



Research article

The use of essential oil-based pharmaceutical products to control cattle ticks

Apichart Manwicha¹, Wiwat Pattanawong¹, Nattha Vigad¹, Sunee Chansakaow²,
Pramote Tipduangta² and Kridda Chukiatsiri^{1,*}

¹Faculty of Animal Science and Technology, Maejo University, Chiang Mai 50290, Thailand

²Department of Pharmaceutical Sciences, Faculty of Pharmacy, Chiang Mai University, Chiang Mai 50200 Thailand

Abstract

The purpose of this study was to compare the efficacy of six essential oils obtained from sour orange (*Citrus aurantium L.*), ginger (*Zingiber officinale Roscoe*), lemongrass (*Cymbopogon citratus* (DC.) Stapf.), litsea (*Litsea cubeba* (Lour.) Pers.), clove (*Eugenia caryophyllata* Thunb.) and curcumin (*Curcuma longa* Linn.) against cattle ticks. Two essential oils demonstrated the greatest significant efficiency and were chosen for further testing on the cattle skin. The results showed that the 8% lemongrass essential oil had the most significant inhibiting effect on the oviposition of engorged female cattle ticks, whereas ginger oil, curcumin oil, sour orange, litsea oil and clove oil, showed lesser effect respectively ($p < 0.05$). There was a significant inverse relationship between lemongrass essential oil concentration and the tick's oviposition index. It was observed that the higher the essential oil concentration (16%), the higher the mortality rate of the larva-stage ticks. Nevertheless, the 4, 8 and 16% of lemongrass essential oil concentrations were also effective against adult ticks, causing a 100% mortality rate. Lemongrass oil can reduce tick number from day 1 after spraying on cattle skin and the tick number was under control for at least 14 days, which is significantly different, comparing to the control group.

Keywords: Cattle, Essential oil, Mortality rate, Oviposition index, Ticks.

Corresponding author: Kridda Chukiatsiri, Faculty of Animal Science and Technology, Maejo University, Chiang Mai 50290, Thailand. Tel: +66-875432, Fax: 053-353830, E-mail: kridda003@hotmail.com.

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INTRODUCTION

Tick is one of the major cattle ectoparasites in the tropics. The most common and important cattle tick is *Rhipicephalus microplus*, which can also be found in ruminants such as goats, sheep, deer and buffaloes. The problems of blood sucking ticks in cattle herd can lead not only to poor productivity such as reduced weight gain and milk production, but also to fatality in young calves due to severe blood loss. Furthermore, ticks can also act as biological vectors for many kinds of tick-born disease transmission, such as Anaplasmosis, Babesiosis and Theileriosis (Pérez de León et al., 2020). In Thailand farming system, beef and dairy cattle often suffer from tick infestation, no matter the cattle are released naturally to graze on pasture nor kept in confinement and fed on forages. The cattle ticks spend most of their parasitic stages on a host before engorged adult female detaching themselves from the cattle bodies and drop onto pasture, laying up eggs which hatch to larvae infesting the pasture before picking up by a suitable host. As ticks have a life cycle both during which they feed on their host and in pastures, making it quite difficult to get rid of and control completely. To control the cattle ticks, the external and injectable acaricide is commonly used including Ivermectin, which is applied by injecting to cattle to cause systemic effect, distributing the drug throughout host tissues before reaching the tick body. Ivermectin blocks the gamma-aminobutyric acid (GABA), a neurotransmitter in ticks. However, if used in large quantities, it can adversely affect animals and pollute the environment. On the other hand, ticks can develop acaricide resistance ability, demanding the farmers to apply higher acaricide dose to produce the same effect (Chaparro-Gutiérrez et al., 2020; Torrents et al., 2020). Externally used acaricides such as Amitraz or other insecticidal agents are also harmful to users if they are not used properly.

