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Toxic Effects of Leaf and Flower Crude Extracts from *Lantana camara* on *Tetranychus urticae*

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Leaves and flowers of *L. camara* were extracted by soxhlet separation for 8 h by *n*-hexane, dichloromethane and methanol, evaporated to dryness and kept at 4 ºC until toxicity tests. Twenty adult gravid females of *T. urticae* were placed on a mulberry leaf disc. Each type of plant extracts (1 % w/v) was applied on the leaf disc by 2 ways: no choice and choice bioassay. Results from no choice bioassay showed that the crude flower extracts had no toxicity. However, both dichloromethane and *n*-hexane extracts of *L. camara*'s flowers repelled 66.04 ± 5.45 % and 60.83 ± 3.45 % *T. urticae* from the leaf discs. The dichloromethane, *n*-hexane and methanol extracts from *L. camara's* flowers reduced the oviposition per day rate of *T. urticae* to 42.3, 34.7 and 17.4 % when compare to ethanol-treated controls. The repellent effects of *n*-hexane and dichloromethane were observed with $EC_{50's}$ of 0.756 % and 0.902 %, respectively. Results from choice bioassay showed that the 1 % w/v of *n*-hexane and dichloromethane crude flower extracts repelled *T. urticae* to the untreated side at 72 h. The numbers of mites and their eggs on treated side were lower than on the untreated side.

Key Words: *Lantana camara*, Crude extract, Toxicity tests, *Tetranychus urticae*.

INTRODUCTION

The use of plant extract in insect pest control is one of the pest control strategies of integrated pest management. The main goal of this approach is to reduce the use of chemical pesticides. Plants have many secondary metabolites such as alkaloids, flavonoids, terpenoids and tannins. These compounds are often active against specific pests and have possible use in integrated pest management programs for agriculture[1,2]. *Lantana camara* is the native topical and subtopical American plant that has spreads to Europe, Australia and Asia[3]. *L. camara* is regarded as a popular ornamental garden plant and a notorious weed. It is used in folk medicine in many countries[3]. *L. camara* is used for its antipyrogenic, antimutagenic and antibacterial properties[4,5]. Essential oil of *L. camara* inhibited growth of *Pseudomonas aeruginosa*, *Aspergillus niger*, *Fusarium solani*, *Candida albican*, *Bacillus subtilis*, *Staphylococcus typhi* and *Bacillus aureus*[6-10]. It has insecticidal properties that control several pests such as *Sitophilus zeamais*[11] and diamondback moth[12].

The two-spotted spider mite (*Tetranychus urticae* Koch) is a pest insect in agriculture that attacks several economic crops worldwide[13]. It has rapidly developed resistance to many chemical insecticides; therefore, biological control techniques are needed to manage its population[14]. However, there are few reports on the use of botanical insecticides to control this mite. Therefore, this study aimed to investigate toxicity effects of leaf and flower crude extracts from *L. camara* on *T. urticae* in order to address its efficacy for controlling this mite.

EXPERIMENTAL

Extraction: Fresh leaves and flowers of *L. camara* were collected from the Faculty of Liberal Arts and Science, Kasetsart University, Kamphaeng Saen, Nakhonpathom, Thailand and oven-dried at 50 ºC until they were brittle. The oven-dried leaves and flowers were ground to fine powders. Leaves and flower powders (20 g) were extracted with 400 mL of *n*-hexane by soxhlet apparatus for 8 h. The extracts then were evaporated by the vacuum rotary evaporator to dryness. The crudes were then extracted by 400 mL of dichloromethane followed by 400 mL of methanol and the solvents were removed by vacuum evaporator. All crude extracts were kept at 4 ºC. The crude extracts were later dissolved by ethanol and diluted with distilled water to prepare the concentrations of extract used for testing.

