

***In vivo* Insecticidal Activity of Formulations Containing Essential Oils of *Cymbopogon citratus* Stapf and *Eucalyptus globulus* Labill. against *Melophagus ovinus* in Sheep**

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Abstract

Background: National insecticide dipping program has been performed in Ethiopia for the last decade on sheep and goat to control ectoparasites. However, there is still high burden of ectoparasite in the country. On the other hand, the widespread use of chemical insecticides has significant drawbacks including increased cost, handling hazards, threat to human health and environment and insecticide resistance. These facts prompted the search for new alternative insecticides from plants for the control of ectoparasite.

Objective: To determine insecticidal potency of essential oil formulations on sheep naturally infested by *Melophagus ovinus* (sheep ked).

Method: A randomized block design studies were used on naturally ked infested sheep (n=6) as treatment and control (negative/Tween 80 and positive/diazinon) groups to evaluate insecticidal activity of two essential oils from *Cymbopogon citratus* and *Eucalyptus globulus* against *Melophagus ovinus* ectoparasite.

Result: The essential oils showed promising insecticidal activity against sheep ked. *C. citratus* essential oil has higher antiparasitic activity than *E. globulus* essential oils. *C. citratus* oil showed 94.7%, 87%, and 90.2% parasite reduction of sheep ked parasite at a concentration of 0.6254%, 0.3125% and 0.1563%, respectively. The *C. citratus* essential oil has no differential ($p>0.05$) insecticidal activity compared to diazinon at a concentration of 0.6254% and 0.1563%. Moderate antiparasitic activity was recorded in the groups of sheep treated by *E. globulus* essential oil.

Conclusion: Essential oils from *C. citratus* and *E. globulus* are potential plant sources of insecticides that can substitute diazinon for the management of *M. ovinus* in sheep. It is recommended that essential oils from *C. citratus* and *E. globulus* can be potential bio-rational insecticides in eco-friendly control of ectoparasite in sheep.

Key Words: *Cymbopogon citratus*, essential oil, *Eucalyptus globulus*, insecticide, *Melophagus ovinus*

Introduction

Ethiopia has the largest livestock population in Africa with 54.3 million cattle, 25.5 million sheep and 22.78 million goats (CSA 2011). As a result the country has huge leather production potential with 2.4 million hides, 10 million sheepskins and 7.4 million goat skins annually (MoA and ILRI 2013). The sector has been the backbone of the country's economy by contributing large share to the GDP of the country through the export of finished and semi-finished products to the world, beside, supplying 16-18 million skins and hides for local tanneries per annum (MoA and ILRI 2013). However, today, the benefits that this sector provide for the country is deteriorating mainly because of the rejection and down grading due to low quality raw materials (Kebede and Fetene 2012), mainly due to antemortem and postmortem factors, including poor animal husbandry, disease and parasites, bad slaughtering and flaying and bad practicing during curing, collection, transportation, storage and general handling (Abadi 2000). Pre slaughter stage of production was responsible for 65% of these defects of which the majority was due to a disease caused by

ectoparasite (Kebede and Fetene 2012; Teshome, 2016). Ectoparasitic skin diseases of domestic ruminants that are caused by lice, sheep ked, ticks and mange mites are among the major problems that result in serious economic loss to smallholder farmers, the tanning industries and the country at large (Teshome 2016).

The prevalent ectoparasites on ruminants are tick (37.66%), mange (10.38%), lice (29.55%) and sheep ked (23.38%) (Teshome 2016). *Melophagus ovinus* (sheep ked) which accounts for 32.57% infestation of sheep was the main ectoparasite causing defects followed by *Bovicola ovis* (22.28%) (Tadesse et al. 2011). Highly potent insecticides are routinely used in Ethiopia for the control of sheep ked infestation (Zewdie 2010). However, indiscriminate use of chemical insecticides has led to insecticide resistance in parasite populations (Wall 2007; Lifschitz et al. 2008; Kumar et al. 2011). Besides their continues application has led to a number of environmental and health problems (Wall 2007). Cognizant of high prevalence of sheep ked among sheep farmers and problems related to synthetic chemical insecticides;

