
Cymbopogon citratus 1.25%	6,67	,715	4,83	8,50	
Cymbopogon citratus 0.625%	2,83	,401	1,80	3,87	
2%Tween80	2,67	,558	1,23	4,10	
Eucalyptus globulus 5%	10,00	,000	10,00	10,00	
Eucalyptus globulus2.5%	6,33	1,520	2,43	10,24	
Eucalyptus globulus 1.25%	4,00	,730	2,12	5,88	
Eucalyptus globulus 0.625%	1,00	,000	1,00	1,00	
Ximenia160mg/ml	6,50	,619	4,91	8,09	
ximenia80mg/ml	5,17	,792	3,13	7,20	
ximenia40mg/ml	4,83	1,138	1,91	7,76	
ximenia20mg/ml	3,17	,833	1,02	5,31	
Pyrethrum160mg/ml	5,67	1,174	2,65	8,68	
pyrethrum80mg/ml	4,67	,558	3,23	6,10	
pyrethrum40mg/ml	4,50	,563	3,05	5,95	
pyrethrum20mg/ml	4,33	,494	3,06	5,60	
kerosene:2%Tween80:3%Ethanol	2,67	,715	,83	4,50	
Tobaco160mg/ml	,50	,342	-,38	1,38	
Tobaco80mg/ml	1,50	,563	,05	2,95	
Tobaco40mg/ml	1,33	,333	,48	2,19	
Tobaco20mg/ml	1,00	,258	,34	1,66	
Ethanol 3%	,33	,211	-,21	,88	
Diazinon 0.1%	4,33	,955	1,88	6,79	
Distl water	,17	,167	-,26	,60	
Total	4,34	,217	3,92	4,77	

Annex 9: Death 12hrs, Damalinia ovis, Test of Between- Subject Effects

Test materials	Mean	Std. Error	95% Confidence Interval for Mean		P=Value
			Lower Bound	Upper Bound	
Jatropha curcas 160mg/ml	9,50	,342	8,62	10,38	0.000
Jatropha curcas 80mg/ml	7,50	,719	5,65	9,35	
Jatropha curcas 40mg/ml	5,83	,792	3,80	7,87	
Jatropha curcas 20mg/ml	5,50	1,875	,68	10,32	
4%Tween80	4,33	,333	3,48	5,19	
Melia 160mg/ml	1,50	,563	,05	2,95	
Melia 80mg/ml	8,67	,494	7,40	9,94	
Melia 40mg/ml	8,00	,577	6,52	9,48	
Melia 20mg/ml	7,83	,703	6,03	9,64	
4%Tween20	7,50	,719	5,65	9,35	
Cymbopogon citratus 5%	1,67	,558	,23	3,10	
Cymbopogon citratus 2. 5%	10,00	,000	10,00	10,00	
Cymbopogon citratus 1.25%	10,00	,000	10,00	10,00	
Cymbopogon citratus 0.625%	7,00	,577	5,52	8,48	
2%Tween80	3,33	,422	2,25	4,42	
Eucalyptus globulus 5%	4,50	1,232	1,33	7,67	
Eucalyptus globulus 2.5%	10,00	,000	10,00	10,00	
Eucalyptus globulus 1.25%	8,83	,833	6,69	10,98	
Eucalyptus globulus 0.625%	5,67	,882	3,40	7,93	
Ximenia 160mg/ml	1,50	,224	,93	2,07	
ximenia 80mg/ml	7,50	,428	6,40	8,60	
ximenia 40mg/ml	5,83	1,167	2,83	8,83	
ximenia 20mg/ml	5,50	,764	3,54	7,46	
Pyrethrum 160mg/ml	4,17	,477	2,94	5,39	
pyrethrum 80mg/ml	8,83	,654	7,15	10,51	
pyrethrum 40mg/ml	6,33	1,022	3,71	8,96	
pyrethrum 20mg/ml	5,83	,792	3,80	7,87	
kerosene:2%Tween80:3%Ethanol	5,17	,601	3,62	6,71	
Tobaco 160mg/ml	3,67	,919	1,30	6,03	
Tobaco 80mg/ml	4,33	,667	2,62	6,05	
Tobaco 40mg/ml	2,83	,792	,80	4,87	
Tobaco 20mg/ml	2,33	,494	1,06	3,60	
Ethanol 3%	2,17	,601	,62	3,71	
Diazinon 0.1%	1,33	,422	,25	2,42	
Distl water	,17	,167	-,26	,60	
Total	5,56	,222	5,12	6,00	

