Acaricidal and Repellent Activity of *Zanthoxylum myriacanthum* (Rutaceae) Fruit Extracts Against *Tetranychus urticae* and *Tetranychus truncatus* (Acari: Tetranychidae)[1]

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Abstract This work investigated acaricidal and repellent activities of *Zanthoxylum myriacanthum* Wall. ex Hook. f. (Rutaceae) extracts against *Tetranychus urticae* Koch and *Tetranychus truncatus* Ehara (Acari: Tetranychidae). Acaricidal activities were tested by spraying the extracts on adult females and eggs, and repellent activity was assessed in paired-choice tests with filter paper treated with the extracts by using adult female mites. Adult mortality 24 h after application of a 12% (w/v) concentration of the hexane extract was 73.3% for *T. urticae* and *T. truncatus*, whereas application of the methylene chloride extract caused 85.8% mortality of *T. urticae* and 85% mortality of *T. truncatus*. Lower concentrations (6–12%) of the methanol extract yielded low efficacy against *T. urticae* but exhibited high efficacy against *T. truncatus* (73.8–95.8%). In general, egg hatch was reduced $<$50% by 5 d following application of the extracts. Only the 6–12% concentration of the hexane extract caused higher levels of *T. truncatus* egg mortality ranging 55.4–68.7%. All extracts repelled adult mites over 64% from 5 to 72 h after exposure. However, the percentage of repellency showed no statistical differences in all treatments. Gas chromatography-mass spectrometry (GC-MS) analysis identified that the major chemical compounds in the hexane extract were DL-limonene (29.75%) and sabinene (9.76%), whereas limonene (40.70%) and sabinene (16.60%) were the principal constituents of the methylene chloride extract. Our results demonstrate that *Z. myriacanthum* dried fruit extracts have potential for controlling *T. urticae* and *T. truncatus* and might be developed as acaricides for integrated pest management programs.

Key Words makwhaen extracts, mite management, two-spotted spider mite, cassava red mite

The family Tetranychidae is comprised of approximately 1,250 species of spider mites. Over 100 species are pests damaging a variety of plants in field crops, orchards, greenhouses, and nurseries (Le Goff et al. 2009, Migeon et al. 2010). The two-spotted spider mite, *Tetranychus urticae* Koch, is an important mite pest worldwide (Flamini 2006). It is a serious pest of strawberry, shrubs, and ornamental trees in the northern part of Thailand (Charanasri et al. 1988). The cassava red mite, *Tetranychus truncatus* Ehara, is a major mite pest throughout Asia and the Pacific Islands (Bolland et al. 1998). In Thailand, they are known to be serious pests

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in cassava-growing areas in the eastern region. Both mite species are often found on the lower leaf surface and use their stylets to suck fluid from leaf cells resulting in foliar chlorosis, leaf desiccation and drop, and reductions in yield (Kumral et al. 2010).

Alternatives to conventional chemical acaricides are needed. Overuse of synthetic pesticides has negative effects on nontarget organisms, human health, and the environment. To address these problems, plant extracts are applied to control mite pests because these natural products have a short environmental persistence and low mammalian toxicity compared with various chemical pesticides (Attia et al. 2012). *Zanthoxylum myriacanthum* Wall. ex Hook. f., locally known as makwhaen in northern Thailand, belongs to the Rutaceae family (Suksathan et al. 2009) and can be found in tropical and temperate areas (Bubpawan et al. 2015). In northern Thailand, its fruit is normally used as a condiment and/or spice (Sriwichai et al. 2019). Limonene (67.1%), α-pinene (6.4%), β-myrcene (3.8%), and linalool (3.0%) are the major components of makwhaen essential oil (Li et al. 2014). The essential oil from the fruit of a related species, *Zanthoxylum limonella* (Dennst.) Alston, has insecticidal effects on *Aedes aegypti* (L.) fourth instars with a median lethal concentration (LC_{50}) of 24.61 ppm (LC_{95}, 55.81 ppm) in 24 h (Pitasawat et al. 2007). However, little is known about the acaricidal activity of *Z. myriacanthum* extracts on polyphagous mite pests. Therefore, this research was focused on the adulticidal, ovicidal, and repellent properties of makwhaen (*Z. myriacanthum*) crude extracts against two-spotted spider mite and cassava red mite under laboratory conditions.

Materials and Methods

Mite rearing. Two-spotted spider mites and red cassava mites were obtained from the Acarology Laboratory in Department of Entomology, Kasetsart University (Bangkok, Thailand). Mites were reared in plastic boxes (17.5-cm width [W] × 25-cm length [L] × 4-cm height [H]) containing a mulberry, *Morus alba* L., leaf placed on tissue paper on a moistened sponge (13-cm W × 22.5-cm L × 2.5-cm H). A mite-infested mulberry leaf was cut into small pieces and placed on the fresh leaf to allow mites to move to the fresh leaves as previously described by Auamcharoen and Chandrapatya (2015). Each spider mite species was maintained in separate boxes in the laboratory at ambient room conditions (27 ± 2°C, 10-h light [L]: 14-h dark [D]). All experiments were conducted under conditions similar to those for mite rearing.