Due to the growing interest in organic farming system because of the consumer health consciousness, and the appealing value-added livestock product imposes restriction on the use of several acaricides or chemicals, which in turn limits the farm productivity. The study of plant-derived products is therefore one of the alternative strategies that many organic farmers resort to. There have been numerous researches reported on applying plant-based extracts to control cattle ticks *in vitro* (Nogueira et al., 2014; Pazinato et al., 2016; Madreseh-Ghahfarokhi et al., 2019), but ready-to-use plant-based pharmaceutical tick control products have not yet been commercially available. Therefore, this study aimed to study the effective and safety concentration of six essential oil from six herbs, namely sour orange (*Citrus aurantium* L.), ginger (*Zingiber officinale* Roscoe), lemongrass (*Cymbopogon citratus* (DC.) Stapf.), litsea (*Litsea cubeba* (Lour.) Pers.), clove (*Eugenia caryophyllata* Thunb.) and curcumin (*Curcuma longa* Linn.) on controlling ticks obtained from cattle raised in pasture. The study was conducted in organic cattle farming by comparing the result in treatment groups with those treated with commercial insecticide products. This will lead to the creation of pharmaceutical products from essential oils for cattle tick control and elimination.

MATERIALS AND METHODS

Preliminary data were obtained from *in vitro* study conducted to determine the optimum efficacy and concentration of six essential oils: orange, ginger, lemongrass, litsea, clove, and curcumin oil against the cattle ticks. The raw plant materials used for essential oil distillation were collected from the Medicinal Plant Garden located at the Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand. Voucher specimens were deposited at the same location. All essential oils were distilled by in-house procedures. Briefly, all raw plant materials were freshly cut, air-dried in an oven at 70 °C for 24 h, and milled into powder using an herb grinding machine. Powders were screened through an 80-mesh sieve and directly kept in zip-lock plastic bags at 4 °C for further use. A measured amount of 150 g of dried milled powder was immersed in a 1-L clean tank. Then, 70% ethanol solvent was poured into the tank at a ratio of 1:7 and allowed to stand for 48 h. The extracts were decanted into another container, filtered through filter paper, and evaporated over a water bath at 60 °C to obtain the essential oil. Two most effective essential oils against cattle ticks were chosen for further *in vivo* experiment in dairy and native cattle. These two-step experiments are as following:

Study 1 An *in vitro* study of the effectiveness of essential oils on cattle ticks

Essential oils obtained from the six plant extracts were diluted by mixture solution of distilled water, 99.5% alcohol and tween 20 to the final concentrations of 1, 2, 4, 8 and 16%. The protocol for testing essential oil effectiveness, the Adult Immersion Test (AIT) and Larvae Immersion Test (LIT), was adapted from Ribeiro et al. (2008). The control group, treatment with Amitraz, and experimental group, treatment with essential oils, are compared against each other. Data were recorded and calculated for the oviposition index (IE), egg-laying inhibition percentage and larva mortality percentage.

$$\text{Reproductive Index (RI)} = \frac{\text{Hatched Egg Weight (g)}}{\text{Female Tick Weight (g)}}$$

$$\text{Inhibition of Ovulation Percentage (IO\%)} = \frac{\text{RI (Control)} - \text{RI (Tested with Essential Oils)}}{\text{RI (Control)}} \times 100$$

Adult immersion test

Each experimental group composed of 10 engorged *R. microplus* females with average weight of 0.3 g per tick were submerged in each essential oil solution at concentrations of 1, 2, 4, 8 and 16% for five minutes. After that, ticks were removed, dried and placed in Petri dishes laid with filter paper and maintained in incubators at 28°C with a relative humidity of 65 – 85%. Observed every 24 hours for a period of 15 days. Survival rate of the ticks were recorded each day after the 1st to 15th day. The tick eggs were weighed to calculate reproductive performance and egg inhibition percentage.

$$\text{Index of Egg Laying (IE)} = \frac{\text{egg weight placed (g)}}{\text{pre-trial tick mother weight (g)}}$$

$$\% \text{ Egg Inhibition} = \frac{\text{IE Control Group} - \text{Trial Group IE}}{\text{IE Control Group}} \times 100$$

Study 2 An *in vivo* study of the acaricidal effect of essential oils on the cattle ticks

