

## Acaricidal Activity of Peel Oil of *Citrus* spp. on *Boophilus microplus*

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### ABSTRACT

Peel oil from three varieties of *Citrus maxima* Merr. (pomelo): Khaao-phuaing (immature fruit), Thong-dee (immature and mature fruits) and Khaao-nam-phueng (mature fruits); *Citrus reticulata* Blanco., *Citrus suncris* Linn., *Citrus sinensis* Linn. and *Citrus hystrix* DC. were collected by mechanical pressing. These peel oil and the *d*-limonene substance were diluted with 95% ethanol into 1:5, 1:10 and 1:15 (V/V) and tested the acaricidal activity on the engorged female cattle tick (*Boophilus microplus*) by dipping method. The acaricidal activity on the tick larvae was tested with these oil and *d*-limonene dilution of 1:10 and 1:15 (V/V). The peel oil of *C. reticulata* and *C. maxima* variety thong-dee (immature and mature fruits) showed high acaricidal activity at the concentration of 1:10 (V/V) on engorged female ticks. These peel oil activity were 2 times higher than that of *d*-limonene. The peel oil concentration 1:10 of *C. sinensis* and *C. maxima* (mature fruits) exhibited high larvicidal activity, while the oil of *C. hystrix*, *C. reticulata*, *C. suncris* and *C. maxima* (immature fruits) showed relatively high larvicidal activity. Their larvicidal activity were 1.5 times stronger than *d*-limonene. The peel oil of the other citrus spp. exhibited weaker acaricidal activity.

This indicated that the peel oil from the citrus fruits could be directly applied to control the cattle tick. The waste of the over fruiting immature pomelo, would be of potential usage as a biocontrol substance.

**Key words:** acaricidal activity, peel oil, *Citrus* spp., tropical cattle ticks.

### INTRODUCTION

The tropical cattle ticks (*Boophilus microplus*) suck the cattle blood caused the loss of body weight and milk production, and can be the vector to transmit protozoan and infectious diseases. The eradication of ticks need time, labor and the cost of acaricides. Thus, ticks make a large economic loss in developing dairy cattles. It needs a large amount of research budget to find new acaricides,

since ticks can develop resistant strains against synthetic insecticides (Drummond, 1977). In addition, the synthetic acaricides leave toxic residue in the environment because of their delay degradation. Therefore, the original use of crude-extract from plants having the acaricidal activity seems to come up with promising results. Previous works revealed that there are many plants showing high acaricidal activity (Chungsamarnyart *et al.*, 1991 a, b, Chungsamarnyart and Jansawan, 1994)

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and some of them can be practical use; the crude-extracts of sugar apple seeds (Chungsamarnyart *et al.*, 1991 c), the volatile oil from lemon and citronella grasses (Chungsamarnyart and Jiwajinda, 1992; Chungsamarnyart and Jansawan, 1992) and the yam bean seeds (Chungsamarnyart and Jansawan, 1993). The peel oil of *Citrus maxima*, *Citrus reticulata* and *Citrus aurantium* has shown insecticidal activity to cowpea and bean beetles (Grainge and Ahmed, 1988), but the acaricidal activity of the peel oil of citrus spp. has not yet been reported. The *d*-limonene in the citrus peel oil constituents has insecticidal properties (Taylor and Vickery, 1974; Hink and Fee, 1986; Karr and Coat, 1988; Power *et al.*, 1988; Schick and Schick, 1986; Karr *et al.*, 1990). Therefore, this study compared acaricidal activity of the citrus peel oils with *d*-limonene to find out the effective peel oil of local citrus spp. for controlling of tropical cattle ticks.

## MATERIALS AND METHODS

The fruits of three varieties of *Citrus maxima* Merr. (pomelo): Khaao-phuaing (immature fruit), Thong-dee (immature and mature fruits) and Khaao-nam-phueng (mature fruits); *Citrus reticulata* Blanco. (mandarin orange), *Citrus suncri*s Linn. (sour orange), *Citrus sinensis* Linn. (sweet orange) and *Citrus hystrix* DC. (Ma-kruut) were twice washed with water and wiped with cloth. The immature fruits of pomelo were the over fruiting which the farmer took them off and left them waste. The peel of these citrus fruits were peeled and mechanical pressed to collect the oil. The oil yield was the mean (% V/W) of 5 collections. The peel oil and the pure *d* (+)-limonene (Wako Pure Chemical Industries. LTD.) were diluted with 95% ethanol into 1:5, 1:10 and 1:15 (V/V) and tested the acaricidal activity on the engorged female of *Boophilus microplus* by dipping method. The mortality of ticks were observed at 24 h, 48 h and

7 days after dipping (Chungsamarnyart and Jansawan., 1990). The acaricidal activity on the larvae was tested with their dilution of 1:10 and 1:15 (V/V). The mortality of larvae were checked at 1-2 h after dipping. Both adults and larvae of the control replications were dipping with 95% ethanol. The corrected mortality of the ticks were calculated by Abbott's formula (Abbott, 1925). The mean (%) of corrected mortality was the average of 5 replications (20 adult ticks / replication and 200-400 larvae /replication).