Crude extract preparation. Makwhaen dried fruits were collected from the local orchard in Mueang District, Lampang Province, Thailand, in September 2018. The extraction method was that of Janlaor and Auamcharoen (2021). Briefly, 1 kg of ground dried makwhaen fruit was immersed separately with 2 L of each organic solvent (hexane, methylene chloride, or methanol) in a glass bottle (5-L capacity) at room temperature for 3 d. During this time, the extract glass bottle was shaken every day. The residue was extracted two more times. The combined solvent solution from each extraction was filtered through a cheesecloth and refiltered again with Whatman no. 1 filter paper (GE Healthcare UK Limited, Amersham Place, Little Chalfont, Buckinghamshire, UK). The hexane, methylene chloride, and methanol solutions were evaporated separately using a rotary evaporator under reduced

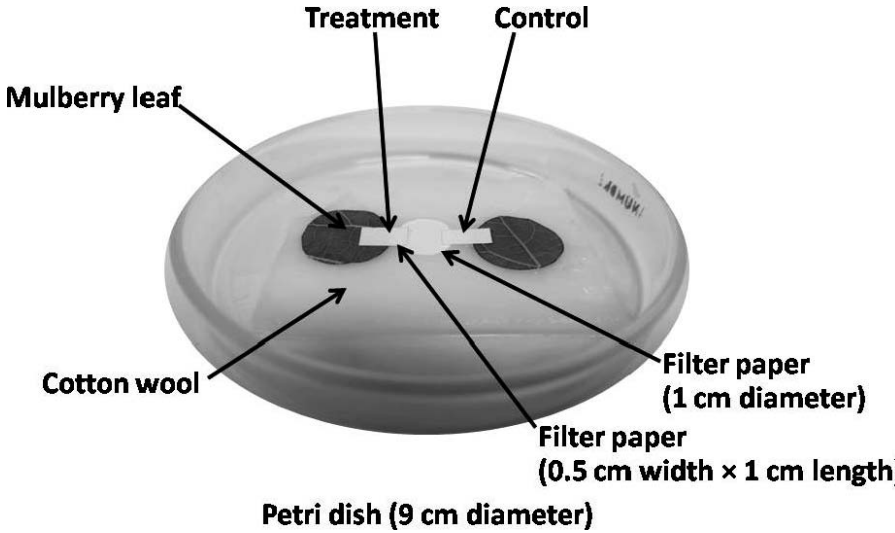

Fig. 1. Diagram of the assay arena used for evaluation of repellent activity of *Z. myriacanthum* extract.

pressure to receive the hexane extract, methylene chloride extract, and methanol extract. The crude extracts were kept separately in amber bottles in the refrigerator (10 ± 2°C) until used in bioassays.

Adulticidal bioassays. A cork borer was used to cut 2-cm diameter discs from fresh mulberry leaves. Three discs each were placed abaxial surface up on moistened cotton in a 9-cm-diameter glass petri dish. Twenty adult females of the same age of each mite species were introduced to each disc by using a fine paint brush. Mites on the 3 leaf discs were sprayed with 500 μL of 6, 8, 10, and 12% (w/v) concentrations of makwhaen extracts using a plastic atomizer. Mites on the mulberry leaf discs in the control were treated with 1% (v/v) Tween-20 (BDH Laboratory Supplies, Poole, UK) plus water. Each treatment was replicated four times with three leaf discs per replicate. The number of dead mites on each leaf disc adult was counted at 24 h after exposure.

Ovicidal bioassays. The bioassay arenas with the three leaf discs were established as in the previous bioassay. Twenty adult females of the same age of each mite species were transferred to each disc by using a fine brush, where they remained for 24 h to lay eggs. The adults were then removed from each disc after 24 h. Leaf discs and eggs were treated with 500 μL of the appropriate extract solution at concentrations of 6, 8, 10, and 12% (w/v) using a plastic atomizer. Controls were treated with 1% Tween-20 in water as done in the previous bioassay. Each treatment was replicated four times with three leaf discs per replicate. The number of hatched eggs on each leaf disc was recorded 5 d after treatment.

Repellent bioassays. The repellency of the extracts was assessed using the methods of Da Camara et al. (2015) and Sararit and Auamcharoen (2020) (Fig. 1). Each assay arena was a 9-cm glass petri dish with a moistened rectangular pad of cotton on the bottom. Two 2-cm-diameter mulberry leaf discs and a 1-cm-diameter

filter paper disc (Whatman no. 1) were arranged linearly on the cotton filter paper that was cut into 1-cm lengths, coated on the bottom with laminating film, and placed between two mulberry leaf discs. Two rectangles of filter paper (Whatman no. 1) measuring 0.5-cm W $\times$ 1-cm L were positioned so that each served as a bridge from the center-positioned filter paper disc to an outer leaf disc. One of the bridges was treated with 15 μL of an appropriate extract solution (6, 8, 10, and 12% [w/v] concentrations), and the other was treated with 1% Tween-20 plus water to serve as a control. Solutions on both bridges were allowed to evaporate for 10 min, after which 20 adult female mites were placed on the middle filter paper disc in the arena. The number of mites on each mulberry leaf disc was counted at 1, 2, 3, 4, 5, 24, 48, and 72 h after treatment. The percent repellence was calculated using the following formula: percent repellency $= [(C - T)/(C + T)] \times 100$. In the formula, C is the number of mites on the leaf disc on the control side and T is the number of mites on the leaf disc on the treated side (Akhtar et al. 2012). Each treatment was replicated four times.

