



HOKKAIDO UNIVERSITY

Title	Enhancement of acaricide activity of citronella oil after microemulsion preparation
Author(s)	Chaisri, Wasana; Chaaryana, Wantida; Pikulkaew, Surachai et al.
Citation	Japanese Journal of Veterinary Research, 67(1), 15-23
Issue Date	2019-02
DOI	https://doi.org/10.14943/jjvr.67.1.15
Doc URL	https://hdl.handle.net/2115/72727
Type	departmental bulletin paper
File Information	015-023 Witaya Suriyasathaporn.pdf



Enhancement of acaricide activity of citronella oil after microemulsion preparation

Wasana Chaisri¹⁾, Wantida Chaiyana²⁾, Surachai Pikulkaew¹⁾,
Siriporn Okonogi²⁾ and Witaya Suriyasathaporn^{1,*}

¹⁾ Department of Food Animal Clinics, Faculty of Veterinary Medicine, Chiang Mai University, Mueang, Chiang Mai, Thailand

²⁾ Department of Pharmaceutical Sciences, Faculty of Pharmacy, Chiang Mai University, Mueang, Chiang Mai, Thailand

Received for publication, February 27, 2018; accepted, June 14, 2018

Abstract

The objectives of this study were to evaluate the preparation of microemulsions for citronella oil and subsequently to compare their acaricide efficacy as against conventional citronella oil. The chemical analysis of the commercial citronella oil used was carried out using an Agilent 6890 gas chromatograph (GC) coupled to an electron impact ionization (EI, 70 eV) with an HP 5973 mass selective detector (MSD). In comparison among the emulsifier systems, the mixture of Tween 20 with propylene glycol in ratios 3:1, 2:1, 1:1, 1:2, and 1:3, the pseudoternary phase diagrams were conducted and used to define the most suitable system for preparing citronella oil microemulsions and using them in acaricide efficacy testing. The citronella microemulsions in concentrations ranging from 0.39–25% w/w were prepared and tested for their acaricide efficacies using Adult Immersion Test and Larval Package Test. The acaricide efficacies were determined by percentages of mortalities of larval and adult ticks and the values of LC_{50} and LC_{99} . Results show that the most suitable emulsifier system was in a ratio of 3:1. The physicochemical characteristics indicated that the size of the microemulsions were in the range of 19.6 ± 0.4 nm to 47.3 ± 2.3 nm with moderate polydispersity index (0.3–0.7). The microemulsions had stronger acaricide efficacy than the conventional citronella oil, indicated by significant lower LC_{50} and LC_{99} values. The LC_{99} of the microemulsions against larval and adult ticks at 24 h were 0.78% w/w (0.56–1.02) and 28.4% w/w (23.27–37.43), respectively. These results demonstrated that microemulsion preparation can be used to improve the acaricide efficacy of citronella oil.

Key Words: microemulsion, citronella oil, acaricide efficacy, *Rhipicephalus microplus*

Introduction

In tropical countries, tick-borne diseases, especially bovine babesiosis and anaplasmosis, can cause sudden death of severely infected animals. The cattle tick *Rhipicephalus microplus* (*R. microplus*) is a significant vector of these deadly diseases¹¹⁾. In general practice, many

dairy farmers use synthetic insecticides, such as pyrethroids, organophosphates, carbamates, and macrocyclic lactones, to control external parasites⁹⁾. However, the extensive use of these insecticides increases the risk of insecticide residues in dairy products and the environment^{22,24)}. From 96 milk samples collected from Mexico, 39.6% contained detectable level of organophosphorus

*Corresponding author: Witaya Suriyasathaporn, Department of Food Animal Clinics, Faculty of Veterinary Medicine, Chiang Mai University, Mueang, Chiang Mai, 50100, Thailand
Phone: +66-53-94-8023. Fax: +66-53-94-8065. E-mail: suriyasathaporn@hotmail.com
doi: 10.14943/jjvr.67.1.15

pesticide residues²⁴). In Brazil, 20% of milk samples were contaminated with organophosphate, 16.7% with carbamate and some samples with both pesticides⁶). Similarity, approximate 14% of 132 raw milk samples from dairy farms in Minas Gerais State, Brazil was contaminated with pyrethroids and 20% with macrocyclic lactones²²). In response to the insecticides residue problems, many researchers attempted to develop bioinsecticide products especially from the essential oil of *Cymbopogon winterianus*, the so-called citronella oil^{14,15,23}), that possesses sufficient biological properties, including repellent, acaricidal, and larvicidal^{8,16,17}) and which in particular acts against *R. microplus*^{4,16}). However, the acaricide efficacy of the natural oil has not been sufficient for it to replace synthetic insecticides in livestock industries.

