Contact Toxicity of an Essential Oil from *Acorus calamus* (Acoraceae) Rhizomes against *Tetranychus urticae* and *Tetranychus macfarlanei* (Acari: Tetranychidae) and *Amblyseius longispinosus* (Acari: Phytoseiidae)[1]

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Abstract The contact toxicity activity of an essential oil extracted from *Acorus calamus* (L.) (Acoraceae) was evaluated against the phytophagous spider mites *Tetranychus urticae* Koch and *Tetranychus macfarlanei* Baker & Pritchard (Acari: Tetranychidae) and the predatory mite *Amblyseius longispinosus* (Evans) (Acari: Phytoseiidae). Adult mortality 24 h following application of 5% (v/v) concentration of the essential oil exceeded 90% for *T. urticae* and *T. macfarlanei*. Application of 1.2–5% concentrations of the essential oil to mite eggs reduced egg viability, with 0–54% hatch of *T. urticae* eggs and 0% hatch of *T. macfarlanei* eggs 6 d following treatment. At 2.5%, the essential oil was toxic to *A. longipinosus* by residual contact toxicity (58% mortality) and direct contact toxicity (0% mortality). No eggs and 47.6 eggs of *A. longispinosus* were oviposited with residual contact toxicity and direct contact toxicity, respectively. The chemical constituents of the essential oil, as determined with gas chromatography–mass spectrometry, showed that camphor (41.07%) and 5,5-dimethyl-2-ethynylcyclopent-2-en-1-ol (27.96%) were the major chemical compounds of the essential oil. These results indicate that this essential oil extracted from fresh *A. calamus* rhizomes could prove useful in controlling *T. urticae* and *T. macfarlanei*. Our findings also showed that the essential oil had no deleterious effects against *A. longispinosus* by direct contact toxicity test; however, *A. longispinosus* consuming spider mite eggs treated with essential oil were negatively impacted.

Key Words plant essential oil, twospotted spider mite, predatory mite

Tetranychid mites (Acari: Tetranychidae) are serious pests of various host plants (Helle and Sabelis 1985). The twospotted spider mite, *Tetranychus urticae* Koch, is an economically important mite pest with a global distribution (Flamini 2006). It feeds on at least 150 host plants of economic value in both greenhouse and field settings, resulting in serious economic losses (Da Camara et al. 2015). *Tetranychus macfarlanei* Baker & Pritchard is reported as a serious mite pest of okra (*Abelmoschus esculentus* [L.]), cotton (*Gossypium* spp.), cucurbits (*Cucurbita* spp.), soybean (*Glycine max* [L.] Merrill), and brinjal (eggplant; *Solanum melongena* L.). Mites damaging soybean fields during late vegetative and early reproductive growth caused a yield reduction of 40–60% (Satish et al. 2018). A

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large population of either mite can leaf chlorosis, leaf curling, and extensive webbing, leading to decreased yields (Kumral et al. 2010).

Phytoseiid mites have been effectively applied in biocontrol programs worldwide (Jeppson et al. 1975, McMurtry et al. 1970), and *Amblyseius longispinosus* (Evans) (Acari: Phytoseiidae) has potential as a predatory mite for reducing populations of phytophagous mite species (De Leon-Facundo and Corpuz-Raros 2005). This natural enemy has been reported occurring naturally on field crops, fruit crops, and ornamental plants (Corpuz-Raros 1989, Schicha and Corpuz-Raros 1992).

Synthetic acaricides have been routinely used to manage mite pests, but these chemistries can have negative repercussions to the environment, workers, and nontarget species and may result in development of mite resistances to the chemicals (Assouguem et al. 2022). Predatory mites are also susceptible to synthetic acaricides. Reduction or elimination of predatory mites with acaricidal applications resulted in lack of control of phytophagous spider mite populations (Alhewairini and Al-Azzazy 2021).