Extract analysis. Gas chromatography-mass spectrometry (GC-MS) analysis was performed with a 5973 Agilent GC 6890N Series autosampler (Agilent, Santa Clara, CA). Each sample contained 2 μL of extract. The initial temperature was programmed at 70°C, then increased to 160°C at the rate of 2°C/min, then increased to 220°C at a rate of 2°C/min, and held for 10 min for total run time of 85 min. Other operating parameters were high purity helium as the gas carrier and a flow rate of 1 mL/min, and the injector and ion source temperatures were 230 and 280°C, respectively. A mass spectra (MS) 40-550 Atomic Mass Unit was used. The MS and retention indices of extract constituents were identified by comparison to a computer library (National Institute of standard and Technology [NIST], Mass Spectral Search Program and Chemstation Wiley Spectral Library, Gaithersburg, MD).

Statistical analyses. Each of the bioassays was performed under a completely randomized design, and data were subjected to analysis of variance. Data reported as a percentage were transformed using the arcsine square root transformation before analysis. Tukey's honestly significant difference test was used for the separation of the treatment means (R Development Core Team 2016). The LC_{50}s were calculated by probit analysis (Finney 1971) using SPSS software version 19.0 (Statistical Package for the Social Sciences, Version 19.0, Armonk, NY).

Results

Adulticidal bioassays. The mean $\pm$ SE mortality of *T. urticae* adults at 24 h after exposure ranged from 52.1 $\pm$ 4.3 to 73.3 $\pm$ 5.1% with the hexane extract and 45.4 $\pm$ 5.8 to 85.8 $\pm$ 4.5% with the methylene chloride extract; however, mortality with the methanol extract was only 14.2 $\pm$ 2.1 to 42.5 $\pm$ 8.4% (Table 1). Similar levels of mortality were observed with *T. truncatus* treated with the hexane (36.3 $\pm$ 6.0 to 73.3 $\pm$ 6.6%) and methylene chloride (51.3 $\pm$ 6.6 to 85.0 $\pm$ 3.5%) extracts. Over the four concentrations (6, 8, 10, and 12%), mortality following exposure to the methanol extract was markedly higher in *T. truncatus* adults (73.8 $\pm$ 8.8 to 95.8 $\pm$ 1.5%) than that in *T. urticae* adults (14.2 $\pm$ 2.1 to 42.5 $\pm$ 8.4%). Statistically significant differences were detected among treatment means within extract

Table 1. Mean ± SE percent mortality of *T. urticae* and *T. truncatus* adult females following exposure to different concentrations of *Z. myriacanthum* extracts.

Spider Mite	Extract Concentration (%)	Mean ± SE Percent Mortality at 24 h Postexposure*		
		Hexane Extract	Methylene Chloride Extract	Methanol Extract
T. urticae	6	58.8 ± 6.0ab	45.4 ± 5.8c	14.2 ± 2.1b
	8	60.8 ± 7.3ab	51.3 ± 8.4bc	18.8 ± 2.8b
	10	52.1 ± 4.3b	71.3 ± 7.3ab	38.8 ± 6.8a
	12	73.3 ± 5.1a	85.8 ± 4.5a	42.5 ± 8.4a
	Control (1% Tween-20)	0 ± 0c	0 ±0d	0 ± 0b
	Control (untreated)	0 ± 0c	0 ± 0d	0 ± 0b
T. truncatus	6	36.3 ± 6.0b	51.3 ± 6.6b	73.8 ± 8.8b
	8	62.5 ± 6.6a	68.3 ± 7.0ab	81.7 ± 7.2ab
	10	66.7 ± 9.5a	70.8 ± 7.7ab	80.8 ± 5.7ab
	12	73.3 ± 6.6a	85.0 ± 3.5a	95.8 ± 1.5a
	Control (1% Tween-20)	0.4 ± 0.4c	0.4 ± 0.4c	0 ± 0c
	Control (untreated)	0 ± 0c	0 ± 0c	0 ± 0c

*Treatment means within the same column and within spider mite species that are followed by the same lowercase letter are not significantly different ($P > 0.05$; Tukey's honestly significant difference [HSD]) ($n = 240$ mites). Means (± SE) of untransformed data are reported.

treatments and mite species; however, statistical separation among the multiple means was affected by the variation in the data. Regardless, the mortality response of the two mite species was, in general, positively related to the concentration of the extract.

The LC_{50} for the hexane extract against *T. truncatus* was 7.94% (95% fiducial limits [FL] = 5.15–10.30; slope ± SE, 0.22 ± 0.02). For the methylene chloride extract, the LC_{50}s were 7.55% (95% FL = 5.71–9.00; slope ± SE, 0.25 ± 0.02) with *T. urticae* and 6.88% (95% FL = 3.67–8.89; slope ± SE, 0.24 ± 0.02) with *T. truncatus*, which were not statistically different based on overlapping 95% FL of these values. To the contrary, the LC_{50}s of the methanol extract against *T. urticae* and *T. truncatus* were 12.34% (95% FL = 11.41–13.76; slope ± SE, 0.19 ± 0.03) and 5.36% (95% FL = −3.53–8.02; slope ± SE, 0.28 ± 0.02), respectively, which were statistically different based on nonoverlapping 95% FL values.