Bioassay: *Tetranychus urticae* were collected from the natural mulberry leaf in Kasetsart University, Kamphaeng Saen, Nakhonpathom, Thailand. They were cultivated on mulberry leaves of 2.5 × 2.5 cm^2 and moist cotton 1 cm thick in the laboratory. The second generation of *T. urticae* was used for testing.

Toxicity, repellent and oviposition-deterrent effects of the *L. camara's* crude extracts were determined in no-choice and

choice bioassays by applying the extracts and controls (water and 10 % ethanol) to mulberry leaf discs of 2 cm in diameter. These were maintained on moist cotton wool at 27 ± 2 ºC, relative humidity of 73 ± 10 % and a photoperiod of 12 L :12 D.

No-choice test: Each crude extract from one part of the plants (leaf or flower) and one type of solvents (*n*-hexane, dichloromethane or methanol) at 1 % w/v was applied on the testing arena. The 50 µL of each extract and each control were applied on the entire mulberry leaf discs and allowed to dry. Then, 20 gravid females were transferred to the mulberry leaf discs. Dead mites, mites that survived and ran off the leaf discs and the eggs that were laid at 24, 48 and 72 h were counted. The crude extracts that showed the toxic effect at 1 % w/v were further evaluated to determine median effective concentrations (EC_{50}). The experiment was arranged by completely randomized design (CRD). Each experiment was conducted with 12 replicates.

Choice test to test the repellent and oviposition deterring effects: Each leaf disc was divided into two halves by its leaf vein. Each extract was prepared at the concentrations of extract used for testing of 1 % w/v and the solvent control was 10 % (v/v) ethanol. The 25 µL of each concentration of crude extract were separately applied on only one half of the mulberry leaf disc. To the other half of the leaf disc was applied 10 % (v/v) of ethanol. Distilled water was applied on the half leaf disc as the blank control[15]. All leaf discs were allowed to dry. Then, 20 gravid females were placed on the leaf discs. Mites that survived and the eggs that they laid during 24, 48 and 72 h were counted. The experiment was arranged by completely randomized design. Each experiment was conducted with 12 replicates.

Data analysis: The per cent mortality was calculated in the uniform population of mites using Abbott's formula[16], which considers the natural mortality of untreated controls in the denominator. Probit analysis was used to estimate EC_{50} value. ANOVA was computed using the SPSS version 15.0 software package. The means of each treatment were compared by ANOVA univariate analysis and Duncan's multiple range test (DMRT), with a predetermined significance level $\alpha < 0.05$. Binomial analysis of deviance was used to analyze the deterrent effects of the survival mites and to investigate possible oviposition deterrent effects of the treatments.

RESULTS AND DISCUSSION

Extraction of dry flowers of *L. camara* using Sohxlet produced *n*-hexane fraction 3.16 % w/w, dichloromethane extract 1.08 % w/w and methanol extract 21.07 % w/w.

No choice test: The 1 % w/v of *L. camara's* leaf crude extracts were tested for the toxicity, repellent and oviposition deterrent effects on *T. urticae*. During the 72 h *T. urticae* lived on the treated leaf discs, the numbers of dead mites and of the mites that ran off the leaf disc were not significantly different from those of controls. The leaf crude extracts of all solvent extractions were slightly less likely to deter oviposition of the mite than the ethanol control. These results suggested that the crude leaf extracts at 1 % w/v concentrations had no major toxicity, repellent or oviposition effects on the two-spotted spider mites (Table-1).

