Residual and Direct Contact Toxicities of Crude Extracts and Essential Oils from *Acorus calamus* L. (Acoraceae) Rhizomes against Cassava Red Mites (Acari: Tetranychidae)[1]

Kitiya Janlaor and Wanida Auamcharoen[2]

Department of Entomology, Faculty of Agriculture, Kasetsart University, Bangkok 10900, Thailand

J. Entomol. Sci. 56(2): 185–197 (April 2021)

Abstract *Tetranychus truncatus* Ehara (Acari: Tetranychidae) is a serious economic pest of many plants in Thailand and other countries. The use of plant extracts is an alternative to conventional synthetic pesticides for controlling mite pests. This study was conducted to evaluate the residual and direct contact toxicities of crude extracts and essential oils obtained from *Acorus calamus* L. (Acoraceae) rhizomes against *T. truncatus* eggs and adult females under laboratory conditions. Residual toxicity was assayed by applying compounds on leaf discs and then releasing adult female mites on the discs; direct contact toxicity was assayed by spraying the compounds on eggs and adult females. In residual assays, a 10% (v/v) concentration of essential oils extracted from fresh *A. calamus* rhizomes caused 73.8% mortality of *T. truncatus* adults, while treatment with oils from dried rhizomes caused 91.8% mortality of adults. In direct-contact toxicity assays, essential oils from fresh rhizomes reduced egg hatch by 96.3% at 5% (v/v) concentration and 100.0% at 10%. Oils extracted from dried rhizomes reduced egg hatch by 28.8% at 5% and 91.8% at 10%. The respective median lethal concentrations (LC_{50}s) were 2.18% and 5.91%, based on cumulative mortality at 7 d after treatment. Methylene chloride extracts from fresh and dried rhizomes (individual extraction method) caused a cumulative adult mite mortality of 100% and 91.4% at 5% (v/v) concentration, with LC_{50}s of 1.31% and 2.52%. Based upon our results, essential oils and methylene chloride extracts from *A. calamus* rhizomes appear as suitable botanical acaricides for further development for the management of *T. truncatus*.

Key Words plant extracts, spider mites, mite management

The cassava red mite, *Tetranychus truncatus* Ehara (Acari: Tetranychidae), is a serious spider mite pest of cassava (*Manihot esculenta* Crantz) and mulberry (*Morus alba* L.). These mites have a high reproductive rate and also a short life cycle (Sakunwarin et al. 2003), which contributes to the pest status. Their damage to plant leaves appears as chlorotic spots or areas which affect photosynthetic processes (Tomczyk and Kropczynska 1985).

Spider mites are routinely managed by applying conventional synthetic pesticides that are efficacious and easily available (Nauen et al. 2001). Nevertheless, chemistries used repeatedly and/or for extended times may result in the development of resistance in target populations, negative impacts on natural

[1]Received 08 May 2020; accepted for publication 09 June 2020.
[2]Corresponding author (email: fagrwda@ku.ac.th).

enemies, and deposition of harmful residues in the environment (Leeuwen et al. 2010; Ullah and Gotoh 2013). The use of natural products from plants (e.g., crude extracts, essential oils) is an alternative to conventional acaricides and their negative impacts.

Acorus calamus L. (Acoraceae), commonly known as sweet flag or calamus, is a perennial wetlands monocot that is native to India (Amit and Vandana 2013). Previous studies have shown that it has antibiotic effects against bacteria and fungi and insecticidal properties against some insects (Eswara Reddy et al. 2016; Koul 1987; Lee et al. 2004; Phongpaichit et al. 2005; Singh et al. 2010; Thaenthanee et al. 2014). The essential oil of *A. calamus* contains various chemical constituents depending on the plant parts (leaves or rhizomes) extracted, with β-asarone as one of the major chemicals identified (Liu et al. 2013; Lohani et al. 2012). This study was conducted to determine residual and direct contact toxicities of crude extracts and essential oils obtained from *A. calamus* rhizomes in laboratory bioassays against eggs and adult females of *T. truncatus*.

Materials and Methods

Mite colony. The stock colony of *T. truncatus* was initiated with from mites collected from infested cassava, *Ma. esculenta*, leaves collected at Kasetsart University (Bangkok, Thailand). Mites were fed on mulberry, *Mo. alba*, leaves placed on tissue paper on a moistened sponge in a plastic box (15 × 21 × 4 cm; width × length × height) and maintained at room temperature (27 ± 2°C and 10L : 14D). Leaves were replenished periodically by placing the infested leaf on a fresh leaf to allow mites to transfer to the fresh leaf as per methods of Auamcharoen and Chandrapatya (2015). All bioassays were conducted and maintained at the same conditions as for mite rearing.