Ovicidal activity. The only extract that exhibited appreciable ovicidal activity was the hexane extract against *T. truncatus* that reduced egg hatch by 55.4%,

62.4%, 59.8%, and 68.7% at the 6%, 8%, 10%, and 12% concentrations, respectively, at 5 d after exposure (Table 2). These means did not differ statistically from each other, but they differed significantly ($F = 61.74$; df $= 5, 66$; $P < 0.001$) from the controls. All other treatments limited egg hatch by only 45% or less.

Repellent bioassays. The repellency of the concentrations of the three extracts was determined by mite response to filter paper strips treated with the chemical. The filter paper strips acted as bridges to an untreated leaf disc from an untreated filter paper disc. Mites were allowed paired choices between untreated leaf discs via the treated bridge and an untreated bridge. Numbers of mites were counted on each leaf disc and each bridge at times following treatment for a period of 72 h. Based on a percentage of repellency derived from those counts, we found no statistical differences in the concentrations or types of extracts for both *T. urticae* and *T. truncatus* adult females at 1, 5, 24, 48, and 72 h after treatment (Table 3).

Extract analysis. Fifteen and 18 constituents were identified from the hexane and methylene chloride extracts of *Z. myriacanthum* dried fruits, respectively, with different elution time and amount (Tables 4, 5). The main components in the hexane extract were DL-limonene (29.75%), sabinene (9.76%), caryophyllene oxide (4.70%), 1,3-cyclooctane (3.85%), and α-terpineol (2.71%) (Table 4). Limonene (40.70%), sabinene (16.60%), 1,3-cyclooctane (3.71%), trans-sabinene hydrate (3.11%), and (-)-caryophyllene oxide (2.79%) were the main constituents of the methylene chloride extract (Table 5).

Discussion

Our results revealed that the 10% and 12% concentrations of the methylene chloride and hexane extracts of *Z. myriacanthum* fruit caused >70 % mortality of *T. urticae* and *T. truncatus* adult mites 24 h after exposure. Based on the LC_{50}s, the methylene chloride extract exhibited a stronger adulticidal effect on *T. urticae* than the methanol extract. Against *T. truncatus*, three extracts presented similar adulticidal activity. Our results were similar to those of other studies. Tewary et al. (2005) reported that 5,000 and 10,000 ppm of petroleum ether extract of *Zanthoxylum armatum* L. leaves caused 35 and 38% *T. urticae* adult mortality, respectively, at 48 h after treatment. Attia et al. (2012) reported the contact toxicity property of extracts in three plant species belonging to Rutaceae family as well as *Z. myriacanthum*. The distillate of *Haplophyllum tuberculatum* Forssk. demonstrated high activity against larvae and adult females of *T. urticae* (94% and 93% mortality, respectively) at 72 h posttreatment, followed by *Ruta chalepensis* L. (66% and 61% mortality, respectively), and *Citrus aurantium* L. (63% and 55% mortality, respectively).

Moreover, an essential oil in other *Zanthoxylum* species also possesses biological activities on the red flour beetle (*Tribolium castaneum* [Herbst]) and mosquito species. Wanna and Satongrod (2020) reported that a 5% concentration of essential oil from dried seeds of *Z. limonella* Alston showed the highest activity on *T. castaneum* at 120 and 48 h for adults and larvae, respectively, whereas 10% concentration caused 100% mortality of *T. castaneum* eggs at 14 d. The *Z. limonella* essential oil hydrolate presented strong larvicidal activity on *Aedes albopictus* (Skuse) and *Culex quinquefasciatus* Say after 24 h. Against *Ae.*

Table 2. Mean (± SE) percentage of unhatched eggs of *T. urticae* and *T. truncatus* treated with different concentrations of *Z. myriacanthum* extracts.

Extract	Spider Mite	Extract Concentration (%)	Mean Total Eggs	Mean (± SE) Percent Unhatched Eggs at Different Days Postexposure*		
				3	4	5
Hexane	*T. urticae*	6	16	32.3 ± 2.8cd	11.8 ± 2.7c	5.7 ± 2.4c
		8	19	41.6 ± 5.2bc	18.2 ± 4.8bc	13.2 ± 4.1bc
		10	19	49.9 ± 3.7ab	30.1 ± 4.1ab	23.4 ± 4.3ab
		12	19	61.5 ± 6.5a	43.7 ± 6.1a	35.4 ± 7.2a
		Control (1% Tween-20)	21	19.4 ± 2.8de	12.6 ± 2.7c	10.2 ± 2.5bc
		Control (untreated)	21	8.6 ± 2.0e	5.9 ± 1.6c	6.8 ± 2.0bc
	T. truncatus	6	65	58.8 ± 4.9a	56.8 ± 5.1a	55.4 ± 2.1a
		8	52	71.4 ± 3.8a	67.7 ± 4.3a	62.4 ± 4.6a
		10	68	66.8 ± 4.5a	62.8 ± 5.1a	59.8 ± 5.2a
		12	64	72.4 ± 3.3a	70.6 ± 3.5a	68.7 ± 3.6a
		Control (1% Tween-20)	78	9.4 ± 2.8c	5.9 ± 1.6b	4.5 ± 1.2b
		Control (untreated)	77	27.3 ± 1.3b	3.0 ± 1.0b	2.8 ± 1.0b

Table 2. Continued.