Two kinds of most effective acaricidal essential oils from study 1 were chosen based on oviposition index (IE), egg inhibition and larva mortality percentage. The preparation of the effective essential oil concentration to try on cattle was based on the result in the *in vitro* test of study 1. This study was approved of by the Animal Ethics Committee, Maejo University, Chiang Mai, Thailand under approval number: MACUC 027A/2561. Four experimental groups of tick-infested beef cattle containing 20 cattle in each group were sprayed with essential oils or control substances on bodies around dewlaps, brisket area, flank, and testes areas. Group 1 to 4 of cattle were sprayed with essential oil 1, essential oil 2, essential oil diluter (negative control), and Amitraz (control) respectively. The individual experimental result was recorded by counting the tick number for 4 times, ie before spraying, after spraying on day 1, 7 and 14 respectively.

Statistical analysis

The tick mortality data was collected and used to analyze the variance of the means according to Complete Randomized Design. The experimental group means were compared based on Duncan' New Multiple Range Test (DMRT), and the reduction of tick number was analyzed using Repeated Measurements in CRD by SAS® University Edition

RESULTS

An *in vitro* study of the effectiveness of essential oils on cattle ticks

All 6 essential oils tested; 8% lemongrass essential oil showed initial effective concentration on inhibiting oviposition of engorged female cattle ticks. The following significantly effective essential oils are ginger, curcumin, sour orange, litsea and clove oil, respectively. However, at the concentration of 16%, all essential oils demonstrated similar result.

Table 1 The effect of 6 essential oils at different concentrations on egg-laying inhibition of cattle Ticks

Treatment	Concentration (%)					SEM	p-value
	1	2	4	8	16		
Amitraz	98.08						
Sour orange	23.22 ^c	30.98 ^c	38.01 ^{bc}	61.80 ^b	86.98 ^a	8.09	0.006
Ginger	27.21 ^c	54.93 ^b	71.69 ^b	75.33 ^{ab}	100.00 ^a	8.40	0.007
Lemongrass	23.58 ^b	46.04 ^b	63.09 ^{ab}	100.00 ^a	100.00 ^a	11.00	0.037
Litsea	23.50	40.65	52.26	63.34	100.00	10.07	0.115
Clove Oil	13.96	25.84	39.96	60.09	75.85	8.70	0.098
Curcumin	28.11 ^c	39.92 ^c	43.51 ^c	72.62 ^b	100.00 ^a	8.99	0.004
SEM	2.97	4.10	5.74	6.23	4.39	2.97	4.10
p-value	0.878	0.430	0.545	0.133	0.570		

^{A,C} means with different superscripts in the same row indicate significantly difference.

Adult immersion test

Table 2 shows that the greater concentration of the essential oil, the less the egg laying index. IE value of 0 was found in each experimental group treated with 16% concentration of ginger, lemongrass, or curcumin essential oil, similar to that of 8% litsea essential oil. Although with the same concentration, each essential oil had a statistically insignificant difference in IE ($p>0.05$), at a concentration of 4%, the ginger and litsea essential oils tended to have insignificantly lower IE values than others ($p>0.05$). 16% of ginger, litsea, lemongrass and curcumin essential oils showed 100% of egg inhibitor percentage, similar to 8% litsea essential oils. At the same concentration. However, at a concentration of 4%, the ginger essential oils and litsea had as insignificant percentage of egg inhibitors than other types ($p>0.05$).

Table 2 Performance of all 6 essential oils in different concentrations on oviposition index (Index of egg laying) of cattle ticks

Treatment	Concentration (%)					SEM	p-value
	1	2	4	8	16		
Amitraz	0.0049						
Sour orange	0.196 ^c	0.176 ^c	0.158 ^{bc}	0.097 ^{ab}	0.033 ^a	0.02	0.01
Ginger	0.186 ^c	0.115 ^b	0.072 ^b	0.063 ^{ab}	0.00 ^a	0.02	0.01
Lemongrass	0.195 ^b	0.138 ^b	0.094 ^{ab}	0.00 ^a	0.00 ^a	0.03	0.04
Litsea	0.20	0.15	0.12	0.09	0.00	0.03	0.12
Clove Oil	0.22	0.19	0.15	0.10	0.06	0.02	0.10
Curcumin	0.184 ^b	0.153 ^b	0.144 ^b	0.150 ^b	0.00 ^a	0.02	0.01
SEM	0.01	0.01	0.01	0.01	0.01		
p-value	0.88	0.44	0.56	0.48	0.57		