Plant-based acaricides, such as essential oils (EOs) and plant extracts, have been considered as management agents for mite pests. They possess contact, fumigant, and repellency toxicity activities against spider mites (Attia et al. 2012, Ghaderi et al. 2013, Sararit and Auamcharoen 2020). Botanical acaricides rapidly biodegrade, have low toxicity to the environment, and may be an alternative to persistent chemical acaricides.

Acorus calamus (L.) (Acoraceae), commonly known as sweet flag, is native to central Asia and eastern Europe and grows along swamps, rivers, and lakes (Gilani et al. 2006, Kim et al. 2009). An EO from *A. calamus* rhizomes injured plasmatocytes and granular hemocytes, thus altering the hemogram of *Spodoptera litura* (F.) (Sharma et al. 2008). This EO could be an alternative for *T. urticae* and *T. macfarlanei* control because it contains many bioactive chemicals (Liu et al. 2013, Lohani et al. 2012). Its toxicity to *A. longispinosus*, a predatory mite of *T. urticae* and *T. macfarlanei*, has not been fully explored or reported. Our objectives in this study were to (a) determine the contact toxicity activity of the EO extracted from fresh *A. calamus* rhizomes against *T. urticae* and *T. macfarlanei* adults and eggs, (2) determine the residual and direct contact toxicity of the EO against the predatory mite *A. longispinosus*, and (c) identify the chemical constituents of the EO.

Materials and Methods

Mite rearing. Twospotted spider mites and *T. macfarlanei* were obtained from the Acarology Laboratory in the Department of Entomology, Kasetsart University (Bangkok, Thailand). Each mite species was reared in a separate plastic box (17.5 cm × 25 cm × 4 cm, width × length × height) containing a clean mulberry (*Morus alba* L.) leaf placed on tissue paper on a moistened sponge (13 cm × 22.5 cm × 2.5 cm, width × length × height) and maintained in laboratory at room temperature (27 ± 2°C) and a photoperiod of 10:14 L:D h. A yellow mulberry leaf infested with mites was cut into small pieces and placed on the fresh leaf to allow mites to move to the fresh leaf (for details, see Auamcharoen and Chandrapatya 2015). All tests were conducted and stored under conditions similar to those for mite rearing.

Distillation of EO. The methods for EO distillation were described by Janlaor and Auamcharoen (2021). In brief, fresh *A. calamus* rhizomes (1 kg) were distilled by water (2.5 L) for 8 h by using a Clevenger-type apparatus. The EO layer was separated from the water layer by using a glass pipette dropper and transferred to amber glass vials for storage in a refrigerator at 10 ± 2°C until used in the tests.

Direct contact toxicity against adult female spider mites. A cork borer was used to cut 2-cm-diameter discs of mulberry leaves. Three discs were deposited, abaxial surface up, on moistened cotton pads in a 9-cm-diameter glass Petri dish to establish a bioassay arena for each mite species and EO concentration. Twenty adult females of the same age of each spider mite species were moved to each leaf disc by using a fine paint brush. The three leaf discs and mites were sprayed with 500 μl of a concentration of EO by using a plastic atomizer. Treatment concentrations were 0.15, 0.3, 0.6, 1.2, 2.5, and 5% (v/v). Mites on leaf discs in the control were sprayed with 1% (v/v) Tween 20 (BDH Laboratory Supplies, Poole, United Kingdom) plus water. Each treatment was replicated three times with three leaf discs per replication. The number of dead mites on each leaf disc was recorded 24 h following treatment.

Direct contact toxicity against eggs of spider mites. Test arenas with the three mulberry leaf discs were performed as described in the prior test. Twenty adult females of the same age of each spider mite species were moved to each leaf disc by using a fine paint brush. Females had the opportunity to lay eggs for 24 h, after which the adults were removed from each leaf disc. Three leaf discs containing eggs were sprayed with 500 μl of the EO solution at a concentration of either 0.15, 0.3, 0.6, 1.2, 2.5, or 5% (v/v) by using a plastic atomizer. Eggs on leaf discs in the control were sprayed with 1% (v/v) Tween 20 in water as described in the prior test. Each treatment was replicated three times with three leaf discs per replication. The number of hatched eggs on each leaf disc was recorded 6 d following exposure.