Extract	Spider Mite	Extract Concentration (%)	Mean Total Eggs	Mean (± SE) Percent Unhatched Eggs at Different Days Postexposure*		
				3	4	5
Methylene chloride	*T. urticae*	6	95	29.3 ± 2.3b	23.3 ± 2.3bc	19.6 ± 2.3bc
		8	84	52.3 ± 6.0a	37.5 ± 6.4ab	32.4 ± 5.9ab
		10	77	49.8 ± 6.0a	40.2 ± 5.9a	34.7 ± 5.4ab
		12	90	51.9 ± 3.7a	42.3 ± 3.3a	39.7 ± 3.5a
		Control (1% Tween-20)	92	9.5 ± 1.1c	8.0 ± 1.0cd	7.2 ± 1.0c
		Control (untreated)	98	5.7 ± 1.5c	4.8 ± 1.6d	4.4 ± 1.4c
	T. truncatus	6	69	54.3 ± 3.0bc	24.6 ± 3.1b	24.6 ± 3.1b
		8	29	48.6 ± 5.6c	37.5 ± 5.1b	29.6 ± 5.2ab
		10	49	71.1 ± 3.2a	52.8 ± 3.6a	38.8 ± 2.9ab
		12	48	66.3 ± 2.8ab	55.9 ± 3.8a	41.6 ± 5.1a
		Control (1% Tween-20)	62	9.4 ± 1.3d	4.2 ± 0.8c	2.7 ± 0.9c
		Control (untreated)	78	4.7 ± 0.7d	2.3 ± 0.6c	1.9 ± 0.5c

Table 2. Continued.

Extract	Spider Mite	Extract Concentration (%)	Mean Total Eggs	Mean (± SE) Percent Unhatched Eggs at Different Days Postexposure*		
				3	4	5
Methanol	*T. urticae*	6	44	24.2 ± 1.2bc	18.8 ± 1.9c	17.0 ± 1.8bc
		8	46	20.0 ± 3.0cd	16.6 ± 2.9c	14.7 ± 2.7c
		10	43	40.2 ± 3.3b	34.3 ± 3.3b	31.5 ± 3.0ab
		12	39	60.0 ± 5.1a	51.0 ± 4.9a	45.2 ± 4.9a
		Control (1% Tween-20)	36	11.8 ± 2.1cd	7.0 ± 2.0c	6.2 ± 2.0c
		Control (untreated)	51	6.3 ± 1.1d	4.9 ± 1.1c	4.9 ± 1.0c
	T. truncatus	6	49	12.2 ± 3.7bc	9.2 ± 2.8bc	8.5 ± 2.7bc
		8	64	23.8 ± 3.6ab	20.1 ± 3.6b	18.5 ± 3.5b
		10	48	24.4 ± 5.2ab	20.2 ± 4.5b	18.3 ± 4.3b
		12	68	36.2 ± 6.5a	33.0 ± 6.1a	32.3 ± 6.4a
		Control (1% Tween-20)	74	12.6 ± 2.0bc	10.3 ± 1.8bc	9.8 ± 1.7bc
		Control (untreated)	70	3.8 ± 1.2c	2.8 ± 1.0c	2.4 ± 1.0c

*Treatment means within the same column, within extract, and within spider mite species that are followed by the same lowercase letter are not significantly different ($P > 0.05$; Tukey's HSD). Means ± SE of untransformed data are reported.

Table 3 Mean (± SE) percent repellency of *Z. myriacanthum* extracts to *T. urticae* and *T. truncatus* adult females on filter paper.

Extract	Spider Mite	Extract Concentration (%)	Mean ± SE Percent Repellency at Different h Postexposure*				
			1	5	24	48	72
Hexane	*T. urticae*	6	80 ± 20	100 ± 0	100 ± 0	100 ± 0	92 ± 8
		8	40 ± 24.5	100 ± 0	100 ± 0	96 ± 4	93.6 ± 6.3
		10	100 ± 0	100 ± 0	100 ± 0	100 ± 0	93.8 ± 4.2
		12	100 ± 0	100 ± 0	100 ± 0	100 ± 0	97.8 ± 2.1
	T. truncatus	6	100 ± 0	95.6 ± 4.4	80 ± 17.4	87.4 ± 10.2	87.2 ± 10.2
		8	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0
		10	100 ± 0	100 ± 0	92 ± 5.2	94 ± 3.8	90.4 ± 6.2
		12	60 ± 24.5	94.2 ± 5.7	88.2 ± 8.7	90 ± 7.8	88 ± 9.7
Methylene chloride	*T. urticae*	6	100 ± 0	100 ± 0	100 ± 0	100 ± 0	94 ± 4
		8	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0
		10	97.5 ± 2.5	98 ± 2	98 ± 2	98 ± 2	98 ± 2
		12	100 ± 0	100 ± 0	100 ± 0	100 ± 0	98 ± 2
	T. truncatus	6	80 ± 20	92 ± 8	97.6 ± 2.5	87.6 ± 9.7	95.4 ± 2.9
		8	60 ± 24.5	72.8 ± 18.8	90.4 ± 4.6	76.8 ± 11.6	64.2 ± 9.3
		10	100 ± 0	97.6 ± 2.4	97.8 ± 2.1	97.8 ± 2.1	95.2 ± 4.7
		12	94 ± 3.8	95.2 ± 2.9	95.4 ± 2.9	95.2 ± 2.9	95.2 ± 2.9

Table 3 Continued.