^{a-c} means with different super script are significant difference ($p<0.05$)

The acaricidal effect at the larval stage of essential oils demonstrated that the greater concentration resulted in higher tick mortality at the larval stage (Table 3). At the essential oil concentration of 4, 8 and 16%, tick mortality reached 100%. The lowest fatal concentration of essential oils that caused 100% mortality in ticks by orange, ginger, lemongrass, lemongrass, cloves and curcumin essential oils are 4, 2, 1, 4, 4 and 4%, respectively.

Table 3 Effectiveness of all 6 essential oils in different concentrations in the killing of cattle ticks at the larval stage (Larvae)

Treatment	Concentration (%)					SEM	p-value
	1	2	4	8	16		
Amitraz	100.00						
Control	12.29						
Sour orange	60.10 ^{Bb}	97.22 ^A	100.00 ^A	100.00 ^A	100.00 ^A	6.03	0.1245
Ginger	99.32 ^a	100.00	100.00	100.00	100.00	0.14	0.4516
Lemongrass	100.00 ^a	100.00	100.00	100.00	100.00	-	-
Litsea	61.41 ^{Bb}	94.67 ^A	100.00 ^A	100.00 ^A	100.00 ^A	4.05	<.0001
Clove Oil	59.82 ^{Bb}	90.38 ^A	100.00 ^A	100.00 ^A	100.00 ^A	4.63	0.0014
Curcumin	59.91 ^{Bb}	92.97 ^A	100.00 ^A	100.00 ^A	100.00 ^A	4.25	<.0001
SEM	5.92	1.52	-	-	-		
p-value	0.0426	0.4	-	-	-		

^{A-B} means with different superscripts in the same row are significantly different

^{a-b} means with different superscripts in the same column are significantly different

Based on the evaluation that 8% litsea or ginger essential oil had significant effect on Egg Laying Index (IE), oviposition inhibition percentage and mortality rates of larval ticks, they were chosen to further experiment on cattle. The result showed that both litsea and ginger essential oils reduced tick numbers on cattle body from 1st day after spraying until 14th day, similar to those treated with Amitraz (Table 4).

Table 4 The overall prevalence of ticks infestation in cattle after spraying with lemongrass essential oil ginger and orange skin at a concentration of 8%

Trial Group	day			SEM	p-value		
	1	7	14		T ¹	D ²	T*D
Lemongrass	77.39	4.67	0.00				
Ginger	64.381	13.00	2.00	5.75	<.0001	<.0001	0.0005
Sour orange	88.67	14.50	20.00				
Control	100.00	95.00	77.28				
Amitraz	32.50	4.00	0.00				

DISCUSSION

Essential oils from litsea and ginger at a concentration of 8% had oviposition inhibition effect on engorged female tick of 100% and 75.33% respectively. Whereas the control group treated with 0.2% Amitraz had inhibition effect of 98.08%. This result is in accordance with Ribeiro et al. (2010) who reported that 50 µl/ml *Hesperozygis ringens* essential oil had oviposition inhibition rate of 76.4 %.

The higher essential oil concentration, the lower the tick's oviposition index was observed. At a concentration of 4%, ginger and lemongrass essential oil showed index of 0.115 and 0.138 which implied higher oviposition inhibition than that in control group respectively. The result was higher than those reported by [Goncalves et al. \(2007\)](#) who examined the effect of solvents and surfactants on larvae and adult cattle ticks by comparing treatments with 100% Methanol and 100% Ethanol which resulted in ovulation index of 0.285 and 0.447, respectively. One percent of the litsea and ginger essential oil effectively killed tick larvae at 100% and 99.32%, respectively, which were close to 100% in the control group (Amitraz). This finding is in consistent with a study by [Ribeiro et al. \(2011\)](#) who reported that the concentrations of 5, 10 and 20 μ l/ml *Calea serrata* (Asteraceae) essential oil were 100% effective against larval stage ticks.