Microemulsions, isotropic mixtures of oil, water and surfactant providing thermodynamic stability, are potential drug delivery vehicles. In pharmaceutical applications, the vehicles were used to improve acaricide efficacies in both some natural products^{3,28}) and synthetic pesticides^{10,20}). However, knowledge on both preparation of microemulsions for citronella oil and its acaricide efficacies were not reported. Therefore, the objectives of this study were to evaluate the preparation of microemulsions for citronella oil and subsequently to compare their acaricide efficacy with citronella oil with conventional preparation.

Materials and Methods

Chemical analysis of citronella oil: Citronella oil was obtained from Thai-China Flavors and Fragrances Industry Co., Ltd., Bangkok, Thailand. The chemical analysis of the oil was carried out using an Agilent 6890 gas chromatograph (GC) coupled to an electron impact ionization (EI, 70 eV) with an HP 5973 mass selective detector (MSD). An HP-5MS fused silica capillary column (30.0 m × 250 µm, i.d. 0.25 µm film thickness,

HP, USA) was used. The carrier gas was helium (grade 5.0) with a flow rate of 1.0 mL/min. The analytical conditions used were as follows: the injector temperature was set at 250°C; the oven temperature was initially held at 70°C for 3 min, and then raised by 3°C/min to 188°C, further raised to 280°C with a heating rate of 20°C/min (isothermal 3 min). For quality assurance of GC-MS, the programmed-temperature Kováts retention indices (RI) was determined. RI was obtained by GC-MS analysis of an aliquot of the citronella oil spiked with an *n*-alkanes mixture containing each homologue from *n*-C₁₁ to *n*-C₂₇. The active compounds were identified by comparing their mass spectra with reference compounds from the WILEY version 2007 and NIST version 2005 databases. The amount of each active compound was proportional to the area under the peak (% area) when compare with all other substances which found in the same sample. The RI is based on the retention of *n*-alkanes with an even number of carbon atoms, the calculation as follows¹³):

$$I = 100Z + 100 \left(\frac{\log t'_{R(x)} - \log t'_{R(Z)}}{\log t'_{R(Z+1)} - \log t'_{R(Z)}} \right)$$

where I = Kovats retention index (RI)

t'_R = retention time

x = the compound of interest

Z = number of carbon atoms in the *n*-alkane

Z + 1 = number of carbon atoms in the *n*-alkane + 1

Microemulsion preparation: For comparison among the emulsifier systems, the mixture of Tween 20 with propylene glycol in ratios 3 : 1, 2 : 1, 1 : 1, 1 : 2, and 1 : 3, the pseudoternary phase diagrams were conducted and used to define the most suitable system for preparing citronella oil microemulsions and testing their acaricide efficacy. Briefly, the solutions were prepared by mixing the appropriate amount of oil with the surfactant mixtures of emulsifier systems and subsequently titrated with water under moderate

agitation using a vortex mixer until homogenous. The solutions were designated as microemulsions when they were flowable and transparent. The pseudoternary phase diagrams were drawn using the Origin 8 program, and the system with the largest microemulsion region was identified as the most suitable form. Microemulsions of concentrations ranging from 0.039% w/w to 25% w/w were prepared according to the selected system.

Characterization and stability testing of microemulsion: The microemulsions obtained were characterized for their particle sizes and size distributions (polydispersity index: PDI) using Zetasizer® version 5.00 (Malvern Instruments, Malvern, UK). The particle size measurements were performed at 25°C with a scattering angle of 173°. Each sample was measured 13 times. The flow characteristics and viscosity of the formulations were measured in triplicate using a Brookfield viscometer. The pH values of the microemulsions were measured using the Mettler Toledo 320 pH meter (Switzerland). The stability of the microemulsions was assessed using the heating-cooling cycle method³⁾. The microemulsions were kept at 4°C for 24 h and moved to 45°C for another 24 h for 12 cycles. The physical appearance and the characteristics of all microemulsions were determined after their last cycles.

Collection and identification of R. microplus: Engorged female ticks were collected from naturally infested cattle at a smallholder dairy farm in Chiang Mai Province, Thailand. They were placed in plastic boxes with orifices, and immediately transported to a laboratory for identification. All ticks were specific identified using stereomicroscope based on their morphologies for *R. microplus* as described by Walker *et al.*²⁷⁾. Briefly, adult female *R. microplus* has broad oval porose areas. The hypostomal teeth are in 4 + 4 column arrangement. The internal margin of palp article has no protuberance and is

short and distinctly concave. Coxa I spurs have distinct, whereas coxae 2 and 3 spurs are present. The genital aperture posterior lips form a broad U shape.