Extract	Spider Mite	Extract Concentration (%)	Mean ± SE Percent Repellency at Different h Postexposure*				
			1	5	24	48	72
Methanol	*T. urticae*	6	40 ± 24.5	80 ± 20	100 ± 0	100 ± 0	98 ± 2
		8	60 ± 24.5	100 ± 0	93.8 ± 4.1	90 ± 7.8	88 ± 8
		10	80 ± 20	100 ± 0	100 ± 0	98 ± 2	98 ± 2
		12	60 ± 24.5	100 ± 0	98 ± 2	100 ± 0	91.8 ± 3.8
	T. truncatus	6	80 ± 20	95.6 ± 4.4	95.6 ± 4.4	95.6 ± 4.4	95.8 ± 4.2
		8	80 ± 20	100 ± 0	100 ± 0	100 ± 0	100 ± 0
		10	60 ± 37.4	92 ± 2.4	97.6 ± 2.4	98 ± 2	97.8 ± 2.1
		12	100 ± 0	100 ± 0	81.8 ± 15.6	94 ± 4	94 ± 4

*Treatment means within the same column, within extract, and within spider mite species are not significantly different ($P > 0.05$; Tukey's HSD) (n = 80 mites). Means ± SE of untransformed data are reported.

Table 4. Chemical composition of hexane extract of dried *Z. myriacanthum* fruits.

No.	Compound	Retention Index	% Area
1	Sabinene	5.49	9.76
2	β-Myrcene	5.87	1.14
3	1,1-Dimethyl-2-(3-methyl-1,3-butadienyl)cyclopropane	6.44	0.26
4	DL-limonene	7.04	29.75
5	β-cis-Ocimene	7.64	0.76
6	cis-β-Terpineol	8.61	1.14
7	L-4-terpineneol	13.49	0.86
8	Cryptone	14.09	1.82
9	α-Terpineol	14.40	2.71
10	Decanal	14.68	1.04
11	1,3-Cyclooctane	14.88	3.85
12	α-Terpinolene	16.69	0.88
13	Geranyl acetate	24.65	2.33
14	Caryophyllene oxide	36.01	4.70
15	Linoleic acid	62.09	1.19

albopictus, the LC_{50} and LC_{90} values were 11 and 19.4% (v/v), whereas those values were 15.5 and 25.8% (v/v) against *Cx. quinquefasciatus* (Rabha et al. 2012). Soonwera and Phasomkusolsil (2017) reported that essential oil from *Z. limonella* dried fruit yielded an LC_{50} of 6.0% and 5.7% after 24 h for *Ae. aegypti* and *Cx. quinquefasciatus* adults, respectively. The concentration of 10% caused 100% mortality after 12 and 24 h of *Ae. aegypti* and *Cx. quinquefasciatus* larvae, respectively.

Among three tested extracts of *Z. myriacanthum*, the hexane extract at all tested concentrations (6–12%) exhibited ovicidal activity against *T. truncatus*, with >50% of eggs remaining unhatched eggs at the end of experiment. This finding suggests that the hexane extract is more suitable as an ovicide for *T. truncatus* than that for *T. urticae*. Unfortunately, the methylene chloride and methanol extracts had less ovicidal activity against the eggs of both species. Further work should be directed to the oviposition activity of the three extracts of *Z. myriacanthum* against *T. urticae* and *T. truncatus*, especially in light of the results of Soonwera and Phasomkusolsil (2017) who reported that *Z. limonella* essential oil deterred the ovipositional activity of *Ae. aegypti* and *Cx. quinquefasciatus* gravid females.

Hexane, methylene chloride, and methanol extracts of *Z. myriacanthum* repelled *T. urticae* and *T. truncatus* adults at levels >60% from 5 to 72 h postexposure. Da

Table 5. Chemical composition of methylene chloride extract of dried *Z. myriacanthum* fruits.

No.	Compound	Retention Index	% Area
1	Sabinene	5.48	16.60
2	β-Myrcene	5.86	1.94
3	L-phellandrene	6.30	0.25
4	δ 3-Carene	6.43	0.38
5	Limonene	7.05	40.70
6	2-β-Pinene	7.25	2.53
7	β-cis-Ocimene	7.62	1.87
8	cis-β-Terpineol	8.58	1.01
9	Trans-sabinene hydrate	9.86	3.11
10	L-4-terpineol	13.44	0.80
11	Crypton	14.02	1.33
12	α-Terpineol	14.34	2.26
13	Decanal	14.64	0.89
14	1,3-Cyclooctane	14.85	3.71
15	α-Terpinolene	16.94	0.94
16	Nerol acetate	24.60	1.82
17	trans-Caryophyllene	26.20	2.32
18	(-)-Caryophyllene oxide	35.94	2.79

Camara et al. (2015) demonstrated that essential oils from *Citrus sinensis* Osbeck var. *pera* and *C. aurantium* fruit skins were repellent at levels of 43.4 and 90.6%, respectively, against *T. urticae* adults at 1 h after exposure, which is a similar repellency effect to those obtained in our study. Furthermore, the essential oils from *Z. limonella* act as a repellent against mosquitoes. The fruit oil at a 30% concentration in mustard oil mixture repelled *Ae. albopictus* with the longest protection time (296–304 min) (Das et al. 2003). The fruit essential oil protected experiment participants from *Ae. aegypti*, *Cx. quinquefasciatus*, and *Anopheles dirus* Peyton & Harrison for 2 h using an arm-in-cage test (Trongtokit et al. 2005). The *Z. limonella* essential oil at a 10% concentration demonstrated 100% and 99.5% repellency against *Ae. aegypti* and *Cx. quinquefasciatus*, respectively (Soonwera and Phasomkusolsil 2017).