Plant materials. Sweet flag, *A. calamus*, rhizomes were collected from Nonthaburi province, Thailand, in August 2016. The plant material was identified by Mr. Sukid Rueangruea (Department of National Parks, Wildlife and Plant Conservation, Bangkok, Thailand), where the voucher specimen (BKF No. 194351) was deposited in the Forest Herbarium of that facility. Extracts were taken from fresh rhizomes immediately after collection and transported to the laboratory. Extracts from dried rhizomes were from those that had been air-dried for 1 wk after collection.

Crude extract preparation. Crude extracts utilized in the bioassays were obtained by two extraction methods—the polar sequential extraction method and the individual extraction method. The process of the polar sequential extraction method was that of Aryani and Auamcharoen (2016) and Pancharoen et al. (2014). Briefly, 1 kg of ground fresh or dried rhizomes was placed in a glass bottle (5-L capacity) with 2 L of hexane for 3 d. The resulting residue was subjected to the same extraction process two additional times. The combined hexane solutions from these extractions were filtered using a Whatman No. 1 filter paper (GE Healthcare U.K. Limited, Amersham Place, Little Chalfont, Buckinghamshire, U.K.) and dried using a rotary evaporator under reduced pressure to receive the hexane extract. The residue from hexane extraction was re-extracted with methylene chloride and, finally, the methylene chloride extraction residue was re-extracted with methanol.

The methylene chloride and methanol solutions were separately evaporated to yield methylene chloride and methanol extracts, respectively. The individual extraction method did not involve sequential extractions. Fresh or dried rhizomes were extracted separately using only hexane, methylene chloride, or methanol at the same conditions previously described. Each of the crude extracts was placed in refrigeration (10 ± 2°C) until used in the bioassays.

Essential oils distillation. The essential oil distillation process is described by Wongtong and Nawanich (2001) and Torres et al. (2014). Briefly, fresh (1 kg) or dried (300 g) rhizomes were distilled by water (2.5 L) for 8 h using a Clevenger-type apparatus. The essential oils layer was separated from the water layer using a glass pipette dropper and stored in glass vials at 10 ± 2°C until used in the bioassays.

Preliminary screening of extracts and oils. Crude extracts and essential oils were screened for residual and direct contact toxicities against *T. truncatus* mites at concentrations of 2.5 and 5% (v/v). The materials showing efficacy against the mites were further evaluated for biological activity against *T. truncatus* eggs and adult females.

Residual contact toxicity bioassays. Mulberry leaf discs (2-cm diam) were placed lower-surface up on moistened cotton in 9-cm diam glass Petri dishes with 3 discs per dish. Droplets (50 μl) of the essential oil solution at concentrations of 0.625, 1.25, 2.5, 5 and 10% (v/v) were deposited on appropriate leaf discs. Mulberry leaf discs treated with Tween-20 (BDH Laboratory Supplies, Poole, BH15 1TD, U.K.) (1%, v/v) in water were used as the untreated controls. The solvent was allowed to evaporate, after which 20 *T. truncatus* adult females from the colony were placed on each disc using a fine paint brush. All treatments were replicated three times with three leaf discs per replication (Petri dish). The number of dead mites on each leaf disc was recorded every 24 h for three consecutive days.

Direct contact toxicity bioassays. For egg bioassays, mulberry leaf discs (2-cm diam) were placed on moistened cotton in 9-cm diam glass Petri dishes. Twenty *T. truncatus* adult females were placed on each leaf disc allowing them to oviposit on the discs. Adults were removed from the discs after 24 h. Three leaf discs with eggs were sprayed with 500 μl of 0.625, 1.25, 2.5, 5, and 10% (v/v) concentrations of the crude extract or the essential oil solution using a plastic atomizer. Eggs treated with Tween-20 (1%, v/v) in water served as controls. The number of unhatched eggs was counted 7 d later.

For adult mite bioassays, 20 adult females from the colony were placed on each 2-cm diam leaf disc in glass Petri dishes. These were then sprayed with 500 μl of crude extract solution at concentrations of either 0.625, 1.25, 2.5, 5, or 10% (v/v) using a plastic atomizer. Tween-20 (1%, v/v) in water was sprayed on leaf discs for the control. The number of dead mites on each leaf disc was counted at 24, 48, and 72 h after treatment.