Direct contact toxicity against adult predatory mites. Mulberry leaf discs (4-cm-diameter) were cut from leaves by using a cork borer. One leaf disc (abaxial surface facing up) was placed on moistened cotton in a 9-cm-diameter plastic Petri dish. Fifty adult female spider mites were introduced to the leaf disc by using a fine paint brush and allowed to lay eggs for 24 h. The adults were then removed from leaf disc. Ten predatory mites on the leaf disc were treated with 500 μl of the 2.5% (v/v) concentration of EO by using a plastic atomizer. The control was treated with 1% Tween 20 in water. Next, treated predatory mites were transferred to the prepared leaf disc containing eggs of spider mites in a plastic Petri dish. The Petri dish was covered. Each treatment was replicated five times, with one leaf disc per replication. The numbers of dead predatory mites and eggs on the leaf disc were counted 24 h following treatment.

Residual contact toxicity against adult predatory mites. This test was established as in the direct contact toxicity test on predatory mites. Fifty adult female spider mites were introduced to each leaf disc and allowed to lay eggs for 24 h. Adults were then removed from the leaf disc. Five hundred microliters of 2.5% (v/v) EO solution was applied to the spider mite eggs by using a plastic atomizer. The control was treated with 1% Tween 20 in water. Ten predatory mites were introduced to leaf disc containing treated spider mite eggs in each plastic Petri dish. The Petri dish was then covered. Each treatment was replicated five

times, with one leaf disc per replication. The numbers of dead predatory mites and eggs on the leaf disc were counted 24 h following treatment.

EO analysis. Gas chromatography–mass spectrometry analysis of the EO was conducted with an Agilent 6890N gas chromatograph and 5973N mass selective detector with 7683 series autosampler (Agilent, Santa Clara, CA). Each sample contained 2 μl of EO. The initial temperature was programmed at 70°C and then increased to 160°C at the rate of 2°C/min and then to 220°C at a rate of 2°C/min and finally held for 10 min, for total run time of 85 min. Other operating parameters were high-purity helium as the gas carrier, a flow rate of 1 ml/min, DB-5MS, 5% phenyl/95% dimethyl polysiloxane fused-silica capillary column (0.25 mm I.D., 0.25-μm film thickness, 30.0-m length), and injector and ion source temperature of 230 and 280°C, respectively. Mass spectra (MS), 40–550 atomic mass units, were used. The MS and retention indices of EO constituents were identified by comparison to MS computer library (National Institute of standard and Technology, Mass Spectral Search Program and Chemstation Wiley Spectral Library).

Statistical analysis. All acaricidal experiments were performed under a completely randomized design. Data are reported as percentages and were transformed using the arcsine square root transformation before analysis. Analysis of variance was used to analyze all data. Tukey's honestly significant difference test was applied to compare the treatment means at $P = 0.05$ (R Development Core Team 2016). Median lethal concentrations (LC_{50}s) were calculated by probit analysis (Finney 1971) by using SPSS, Version 19.0 (Statistical Package for the Social Sciences, Armonk, NY).