Annex 10: Death 10 min, Linognathus ovillus, Test of Between- Subject Effects

Test materials	Mean	Std. Error	95% Confidence Interval for Mean		P=Value
			Lower Bound	Upper Bound	
Jatropha curcas 160mg/ml	,17	,167	-,26	,60	
Jatropha curcas 80mg/ml	,00	,000	,00	,00	
Jatropha curcas 40mg/ml	,00	,000	,00	,00	
Jatropha curcas 20mg/ml	,00	,000	,00	,00	
4%Tween80	,00	,000	,00	,00	
Melia160mg/ml	2,33	,667	,62	4,05	
Melia80mg/ml	,17	,167	-,26	,60	
Melia40mg/ml	,00	,000	,00	,00	
Melia20mg/ml	,00	,000	,00	,00	
4%Tween20	,00	,000	,00	,00	
Cymbopogon citratus 5%	1,00	,516	-,33	2,33	
Cymbopogon citratus 2. 5%	,33	,333	-,52	1,19	
Cymbopogon citratus 1.25%	,00	,000	,00	,00	
Cymbopogon citratus 0.625%	,00	,000	,00	,00	
2%Tween80	,67	,667	-1,05	2,38	
Eucalyptus globulus 5%	3,00	1,033	,35	5,65	
Eucalyptus globulus2.5%	,67	,494	-,60	1,94	
Eucalyptus globulus 1.25%	,00	,000	,00	,00	
Eucalyptus globulus 0.625%	,00	,000	,00	,00	
Ximenia 160mg/ml	,17	,167	-,26	,60	
ximenia80mg/ml	,00	,000	,00	,00	
ximenia40mg/ml	,00	,000	,00	,00	
ximenia20mg/ml	,17	,167	-,26	,60	
Pyrethrum 160mg/ml	,17	,167	-,26	,60	
pyrethrum80mg/ml	,17	,167	-,26	,60	
pyrethrum40mg/ml	,00	,000	,00	,00	
pyrethrum20mg/ml	,00	,000	,00	,00	
kerosene:2%Tween80:3%Ethanol	,00	,000	,00	,00	
Tobaco 160mg/ml	,17	,167	-,26	,60	
Tobaco80mg/ml	,00	,000	,00	,00	
Tobaco40mg/ml	,00	,000	,00	,00	
Tobaco20mg/ml	,00	,000	,00	,00	
Ethanol 3%	,00	,000	,00	,00	
Diazinon 0.1%	,00	,000	,00	,00	
Distl water	,00	,000	,00	,00	
Total	,26	,062	,14	,38	