TABLE-1
TOXICITY AND REPELLENT EFFECTS OF *Lantana camara*'s LEAF EXTRACTS AT 1% W/V CONCENTRATION ON *Tetranychus urticae* FOR 72 h

Solvents	*T. urticae*		
	Per cent Mortality	Percent of mites that ran off the disc	Oviposition rate (mites/day)
Water control	3.75 ± 0.84^{a}	1.88 ± 0.69^{a}	9.71 ± 0.16^{bc}
Ethanol control	3.96 ± 1.32^{a}	3.33 ± 0.83^{a}	9.35 ± 0.16^{a}
Methanol	5.00 ± 0.97^{a}	2.50 ± 0.62^{a}	9.80 ± 0.06^{c}
Dichloromethane	5.20 ± 0.72^{a}	2.50 ± 0.87^{a}	9.58 ± 0.07^{abc}
n-Hexane	4.79 ± 0.84^{a}	1.67 ± 0.47^{a}	9.37 ± 0.08^{ab}

Means ± standard error within columns followed by the same letter were not significantly different by DMRT ($p < 0.05$)

L. camara flower extracts had no acute toxicity, as noted by no significant differences of mortality between treatments and controls (Table-2). However, the dichloromethane and *n*-hexane extracts of *L. camara's* flowers repelled 66.04 ± 5.45 % and 60.83 ± 3.45 % *T. urticae* from the leaf discs, respectively (about 12 and 11 times compared with the ethanol control). The dichloromethane, *n*-hexane and methanol extracts from *L. camara's* flowers significantly reduced oviposition rate of *T. urticae* to 5.85 ± 0.56, 6.62 ± 0.23, 8.38 ± 0.22 mites/day (reduction of 42.3, 34.7 and 17.4 % compared to ethanol control), respectively. Therefore, the most effective extracts for repelling mites and deterring their oviposition were dichloromethane and *n*-hexane extracts of *L. camara's* flowers. These *n*-hexane and dichloromethane crude extracts were further tested at 0.5, 0.7 and 0.9 % w/v concentrations to determine the median effective concentration (EC_{50}).

Median effective concentration (EC_{50}) bioassay: The factorial design was used with treated plant parts and different solvents as factors to investigate the corrected percentage of the mites that ran off the leaf disc[16]. Repellent effects of *n*-hexane and dichloromethane extracts of *L. camara's* flowers

TABLE-2
TOXICITY AND REPELLENT EFFECTS OF *Lantana camara*'s FLOWER EXTRACTS AT 1% w/v CONCENTRATION ON *T. urticae*

Solvents	*T. urticae*			
	Per cent mortality	Per cent of mites leaving the disc	Oviposition rate (mites/day)	Reduction of oviposition compared with ethanol control (%)
Water control	9.38 ± 1.38^{a}	8.33 ± 1.80^{a}	10.41 ± 0.42^{a}	
Ethanol control	10.21 ± 1.23^{a}	5.63 ± 1.27^{a}	10.14 ± 0.27^{a}	
Methanol	9.58 ± 1.30^{a}	13.54 ± 1.88^{a}	8.38 ± 0.22^{b}	17.4
Dichloromethane	$12.71 \pm 2.55^{a,b}$	66.04 ± 5.45^{b}	5.85 ± 0.56^{c}	42.3
n-Hexane	15.83 ± 2.05^{b}	60.83 ± 3.45^{b}	6.62 ± 0.23^{c}	34.7

Means ± standard error within columns followed by the same letter were not significantly different by DMRT ($p < 0.05$)

TABLE-3
PER CENT OF *T. urticae* AND THEIR EGGS ON TREATED SIDE AFTER APPLICATION OF *L. camara*'s LEAF EXTRACTS ON THE MULBERRY LEAF DISC

Treatment	Per cent of *T. urticae* on treated side			Per cent of *T. urticae*' eggs on treated side		
	24 h	48 h	72 h	24 h	48 h	72 h
Methanol	4.75* (21/442)	25.61* (105/410)	47.34 (178/376)	1.67* (36/2159)	21.91* (619/2825)	37.33* (1096/2936)
Dichloromethane	44.09* (205/465)	31.17* (139/446)	56.67 (242/427)	54.67 (1509/2760)	25.79 (836/3241)	41.12 (1266/3079)
n-Hexane	75.8 (354/467)	47.23 (213/451)	43.42 (185/426)	79.25 (2677/3378)	56.09 (1888/3366)	38.36 (1252/3264)
Ethanol control	56.56 (263/465)	44.06 (189/429)	55.5 (227/409)	53.54 (1550/2895)	45.00 (1642/3649)	52.92 (2075/3921)