Statistical analyses. Data were subjected to analysis of variance (ANOVA) following correction for control mortality (Abbott 1925). A Tukey's honestly significant difference (HSD) test was applied to compare the treatment means (R Development Core Team 2016). Median lethal concentrations (LC_{50}s) were estimated by probit analysis (Finney 1971) using SPSS (Statistical Package for the Social Sciences, Version 19.0, Armonk, NY).

Table 1. Cumulative mean (±SE) percent mortality of *T. truncatus* adult females following exposure to leaf surfaces treated with essential oils extracted from fresh and dried rhizomes of *A. calamus*.

Rhizome	Essential Oil Concentration (%)	Mean (±SE) Percent Mortality at Days Postexposure*		
		1 d	2 d	3 d
Fresh	0.625	6.23 ± 5.66d	3.90 ± 4.74d	4.84 ± 13.53c
	1.25	16.45 ± 4.18cd	16.51 ± 5.98cd	22.58 ± 13.52bc
	2.5	23.84 ± 3.70c	25.65 ± 4.48c	25.94 ± 10.22bc
	5.0	54.52 ± 4.00b	57.51 ± 5.18ab	62.07 ± 8.63ab
	10.0	72.29 ± 3.56a	75.27 ± 3.39a	73.85 ± 4.06a
Dried	0.625	4.44 ± 2.45d	11.76 ± 4.95c	17.10 ± 7.27c
	1.25	12.59 ± 4.43cd	16.79 ± 6.96c	19.90 ± 10.14bc
	2.5	20.85 ± 4.49bc	23.98 ± 6.13bc	26.18 ± 7.98bc
	5.0	34.95 ± 5.90b	41.93 ± 5.39b	47.42 ± 6.13b
	10.0	90.94 ± 2.45a	91.33 ± 2.61a	91.79 ± 2.27a
Control (untreated)		0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Control (1% Tween-20)		0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

* Treatment means within the same column and within fresh or dried rhizome that are followed by the same lowercase letter are not differ significantly ($P > 0.05$) under Tukey's HSD test ($n = 180$ mites).

Results

Residual toxicity. Mortality of *T. truncatus* adult females placed on leaf discs previously treated with essential oils extracted from *A. calamus* rhizomes varied with concentration and source (dried versus fresh) of the oil (Table 1). Cumulative percentage mortality 3 d after placement of female mites on the treated surfaces ranged from a mean (±SE) of 4.8 ± 13.5% at the 0.625% concentration to 73.8 ± 4.1% at the 10% concentration using oil from fresh rhizomes. Mean cumulative mortality following exposure to oils extracted from dried rhizomes was 17.1 ± 7.3% at the 0.625% concentration and 91.8 ± 2.3% at the 10% concentration. Mortality in the 5 and 10% concentrations did not differ statistically with the oil extracted from fresh rhizomes, but mortality with the 10% concentration (91.8 ± 2.3%) of the oil from dried rhizomes differed significantly ($F = 18.4$; df = 4, 40; $P < 0.001$) from that with the 5% concentration (47.4 ± 6.1%). The LC_{50}s of the respective preparations were 5.75% (95% fiducial limits [FL] = 3.39–12.01; Slope ± SE = 0.23 ± 0.02) for oils from fresh rhizomes and 4.97% (95% FL = 3.76–6.81; Slope ± SE = 0.28 ± 0.02) for oils from dried rhizomes, which are not statistically significant based on overlapping 95% FL of these values.

Table 2. Mean (±SE) percent hatched eggs of *T. truncatus* treated with essential oils extracted from fresh and dried rhizomes of *A. calamus*.

Rhizome	Essential Oil Concentration (%)	Mean Total Eggs	Mean (±SE) Percent Hatched Eggs at 7 Days Postexposure*
Fresh	0.625	69	88.38 ± 2.37d
	1.25	52	74.55 ± 3.55c
	2.5	120	33.37 ± 8.45b
	5.0	92	3.66 ± 1.66a
	10.0	121	0.00 ± 0.00a
Dried	0.625	75	94.26 ± 2.27d
	1.25	79	87.54 ± 3.60cd
	2.5	92	79.65 ± 4.83bc
	5.0	87	71.17 ± 3.90b
	10.0	73	8.24 ± 2.60a
Control (untreated)		124	100.00 ± 0.00
Control (1% tween-20)		103	100.00 ± 0.00

* Treatment means within the same column and within fresh or dried rhizome that are followed by the same lowercase letter are not differ significantly ($P > 0.05$) under Tukey's HSD test.