Annex 11: Death 30 min, Linognathus ovillus, Test of Between- Subject Effects

Test materials	Mean	Std. Error	95% Confidence Interval for Mean		P=Val
			Lower Bound	Upper Bound	
Jatropha curcas 160mg/ml	,17	,167	-,26	,60	
Jatropha curcas 80mg/ml	,00	,000	,00	,00	
Jatropha curcas 40mg/ml	,00	,000	,00	,00	
Jatropha curcas 20mg/ml	,00	,000	,00	,00	
4%Tween80	,17	,167	-,26	,60	
Melia160mg/ml	3,67	,615	2,09	5,25	
Melia80mg/ml	1,50	,563	,05	2,95	
Melia40mg/ml	1,00	,516	-,33	2,33	
Melia20mg/ml	,00	,000	,00	,00	
4%Tween20	,00	,000	,00	,00	
Cymbopogon citratus 5%	3,83	1,424	,17	7,49	
Cymbopogon citratus 2. 5%	,00	,000	,00	,00	
Cymbopogon citratus 1.25%	,00	,000	,00	,00	
Cymbopogon citratus 0.625%	,00	,000	,00	,00	
2%Tween80	,83	,654	-,85	2,51	
Eucalyptus globulus 5%	4,50	,992	1,95	7,05	
Eucalyptus globulus2.5%	2,67	1,585	-1,41	6,74	
Eucalyptus globulus 1.25%	,00	,000	,00	,00	
Eucalyptus globulus 0.625%	,00	,000	,00	,00	
Ximenia160mg/ml	,17	,167	-,26	,60	
ximenia80mg/ml	,00	,000	,00	,00	
ximenia40mg/ml	,00	,000	,00	,00	
ximenia20mg/ml	,17	,167	-,26	,60	
Pyrethrum160mg/ml	1,00	,365	,06	1,94	
pyrethrum80mg/ml	,83	,543	-,56	2,23	
pyrethrum40mg/ml	,33	,211	-,21	,88	
pyrethrum20mg/ml	,33	,211	-,21	,88	
kerosene:2%Tween80:3%Ethanol	,33	,211	-,21	,88	
Tobaco160mg/ml	,33	,211	-,21	,88	
Tobaco80mg/ml	,17	,167	-,26	,60	
Tobaco40mg/ml	,00	,000	,00	,00	
Tobaco20mg/ml	,00	,000	,00	,00	
Ethanol 3%	,00	,000	,00	,00	
Diazinon 0.1%	,00	,000	,00	,00	
Distl water	,00	,000	,00	,00	
Total	,63	,109	,41	,84	

Annex 12: Death 1hr, *Linognathus ovillus*, Test of Between- Subject Effects

Test materials	mean	Std. Error	95% Confidence Interval		P=Value
			Lower Bound	Upper Bound	
Jatropha curcas 160mg/ml	,17	,167	-,26	,60	0.000
Jatropha curcas 80mg/ml	,50	,342	-,38	1,38	
Jatropha curcas 40mg/ml	,00	,000	,00	,00	
Jatropha curcas 20mg/ml	,00	,000	,00	,00	
4%Tween80	,17	,167	-,26	,60	
Melia160mg/ml	4,83	,601	3,29	6,38	
Melia80mg/ml	1,67	,667	-,05	3,38	
Melia40mg/ml	2,00	,632	,37	3,63	
Melia20mg/ml	,33	,211	-,21	,88	
4%Tween20	,17	,167	-,26	,60	
Cymbopogon citratus 5%	6,67	,760	4,71	8,62	
Cymbopogon citratus 2. 5%	,00	,000	,00	,00	
Cymbopogon citratus 1.25%	,00	,000	,00	,00	
Cymbopogon citratus 0.625%	,00	,000	,00	,00	
2%Tween80	,83	,654	-,85	2,51	
Eucalyptus globulus 5%	5,33	,989	2,79	7,88	
Eucalyptus globulus2.5%	3,17	1,493	-,67	7,00	
Eucalyptus globulus 1.25%	,00	,000	,00	,00	
Eucalyptus globulus 0.625%	,00	,000	,00	,00	
Ximenia160mg/ml	,17	,167	-,26	,60	
ximenia80mg/ml	,00	,000	,00	,00	
ximenia40mg/ml	,00	,000	,00	,00	
ximenia20mg/ml	,17	,167	-,26	,60	
Pyrethrum160mg/ml	1,00	,516	-,33	2,33	
pyrethrum80mg/ml	,83	,543	-,56	2,23	
pyrethrum40mg/ml	,83	,307	,04	1,62	
pyrethrum20mg/ml	,83	,401	-,20	1,87	
kerosene:2%Tween80:3%Ethanol	,50	,224	-,07	1,07	
Tobaco160mg/ml	,67	,333	-,19	1,52	
Tobaco80mg/ml	,50	,224	-,07	1,07	
Tobaco40mg/ml	,00	,000	,00	,00	
Tobaco20mg/ml	,00	,000	,00	,00	
Ethanol 3%	,00	,000	,00	,00	
Diazinon 0.1%	,00	,000	,00	,00	
Distl water	,00	,000	,00	,00	
Total	,90	,130	,64	1,15	