calls for search for alternative bio rational
insecticides from natural sources which are safe,
effective and environmental friendly. The use of
herbal medicines

by traditional healers and the rural community at large for treatment of sheep ked parasite remains the main stay of health care system for the majority of the population in Ethiopia. A previous study reported promising insecticidal activity of *Cymbopogon citratus*, and *Eucalyptus globulus* essential oils against *M. ovinus* (Gemeda et al. 2014). However, the study was conducted *in vitro* and no data is available on the insecticidal activity of these essential oils against *M. ovinus* in sheep (*in vivo*). This study was therefore conducted to evaluate the insecticidal activity of *C. citratus* (DC.) Stapf (Poaceae), *Eucalyptus globulus* Labill (Myrtaceae) essential oils in naturally *M. ovinus* sheep ked infested sheep.

Material and methods

Plant material collection, identification and extraction: *C. citratus* and *E. globulus* essential oils were extracted from aerial and leaf part, respectively in Wondo-Genet Agricultural Research Center, EIAR, Wondo Genet, Ethiopia. Fresh plant materials (250g) were placed in a 5L round-bottom distillation flask and the plant material was wetted with 3L distilled water. The essential oils were obtained by hydro-distillation using Clevenger-type apparatus continuously for 3 hours. The volatile oils were taken from the upper layer. The aqueous layer was further portioned using dichloromethane to extract and enrich the essential oil from the water layer. The organic layer (dichloromethane extract) was filtered and dried with anhydrous sodium sulfate and concentrated using rotary evaporator to give the crude essential oil.

Formulations: The formulations recipe of essential oils were optimized in the Formulation Laboratory of the Traditional and Modern Medicine Directorate, EPHI. The formulation recipes are as follows: Formulation 1: 2.5% *E. globulus* oil in 2% aqueous Tween 80; Formulation 2: 1.25% *E. globulus* oil in 2% aqueous Tween 80; Formulation 3: 0.625% *E. globulus* oil in 2% aqueous Tween 80; Formulation 4: 0.625% *C. citratus* oil in 2% aqueous Tween 80; Formulation 5: 0.3125% *C. citratus* oil in 2% aqueous Tween 80 and Formulation 6: 0.156% *C. citratus* oil in 2% aqueous Tween 80.

In Vivo Insecticidal Assay: Experimental study design recommended by World Association for the Advancement of Veterinary Parasitology was followed in this study (Holdsworth et al. 2006). Sheep of the same weight and age (6-12 months) with adequate natural infestation were bought from the Fiche Local market and housed indoors in individual cages and fed daily. Animals were acclimatized to the facilities and the feeding /regime for 14 days prior to the treatment. The animals were identified by

uniquely numbered ear tag. A randomized block design was used to randomly allocate the animals (n=6) into treatment and control (negative/Tween 80 and positive/diazinon) groups. The groups were maintained in isolation for the duration of the study to avoid contact with animals in any other group. On day 0, the numbers of live sheep ked found on 40 hair partings (10 cm wide) on four body sites (neck, shoulder, withers, flank and rump) were counted and recorded for each animal. All the treatment groups were treated by the essential oils at day-0 and day-14. Positive controls were treated only once with Diazinon at the start of the experiment (day 0) at a dose rate of 0.001%. The groups were selected by lottery method for each treatment as follows: Group 1: 1.25% *E. globulus*; Group 2: Diazinon; Group 3: 0.625% *C. citratus*; Group 4: 0.15625% *C. citratus*; Group 5: 2% Tween 80; Group 6: 2.5% *E. globulus*; Group 7: 0.3125% *C. citratus*; and Group 8: 0.625% *E. globulus*. Sheep ked parasite counting was done every week for 56 days. At each time point the mean live sheep ked count was taken for each group and the results compared with that of the untreated control group and positive control group. Responses to the treatment were monitored on alternate days at the time of drug application in terms of mean parasite count, and percent parasite reduction. Personnel involved in the collection of efficacy data were masked to the treatment assignment of the animals.