Ovicidal activity. Contact toxicity of essential oils and crude methanol extracts against *T. truncatus* eggs was determined by applying oils or extracts directly on the eggs. No eggs hatched following treatment with a 10% concentration of essential oil extracted from fresh rhizomes, while only 3.7 ± 1.7% of eggs hatched following treatment with 5% concentration of the oil (Table 2). These treatments did not differ statistically, but the higher concentrations differed significantly ($F = 88.02$; df = 4, 40; $P < 2e\text{-}16$) from the remaining concentrations tested. Oil extracted from dried rhizomes appeared be less effective as an ovicide with percentage hatch ranging from 94.3 ± 2.3% to 71.2 ± 3.9% following treatment with oil concentrations ≤5% (Table 2). Ovicidal activity was noted only with the 10% concentration of essential oil extracted from dried rhizomes with percentage hatch of 8.2 ± 2.6% (Table 2). The LC_{50}s of essential oil from fresh and dried rhizomes were 2.18% (95% FL = 1.74–2.78; Slope ± SE = 0.79 ± 0.06) and 5.91% (95% FL = 4.69–7.78; Slope ± SE = 0.31 ± 0.02), respectively, which are statistically significant based on nonoverlapping 95% FL of these values.

The crude methanol extract derived by the individual extraction method with dried rhizomes reduced egg hatch by 73.7 ± 5.1% at the 10% concentration (Table 3). Other concentrations of this extract were less effective in reducing egg hatch (82.6–42.2%). Extracts using polar sequential extraction methods with hexane and

Table 3. Mean (±SE) percent hatched eggs of *T. truncatus* treated with extracts from dried rhizomes of *A. calamus*.

Solvent (Rhizome, Extraction Method)	Extract Concentration (%)	Mean Total Eggs	Mean (±SE) Percent Hatched Eggs at 7 Days Postexposure*
Methanol (dried, individual)	0.625	39	82.58 ± 4.98c
	1.25	63	62.24 ± 7.59bc
	2.5	66	58.34 ± 4.81bc
	5.0	56	42.19 ± 9.52ab
	10.0	76	26.14 ± 5.12a
Hexane (dried, polar sequential)	0.625	95	96.17 ± 1.44b
	1.25	127	90.65 ± 1.72b
	2.5	82	65.26 ± 5.05a
	5.0	80	65.14 ± 5.18a
	10.0	66	49.18 ± 8.55a
Methylene chloride (dried, polar sequential)	0.625	170	98.93 ± 0.56c
	1.25	175	98.52 ± 0.91bc
	2.5	177	95.97 ± 0.63abc
	5.0	190	94.48 ± 1.05ab
	10.0	161	93.35 ± 1.75a
Control (untreated)		124	100.00 ± 0.00
Control (1% tween-20)		103	100.00 ± 0.00

* Treatment means within the same column and within solvent that are followed by the same lowercase letter are not differ significantly ($P > 0.05$) under Tukey's HSD test.

methylene chloride demonstrated low ovicidal activity (<51% reduction in hatch) on eggs of *T. truncatus* (Table 3).

Direct contact toxicity against adult females. Mortality of adult female mites was determined following exposure to various concentrations of compounds extracted from dried or fresh rhizomes by different extraction methods and solvents (Table 4). Mortality exceeded 90% with treatments of 2.5 and 5% concentrations of methylene chloride (individual method) extracts from fresh rhizomes, 5% concentration of methylene chloride (individual method) extract from dried rhizomes, and 5 and 10% concentrations of methanol (individual method) extracts from dried rhizomes. Mortality following treatment with 1.25, 2.5, 5.0, and 10.0% of the methylene chloride (polar sequential method) extracts from fresh rhizomes was >91% (Table 4). The LC_{50}s for the extracts ranged from 1.31% (95% FL = 1.03–1.65; Slope ± SE = 1.37 ± 0.11) for the methylene chloride extracts from fresh

Table 4. Cumulative mean (±SE) percent mortality of *T. truncatus* adult females following exposure to various concentrations of extracts from fresh and dried rhizomes of *A. calamus*.