Preparation of R. microplus adults and larvae: After identification, all female ticks were transferred into small plastic boxes with holes and incubated at 27–28°C with 80–85% relative humidity for 14–18 days until eggs were laid. The eggs laid were separated and transferred to polypropylene tubes with orifices on the lid. Unfed live larvae of 14–21 days of age after hatchability were used for acaricide efficacy testing.

Acaricide efficacy test: Citronella oil was serially diluted in 2% Tween 20 to obtain concentrations of 25.0, 12.5, 6.25, 3.125, 1.5625, 0.78, and 0.39% w/w. The acaricide efficacies against ticks of citronella oil and citronella microemulsion were evaluated using the Larva Package Test (LPT) and Adult Immersion Test (AIT), according to Drummond *et al.*⁵⁾, with some modifications. For LPT, approximately 100 larvae were placed onto each filter paper in 4 cm² envelopes (Whatman, 80 g, No. 4) moistened with 2 ml each of either citronella oil or citronella microemulsion. The borders of the envelopes were then folded over and fastened with metallic clips. The packages were incubated at 27–28°C and 85–95% relative humidity for 48 h.

For AIT, 10 female ticks were immersed for 10 min in each of the respective concentrations of citronella oil and citronella microemulsion. Tween 20 at 2% w/w in distilled water was used for the negative control group. Cypermethrin was used for the positive control. After immersion, the ticks were dried on tissue paper before being placed in plastic boxes with holes and incubated at 27–28°C with 70–80% relative humidity for 48 h.

Statistical analyses: The acaricide efficacies were determined in 2 ways as 1) mean $\pm$ standard

Table 1. Characteristics of chemical components of citronella oil in this study

Peak No.	RT. ^a	RI. (Lit.) ^b	RI.(Oil) ^c	Compounds	% Area	% QA
1	10.53	1153	1159	citronellal	31.4	98
2	13.64	1228	1237	L-citronellol	12.1	97
3	14.74	1255	1264	geraniol	19.4	97

^aRetention time (minutes)^bKováč Retention Index from literature data^cKováč Retention Index obtained from this study

QA = %similarity or quality match with NIST and WILEY databases

deviation (SD) of mortality rate of adult and larval ticks at 24 and 48 h after treatment and 2) the value of lethal concentrations (LC) of conventional citronella oil and citronella microemulsion that killed 50% (LC₅₀) and 99% (LC₉₉) of larvae and adult ticks. The dead larvae were reconfirmed as dead using a stereomicroscope. Each treatment was performed in three replicates. The percentage of mortality was calculated as follows:

$$\% \text{ mortality} =$$

$$\frac{\% \text{ mortality of experimental group} - \% \text{ mortality of control group}}{100 - \% \text{ mortality of control}}$$

$$\times 100$$

Comparisons of acaricide efficacies were evaluated in two ways including 1) comparisons within the same formulation and same time and 2) comparison within the same concentration, using analysis of variance with Tukey test comparison. The lethal concentrations (LC) of citronella oil and citronella microemulsion that killed 50% and 99% of larvae and adult ticks were calculated by probit analysis using SAS program. Significant levels in all tests were defined at $P < 0.05$.

Results

The main chemical components of citronella oil, kovats retention index (RI) and % quality match (QA) are shown in Table 1. RI is useful references for tentative compound identification by GC-MS. In this study, RI of main compounds

found in citronella oil nearly the same value on any GC system under any set of parameters from references. Mass spectra of main compounds match closely with the corresponding standard library of 97–98%.

The chromatogram of citronella oil from GC-MS is shown in Fig. 1. Three main chemical compounds of citronella oil were separately identified: citronellal (31.4%), geraniol (19.4%), and L-citronellol (12.1%) (Table 1). The microemulsion regions are indicated by the dark areas in the phase diagrams shown in Fig. 2. Among the tested systems in this study, system containing Tween 20 and propylene glycol with a weight ratio of 3:1 exhibited the largest microemulsion region (34.62%), indicating it was the most suitable form of citronella microemulsion. Therefore, this system was selected for the acaricide efficacy testing. Overall characteristics of all formulations were transparent yellow with a pH of 5.0. Means and SD of particle sizes were in between 19.6 ± 0.4 nm and 47.3 ± 2.3 nm, with moderate PDI (0.3–0.7) ($n = 13$). All formulations exhibited good stability and low viscosity (0.035–0.0642 mPS), with Newtonian flow behavior.