Results

Direct contact toxicity against adult female spider mites. Mortality of adult female spider mites was evaluated following exposure to various concentrations of EO (0.15, 0.3, 0.6, 1.2. 2.5, and 5%) extracted from fresh rhizomes of *A. calamus* (Table 1). Mortality of *T. urticae* and *T. macfarlanei* adults 24 h following exposure exceeded 90% with treatments of the 5% concentration. Mortality (mean ± SE) of *T. urticae* (99.44 ± 0.56%) and *T. macfarlanei* (95.00 ± 5.00%) treated with the 5% concentration differed statistically ($F = 105.75$; df = 7, 16; $P < 0.001$ for *T. urticae* and $F = 31.30$; df = 7, 16; $P < 0.001$ for *T. macfarlanei*) from that of the remaining concentrations tested and the controls. The LC_{50}s of EO were 2.93% (95% fiducial limits [FL] = 2.59–3.46; slope ± SE = 1.28 ± 0.15) for *T. urticae* and 3.10% (95% FL = 2.53–3.82; slope ± SE = 0.77 ± 0.05) for *T. macfarlanei*, which were not statistically significant based on overlapping 95% FL of these values.

Direct contact toxicity against eggs of spider mites. Contact toxicity of EO from fresh rhizomes of *A. calamus* against spider mite eggs was determined by applying oils directly on the eggs (Table 2). No eggs of *T. urticae* hatched following treatment with a 5% concentration, whereas 54.44 ± 9.69 and 30.89 ± 6.61% of eggs hatched following treatment with 1.2 and 2.5% concentrations, respectively, of the EO. These two treatments did not differ statistically, whereas the highest concentration (5%) differed significantly ($F = 51.23$; df = 7, 16; $P < 0.001$) from the lower concentrations and the controls. The EO showed effectiveness as an ovicide against *T. macfarlanei* with concentrations ≥1.2%, and these concentrations differed significantly ($F = 312.39$; df = 7, 16; $P < 0.001$) from those of other concentrations tested and the controls. No eggs hatched following treatment with 1.2, 2.5, and 5% concentrations, whereas

Table 1. Percent mortality of *Tetranychus urticae* and *Tetranychus macfarlanei* adult females following exposure to various concentrations of essential oil from fresh rhizomes of *Acorus calamus*.

	Percent Mortality (Mean ± SE) at 24 h Postexposure*	
Concentration (%)	***T. urticae***	***T. macfarlanei***
0.15	1.11 ± 0.56c	2.22 ± 1.47c
0.3	1.69 ± 0.98c	4.44 ± 0.56bc
0.6	0.56 ± 0.56c	8.89 ± 0.56bc
1.2	1.13 ± 1.13c	17.22 ± 8.30bc
2.5	31.67 ± 7.64b	25.56 ± 2.00b
5	99.44 ± 0.56a	95.00 ± 5.00a
Control (1% Tween 20)	1.11 ± 0.56c	3.89 ± 3.09bc
Control (untreated)	0.00 ± 0.00c	0.55 ± 0.55c

* Treatment means within the same column that are followed by the same lowercase letter do not differ significantly ($P > 0.05$) via Tukey's honestly significant difference test ($n = 180$ mites).

concentrations ≤0.6% appeared to be less effective, with the percentage of egg hatch ranging from 98.80 ± 0.69 to 93.22 ± 3.07%. The LC_{50}s of EO against *T. urticae* and *T. macfarlanei* eggs were 1.68% (95% FL = 1.51–1.87; slope ± SE = 0.79 ± 0.06) and 0.78% (95% FL = 0.36–6.80; slope ± SE = 2.93 ± 0.21), respectively, and were not statistically significant based on overlapping 95% FL of these values.

Direct contact toxicity against predatory mite adults. Eggs of adult predatory mites were observed after adults were treated with the 2.5% concentration of EO and extract from fresh rhizomes of *A. calamus* (Table 3). Exposure to an EO, methylene chloride extract (MCE), and the mixture of EO and MCE did not cause mortality to the predatory mite *A. longispinosus* (data not shown). Numbers of *A. longispinosus* eggs laid averaged 47.6 ± 5.32, 53.2 ± 5.06, and 55.4 ± 3.28 in EO, MCE, and the mixture of EO and MCE treatments, respectively. These numbers were not statistically significant from those of the controls.