Data analysis: The *in vivo* sheep ked counts (every week for 56 days after treatment application) were recorded. Repeated measure analysis of variance was used to test for the effects of time and the time-treatment interaction. One-way analysis of variance (using Minitab 16.0 software) was used to test for treatment effects on each day and treatment groups were compared to the controls using Tukey test with a family error rate of 0.05. A probability of $p < 0.05$ was considered to be significant.

Results

Table 1 shows *M. ovinus* reduction effect of *C. citratus* oil at different concentration on sheep on various days' post treatment. As can be seen in Table1, differences between mean percent reductions as identified by Tukey's Tests are displayed. Different concentration of *C. citratus* essential oils has comparable effect of reducing *M. ovinus* parasite with Diazinon after 7, 14, 28 and 49 days post treatment of sheep. Compared to standard drug used in the study, *C. citratus* essential oil at a concentration of 0.3125% has statistically lower ($p < 0.05$) toxic effects on *M. ovinus* parasite in sheep on 7, 21, 35, 42 and 56 days of post treatment. On the

other hand, at all study points in post treatment day,
all essential oil concentrations have statistically

no differential parasite reduction potency. The mean percentage reduction of parasite in sheep treated with *C. citratus* oils ranged between 73.8% – 94.7%,

38.2%–87.0%, 48.2%–90.2% at a concentration of 0.6254%, 0.3125% and 0.1563 respectively.

Table1: *In vivo* insecticidal activity of *C. citratus* essential oil formulation on naturally *M. ovinus* infested Sheep, Fiche, Oromia region, Ethiopia

	Day	Treatment dose (v/v)				
		2%T80	0.1000% D	0.6254% C	0.3125% C	0.1563% C
Sheep Ked percent reduction	7 (PT)	39.5±26.4 ^c	100.0±0.0 ^a	75.5±15.6 ^{ab}	38.2±21.2 ^c	48.2±24.6 ^{bc}
	14(PT)	36.1±21.9 ^c	100.0±0.0 ^a	73.8±14.9 ^{ab}	87.0±12.5 ^{ab}	61.6±22.1 ^{bc}
	21(PT)	25.2±20.3 ^c	100.0±0.0 ^a	91.5±9.0 ^{ab}	75.5±7.4 ^b	81.3±8.5 ^{ab}
	28(PT)	43.3±20.6 ^c	100.0±0.0 ^a	86.0±10.5 ^{ab}	72.9±13.6 ^{ab}	80.4±13.2 ^b
	35(PT)	36.1±16.8 ^c	100.0±0.0 ^a	93.2±5.5 ^a	61.5±6.8 ^b	84.4±10.1 ^a
	42(PT)	32.6±21.7 ^c	100.0±0.0 ^a	84.7±11.8 ^{ab}	66.5±10.6 ^b	65.6±23.2 ^b
	49(PT)	33.3±18.6 ^c	100.0±0.0 ^a	90.0±6.2 ^{ab}	70.9±15.9 ^b	86.6±13.7 ^{ab}
	56(PT)	38.0±16.3 ^c	100.0±0.0 ^a	94.7±4.6 ^a	71.9±7.7 ^b	90.2±11.9 ^a

C: *C. citratus*; D: Diazinon; PT: post treatment; T: Tween;. Values are expressed as percent reduction mean ± SD; mean percent reductions that do not share letter in the same rows are significantly different ($P<0.05$)

As indicated in Table2, *E. globulus* essential oil has moderate insecticidal activity on *M. ovinus* on sheep. Analysis of parasite on sheep on 14th and 28th days after spray of different concentrations of *E. globulus* essential oil formulations in 2% Tween 80 on sheep have no differential ($p<0.05$) sheep ked reduction activity compared to Diazinon. The percent reduction

of *M. ovinus* parasite in sheep at lowest concentration of *E. globulus* (0.625%) was statistically lower compared to Diazinon. The highest percentage of parasite reduction (82%) by this oil was observed at a concentration of 2.5% on 28th day post treatment of sheep, while the lowest was observed at 1.25% concentration of *E. globulus* oil on 7th day of post-treatment.