Percentages of larval mortality from all concentrations of citronella oil and citronella microemulsions are shown in Table 2. Mortality rates with citronella oil of 0.39% w/w were significant lower than the rate obtained from microemulsion at the same concentration at 24 h. A concentration of microemulsion (0.78%) causing 100% larval mortality rate at 24 h was less than that of conventional citronella oil (3.125%), indicating higher acaricide efficacies of the microemulsion. The acaricide efficacies against

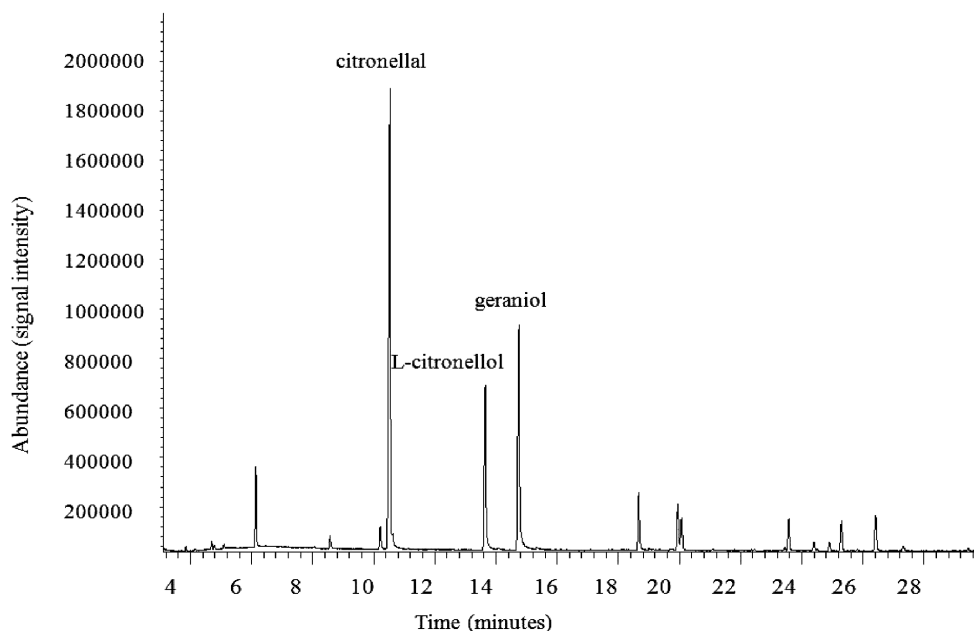


Fig. 1. Chromatogram of citronella oil.

adult cattle ticks are shown in Table 3. Mortality rates with microemulsion at 25% w/w at both 24 and 48 h were higher than the rate obtained from cypermethrin significantly. Values of LC_{50} and LC_{99} and their 95% confidence intervals of both citronella oils are shown in Table 4. For AIT, LC_{99} of conventional citronella oil (54.29% w/w) were significantly higher than that of citronella microemulsion (28.44%). However, no significant difference of LC_{99} for LPT was observed between conventional citronella oil and citronella microemulsion.

Discussion

The three main active compounds—citronellal, geraniol, and L-citronellol—found in this study (Fig. 1) were classified as monoterpenes that are known to have acaricide properties especially citronellal^{15,25)} and geraniol^{15,23)}. The variation in the main compound of citronella oil is due to differences of environment and plant management across regions¹⁹⁾.

In order to obtain the suitable system for generated citronella microemulsion, different

weight ratios of Tween 20 and propylene glycol were used. In this study, the ratio of 3 : 1 was the most suitable system since it could produce the largest region of microemulsion. It was noted that surfactant plays an important role in the formation of microemulsions. The role of the surfactants is to reduce the interfacial tension between the oil and the water phase which is crucial for the formation of microemulsions, hence, the higher proportion of surfactant in the surfactant mixtures could produce the larger region of microemulsion in the pseudoternary phase diagram^{3,28)}.

This study obtained the ticks (*R. microplus*) from small holder dairy farms that have routinely used insecticides, especially cypermethrin, for a long time. Improper use of cypermethrin, including incorrect dosage, frequency and duration, can lead to cypermethrin resistance in *R. microplus*⁷⁾. According to Veiga *et al.*²⁶⁾, the mortality rate of ticks less than 95% was considered as ineffectiveness of acaricide. Therefore, the approximately 50% mortality of adult ticks using cypermethrin (Table 2) indicated some degree of resistance to insecticides by the ticks used in this study. This agreed with Mendes *et al.*¹⁸⁾ and