Residual contact toxicity against predatory mite adults. Mortality of adult predatory mites consuming eggs of spider mites treated with the 2.5% concentration of EO and extract from fresh rhizomes of *A. calamus* is shown in Table 4. The treatments of EO (58.0 ± 10.68%) and the mixture of EO and MCE (84.0 ± 6.0%) showed high toxicity to *A. longispinosus* compared with the MCE treatment (28.0 ± 6.63%). These treatments differed significantly ($F = 34.95$; df = 4, 20; $P < 0.001$) from those of the controls. No eggs of *A. longispinosus* were laid following treatment with EO and the mixture of EO and MCE, whereas 10.8 ± 3.44 eggs were observed in the MCE treatment (Table 4). These treatments did not differ statistically from each other, but differed significantly ($F = 21.91$; df = 4, 20; $P < 0.001$) from the controls.

EO analysis. Twelve constituents were identified from the EO of *A. calamus* fresh rhizomes, with different relation times and amounts (Table 5). The main components

Table 2. Percent hatched eggs of *Tetranychus urticae* and *Tetranychus macfarlanei* treated with different concentrations of essential oil from fresh rhizomes of *Acorus calamus*.

Concentration (%)	Mean Total Eggs		Percent Hatched Eggs (Mean ± SE) at 6 d Postexposure*	
	T. urticae	*T. macfarlanei*	*T. urticae*	*T. macfarlanei*
0.15	94	38	87.29 ± 4.97a	98.80 ± 0.69a
0.3	93	40	86.97 ± 4.01a	93.22 ± 3.07ab
0.6	84	41	84.33 ± 6.04a	94.94 ± 3.26ab
1.2	84	26	54.44 ± 9.69b	0.00 ± 0.00c
2.5	78	26	30.89 ± 6.61b	0.00 ± 0.00c
5	43	25	0.00 ± 0.00c	0.00 ± 0.00c
Control (1% Tween 20)	82	25	92.16 ± 1.79a	89.14 ± 1.63b
Control (untreated)	81	30	97.95 ± 0.63a	96.32 ± 1.28ab

* Treatment means within the same column that are followed by the same lowercase letter do not differ significantly ($P > 0.05$) via Tukey's honestly significant difference test (n = 180).

were camphor (41.07%), 5,5-dimethyl-2-ethynylcyclopent-2-en-1-ol (27.96%), 2,6,6-trimethyl-3-methylenecyclohexane (7.50%), L-4-terpineol (7.32%), and α-pinene (3.50%).

Discussion

Our results indicate that the 5% concentration of EO from *A. calamus* fresh rhizomes killed >90% of *T. urticae* and *T. macfarlanei* adult spider mites 24 h

Table 3. Egg number of *Amblyseius longispinosus* following exposure to 2.5% concentrations of essential oil (EO) and methylene chloride extract (MCE) from fresh rhizomes of *Acorus calamus*.

Treatment	Egg No. (Mean ± SE) at 24 h Postexposure*
EO	47.6 ± 5.32a
MCE	53.2 ± 5.06a
EO + MCE	55.4 ± 3.28a
Control (1% Tween 20)	48.8 ± 4.19a
Control (untreated)	54.6 ± 7.38a

* Treatment means within the same column that are followed by the same lowercase letter do not differ significantly ($P > 0.05$) via Tukey's honestly significant difference test ($n = 50$ mites).

Table 4. Percent mortality of *Amblyseius longispinosus* adult females following exposure to spider mite eggs treated with essential oil (EO) and methylene chloride extract (MCE) from fresh rhizomes of *Acorus calamus*.