Parenthesis indicate the number of *T. urticae* or *T. urticae*'s eggs on the treated side when compared to those on the water side of the leaf
Asterisks indicate that the percent of *T. urticae* or their eggs on the treated side were significantly different from 50 % ($p < 0.05$)

TABLE-4
PER CENT OF *T. urticae* AND THEIR EGGS ON TREATED SIDE AFTER APPLICATION OF *L. camara* FLOWER EXTRACTS

Treatment	Per cent of *T. urticae* on treated side			Per cent of *T. urticae*'s eggs on treated side		
	24 h	48 h	72 h	24 h	48 h	72 h
Methanol	11.77* (48/408)	37.40* (144/385)	53.82 (162/301)	8.02* (197/2457)	25.99* (741/2851)	41.58* (987/2374)
Dichloromethane	1.65* (7/424)	21.13* (75/355)	34.74* (99/285)	0.52* (12/2320)	6.53* (142/2173)	37.13* (551/1484)
n-Hexane	0.25* (1/399)	6.95* (21/302)	15.86* (23/145)	0.05* (1/2125)	0.75* (15/1994)	7.19* (64/890)
Ethanol control	51.75 (207/400)	50.66 (192/379)	48.57 (170/350)	51.36 (1629/3172)	59.53 (1902/3195)	40.59 (1296/3193)

Parenthesis indicates the number of *T. urticae* or *T. urticae*'s eggs on the treated side compared to these values on the water-treated side of the test leaf. Asterisks indicate that the percent of *T. urticae* and their eggs on the treated side were significantly different from 50 % ($p < 0.05$)

on *T. urticae* are shown in Fig. 1. The median effective concentrations of *n*-hexane and dichloromethane extracts of *L. camara's* flowers on *T. urticae* were 0.756 % and 0.902 % w/v, respectively.

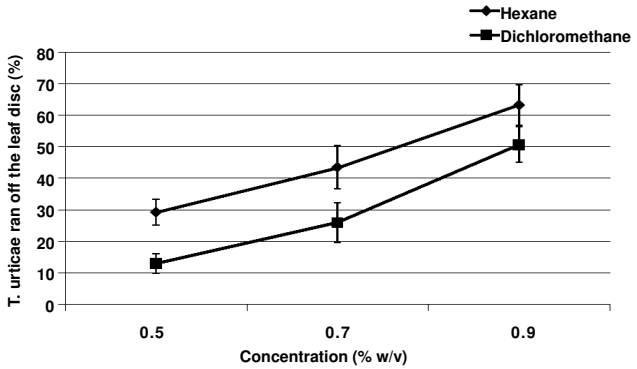

Fig. 1. Repellent effects of *n*-hexane and dichloromethane extracts of *L. camara's* flower on *T. urticae* at 72 h

Choice test: The dichloromethane and methanol leaf extracts repelled *T. urticae* at 24 and 48 h and the methanol extract deterred their oviposition at 24, 48 and 72 hs (Table-3) when effects were compared to the ethanol control side of the leaf. Only 4.75 % of the *T. urticae* lived on methanol side in the first 24 h and only 25.61 % were there at 48 h. The mites on dichloromethane side were about 44 % and 31 % at 24 and 48 h, respectively. About 1.7, 22 and 37 % of the *T. urticae*'s eggs were on the methanol side when observed at 24, 48 and 72 h, respectively.

The flower crude *L. camara's* extracts (1 % w/v) were tested to determine the percent of *T. urticae* remaining on the treated side of the test leaf. All three solvent extracts repelled and deterred oviposition of *T. urticae*. The *n*-hexane extract was the most effective, followed by the dichloromethane and the methanol extracts, respectively (Table-4). Only 0.25 % of *T. urticae* were on the *n*-hexane side at 24 h, although this increased to 15.86 % at 72 h. Only 7.19 % of the total eggs were on that *n*-hexane side at 72 h. Similar to the *n*-hexane effects, the numbers of *T. urticae* and their eggs on treated side of leaves treated with dichloromethane and methanol extracts were less in first period and increased later. This effect might occur because the efficacy of the extracts decreased with time.