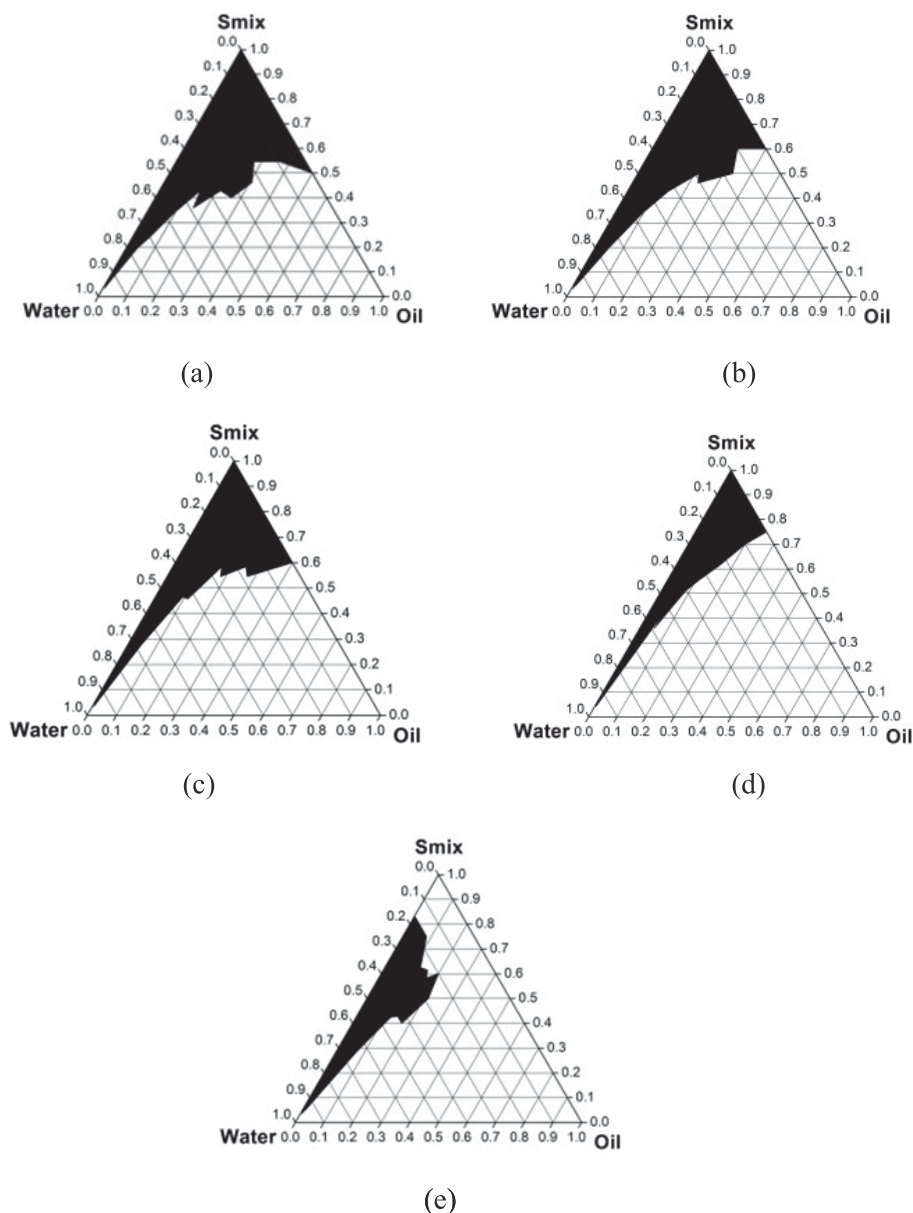


Fig. 2. The pseudoternary phase diagrams of the system of Tween 20 mixed with propylene glycol (Smix) in the ratios of 3 : 1 (a), 2 : 1 (b), 1 : 1 (c), 1 : 2 (d), and 1 : 3 (e).

Veiga *et al.*²⁶⁾, who also found resistance to cypermethrin by *R. microplus*.

The standard citronella oil prepared in this study had similar ranges of acaricide activity for larval ticks as reported by a previous study²⁾, and was in between the acaricide efficacies for adults ticks reported by two other studies^{16,17)}. For larvae with the concentrations of citronellal substance (35%) in citronella oil at 25%, Chagus *et al.*²⁾ reported a 97% mortality rate of *R. microplus*,

but they found a low acaricide efficacy for adult ticks. Martin¹⁶⁾ reported 100% acaricide efficacy against adult ticks, more than 85% found in this study, using 12% to 50% w/w of citronella oil enriched by citronellal (41.15%). In contrast, Mello-Peixoto *et al.*¹⁷⁾ reported lower acaricide efficacy (killing 87% of gravid female ticks) using 100% w/w citronella oil with citronellal at 39.42%. The lower acaricide efficacy found in our study might relate to the use of suspected

Table 2. Percentages of mortality of larval ticks in either conventional citronella oil or citronella microemulsion