In further experiment of spraying essential oils on cattle was based on the *in vitro* result, using litsea and ginger essential oils at concentrations of 8% which showed the highest inhibition effect on cattle tick oviposition, oviposition index and the larval stage mortality rate. Two essential oils were sprayed on the cattle around the dewlap, brisket area, flank, and around the testicle areas. It was found that on the 7th day after spraying, cattle ticks tended to decrease, with litsea essential oils having a similar deterrent effect to the control group (Amitraz). And on the 14th day after spraying, there was 100% deterrent effect on cattle ticks. The result was in line with a study by [Jailanga et al. \(2009\)](#) who stated that derris (rotenone), tobacco, *Annona reticulata* leaves, and citronella grass (*Cymbopogon nardus*) extractions could kill cattle ticks at concentrations of 10%, 5%, 100% and 100%, respectively.

According to [Chungsamarnyart and Jansawan \(1996\)](#) who studied the effectiveness of acaricides, they found that the most active ingredients in citronella grass and lemongrass oil was d-limonene, followed by 5 other substances: citral, linalool, borneol, Citronellal and citronellol respectively. According to [Yang et al. \(2004\)](#), they found that rutaceae oil, containing the main substances called α -citral (33.50%) and citral (35.77%), could effectively kill *Culex pipiens quinquefasciatus* mosquitoes within half an hour of fumigation (LC50 0.0012%). Citronella has insect repellent properties because it contains an important active ingredient called citronellal, which is acyclic monoterpene, and it has been used as a substitute for N, N-diethyl-mtoluamide (DEET) for mosquito and other insect repellants. Limonene was also used for flea and mite control ([Laohakunjit et al., 2007](#)). Diterpenoids and triterpenoids, including columbin, solidagenone, limonene, nomilin and helvolic acid, can also be used as appetite suppressant and growth inhibitor substances in insect ([Singh and Upadhyay, 1993](#)).

The main chemical components of ginger essential oils are α -Zingiberene, β -Sesquiphellandrene and ar-Curcumene. It has been reported that these herbal oils family can kill *Staphylococcus aureus* bacteria and affect the *Culex quinquefasciatus* mosquito species, and it also has repellent effect on leptomastix mite larvae, which is consistent with previous study by [Pushpanathan et al. \(2008\)](#) who reported the insecticidal and repellent effect of ginger (*Zingiber officinale*) essential oil on mosquitoes (*C. quinquefasciatus*). Ginger and eucalyptus essential oils have a greater mosquito-killing effect than the control group, especially when the concentration of the substance increases

(Madreseh-Ghahfarokhi et al., 2018). Ginger and eucalyptus essential oils have a greater killing effect on *Culex theileri* Theobald mosquitoes and *Rhipicephalus bursa* ticks in experiment groups than those in negative control groups (Madreseh-Ghahfarokhi et al., 2019), and they also show insecticidal, repelling and oviposition inhibition effects on *Anopheles stephensi* (Prajapati et al., 2005). Ginger oil at a level of 100 ppm can kill mosquito larvae and lesser grain borer (*Rhyzopertha dominica*) (Pavela, 2005).

CONCLUSIONS

Litsea and ginger essential oils at a concentration of 8% are effective in eliminating cattle ticks, inhibiting oviposition and larvae hatching, in in vitro experiments, and can be effectively used to get rid of ticks by spraying on cattle, but research should be carried out to increase the concentration capacity of essential oils for further development.

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AUTHOR CONTRIBUTIONS

AM, WP, NV, SC, PT and KC conceived and designed the experiments. NV executed the lab experiments and analyzed the data. AM and KC prepared the first draft of manuscript. KC is the principle investigator of the project who was responsible for preparation of project proposal, procure funding, resource allocation, and manage human resource and along with WP PT and SC. All authors approved the final draft of manuscript.

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