Table 2: *In vivo* insecticidal activity of *E. globulus* essential oil formulation on naturally *M. ovinus* infested sheep, Fiche, Oromia region, Ethiopia

	Day	Treatment dose (v/v)				
		2%T80	0.1000%D	2.5000%E	1.2500%E	0.6250%E
Sheep Ked percent reduction	7 (PT)	39.5±26.4 ^{bc}	100.0±0.0 ^a	43.9±7.6 ^b	9.5±12.3 ^c	49.8±26.3 ^b
	14(PT)	36.1±21.9 ^c	100.0±0.0 ^a	78.1±13.2 ^{ab}	38.9±29.3 ^c	53.9±29.7 ^{bc}
	21(PT)	25.2±20.3 ^c	100.0±0.0 ^a	62.5±17.8 ^b	61.5±17.0 ^b	54.2±21.9 ^{bc}
	28(PT)	43.3±20.6 ^d	100.0±0.0 ^a	82.3±14.5 ^{ab}	54.4±14.4 ^{cd}	43.2±20.6 ^{bc}
	35(PT)	36.1±16.8 ^c	100.0±0.0 ^a	68.6±14.0 ^b	48.4±25.3 ^{bc}	51.1±15.4 ^{bc}
	42(PT)	32.6±21.7 ^c	100.0±0.0 ^a	69.5±21.8 ^b	60.4±14.3 ^b	56.0±13.4 ^c
	49(PT)	33.3±18.6 ^c	100.0±0.0 ^a	71.2±17.5 ^b	67.7±9.3 ^b	54.0±18.4 ^{bc}
	56(PT)	38.0±16.3 ^c	100.0±0.0 ^b	78.9±16.6 ^{ab}	58.8±11.6 ^{bc}	38.0±16.3 ^c

E: *E. globulus*; D: Diazinon; PT: post treatment; T: Tween. Values are expressed as percent reduction mean ± SD; mean percent reductions that do not share letter in the same rows are significantly different ($P<0.05$)

The two aromatic plant essential oils tested in this study showed parasite reduction activity in sheep against *M. ovinus* (Fig 1). The figure demonstrates that *E. globulus* oil has considerable lower sheep ked reduction activity compared to *C. citratus*, though the concentration used for *E. globulus* (0.625%) is fourfold higher than that of *C. citratus* (0.1563%).

A significantly high sheep ked parasite reduction was observed on day 35 and 49 by *C. citratus* essential oil compared to *E. globulus* oil. Analysis of the data revealed that *C. citratus* essential oil has comparable reduction effect to Diazinon while *E. globulus* essential oil showed significantly lower ($p<0.05$) percent reduction.