Annex 13: Death 2hrs, Linognathus ovillus, Test of Between- Subject Effects

Test materials	Mean	Std. Error	95% Confidence Interval		P=Value
			Lower Bound	Upper Bound	
Jatropha curcas 160mg/ml	,17	,167	-,26	,60	0.000
Jatropha curcas 80mg/ml	1,67	,494	,40	2,94	
Jatropha curcas 40mg/ml	,67	,333	-,19	1,52	
Jatropha curcas 20mg/ml	,17	,167	-,26	,60	
4%Tween80	,33	,211	-,21	,88	
Melia160mg/ml	5,00	,632	3,37	6,63	
Melia80mg/ml	3,17	1,108	,32	6,02	
Melia40mg/ml	2,67	,882	,40	4,93	
Melia20mg/ml	,83	,307	,04	1,62	
4%Tween20	,50	,342	-,38	1,38	
Cymbopogon citratus 5%	9,50	,500	8,21	10,79	
Cymbopogon citratus 2. 5%	9,00	,516	7,67	10,33	
Cymbopogon citratus 1.25%	1,83	1,276	-1,45	5,11	
Cymbopogon citratus 0.625%	,00	,000	,00	,00	
2%Tween80	5,67	1,054	2,96	8,38	
Eucalyptus globulus 5%	3,67	1,382	,11	7,22	
Eucalyptus globulus2.5%	,00	,000	,00	,00	
Eucalyptus globulus 1.25%	,00	,000	,00	,00	
Eucalyptus globulus 0.625%	,33	,211	-,21	,88	
Ximenia160mg/ml	,17	,167	-,26	,60	
ximenia80mg/ml	,17	,167	-,26	,60	
ximenia40mg/ml	,17	,167	-,26	,60	
ximenia20mg/ml	1,33	,667	-,38	3,05	
Pyrethrum160mg/ml	,83	,543	-,56	2,23	
pyrethrum80mg/ml	1,17	,307	,38	1,96	
pyrethrum40mg/ml	,83	,401	-,20	1,87	
pyrethrum20mg/ml	,50	,224	-,07	1,07	
kerosene:2%Tween80:3%Ethanol	1,17	,477	-,06	2,39	
Tobaco160mg/ml	,83	,167	,40	1,26	
Tobaco80mg/ml	,17	,167	-,26	,60	
Tobaco40mg/ml	,33	,211	-,21	,88	
Tobaco20mg/ml	,00	,000	,00	,00	
Ethanol 3%	,50	,342	-,38	1,38	
Diazinon 0.1%	,00	,000	,00	,00	
Distl water	1,57	,186	1,20	1,94	