Concentration of oil (% w/w)	Citronella oil		Citronella microemulsion	
	24 h	48 h	24 h	48 h
2% Tween	0.0 ± 0.0 ^{a, x}	32.7 ± 14.04 ^{a, y}	0.0 ± 0.0 ^{a, x}	32.7 ± 14.04 ^{a, y}
0.39	30.3 ± 15.82 ^{b, x}	79.3 ± 18.10 ^{b, y}	96.7 ± 1.55 ^{b, y}	100.0 ± 0.0 ^{b, y}
0.78	82.5 ± 17.73 ^c	98.3 ± 2.17 ^c	100.0 ± 0.0 ^c	100.0 ± 0.0 ^b
1.5625	99.6 ± 0.63 ^c	100.0 ± 0.0 ^c	100.0 ± 0.0 ^c	100.0 ± 0.0 ^b
3.125	100.0 ± 0.0 ^c	100.0 ± 0.0 ^c	100.0 ± 0.0 ^c	100.0 ± 0.0 ^b
6.25	100.0 ± 0.0 ^c	100.0 ± 0.0 ^c	100.0 ± 0.0 ^c	100.0 ± 0.0 ^b
12.5	100.0 ± 0.0 ^c	100.0 ± 0.0 ^c	100.0 ± 0.0 ^c	100.0 ± 0.0 ^b
25	100.0 ± 0.0 ^c	100.0 ± 0.0 ^c	100.0 ± 0.0 ^c	100.0 ± 0.0 ^b
Cypermethrin [*]	100.0 ± 0.0 ^c	100.0 ± 0.0 ^c	100.0 ± 0.0 ^c	100.0 ± 0.0 ^c

Data are expressed as the mean ± SD (n = 100 larval ticks/group)

^{a, b, c}Different letters within the same column indicate statistically significant ($P < 0.05$)

^{x, y}Different letters within the same row indicate statistically significant ($P < 0.05$)

^{*}Cypermethrin data were used to compare both Citronella oil and Citronella microemulsion

Table 3. Percentages of mortality of adult ticks in either conventional citronella oil or citronella microemulsion

Concentration of oil (% w/w)	Citronella oil		Citronella microemulsion	
	24 h	48 h	24 h	48 h
2% Tween	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a
1.56	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	3.7 ± 6.42 ^a	4.2 ± 7.22 ^a
3.125	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	18.5 ± 12.83 ^{ab}	18.9 ± 12.45 ^{ab}
6.25	7.4 ± 12.83 ^{ab, x}	11.1 ± 11.11 ^{ab, xy}	33.3 ± 11.11 ^{abc, xy}	38.4 ± 5.61 ^{b, c, y}
12.5	31.9 ± 28.66 ^{ab}	42.5 ± 28.51 ^{bc}	59.3 ± 16.97 ^{cd}	61.1 ± 14.70 ^c
25	85.2 ± 16.97 ^{bc}	88.4 ± 11.14 ^d	88.9 ± 11.11 ^d	92.1 ± 6.85 ^d
Cypermethrin [*]	51.7 ± 20.71 ^c	62.0 ± 8.83 ^{cd}	51.7 ± 20.71 ^{bc}	62.0 ± 8.83 ^c

Data are expressed as the mean ± SD (n = 10 adults ticks/group)

^{a, b, c, d}Different letters within the same column indicate statistically significant ($P < 0.05$)

^{x, y}Different letters within the same row indicate statistically significant ($P < 0.05$)

^{*}Cypermethrin data were used to compare both Citronella oil and Citronella microemulsion

Table 4. Lethal Concentrations (50% and 99%) of conventional citronella oil and citronella microemulsion for larval ticks at 24 h, with a 95% confidence interval

	LC ₅₀		LC ₉₉	
	Mean	Confidence Interval	Mean	Confidence Interval
LPT				
Citronella oil	0.53	0.39–0.69	1.13	0.89–1.78
Microemulsion	–*	–	0.75	0.58–1.10
AIT				
Citronella oil	19.73 ^a	15.19–25.99	54.29 ^a	43.18–75.99
Microemulsion	11.46 ^b	9.35–14.48	28.44 ^b	23.27–37.43

^{a, b}Different letters within the same column indicate statistically significant ($P < 0.05$)

^{*}LC₅₀ could not be calculated because the tested concentrations can kill ticks more than 50%

acaricide resistant adult ticks, as previously mentioned. In addition, differences in drug absorption due to variations in the content and the composition of a tick's cuticular lipids in its different life stages may contribute to the differing efficacies reported²¹⁾.