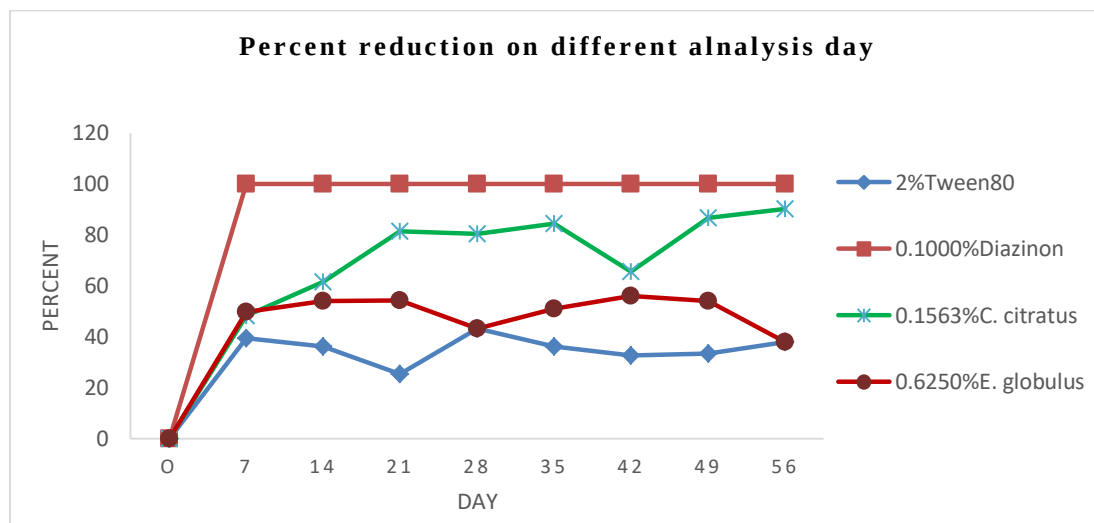


Figure 1: Percent reduction of *M. ovinus* parasite in sheep treated with *C. citratus*, *E. globulus* and Diazinon

Discussions

In the present study, six essential oil formulations from two medicinal plants were examined on sheep naturally infested by sheep ked. *C. citratus* essential oil has statistically comparable insecticidal activity in vivo with synthetic insecticide Diazinon. Synergy between the complex chemical constituents in the essential oil may be responsible for this insecticidal activity or may be due to potency of major compounds against *M. ovinus*. This finding showed *C. citratus* essential oil have similar activity with that of synthetic insecticide and can be the best candidate to alternate synthetic insecticides which corroborates the previous study in which showed *C. citratus* oil has comparable results with a synthetic insecticide Ivermectin (Gemedá et al. 2014). This finding also corroborates with the previous study of various researchers. Freitas et al. (2010), Vera et al. (2014) and Kabera et al (2011) reported insecticidal activity of *C. citratus* essential oils and its major constituents against adult and larvae of insects. The insecticidal activity of *C. citratus* attributed to its major constituents citral, or its isomers geranial and neral (Freitas et al. 2010). The essential oil and its constituents have recognized wide variety of biological activity against house fly (Sinthusiri and Soonwer 2013), tick (Singh et al. 2014), mite (Hanifah et al. 2011), pest (Ayvaz et al. 2010, Olorunnisola et al. 2014, Sonker et al. 2014), antimicrobial (Sonker et al. 2014), mosquito (Soonwera and Phasomkusolsi 2014) and leishmania (Machado et al. 2012).

Insecticidal activity was observed against *M. ovinus* sheep ked parasite by *E. globulus* essential oil in *in-vivo* model. Moderate parasite reduction by the essential oil from the eucalyptus may be due to phytochemicals including the major constituents 1, 8-cineole that could affect the nerve of the sheep ked

leading to a disruption of vital system (Slimane et al. 2014). This finding was in line with previous study of Gemedá et al. (2014) that reported insecticidal activity of *E. globulus* oil against sheep ked. Song et al. (2016) also reported the effect of eucalyptus oils against stored mite and grain insects where they found fifty percent toxicity. The plant has insecticidal activity against egg, larvae and adult stage of insects (Maciel et al. 2009; Kumar et al. 2012). Moreover, other biological activities were reported including acaricidal activity (Pirali-Kheirabadi et al. 2009).

Superior insecticidal activity by *C. citratus* essential oils may be attributed to major constituents citral and other active compounds including myrcene, citronellal and neral found in the oils. *C. citratus* oil has better insecticidal activity against sheep ked in the present study than *E. globulus* essential oil. The same conclusion has been reported in the study conducted by a previous study (Gemedá et al. 2014), where they reported high insecticidal activity of *C. citratus* essential oils against *M. ovinus*. In a similar study, Bossou et al. (2013), *C. citratus* has the best insecticidal activity against *Anopheles gambiae* compared with other tested essential oils.

Conclusion

This study that was conducted to evaluate *in vivo* insecticidal activity of two aromatic medicinal plants at three different concentrations against sheep ked showed promising results. The essential oil from *C. citratus* showed the highest insecticidal activity against *M. ovinus* at lowest concentration tested. Moreover, moderate insecticidal activity was observed by *E. globulus* essential oil against *M. ovinus*. These aromatic plants could be potential sources of biorational insecticides against ectoparasites.

Further study is needed to evaluate the stability of the formulations and dosage validation using community based study.

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