## RESULTS

The peel oil yield from the *Citrus suncri*s was the highest yield (1.11%, V/W). The oil yield of the immature fruits of *Citrus maxima* (som-o-thong-dee; 0.88%, V/W) and the *Citrus sinensis* (0.85%, V/W) were relatively high collection. The moderate oil yield were the oil from mature fruit of *Citrus maxima* (som-o-thong-dee; 0.59%, V/W), *Citrus hystrix* (0.57%, V/W) and *Citrus reticulata* (0.55%, V/W). The less oil yield were the oil from the immature fruits of *Citrus maxima* variety of khaao-phuaing (0.37%, V/W) and the mature fruit of khaao-nam-phueng (0.34%, V/W).

The acaricidal activity of peel oil and *d*-limonene on adult tropical cattle ticks was shown in Table 1. The peel oil of *Citrus maxima* variety thong-dee (mature and immature fruits) and *Citrus reticulata* showed the highest acaricidal activity, since their concentration of 1:10 dilution showed 98%, 96% and 95% corrected mortality at 24 h after dipping, respectively. The peel oil of *Citrus sinensis*, *Citrus maxima* variety khaao-nam-phueng and khaao-phuaing and *d*-limonene showed high acaricidal activity (99%, 89%, 98% and 100% mortality, respectively) at the concentration of 1:5 dilution. The peel oil concentration of 1:5 dilution of *Citrus hystrix* and *Citrus suncri*s showed high activity at 48 h after dipping.

**Table 1** Acaricidal activity of the peel oil of *Citrus* spp. and *d*(+)-limonene on engorged female tropical cattle ticks.

Scientific name (Local name) of <i>Citrus</i> spp. and chemical	Dilution (V/V) oil:ethanol	Corrected mortality (Mean, %)*		
		24 h <sup>1</sup>	48 h <sup>2</sup>	7 days <sup>3</sup>
<i>Citrus hystrix</i> DC. (Ma-kruut)	1: 5	80 def	98 a	98 ab
	1:10	61 g	79 cde	79 cde
	1:15	47 h	70 ef	70 fgh
<i>Citrus reticulata</i> Blanco. (Som-khiea-waan)	1: 5	100 a	100 a	100 a
	1:10	95 abc	95 ab	95 ab
	1:15	78 ef	78 cde	78 efg
<i>Citrus sinensis</i> L. (Som-cheng)	1: 5	99 ab	99 a	99 ab
	1:10	79 def	79 cde	79 def
	1:15	49 h	49 h	49 i
<i>Citrus maxima</i> L. (Som-o-thong-dee)	1: 5	100 a	100 a	100 a
	1:10	98 ab	98 a	98 ab
	1:15	74 f	74 de	74 efg
<i>Citrus maxima</i> L. (Som-o-thong-dee), immature	1: 5	9 8ab	99 a	100 a
	1:10	96 abc	96 ab	97 ab
	1:15	81 def	82 cd	82 cde
<i>Citrus maxima</i> L. (Som-o-khaao-nam-phueng)	1: 5	89 bcd	89 bc	89 bcd
	1:10	76 ef	79 cde	89 bcd
	1:15	51 gh	63 fg	67 h
<i>Citrus maxima</i> L. (Som-o-khaao-phuaing), immature	1: 5	98 ab	100 a	100 a
	1:10	4 8h	56 gh	68 gh
<i>Citrus suncris</i> L. (Som-san-cris) <i>d</i> (+)-limonene	1: 5	86 cde	97 ab	98 ab
	1:10	27 i	30 i	46 i
	1: 5	100 a	100 a	100 a
	1:10	73 f	76 de	79 def
	1:15	14 j	15 j	17 j

\* Mean (%) with the same letter are not significantly different of corrected mortality of 5 replications (20 ticks/rep.).

<sup>1</sup> LSD .01 = 5.5027,    <sup>2</sup> LSD .01 = 10.6513,    <sup>3</sup> LSD .01 = 10.2988

The acaricidal activity of the peel oil of citrus spp. and *d*-limonene on the tick larvae were shown in Table 2. The oil of *Citrus sinensis* and *Citrus maxima* variety thong-dee (mature fruits) showed the highest larvicidal activity at the

concentration of 1:10 dilution. The relatively high larvicidal activity were the oil dilution 1:10 of *Citrus hystrix*, *Citrus reticulata*, *Citrus maxima* variety thong-dee (immature fruit) and *Citrus suncris*. The dilution 1:10 of *d*-limonene showed

**Table 2** Acaricidal activity of the peel oil of *Citrus* spp. and *d* (+)-limonene on larvae of tropical cattle ticks.