Solvent (Rhizome, Extraction Method)	Extract Concentration (%)	Mean (±SE) Percent Mortality at Days Postexposure*		
		1 d	2 d	3 d
Methylene chloride (fresh, individual)	0.625	9.47 ± 2.55d	19.44 ± 5.15c	22.06 ± 4.24c
	1.25	32.57 ± 6.28c	43.04 ± 6.55b	49.36 ± 7.29b
	2.5	76.64 ± 3.98b	89.69 ± 3.72a	92.81 ± 3.83a
	5.0	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a
Methylene chloride (fresh, polar sequential)	0.625	25.31 ± 4.62c	40.16 ± 5.88c	46.01 ± 7.64b
	1.25	70.81 ± 3.44b	86.63 ± 3.00ab	91.42 ± 3.56a
	2.5	75.33 ± 3.85b	87.13 ± 2.42ab	92.10 ± 1.85a
	5.0	77.67 ± 4.67b	90.00 ± 3.39ab	93.72 ± 2.90a
	10.0	98.89 ± 0.73a	98.86 ± 0.75a	98.85 ± 0.77a
Methylene chloride (dried, individual)	0.625	5.44 ± 4.70c	15.92 ± 4.87bc	25.58 ± 6.21bc
	1.25	13.76 ± 4.54bc	19.88 ± 4.76bc	27.01 ± 6.30bc
	2.5	27.81 ± 6.18b	37.13 ± 8.68b	44.96 ± 7.78b
	5.0	77.73 ± 6.39a	89.53 ± 6.10a	91.36 ± 4.77a

Table 4. Continued.

Solvent (Rhizome, Extraction Method)	Extract Concentration (%)	Mean (±SE) Percent Mortality at Days Postexposure*		
		1 d	2 d	3 d
Methylene chloride (dried, polar sequential)	0.625	8.02 ± 5.53c	17.45 ± 6.07c	24.60 ± 7.35c
	1.25	11.92 ± 3.76c	24.02 ± 4.72c	30.27 ± 5.06bc
	2.5	21.71 ± 7.07bc	31.40 ± 7.34bc	35.57 ± 8.51bc
	5.0	44.31 ± 8.73ab	57.37 ± 8.37ab	60.21 ± 8.10ab
	10.0	59.97 ± 9.04a	76.29 ± 7.99a	75.39 ± 8.67a
Methanol (fresh, individual)	0.625	16.56 ± 5.24d	19.34 ± 5.53c	27.93 ± 6.93c
	1.25	18.32 ± 4.32cd	25.30 ± 5.90c	30.56 ± 6.15c
	2.5	34.14 ± 4.14bc	50.80 ± 8.07b	52.09 ± 8.10bc
	5.0	39.55 ± 7.88bc	49.81 ± 6.58b	58.60 ± 6.58b
	10.0	68.06 ± 6.04a	80.39 ± 4.01a	85.94 ± 4.19a
Methanol (fresh, polar sequential)	0.625	20.65 ± 4.07c	28.92 ± 5.53c	36.41 ± 7.39bc
	1.25	33.63 ± 5.48bc	37.57 ± 5.41bc	42.64 ± 5.92bc
	2.5	34.48 ± 3.28bc	43.18 ± 5.10bc	46.87 ± 3.77bc
	5.0	49.02 ± 7.92ab	51.93 ± 7.85ab	52.46 ± 7.81ab
	10.0	71.01 ± 5.59a	73.14 ± 4.17a	74.48 ± 4.24a

Table 4. Continued.

Solvent (Rhizome, Extraction Method)	Extract Concentration (%)	Mean (±SE) Percent Mortality at Days Postexposure*		
		1 d	2 d	3 d
Methanol (dried, individual)	0.625	8.45 ± 2.89d	13.54 ± 3.00b	27.55 ± 4.73bc
	1.25	18.41 ± 5.81d	31.29 ± 7.22b	40.75 ± 7.96bc
	2.5	26.19 ± 5.53cd	31.55 ± 7.38b	46.11 ± 5.80b
	5.0	74.06 ± 5.04a	83.43 ± 3.80a	91.53 ± 3.42a
	10.0	46.14 ± 6.87bc	81.61 ± 4.43a	93.98 ± 2.61a
Methanol (dried, polar sequential)	0.625	4.15 ± 3.40c	6.35 ± 3.83c	12.16 ± 5.05b
	1.25	15.00 ± 3.63c	19.81 ± 4.17c	25.05 ± 3.59b
	2.5	57.03 ± 9.20ab	69.28 ± 7.43ab	72.94 ± 6.99a
	5.0	63.04 ± 7.01ab	75.41 ± 4.78ab	79.81 ± 4.52a
	10.0	76.93 ± 5.30a	84.80 ± 5.76a	85.97 ± 5.46a
Control (untreated)		0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Control (1% tween-20)		0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

* Treatment means within the same column and within solvent that are followed by the same lowercase letter are not differ significantly ($P > 0.05$) under Tukey's HSD test ($n =$ 180 mites).

rhizomes obtained by the individual extraction method to 2.52% (95% FL = 1.51–4.47; Slope ± SE = 0.55 ± 0.04) for the methylene chloride extracts from dried rhizomes obtained by the individual extraction method (Table 4). The LC_{50} with adult females are not statistically significantly based on overlapping 95% FL of these values.