Dried *Z. myriacanthum* fruit extracted with different solvents in this study showed variations in the chemical compositions. Limonene was the major chemical compound in the hexane extract (29.75%) and the methylene chloride extract (40.70%), corroborating the findings of a number of previous studies in

which limonene was identified as the main chemical constituent of *Zanthoxylum* species (Li et al. 2014, 2016, Sriwichai et al. 2019). Limonene and sabinene were the major components from essential oils of fresh and dried *Z. myriacanthum* fruits (Sriwichai et al. 2019), whereas Li et al. (2014) found that limonene was the main compound in the essential oil of fresh *Z. myriacanthum* fruits. Also, Li et al. (2014, 2016) demonstrated that the main constituent was limonene in *Zanthoxylum schinifolium* Sieb. et Zucc. and *Zanthoxylum bungeanum* Maxim. Moreover, *d*-limonene also was the major chemical compound in *C. sinensis* and *C. aurantium* essential oils (Da Camara et al. 2015). Kim et al. (2013) found limonene (22.83%) in essential oil of *Anethum graveolens* L. This plant essential oil had high repellency and fumigant activities against *T. urticae* and *T. truncatus* (Sararit and Auamcharoen 2020). Consequently, limonene in hexane and methylene chloride extracts of *Z. myriacanthum* in this study may be the causative factors for the observed adulticidal, ovicidal, and repellency activities against *T. urticae* and *T. truncatus*. Limonene (255.44 mg/L) caused moderate toxicity on *T. urticae* by using direct contact application methods, whereas limonene at 125 mg/L displayed high egg mortality (Badawy et al. 2010). *d*-Limonene showed 71.3% repellency activity against *T. urticae* at 1 h of exposure (Da Camara et al. 2015).

Based on our results, the hexane, methylene chloride, and methanol extracts of *Z. myriacanthum* demonstrated potential for use in managing adults and eggs of *T. urticae* and *T. truncatus*. These extracts can be used as acaricides for controlling the population of both mites or can be applied as repellent products to protect plants from spider mite damage. Furthermore, Charoenying et al. (2010) reported that the crude ethyl acetate extract of *Z. limonella* Aston fruits at a 1,000-ppm concentration showed efficacy in inhibiting Chinese amaranth (*Amaranthus tricolor* L.). This result indicates that Z. *limonella* fruit extract may contain allelopathic toxins and may have potential as an allelopathic product. Xanthoxyline, a phenolic compound isolated from *Z. limonella* fruits at 2,500 μM also completely inhibited seed germination and growth of Chinese amaranth, and it inhibited barnyard grass (*Echinochloa crus-galli* [L.] Beauv.) seed germination, shoot length, and root length by 43.6%, 71.6%, and 87.5%, respectively. From their results, we might postulate the allelopathic effects of *Z. myriacanthum* extracts. Further studies should investigate the allelopathic and phytotoxic effects of *Z. myriacanthum* extracts on cultivated plants before developing them as botanical acaricides against spider mites on infested plants. The results will guide producers in the decision to use appropriate plant extracts for controlling target pests.

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References Cited

Akhtar, Y., E. Pages, A. Stevens, R. Bradbury, C.A.G. da Camara and M.B. Isman. 2012. Effect of chemical complexity of essential oils on feeding deterrence in larvae of the cabbage looper. Physiol. Entomol. 37: 81–91.

Attia, S., K.L. Grissa, Z.G. Ghrabi, A.C. Mailleux, G. Lognay and T. Hance. 2012. Acaricidal activity of 31 essential oils extracted from plants collected in Tunisia. J. Essent. Oil Res. 24: 279–288.

Auamcharoen, W. and A. Chandrapatya. 2015. Acaricidal and ovicidal efficacies of *Leucaena glauca* Benth. seed crude extracts on *Tetranychus urticae* Koch (Acari: Tetranychidae). J. Biopest. 8: 68–81.

Badawy, M.E.I., S.A.A. El-Arami and S.A.M. Abdelgaleil. 2010. Acaricidal and quantitative structure activity relationship of monoterpenes against the two-spotted spider mite, *Tetranychus urticae*. Exp. Appl. Acarol. 52: 261–274.

Bolland, H.R., J. Gutierrez and C.H.W. Flechtmann. 1998. World Catalogue of the Spider Mite Family (Acari: Tetranychidae). Brill, Leiden Boston Koln, The Netherlands.

Bubpawan, P., S. Boonphong, C. Sriwattanawarunyoo and V. Udeye. 2015. Characterization of the essential oil and fatty oil from makhwaen fruit (*Zanthoxylum rhetsa* (Roxb.) DC). NU. Int. J. Sci. 12: 1–10.

Charanasri, V., C. Sarinkaphaibul, M. Kongcheunsin, T. Kulpiyawat and N. Wongsiri. 1988. Taxonomic study on mites as pests of tangerine, *Citrus reticulate* Blanco. in Thailand, Pp. 133–177. *In* Insect Taxonomy and Acarology Research, Annual Report 1988. Department of Agriculture, Bangkok (in Thai).

Charoenying, P., M. Teerarak and C. Laosinwattana. 2010. An allelopathic substance isolated from *Zanthoxylum limonella* Alston fruit. Sci. Hort. 125: 411–416.