Treatment	Percent Mortality (Mean ± SE) at 24 h Postexposure*	Egg No. (Mean ± SE) at 24 h Postexposure*
EO	58.0 ± 10.68b	0.0 ± 0.00b
MCE	28.0 ± 6.63c	10.8 ± 3.44b
EO + MCE	84.0 ± 6.00a	0.4 ± 0.24b
Control (1% Tween 20)	0.0 ± 0.00d	31.8 ± 6.36a
Control (untreated)	0.0 ± 0.00d	31.2 ± 2.18a

* Treatment means within the same column that are followed by the same lowercase letter do not differ significantly ($P > 0.05$) via Tukey's honestly significant difference test ($n = 50$ mites).

following exposure. EO at 5 and 1.2–5% concentrations also exhibited ovicidal activity against *T. urticae* and *T. macfarlanei*, respectively, with no eggs hatched by the end of the period of observation. These results indicate these concentrations of the EO are more appropriate for use as an adulticide than an ovicide for controlling both species of phytophagous spider mites. Based on the LC_{50}s, this EO appeared to have similar direct contact toxicity activity against both adults and

Table 5. Chemical composition of essential oil of fresh *Acorus calamus* rhizomes.

Compound	Retention Index	% Area
α-Terpinolene	3.55	0.47
α-Pinene	3.72	3.50
5,5-Dimethyl-2-ethynylcyclopent-2-en-1-ol	4.04	27.96
α-Terpinene	5.63	1.46
p-Cymenene	5.87	2.23
1,3,6-Octatriene, 3,7-dimethyl-, (E)-	6.49	1.66
γ-Terpinene	6.87	1.66
2,6,6-Trimethyl-3-methylenecyclohexane	8.39	7.50
Camphor	10.25	41.07
1-Borneol	11.48	1.97
L-4-Terpineol	11.94	7.32
Mesitol	12.95	0.43

eggs of the two phytophagous spider mite species. These results agree with those of Janlaor and Auamcharoen (2021), who reported that EO extracted from fresh and dried *A. calamus* rhizomes produced mortality against *Tetranychus truncatus* Ehara (Acari: Tetranychidae) adult females (55 and 35%, respectively) at a 5% concentration 24 h following exposure under residual contact toxicity. EO from fresh and dried *A. calamus* rhizomes reduced egg hatch of *T. truncatus* by 96.3 and 29% at a 5% concentration 7 d following exposure, respectively. Moreover, Tewary et al. (2005) reported that EO from *A. calamus* rhizomes caused 72 and 93% mortality against adults of *T. urticae* at 0.5 and 1% concentrations at 48 h following treatment, respectively. Results of Eswara Reddy and Dolma (2018) showed that *A. calamus* EO was toxic to *T. urticae* adults ($LC_{50} = 103.40$ mg L^{-1} air) at 20 h following treatment in a fumigant toxicity assay, whereas this oil showed 100% repellency activity at 1 h following treatment to *T. urticae*. A methanolic extract of *A. calamus* at 1 and 2% concentrations revealed 72 and 100% and 91 and 100% mortality of *T. truncatus* adult females at 1 and 3 d following treatment (Laya et al. 2021).

Based upon previous results, MCE from *A. calamus* fresh rhizomes displayed botanical acaricidal activity against *T. truncatus* adult females (Janlaor and Auamcharoen 2021). Consequently, the MCE was used in combination with EO from *A. calamus* to apply on *A. longispinosus*, a potential predator of spider mites in this study. When EO and MCE were combined, mortality of the predatory mite was higher than when either EO or MCE extracts were applied alone. *Amblyseius longispinosus* was more sensitive to EO, MCE, and a mixture of EO and MCE by residual contact toxicity than direct contact toxicity. *Amblyseius longispinosus* sprayed with these treatments directly did not die and laid eggs in numbers that were not statistically different from that of controls, but *A. longispinosus* consuming eggs of spider mites sprayed with all treatments died and did not lay eggs or laid fewer eggs that differed significantly from that of the controls. The results demonstrate that *A. calamus* EO also possesses toxicity to predatory mites via oral toxicity.