Discussion

Many plant extracts have biological activity against tetranychid spider mites in laboratory conditions. Residues of hexane extracts from *Annona vepretorum* Mart. (Annonaceae) yielded an LC_{50} value of 50.61 mg/ml against the two-spotted spider mite, *Tetranychus urticae* (Koch), while methanol extracts exhibited LC_{50}s of 10.96 mg/ml for topical + residue exposure and 22.07 mg/ml for residual toxicity only (Fernandes et al. 2017). Residues of ethanol extracts from *Annona muricata* L. seeds killed 93.3% of *T. urticae* mites, with the residual effects extended 120 h after treatment when >80% mortality was recorded (Maciel et al. 2015). Our results showed similar responses with the essential oils from fresh and dried rhizomes of *A. calamus* having residual activity resulting in >70% *T. truncatus* mite mortality 24 h after application of a 10% concentration of the extract. Based on LC_{50}s, essential oils from fresh and dried rhizomes of *A. calamus* appear to possess a potential for development as a residual miticides.

We also demonstrated that essential oil from rhizomes was an effective ovicide with >91% reduction in egg hatch following exposure to 5 and 10% concentrations of extracts from fresh rhizomes and 10% concentration of extracts from dried rhizomes. Similarly, Choi et al. (2004) also found that essential oils from caraway seed (*Carum carvi* L., Apiaceae), citronella java (*Cymbopogon nardus* (L.) Rendle, Gramineae), lemon eucalyptus (*Eucalyptus citriodora* Hook., Myrtaceae), pennyroyal (*Mentha pulegium* L., Lamiaceae), peppermint (*Mentha piperita* L., Lamiaceae), and spearmint (*Mentha spicata* L., Lamiaceae) had ovicidal effects against *T. urticae*. Erdogan et al. (2012), however, determined that oils from *Helichrysum arenarium* L. (Asteraceae), *Rhododendron luteum* Sweet (Ericaceae), *Veratrum album* L. (Liliaceae), *Tanacetum parthenium* L. (Asteraceae), and *Allium sativum* L. (Amaryllidaceae) had no ovicidal effects on *T. urticae*. In this study, the 10% concentration of methanol (individual method) extract from dried rhizomes of *A. calamus* showed a moderate ovicidal effect with 73.7% reduction in egg hatch. Similarly, Sarmah et al. (2009) reported relatively high ovicidal effects of aqueous extracts from either *Xanthium strumarium* (L.) or *A. calamus* against the spider mite *Oligonychus coffeae* (Nietner); however, extracts from *Polygonum hydropiper* (L.) and *Clerodendron infortunatum* (L.) proved less efficacious.

We observed that direct contact toxicity against adult *T. truncatus* is linked to the polarity of the organic solvents used for extraction. Methylene chloride extracts from fresh and dried rhizomes of *A. calamus* demonstrated higher acaricidal activity than did methanol extracts. This result corresponds to that of Roy et al. (2011) who tested the acaricidal activity of petroleum ether, acetone, and methanol extracts of *P. hydropiper* obtained by cold percolation and sequential extraction methods against *Oligonychus coffeae* with acetone fractions showing the greatest acaricidal activity, followed by petroleum ether and methanol fractions. Activity also showed a

positive concentration-dependent response. Roy et al. (2018) subsequently reported acaricidal properties of karanja oil, mustard oil, olive oil, sesame oil, castor oil, groundnut oil, and rose oil against *O. coffeae*. Water extracts from juazeiro, *Ziziphus joazeiro* von Martius, leaves applied to cotton foliage infested with *Tetranychus ludeni* Zacher caused mortality levels ranging from 17 to 81% (Ferraz et al. 2017).