In this study, microemulsion preparations had higher acaricide efficacies than conventional citronella oil in all parameters, including larval mortality (Table 1), adult mortality (Table 2), LC₅₀ and LC₉₉ (Table 3). In a study of mites on sheep, neem oil prepared as a microemulsion had higher acaricide efficacy than that with a standard preparation²⁸⁾. In addition, Chagas *et al.*¹⁾ reported enhanced activity of oils when formulated in small particle sizes or in emulsifiable concentrates. Xu *et al.*²⁸⁾ have reported that the higher acaricide efficacy of a microemulsion was probably due to the synergistic effect of the surfactants contained in the formulation. In addition, a microemulsion might interrupt the continuity of the waxy layer of the tick's epicuticle, which is comprised of a complex mixture of lipids, and reduce the resistance of its skin to drug penetration^{12,21}.

Conclusion

The microemulsion of citronella oil prepared in this study exhibited higher acaricide efficacies against both larval and adult cattle ticks than pure citronella oil. The emulsifier system that generated the largest microemulsion in this study was composed of citronella oil, Tween 20 and propylene glycol in the ratio 3 : 1.

Acknowledgments

The authors are grateful for the financial support provided by the Research Administration Center, Office of the University, Chiang Mai University.

References

1. Chagas ACS, Passos WM, Prates HT, Leite RC, Furlong J, Fortes ICP. Acaricide effect of *Eucalyptus* spp. essential oils and concentrated emulsion on *Boophilus microplus*. *Braz J Vet Res Anim Sci* 39, 247-253, 2002
2. Chagas ACS, Domingues LF, Fantatto RR, Giglioti R, Oliveira MC, Oliveira DH, Mano RA, Jacob RG. In vitro and in vivo acaricide action of juvenoid analogs produced from the chemical modification of *Cymbopogon* spp. and *Corymbia citriodora* essential oil on the cattle tick *Rhipicephalus* (*Boophilus*) *microplus*. *Vet Parasitol* 205, 277-284, 2014
3. Chaiyana W, Saeio K, Hennink WE, Okonogi S. Characterization of potent anticholinesterase plant oil based microemulsion. *Int J Pharm* 401, 32-40, 2010
4. Chungsamarnyart N, Jiwajindee S. Acaricide activity of volatile oil from lemon and citronella grasses on tropical cattle ticks. *Kasetsart J (Nat. Sci. Suppl.)* 26, 46-51, 1992
5. Drummond RO. Resistance in ticks and insects of veterinary importance. In: *Pesticide management and insecticide resistance*, 1st ed. Watsan DL and A.W.A. Brown AWA, eds. Academic Press, London, pp. 303-317, 1997.
6. Fagnani R, Beloti V, Battaglini APP, Dunga KS, Tamanini R. Organophosphorus and carbamates residues in milk and feedstuff supplied to dairy cattle. *Pesq Vet Bras* 31, 598-602, 2011
7. Farias NA, Ruas JL, Santos TRB. Acaricides efficacy analysis on *Boophilus microplus* tick, in the last decade in the southern of Rio Grande do Sul. *Cienc. Rural* 38, 1700-1704, 2008
8. Gusmão NMS, de Oliveira JV, Navarro DMJF, Dutra KA, de Silva WA, Wanderley MJA. Contact and fumigant toxicity and repellency of *Eucalyptus citriodora* Hook., *Eucalyptus staigeriana* F., *Cymbopogon winterianus* Jowitt and *Foeniculum vulgare* Mill. Essential oils in the management of *Callosobruchus maculatus* (FABR.) (Coleoptera: Chrysomelidae, Bruchinae). *J Stored Prod Res* 54, 41-47, 2003
9. Ghosh S, Azhahianambi P, de la Fuente J. Control of ticks of ruminants, with special emphasis on livestock farming systems in India: present and future possibilities for integrated control—a review. *Exp Appl Acarol* 40, 49-66, 2006