Annex 14: Death 3hrs, *Linognathus ovillus*, Test of Between- Subject Effects

Test materials	Mean	Std. Error	95% Confidence Interval		P=Value
			Lower Bound	Upper Bound	
Jatropha curcas 160mg/ml	1,50	,342	,62	2,38	0.000
Jatropha curcas 80mg/ml	2,50	,563	1,05	3,95	
Jatropha curcas 40mg/ml	2,00	,516	,67	3,33	
Jatropha curcas 20mg/ml	1,33	,422	,25	2,42	
4%Tween80	1,00	,365	,06	1,94	
Melia160mg/ml	5,67	,667	3,95	7,38	
Melia80mg/ml	4,00	,966	1,52	6,48	
Melia40mg/ml	4,17	1,014	1,56	6,77	
Melia20mg/ml	1,83	,307	1,04	2,62	
4%Tween20	,83	,307	,04	1,62	
Cymbopogon citratus 5%	10,00	,000	10,00	10,00	
Cymbopogon citratus 2. 5%	10,00	,000	10,00	10,00	
Cymbopogon citratus 1.25%	2,00	1,238	-1,18	5,18	
Cymbopogon citratus 0.625%	,00	,000	,00	,00	
2%Tween80	1,17	,601	-,38	2,71	
Eucalyptus globulus 5%	6,33	,843	4,17	8,50	
Eucalyptus globulus2.5%	4,00	1,342	,55	7,45	
Eucalyptus globulus 1.25%	,17	,167	-,26	,60	
Eucalyptus globulus 0.625%	,00	,000	,00	,00	
Ximenia160mg/ml	,83	,307	,04	1,62	
ximenia80mg/ml	1,17	,307	,38	1,96	
ximenia40mg/ml	1,17	,401	,13	2,20	
ximenia20mg/ml	,83	,477	-,39	2,06	
Pyrethrum160mg/ml	1,33	,667	-,38	3,05	
pyrethrum80mg/ml	1,17	,601	-,38	2,71	
pyrethrum40mg/ml	1,17	,307	,38	1,96	
pyrethrum20mg/ml	1,17	,601	-,38	2,71	
kerosene:2%Tween80:3%Ethanol	2,33	,989	-,21	4,88	
Tobaco160mg/ml	2,17	,401	1,13	3,20	
Tobaco80mg/ml	1,50	,342	,62	2,38	
Tobaco40mg/ml	,67	,333	-,19	1,52	
Tobaco20mg/ml	1,00	,258	,34	1,66	
Ethanol 3%	,17	,167	-,26	,60	
Diazinon 0.1%	,67	,422	-,42	1,75	
Distl water	,17	,167	-,26	,60	
Total	2,17	,190	1,80	2,55	

Annex 15: Death 6hrs, Linognathus ovillus, Test of Between- Subject Effects

Test materials	Mean	Std. Error	95% Confidence Interval for Mean		P=Value
			Lower Bound	Upper Bound	
Jatropha curcas 160mg/ml	5,17	,601	3,62	6,71	0.000
Jatropha curcas 80mg/ml	4,67	,494	3,40	5,94	
Jatropha curcas 40mg/ml	4,00	,730	2,12	5,88	
Jatropha curcas 20mg/ml	2,17	,543	,77	3,56	
4%Tween80	1,33	,333	,48	2,19	
Melia160mg/ml	7,50	,764	5,54	9,46	
Melia80mg/ml	5,17	,946	2,74	7,60	
Melia40mg/ml	5,33	,558	3,90	6,77	
Melia20mg/ml	1,67	,667	-,05	3,38	
4%Tween20	1,17	,401	,13	2,20	
Cymbopogon citratus 5%	10,00	,000	10,00	10,00	
Cymbopogon citratus 2. 5%	10,00	,000	10,00	10,00	
Cymbopogon citratus 1.25%	2,00	1,238	-1,18	5,18	
Cymbopogon citratus 0.625%	,17	,167	-,26	,60	
2%Tween80	1,50	,500	,21	2,79	
Eucalyptus globulus 5%	6,83	,833	4,69	8,98	
Eucalyptus globulus2.5%	4,50	1,258	1,27	7,73	
Eucalyptus globulus 1.25%	,67	,333	-,19	1,52	
Eucalyptus globulus 0.625%	,00	,000	,00	,00	
Ximenia160mg/ml	3,17	,792	1,13	5,20	
ximenia80mg/ml	2,83	,477	1,61	4,06	
ximenia40mg/ml	2,50	,563	1,05	3,95	
ximenia20mg/ml	2,17	,654	,49	3,85	
Pyrethrum160mg/ml	1,17	,401	,13	2,20	
pyrethrum80mg/ml	2,83	,946	,40	5,26	
pyrethrum40mg/ml	2,50	,428	1,40	3,60	
pyrethrum20mg/ml	2,17	,307	1,38	2,96	
kerosene:2%Tween80:3%Ethanol	2,17	,946	-,26	4,60	
Tobaco160mg/ml	3,00	1,033	,35	5,65	
Tobaco80mg/ml	2,33	,422	1,25	3,42	
Tobaco40mg/ml	2,17	,477	,94	3,39	
Tobaco20mg/ml	1,67	,333	,81	2,52	
Ethanol 3%	1,50	,224	,93	2,07	
Diazinon 0.1%	1,67	,667	-,05	3,38	
Distl water	,17	,167	-,26	,60	
Total	3,08	,195	2,70	3,47	