Scientific name (Local name) of <i>Citrus</i> spp. & chemical	Dilution (V/V) oil : ethanol	Corrected Mortality <sup>1/</sup> 1-2 h after dipping
<i>Citrus hystrix</i> DC. (Ma-kruut)	1:10	90.29 bc
	1:15	72.61 f
<i>Citrus reticulata</i> Blanco. (Som-kliea-waan)	1:10	90.77 bc
	1:15	72.66 f
<i>Citrus sinensis</i> L. (Som-cheng)	1:10	98.59 a
	1:15	87.32 cd
<i>Citrus maxima</i> L. (Som-o-thong-dee)	1:10	95.07 ab
	1:15	81.78 de
<i>Citrus maxima</i> L. (Som-o-thong-dee), immature	1:10	89.05 bc
	1:15	78.60 ef
<i>Citrus maxima</i> L. (Som-o-khaao-nam-phueng)	1:10	81.88 de
	1:15	54.25 h
<i>Citrus maxima</i> L. (Som-o-khaao-phuaing), immature	1:10	62.61 g
	1:15	45.68 i
<i>Citrus suncri</i> L. (Som-san-cris)	1:10	89.88 bc
	1:15	73.15 f
<i>d</i> (+)-limonene	1:10	76.62 ef
	1:15	11.62 j

<sup>1/</sup> Mean (%) with the same letter are not significantly different of corrected mortality of 5 replications (200-400 larvae/ rep.). LSD .01 = 6.4937

similarly low activity as the oil dilution 1:15 of *Citrus hystrix*, *Citrus reticulata*, *Citrus maxima* variety thong-dee (mature and immature fruits) and *Citrus suncri*.

## DISCUSSION

The acaricidal activity on engorged female ticks, the peel oil of the *Citrus maxima* variety thong-dee (mature and immature fruits) and the *Citrus reticulata* exhibited the acaricidal activity two times higher than the activity of *d*-limonene, since their oils concentration of 1:10 dilution showed similar

activity as the *d*-limonene concentration of 1:5 dilution at 24 h after dipping (Table 1). The larvicidal activity of the oil of *Citrus hystrix*, *Citrus reticulata*, *Citrus sinensis*, *Citrus maxima* variety thong-dee (mature and immature fruits) and *Citrus suncri* were significantly showed 1.5 times stronger activity than the *d*-limonene, since the *d*-limonene concentration of 1:10 dilution caused the similar mortality rate of the larvae by these peel oil dilution 1:15 (Table 2). This might indicate that the active substance in these citrus peel oil is not only *d*-limonene, but the oil also has other synergistic active substances. Therefore, the direct use of these

natural peel oil to control ticks is the best, because their oils activity are stronger than pure *d*-limonene and the waste immature fruits will be useful.

Comparing the acaricidal activity results of these citrus peel oils with lemon and citronella grasses oils, the peel oil of the *Citrus maxima* variety thong-dee (mature and immature fruits) and of the *Citrus reticulata* were 3 times stronger than the lemon and citronella grasses oils (Chungsamarnyart and Jiwajinda, 1992). The odor of the citrus peel oils are better to smell than these grasses oils. Thus these citrus peel oil might be favorably used to control the ticks than the lemon and citronella grasses oils, especially the oil of immature fruits of *C. maxima* variety thong-dee. In addition, the oil yield of immature fruits of *C. maxima* variety thong-dee (0.88%) were higher than that of lemon grass (0.38%) and citronella grass (0.69%) (Chungsamarnyart and Jansawan, 1992). However, this peel oil collecting method was not in practice. The commercial collecting method of the peel oil should be the hydrolic press or cold-press methods (Lane, 1983; Dugo *et al.*, 1992). The proper method for collecting peel oil from the immature fruits of *C. maxima* variety thong-dee should be further studies.

The acaricidal activity on the cattle tick of the peel oil of citrus spp. has not yet been reported, particularly, the effective acaricide of the peel oil of *Citrus maxima* variety thong-dee and *Citrus reticulata*. The insecticidal activity of the peel oil of *Citrus maxima*, *Citrus reticulata* and *Citrus aurantium* has been effected against the cowpea and bean beetles (Grainge and Ahmed, 1988), the house fly and red fire ant (Sheppard, 1984). The active insecticidal substance (*d*-limonene) of the citrus oil has been known (Taylor and Vickery, 1974; Karr and Coat, 1988; Power *et al.*, 1988), and its activity has been only tested on the cat flea (Schick and Schick, 1986; Hink and Fee, 1986) and on the earthworm (Karr *et al.*, 1990). The acaricidal

activity on *Boophilus microplus* of *d*-limonene also has no previous reports.