10. Ghosh S, Bhowmick DN, Pratap AP. Application of neem and karanjia oils as natural pesticide microemulsion systems. *Tenside Surfact Det* 47, 369–375, 2010
11. Ghosh S, Kumar R, Nagar G, Kumar S, Sharma AK, Srivastava A, Kumar S, Ajith Kumar KG, Saravanan BC. Survey of acaricides resistance status of *Rhipicephalus (Boophilus) microplus* collected from selected places of Bihar, an eastern state of India. *Ticks Tick Borne Dis* 6, 668–675, 2015
12. Kogan A, Garti N. Microemulsions as transdermal drug delivery vehicles. *Adv Colloid Interface Sci* 123–126, 369–385, 2006
13. Kovats E. Gas-chromatographische Charakterisierung organischer Verbindungen. Teil 1: Retentionsindices aliphatischer Halogenide, Alkohole, Aldehyde und Ketone. *Helv Chim Acta* 41, 1915–1932, 1958
14. Labinas MA, Crocomo WB. Effect of java grass (*Cymbopogon winterianus*) essential oil on fall army worm *Spodoptera frugiperda* (J. E. Smith, 1979) (Lepidoptera, Noctuidae). *Acta Scientiarum* 24, 1401–1405, 2002
15. Leite BLS, Souza TT, Antonioli AR, Guimarães AG, Siqueira RS, Quintans JSS, Leonardo R, Bonjardim LR, Alves PB, Blank AF, Botelho MA, Almeida JRGS, Lima JT, Adriano AS, Araújo AAS, Quintans-Júnior LJ. Volatile constituents and behavioral change induced by *Cymbopogon winterianus* leaf essential oil in rodents. *Afri J Biotechnol* 10, 8312–8319, 2011
16. Martins RM. In vitro study of the acaricidal efficacy of the essential oil from the Citronella of Java (*Cymbopogon winterianus* Jowitt) to the tick *Boophilus microplus*. *Rev Bras Pl Med* 8, 71–78, 2006
17. Mello-Peixoto ECT, Figueiredo A, Novo SMF, Porto EP, Valadares F, Silva LP, Silva RMG. Application of *Cymbopogon winterianus* Jowitt and *Azadirachta indica* A. Juss in the control of *Rhipicephalus (Boophilus) microplus*. *J Med Plants Res* 7, 2392–2398, 2013
18. Mendes MC, Veríssimo CJ, Kaneto CN, Pereira JR. Bioassays for measuring the acaricides susceptibility of cattle tick *Boophilus microplus* (Canestrini, 1887) in São Paulo State, Brazil. *Arq Inst Biol* 68, 23–27, 2001
19. Morais LAS. Influencia dos fatores abióticos na composição química dos óleos essenciais. *Hortic Bras* 27, 4050–4063, 2009
20. Pratap AP, Bhowmick DN. Pseudo ternary diagrams of pesticide microemulsion systems. *Tenside Surfact Det* 41, 78–82, 2004
21. Pedrini N, Ortiz-Urquiza A, Huarte-Bonnet C, Zhang S, Keyhani NO. Targeting of insect epicuticular lipids by the entomopathogenic fungus *Beauveria bassiana*: hydrocarbon oxidation within the context of a host-pathogen interaction. *Front Microbiol* 4, 1–18, 2013
22. Picinin LCA, Toaldo IM, Hoff RB, Souza FN, Leite MO, Fonseca LM, Diniz SA, Silva MX, Cerqueira MMOP, Bordignon-Luiz MT. Survey of pyrethroid, macrocyclic lactone and antibacterial residues in bulk milk tank from Minas Gerais State, Brazil. *Pesq Vet Bras* 37, 97–104, 2017
23. Quintans-Júnior LJ, Souza TT, Leite BS, Lessa NMN, Bonjardim LR, Santos MRV, Aleves PB, Blank AF, Antonioli AR. Phytochemical screening and anticonvulsant efficacy of *Cymbopogon winterianus* Jowitt (Poaceae) leaf essential oil in rodents. *Phytomedicine* 15, 619–624, 2008
24. Salas JH, González MM, Noa M, Pérez NA, Díaz G, Gutiérrez R, Zazueta H, Osuna I. Organophosphorus pesticide residues in Mexican commercial pasteurized milk. *J Agri Food Chem* 51, 4468–4471, 2003
25. Torres FC, Lucas AM, Ribeiro VLS, Martins JR, Poser G, Guala MS, Elder HV, Cassel E. Influence of essential oil fractionation by vacuum distillation on acaricidal activity against the cattle tick. *Braz Arch Biol Technol* 55, 613–621, 2012
26. Veiga LPHN, Souza AP, Bellato V, Sartor AA, Nunes APO, Cardoso HM. Resistance to cypermethrin and amitraz in *Rhipicephalus (Boophilus) microplus* on the Santa Catarina Plateau, Brazil. *Rev. Bras. Parasitol Vet* 21, 133–136, 2012
27. Walker AR. Bouattour A, Camicas JL, Estrada-Peña A, Horak IG, Latif AA, Pegram RG, Preston PM. Ticks of domestic animals in Africa: a Guide to Identification of Species. 1st ed. Bioscience Reports, Edinburgh Scotland. 2003.
28. Xu J, Fan QJ, Yin ZQ, Li XT, Du YH, Jia RY, Wang KY, Lv C, Ye G, Geng Y, Su G, Zhao L, Hu TX, Shi F, Zhang L, Wu CL, Tao C, Zhang YX, Shi DX. The preparation of neem oil microemulsion (*Azadirachta indica*) and the comparison of acaricidal time between neem oil microemulsion and other formulations in vitro. *Vet Parasitol* 169, 399–403, 2010