Based on our results, using methylene chloride as the organic solvent to extract oil from fresh rhizomes of *A. calamus* by the polar sequential extraction method yielded a product that has greater potential for further development as an acaricide than those derived by the individual extraction method. However, the polar sequential extraction method is a longer process than the individual extraction method. With the polar sequential extraction method, methylene chloride will be used to extract the residues of plant materials after extraction with hexane; yet, methylene chloride can be used immediately to extract plant materials using the individual extraction method. On the other hand, the polar sequential extraction method is more cost effective in that residues of plant materials can be extracted several times with organic solvents before discarding. Under the individual extraction method, the residue of plant material extracted with only one solvent was discarded after extraction. Mite mortality in the control treatments interfered with our ability to discern cumulative *T. truncatus* mortality over longer periods of exposure.

Based on the experimental results, the essential oils from fresh and dried rhizomes of *A. calamus* had residual contact toxicity on *T. truncatus* adult females and direct contact toxicity on *T. truncatus* eggs. The essential oil from fresh rhizomes was more toxic than the essential oil from dried rhizomes against the egg stage of mites. On the contrary, the methylene chloride extracts from fresh *A. calamus* rhizomes extracted by individual extraction method and polar sequential extraction method displayed direct contact toxicity to *T. truncatus* adult females. Future studies should be directed to investigating the activities of the different active constituents in the essential oil and extracts of *A. calamus* rhizomes. This plant product is appropriate to develop as bio-acaricides against mites in the field.

Acknowledgments

This research is supported in part by the Graduate Program Scholarship from The Graduate School, Kasetsart University.

References Cited

Abbott, W.S. 1925. A method of computing the effectiveness of an insecticide. J. Econ. Entomol. 18: 265–267.

Amit, K. and Vandana. 2013. Medicinal properties of *Acorus calamus*. J. Drug Deliv. Ther. 3: 143–144.

Aryani, D.S. and W. Auamcharoen. 2016. Repellency and contact toxicity of crude extracts from three Thai plants (Zingiberaceae) against maize grain weevil, *Sitophilus zeamais* (Motschulsky) (Coleoptera: Curculionidae). J. Biopestic. 9: 52–62.

Auamcharoen, W. and A. Chandrapatya. 2015. Potential control of two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae) by crude extracts of *Duabanga grandiflora* (Lythraceae) and *Diospyros cauliflora* (Ebenaceae). Pak. J. Zool. 47: 953–964.

Choi, W.I., S.G. Lee, H.M. Park and Y.J. Ahn. 2004. Toxicity of plant essential oils to *Tetranychus urticae* (Acari: Tetranychidae) and *Phytoseiulus persimilis* (Acari: Phytoseiidae). J. Econ. Entomol. 97: 553–558.

Erdogan, P., A. Yildirim and B. Sever. 2012. Investigations on the effects of five different plant extracts on the two-spotted mite *Tetranychus urticae* Koch (Arachnida: Tetranychidae). Psyche 2012(1–2): 1–5.

Eswara Reddy, S.G., S.K. Dolma, R. Koundal and B. Singh. 2016. Chemical composition and insecticidal activities of essential oils against diamond back moth, *Plutella xylostella* (L.) (Lepidoptera: Yponomeutidae). Nat. Prod. Res. 30: 1834–1838.

Fernandes, M.H. de A., K.O. de Menezes, A.M. de Souza, J.R.G. da S. Almeida, J.E. de M Oliveira and R. de C.R.G. Gervasio. 2017. Bioactivity of the organic extracts of *Annona vepretorum* on *Tetranychus urticae* (Acari: Tetranychidae). Pesqui. Agropecu. Bras. 52: 707–714.

Ferraz, J.C.B., C.H.C. Matos, C.R.F. de Oliveira, M.D.G.R. de Sa and A.G.C. da Conceicao. 2017. Acaricidal activity of juazeiro leaf extract against red spider mite in cotton plants. Pesqui. Agropecu. Bras. 52: 493–499.

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

Koul, O. 1987. Antifeedant and growth inhibitory effects of calamus oil and neem oil on *Spodoptera litura* under laboratory conditions. Phytoparasitica 15: 169–180.

Lee, J.L., J.Y. Yan and B.S. Hwang. 2004. Antifungal activity of β-asarone from rhizomes of *Acorus gramineus*. J. Agric. Food Chem. 52: 776–780.

Leeuwen, T.V., J. Vontas, A. Tsagkarakou, W. Dermauw and L. Tirry. 2010. Acaricide resistance mechanisms in the two-spotted spider mite *Tetranychus urticae* and other important Acari: A review. Insect Biochem. Mol. Biol. 40: 563–572.