Da Camara, C.A.G., Y. Akhtar, M.B. Isman, R.C. Seffrin and F.S. Born. 2015. Repellent activity of essential oils from two species of citrus against *Tetranychus urticae* in the laboratory and greenhouse. Crop Prot. 74: 110–115.

Das, N.G., I. Baruah, P.K. Talukdar and S.C. Das. 2003. Evaluation of botanicals as repellents against mosquitoes. J. Vect. Borne Dis. 40: 49–53.

Finney, D.J. 1971. Probit Analysis. Cambridge Univ. Press, London.

Flamini, G. 2006. Acaricides of natural origin. Part 2. Review of the literature (2002-2006). Nat. Prod. Commun. 1: 1151–1158.

Janlaor, K. and W. Auamcharoen. 2021. Residual and direct contact toxicities of crude extracts and essential oils from *Acorus calamus* L. (Acoraceae) rhizomes against cassava red mites (Acari: Tetranychidae). J. Entomol. Sci. 56: 185–197.

Kim, S.W., J. Kang and I.K. Park. 2013. Fumigant toxicity of Apiaceae essential oils and their constituents against *Sitophilus oryzae* and their acetylcholinesterase inhibitory activity. J. Asia-Pac. Entomol. 16: 443–448.

Kumral, N.A., S. Cobanoglu and C. Yalcin. 2010. Acaricidal, repellent and oviposition deterrent activities of *Datura stramonium* L. against adult *Tetranychus urticae* (Koch). J. Pest Sci. 83: 173–180.

Le Goff, G., A.-C. Mailleux, C. Detrain, J.-L. Deneubourg, G. Clotuche and T. Hance. 2009. Spatial distribution and inbreeding in *Tetranychus urticae*. C. R. Biologies 332: 927–933.

Li, K., R. Zhou, W.W. Jia, Z. Li, J. Li, P. Zhang and T. Xiao. 2016. *Zanthoxylum bungeanum* essential oil induces apoptosis of HaCaT human keratinocytes. J. Ethnopharmacol. 186: 351–361.

Li, R., J.-j. Yang, Y.-x. Shi, M. Zhao, K.-l. Ji, P. Zhang, Y.-k. Xu and H.-b. Hu. 2014. Chemical compositions, antimicrobial and anti-inflammatory activities of the essential oil from maqian (*Zanthoxylum myriacanthum* var. *pubescens*) in Xishuangbanna, southwest China. J. Ethnopharmacol. 158 (Part A): 43–48.

Migeon, A., E. Nouguier and F. Dorkeld. 2010. Spider mites web: A comprehensive database for the Tetranychidae, Pp. 557–560. *In* Sabelis, M.W. and J. Bruin (eds.), Trends in Acarology: Proc. 12th Intern. Congr., Springer, Dordrecht, The Netherlands.

Pitasawat, B., D. Champakaew, W. Choochote, A. Jitpakdi, U. Chaithong, D. Kanjanapothi, E. Rattanachanpichai, P. Tippawangkosol, D. Riyong, B. Tuetun and D. Chaiyasit. 2007. Aromatic plant-derived essential oil: An alternative larvicide for mosquito control. Fitoterapia 78: 205–210.

Rabha, B., R. Gopalakrishnan, I. Baruah and L. Singh. 2012. Larvicidal activity of some essential oil hydrolates against dengue and filariasis vectors. E3 J. Med. Res. 1: 14–16.

R Development Core Team. 2016. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Sararit, P. and W. Auamcharoen. 2020. Biological activities of essential oils from *Anethum graveolens* L. and *Allium sativum* L. for controlling *Tetranychus truncatus* Ehara and *Tetranychus urticae* Koch. J. Biopest. 13: 01–12.

Soonwera, M. and S. Phasomkusolsil. 2017. Adulticidal, larvicidal, pupicidal and oviposition deterrent activities of essential oil from *Zanthoxylum limonella* Alston (Rutaceae) against *Aedes aegypti* (L.) and *Culex quinquefasciatus* (Say). Asian Pac. J. Trop. Biomed. 7: 967–978.

Sriwichai, T., P. Sookwong, M.W. Siddiqui and S.R. Sommano. 2019. Aromatic profiling of *Zanthoxylum myriacanthum* (makwhaen) essential oils from dried fruits using different initial drying techniques. Ind. Crops Prod. 133: 284–291.

Suksathan, R., C. Trisonthi, P. Trisonthi and P. Wangpakapattanawong. 2009. Notes on spice plants in the genus *Zanthoxylum* (Rutaceae) in northern Thailand. Thai For. Bull. (Bot.) Special Issue: Papers from the 14th Flora of Thailand Meeting. 197–204.

Tewary, D.K. A. Bhardwaj and A. Shanker. 2005. Pesticidal activities in five medicinal plants collected from mid hills of western Himalayas. Ind. Crops Prod. 22: 241–247.

Trongtokit, Y., Y. Rongsriyam, N. Komalamisra and C. Apiwathnasorn. 2005. Comparative repellency of 38 essential oils against mosquito bites. Phytother. Res. 19: 303–309.

Wanna, R. and B. Satongrod. 2020. Potential effects of essential oil from *Zanthoxylum limonella* seeds against *Tribolium castaneum* (Coleoptera: Tenebrionidae). Aust. J. Crop Sci. 14: 1920–1925.