L. camara has insecticidal properties to control several pests. These include *Sitophilus zeamais*[11] and diamondback moth[12] and and *Spodoptera litura*[17]. Our results showed that the flower extracts of *L. camara* had more potential to repel and deter oviposition of the two-spotted spider mite (*T. urticae*) than the leaf extract, especially when *n*-hexane and dichloromethane were used as extraction solvents. However, the methanol leaf crude extract also repelled *T. urticae* at 48 h and deterred their oviposition at 72 h. The results were in agreement with Moussa *et al.*[18], who evaluated 25 local plant species for bactericidal activity and acaricidal activity against the two-spotted spider mite. They noted that 5 methanol leaf crude extracts were active in the following sequence: *Cassia sp.* > *Pittosporum tobira* > *Myrtus communis* > *Lantana camara* > *Acacia salogna*. The LC_{50} of *Lantana camara's* methanol leaf extract was 225 mg/L.

There are few reports on toxic effects of plant extracts on *T. urticae*. Sanguanpong and Schmutterer[19] reported that neem oil and other neem-seed extracts were toxic to the two-spotted spider mite when applied as foliage or contact spray. Pentane extract and cold-pressed neem oil decreased the fecundity and the survival of nymphs hatched from treated eggs. Knapp and Kashenge[20] also reported effects of different neem formulations on the two-spotted spider mite on tomato. All neem formulations except Neemros® expressed strong repellence and oviposition deterrence to *T. urticae*. Furthermore, azadirachtin is the bioactive compound isolated from the neem seed (*Azadirachta indica* A. Juss) affected fecundity and mortality of the two-spotted mites but had no effect on their fertility and offspring development[21]. Although the neem extracts showed bioactivity against *T. urticae*, other plant extracts were also tested.

A mixture of vegetable, essential oils (caraway oil) and fatty acid potassium salts has been reported to have toxic effects on *T. urticae*[22]. This mixture cause moderate mortality of eggs as well as larvae hatched from the treated eggs, also this essential oil mixture caused a delay in the postembryonic development of *T. urticae*. In another study, extracts of *Albizzia coreana* twig and *Pyracantha angustifolia* leaf demonstrated acaricidal activity against *T. urticae* and reduced reproduction of *T. urticae*[23]. From our results, the *n*-hexane and dichloromethane extracts from flowers of *L. camara* both deterred and increased mortality of *T. urticae*. Ghisalberti[3] reviewed about phytochemistry, ethnopharmacolgy and toxicology of *L. camara* and reported that essential oils from flower of *L. camara* contained several triterpene compounds, iridoid glycosides.

Conclusion

In the no choice test, the *n*-hexane and dichloromethane extracts of *L. camara* flowers were more toxic to *T. urticae*, with median effective concentrations of 0.756 % and 0.902 %, respectively. Moreover, the oviposition rate of *T. urticae* was also significantly reduced. The dichloromethane, *n*-hexane and methanol extracts of flowers reduced oviposition by 42.3, 34.7 and 17.4 %, respectively. For the choice test, the 1 % w/v of *n*-hexane or dichloromethane crude extracts of flowers repelled *T. urticae* to the untreated side of the leaf discs at 72 h and caused to lay more eggs on the untreated side higher than their treated side of the leaf. Crude leaf extracts of *L. camara* had no toxicity or repellent effect on *T. urticae* in the no choice test. However, in the choice test, the dichloromethane and methanol leaf extracts repelled *T. urticae* to the untreated side at 48 h, although they were less efficient than the crude extracts of *L. camara* flower.

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