Liu, X.C., L.G. Zhou, Z.L. Liu and S.S. Du. 2013. Identification of insecticidal constituents of the essential oil of *Acorus calamus* rhizomes against *Liposcelis bostrychophila* Badonnel. Molecules 18: 5684–5696.

Lohani, H., H.C. Andola, N. Chauhan and U. Bhandari. 2012. Variations of essential oil composition of *Acorus calamus*: From Uttarakh and Himalaya. J. Pharm. Res. 5: 1246–1247.

Maciel, A.G.S., J.S. Rodrigues, R.C.P. Trindade, E.S. Silva, A.E.G. Sant'Ana and E.E.P. Lemos. 2015. Effect of *Annona muricata* L. (1753) (Annonaceae) seeds extracts on *Tetranychus urticae* (Koch, 1836) (Acari: Tetranychidae). Afr. J. Agric. Res. 10: 4370–4375.

Nauen, R., N. Stumpf, A. Elbert, C.P.W. Zebitz and W. Kraus. 2001. Acaricide toxicity and resistance in larvae of different strains of *Tetranychus urticae* and *Panonychus ulmi* (Acari: Tetranychidae). Pest Manage. Sci. 57: 253–261.

Pancharoen, S., A. Chandrapatya and W. Auamcharoen. 2014. Contact toxicity of sweet flag rhizome (*Acorus calamus* L.) crude extract on maize weevil, *Sitophilus zeamais* Motschusky, Pp. 1099–1103. *In* Proc. 11th International Working Conference on Stored-Product Protection. Chiang Mai, Thailand.

Phongpaichit, S., N. Pujenjob, V. Rukachaisirikul and M. Ongsakul. 2005. Antimicrobial activities of the crude methanol extract of *Acorus calamus* Linn. Songklanakarin J. Sci. Technol. 27: 517–523.

R Development Core Team. 2016. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Roy, S., G. Gurusubramanian and S.K. Nachimuthu. 2011. Anti-mite activity of *Polygonum hydropiper* L. (Polygonaceae) extracts against tea red spider mite, *Oligonychus coffeae* Nietner (Tetranychidae: Acarina). Int. J. Acarol. 37: 561–566.

Roy, S., G. Handique, F.R. Bora and A. Rahman. 2018. Evaluation of certain non-conventional plant based oils against red spider mite of tea. J. Environ. Biol. 39: 1–4.

Sakunwarin, S., A. Chandrapatya and G.T. Baker. 2003. Biology and life table of the cassava mite, *Tetranychus truncatus* Ehara (Acari: Tetranychidae). Syst. Appl. Acarol. 8: 13–24.

Sarmah, M., A. Rahman, A.K. Phukan and G. Gurusubramanian. 2009. Effect of aqueous plant extracts on tea red spider mite, *Oligonychus coffeae*, Nietner (Tetranychidae: Acarina) and *Stethorus gilvifrons* Mulsant. Afr. J. Biotechnol. 8: 417–423.

Singh, S., R. Srivastava and S. Choudhary. 2010. Antifungal and HPLC analysis of the crude extracts of *Acorus calamus*, *Tinospora cordifolia* and *Celestrus paniculatus*. J. Agric. Technol. 6: 149–158.

Thaenthanee, S., J. Sukprasert, S. Daosukho and S. Rodprasert. 2014. The study on efficiency of *Acorus calamus* L. extract against fruit rot fungi isolated from lychee. Bull. Appl. Sci. 3: 88–101.

Tomczyk, A. and D. Kropczynska. 1985. Effects on the host plant, Pp. 317–329. *In* Helle, W. and M.W. Sabelis (eds.), Spider Mites, Their Biology, Natural Enemies and Control. Elsevier, Amsterdam, Netherlands.

Torres, C., G. Silva, M. Tapia, J.C. Rodriguez, I. Figueroa, A. Lagunes, C. Santillan, A. Robles, S. Aguilar and I. Tucuch. 2014. Insecticidal activity of *Laurelia sempervirens* (Ruiz & Pav.) Tul. essential oil against *Sitophilus zeamais* Motschulsky. Chil. J. Agric. Res. 74: 421–426.

Ullah, M.S. and T. Gotoh. 2013. Laboratory-based toxicity of some acaricides to *Tetranychus macfarlanei* and *Tetranychus truncatus* (Acari: Tetranychidae). Int. J. Acarol. 39: 244–251.

Wongtong, S. and S. Nawanich. 2001. Some insecticidal plant extracts for controlling maize weevil, *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae). Kasetsart J. (Nat. Sci.) 35: 259–270.