## CONCLUSION

The peel oil of the *Citrus maxima* variety thong-dee (mature and immature fruits) and *Citrus reticulata* showed the highest acaricidal activity at the concentration of 1:10 (V/V) on both larvae and engorged female ticks. These peel oils activity on the larvae and engorged female ticks were 1.5 and 2 times higher than pure *d*-limonene, respectively. Thus these peel oil can be directly spray to cattles for controlling ticks. In addition, the waste of over immature fruiting pomelo will be useful.

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## LITERATURE CITED

- Abbott, W.S. 1925. A method of computing the effectiveness of an insecticide. *J. Econ. Ent.* 18: 265-267.
- Chungsamarnyart, N. and W. Jansawan. 1990. Bioassay techniques of insecticidal plant-extracts on tropical cattle ticks *Boophilus microplus*. *Kasetsart J. (Nat. Sci. Suppl.)* 24: 24-27.
- Chungsamarnyart, N., S. Jiwajinda, and W. Jansawan. 1991 a. Larvicidal effect of plant crude-extracts on the tropical cattle tick (*Boophilus microplus*). *Kasetsart J. (Nat. Sci. Suppl.)* 25: 80-89.
- Chungsamarnyart, N., S. Jiwajinda, and W.

- Jansawan. 1991 b. Acaricidal effect of plant crude-extracts on tropical cattle ticks (*Boophilus microplus*). *Kasetsart J. (Nat. Sci. Suppl.)* 25: 90-100.
- Chungsamarnyart, N. and W. Jansawan. 1992 . Efficacy of practical steam distillation set for distilling lemon and citronella grasses oils. *Kasetsart J. (Nat. Sci. Suppl.)* 26: 52-56.
- Chungsamarnyart, N. and W. Jansawan. 1993. Acaricidal effect of practical crude-extracts of plants against tropical cattle ticks. *Kasetsart J. (Nat. Sci. Suppl.)* 27: 1-8.
- Chungsamarnyart, N. and W. Jansawan. 1994. Acaricidal activity of the combine of plant crude-extracts to tropical cattle ticks. *Kasetsart J. (Nat. Sci.)* 28, 347-353
- Chungsamarnyart, N., S. Jiwajinda, C. Rattanakreetakul, and W. Jansawan. 1991c. Practical extraction of sugar apple seeds against tropical cattle ticks. *Kasetsart J. (Nat. Sci. Suppl.)* 25: 101-105.
- Chungsamarnyart, N. and S. Jiwajinda. 1992 . Acaricidal activity of volatile oil from lemon and citronella grasses on tropical cattle ticks. *Kasetsart J. (Nat. Sci. Suppl.)* 26: 46-51.
- Drummond, R.O. 1977. Resistance in ticks and insects of veterinary important ce, pp. 303-317. *In* D.L. Watson and A.W.A. Brown (eds). *Pesticide Management and Insecticide Resistance*. Academic Press, New York, San Francisco, London.
- Dugo, G., G. Lamonica, A. Cotroneo, I.S. d' Alcontres, A. Verzera, M.G. Donato, and D. Licandro. 1992. High resolution gas chromatography for detection of adulterations of citrus cold-pressed essential oils. *Perfumer Flavorist* 17, 57-74.
- Grainge, M. and S. Ahmed. 1988. *Handbook of plants with pest-control properties*. John Wiley & Sons Inc. New York, Chichester, Brisbane, Toronto, Singapore.
- Hink, W.F. and B.J. Fee. 1986. Toxicity of *d*-limonene, the major component of citrus peel oil, to all lifestages of the cat flea, *Ctenocephalides felis* (Siphonaptera: Pulicidae). *J. Med. Entomol. Honolulu*. 23: 400-404.
- Karr, L.L. and J.R. Coats. 1988. Insecticidal properties of *d*-limonene. *J. Pest. Sci.* 13: 287-290.
- Karr, L.L., C.D. Drewes, and J.R. Coats. 1990. Toxic effects of *d*-limonene in the earthworm *Eisenia fetida* (Savigny). *Pest. Biochem. Physiol.* 36: 175-186.
- Powers, K.A., S.B. Hooser, J.P. Sundberg, and V.R. Beasley. 1988. An evaluation of the acute toxicity of an insecticidal spray containing linalool, *D*-limonene. *Vet. Human Toxicol.* 30: 206-210.
- Schick, M.P. and R.O. Schick. 1986. Understanding and implementing safe and effective flea control. *JAAHA* 22: 421-434.
- Sheppard, D.C. 1984. Toxicity of citrus peel liquids to the house fly and red imported fire ant. *J. Agri. Entomol.* 1, 95.
- Taylor, W.E. and B. Vickery. 1974. Insecticidal properties of limonene, a constituent of citrus oil. *Ghana J. Agri. Sci.* 7: 61-62.