Annex 16: Death 12hrs, Linognathus ovillus, Test of Between- Subject Effects

Test materials	Mean	Std. Error	95% Confidence Interval for Mean		P=Value
			Lower Bound	Upper Bound	
Jatropha curcas 160mg/ml	7,33	,422	6,25	8,42	0.000
Jatropha curcas 80mg/ml	6,67	,715	4,83	8,50	
Jatropha curcas 40mg/ml	6,17	,946	3,74	8,60	
Jatropha curcas 20mg/ml	4,83	1,014	2,23	7,44	
4%Tween80	1,33	,333	,48	2,19	
Melia160mg/ml	,33	,211	-,21	,88	
Melia80mg/ml	8,17	,543	6,77	9,56	
Melia40mg/ml	7,83	,307	7,04	8,62	
Melia20mg/ml	6,50	,764	4,54	8,46	
4%Tween20	5,33	,558	3,90	6,77	
Cymbopogon citratus 5%	1,17	,401	,13	2,20	
Cymbopogon citratus 2. 5%	10,00	,000	10,00	10,00	
Cymbopogon citratus 1.25%	10,00	,000	10,00	10,00	
Cymbopogon citratus 0.625%	3,00	1,291	-,32	6,32	
2%Tween80	,33	,211	-,21	,88	
Eucalyptus globulus 5%	7,67	,882	5,40	9,93	
Eucalyptus globulus2.5%	4,67	1,174	1,65	7,68	
Eucalyptus globulus 1.25%	1,00	,447	-,15	2,15	
Eucalyptus globulus 0.625%	,33	,211	-,21	,88	
Ximenia160mg/ml	5,00	,683	3,24	6,76	
ximenia80mg/ml	4,83	,401	3,80	5,87	
ximenia40mg/ml	4,33	,843	2,17	6,50	
ximenia20mg/ml	2,83	,307	2,04	3,62	
Pyrethrum160mg/ml	4,67	1,256	1,44	7,90	
pyrethrum80mg/ml	4,00	,365	3,06	4,94	
pyrethrum40mg/ml	3,83	,601	2,29	5,38	
pyrethrum20mg/ml	3,67	1,145	,72	6,61	
kerosene:2%Tween80:3%Ethanol	4,00	1,211	,89	7,11	
Tobaco160mg/ml	3,50	,764	1,54	5,46	
Tobaco80mg/ml	3,00	,775	1,01	4,99	
Tobaco40mg/ml	2,33	,422	1,25	3,42	
Tobaco20mg/ml	1,83	,401	,80	2,87	
Ethanol 3%	,67	,333	-,19	1,52	
Diazinon 0.1%	3,17	,946	,74	5,60	
Distl water	,33	,211	-,21	,88	
Total	4,13	,215	3,71	4,56	

Annex 17: Fofmate for data collection in the field study

Date _____

Zone_____,**District**_____,**PA**_____,**Altitude**_____,**Latitude**_____,**Longitude**

No	wners' name	Animal spp		Age		Sex		Body Condi tion		Lesion status				Lesion, Locations						Ticks, Locations						Lice, Locations						Sh.Keds Location		
		Sheep	Goats	Adult	Youn	Male	Femal	Good	Poor	0	1	2	3	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3
1																																		
2																																		
3																																		
4																																		

9. SIGNED DECLARATION SHEET

This thesis is my original work, has not been presented for a degree in any other university and that all sources of materials used for thesis have been duly acknowledged.

Name: Hailu Wondimu Dr)

Signature: _____

Date of submission: June 22/2010

This thesis has been submitted for examination with our approval as university advisors.

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