El-Sharabasy (2010) revealed that an ethanol extract of *Artemisia judaica* L. leaves was toxic to adult females and immatures of *T. urticae*, followed by acetone, petroleum ether, and aqueous extracts. Nevertheless, these extracts caused mortality to the predatory mite *Phytoseiulus persimilis* Athias Henriot, with lower LC_{50}s than those of *T. urticae*. Vergel et al. (2011) reported that garlic–pepper extract at a 1.25% concentration caused mortality levels of 24 and 9.82% of *P. persimilis* and *Neoseiulus californicus* (McGregor). This extract caused a significant decrease in the fecundity of *N. californicus*. Our results corroborated those results.

EO and MCE of *A. calamus* decreased egg production of *A. longispinosus* compared with that of the controls. By contrast, the predatory mite *Typhlodromus negevi* Swirki & Amitai and *P. persimilis* adult females were more tolerant than *T. urticae* to *Laurus nobilis* L. EO and laurcide (Amer et al. 2016). Ribeiro et al. (2016) demonstrated EOs from stems, flowers, and leaves of *Piper marginatum* Jacq. caused 50–70% mortality of *N. californicus* in a fumigation bioassay. Choi et al. (2004) reported that EOs of caraway seed (*Carum carvi* L.), citronella java (*Cymbopogon nardus* [L.] Rendle), lemon eucalyptus (*Eucalytus citriodora* L.), pennyroyal (*Mentha pulegium* L.), peppermint (*M. piperita* L.), and spearmint

(*M. spicatal* L.) caused >90% mortality at 7.1×10^{-3} μl/ml air against adults of *P. persimilis* by using the filter paper diffusion bioassay. Elhalawany and Dewidar (2017) indicated that LC_{50}s of lemongrass (*Cymbopogon citratus* [Dc] Staph), spearmint, rosemary (*Rosmarinus officinalis* L.), marjoram (*Origanum majorana* L.), fennel (*Foeniculum vulgare* Mill.), coriander (*Coriandrum sativum* L.), and chamomile (*Matricaria recutila* L.) EOs ranged between 7.09 and 9.63% for *P. persimilis* and between 4.94 and 9.63% for *N. californicus*.

The contact toxicity of EO from *A. calamus* fresh rhizomes against adults and eggs of *T. urticae* and *T. macfarlanei* may be attributed to the existence of active chemical constituents such as camphor and other compounds present in the EO. Camphor exhibited acaricidal activity with LC_{50}s of 7.72 and >550 mg/L against *T. urticae* adult females under fumigant and direct contact toxicity exposure, respectively. By contrast, camphor displayed the least contact toxicity against *T. urticae* eggs (Badawy et al. 2010).

Further study should be focused on the acaricidal efficacy of fresh *A. calamus* rhizomes against spider mites and other phytophagous mites growing on plants under greenhouse and field conditions. Gajalakshmi et al. (2016) found that an aqueous rhizome extract of *A. calamus* (5%) + *Sapindus marginatus* L. (5%) and *A. calamus* (10%) caused 56.08 and 47.67% and 51.59 and 41.59% reduction over control of *T. urticae* population on okra plants after the third round of spraying under pot culture and field conditions, respectively. These extracts gave significantly higher okra fruit yield than the untreated check. Ethanol extract of *A. calamus* (10%) showed 79.54 and 78.23% egg reduction and adult plus nymph reduction over untreated check, respectively, against *T. urticae* on tomato (*Solanum lycopersicum* L.) in field conditions; yield increased by 55.91% over the untreated check (Premalatha and Chinniah 2017). From our results, the development of *A. calamus* rhizomes as a botanical acaricide is one alternative in the management of mite pests. Studying the acaricidal activity of *A. calamus* extract under protected and open production systems could reveal an important use of EOs by producers in managing phytophagous mites in agricultural production. However, suitable use of *A. calamus* acaricides in this study is suggested when the mite population reaches the economic threshold level and there are no predaceous mites on host plants for beneficial mite conservation in sustainable agriculture.

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