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Potential Control of Two-Spotted Spider Mite, *Tetranychus urticae* Koch (Acari: Tetranychidae) by Crude Extracts of *Duabanga grandiflora* (Lythraceae) and *Diospyros cauliflora* (Ebenaceae)

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Abstract.- Laboratory bioassays were carried out to determine the toxicity and repellency of *Duabanga grandiflora* and *Diospyros cauliflora* crude extracts against *Tetranychus urticae* female. Toxicity and repellency tests were performed using leaf disc no choice and leaf disc choice tests. Crude methanol extract of *D. grandiflora* was found to have less toxicity with maximum mortality of 35.6% when compared to control while chloroform crude extract of *D. cauliflora* showed 45.6% repellency which is equalled to those of *D. grandiflora* crude methanol extract, 26.7% mortality of *T. urticae* at 8% (w/v) concentration on leaf disc no choice test 3 days after exposure. Both crude extracts were also able to reduce the number of eggs with a maximum of 92.2 and 94.6% at the hightest concentration. Results also indicated that 8% (w/v) concentration of *D. grandiflora* crude methanol extract exhibited the strongest repellency and oviposition deterrent activities to *T. urticae* throughout the experiment with only 0.0-9.5% alive mites and 0.0-3.4% of eggs laid on the treated sides under leaf disc choice test. Besides, 2.2-18.7% and 0.5-6.6% were recorded for alive mites and eggs laid, respectively on the treated sides by highest concentration of *D. cauliflora* crude chloroform extract. The oviposition-deterrent indices decreased as time progressed at all concentrations of the crude extracts, except the highest concentration (8% w/v) of crude methanol extract of *D. grandiflora* and crude chloroform extract of *D. cauliflora* where the ODI is ranging from 92.8-100.0 and 88.3-99.0, respectively during the experiment.

Keywords: Crude extract, *Duabanga grandiflora, Diospyros cauliflora, Tetranychus urticae*

INTRODUCTION

Two-spotted spider mite (TSSM), *Tetranychus urticae* Koch (Acari: Tetranychidae) is a cosmopolitan herbivorous pest of agricultural crops worldwide (Bolland *et al*., 1998; Antonious *et al*., 2007) as it infests 3,877 host plants in both field and greenhouse crops (Migeon and Dorkeld, 2007), especially herbaceous annuals, including beans, fruit trees and ornamental plants (Ho, 2000; Kennedy and Storer, 2000; Lee *et al*., 2003). This mite feeds by penetrating the cells of the leaf with its stylets and sucking out the cell contents that causes cell collapse and manifests as spotting on the upper leaf surface. Heavy infestations by this mite disturb the water balance in leaf and accelerate transpiration resulting in hyper-necrosis, leaf drying and leaf drop (Liesering, 1960; Bolland *et al*., 1998; Lee *et al*., 2003; Landeros *et al*., 2004). Finally, the yield is decreased while the quality is also lower or unacceptable for the market.

Organophosphates, carbamates, dicofol, organotins, hexythiazox, clofentezine, abamectin, bifenthrin and chlorfenapyr are the selective acaricides for controlling this pest (Van Pottelberge *et al*., 2009). Some acaricides can kill *T. urticae* immediately whereas some of them take time before inducing mortality. However, others could effect by inhibiting movement or reducing oviposition rates (Steiner *et al*., 2011). Application of pesticides in agriculture to control various insects and mites not only have a positive effect for controlling pests, crop diseases and weeds but also result in a variety of problems such as pesticide residues in food chain, the effects of pesticides on the natural enemies and pests become resistant to many acaricides (The Insecticide Resistance Action Committee [IRAC], 2008; Van Leeuwen *et al*., 2009; Kumral *et al*., 2010). Consequently, the development of control techniques that provides efficient pest control without serious effects on the product, environment and public health are required.

A botanical pesticide can be employed as an alternative source to control pests where there are concerns of biodegradablility, contamination in

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environment and human health hazards (Grange and Ahmed, 1988; Devlin and Zettel, 1999). Plants contain a rich source of bioactive chemicals which show some biological activities to specific target pests. Many plant extracts are known to be acaricidal on *T. urticae* (Zhu *et al*., 2003; Rasikari *et al*., 2005; Shi *et al*., 2006). Extracts from the subfamilies Ajugoideae, Scutellarioideae, Chloanthoideae, Viticoideae and Nepetoideae have acaricidal activity (Rasikari *et al*., 2005). The ethanol extract of the leaves of *Ailanthus altissima* L. (Simarubaceae) and roots of *Convolvulus krauseanus* Regel. and Schmalh. (Convolvulaceae) showed high acaricidal activity to *T. urticae*. In addition the extracts reduced the population of the next generation of *T. urticae* (Chermenskaya *et al*., 2010). Zhu *et al.* (2003) reported the extract of *Artemisia annua* L. deterred the feeding activity of *T. urticae* while Shi *et al*. (2006) revealed that chloroform extract from *Kochia scoparia* (L.) Schrad caused high mite mortality. Furthermore, the extract of *Capparis aegyptia* L. (Capparaceae) (Hussein *et al*., 2006) and *Nerium oleander* L. (Apogynaceae) (Islam *et al*., 2008) revealed to be effective against *T. urticae*. However, there are many plants which have not been screened for their biological activities on *T. urticae*.

Duabanga grandiflora (Roxb. ex DC.) Walp. which belongs to subfamily Duabangoideae, family Lythraceae is a member of a tropical African and Southeast Asian tree (Graham *et al*., 1998, 2005). Boiled seeds of *D. grandiflora* relieve stomach ache due to indigestible food whereas boiled stem bark is used for peptic ulcer medicine. On the other hand, stem bark of *D. grandiflora* has been used to reduce inflamed gums (Anderson, 1993; Trisonti, 2002). Broth-based (turbidometric (TB)) assay has been able to show the inhibitory effects of *D. grandiflora* on the growth kinetic of two bacterial species, namely *Escherichia coli* and *Staphylococcus aureus* (Othman *et al*., 2011). The methanol extract of the timber of *D. grandiflora* was also examined for its leishmanicidal activities and revealed MLC and MIC > 400 and 400, respectively (Takahashi *et al*., 2004).

Diospyros cauliflora Blume belongs to the genus *Diospyros* which is a large genus of mainly tropical trees within the family Ebenaceae, many of which are economically important plants such as edible fruit (persimmons, *Diospyros kaki* Thunb and *Diospyros virginiana* L.) and ebony (*Diospyros ebenum* J. Koenig). The bark extract (dichloromethane: methanol = 1: 1) of *D. cauliflora* was tested on the malarial parasite, *Plasmodium falciparum* (Gombak A: chloroquine-resistant strain and D 10: chloroquine-sensitive strain) and revealed less inhibition (Khozirah *et al*., 2011). The methanol extract of *D. cauliflora* at 10 μg/well showed no ability to inhibit specific radioligand binding to 5HT1a, $GABA_B$ and dopamine (D2S) central nervous system receptors (Chung *et al*., 2005).

As the information dealing with acaricidal activities of *D. grandiflora* and *D. cauliflora* are still lacking, so in the present paper we report the activity of the extracts from these plants on *T. urticae* under laboratory conditions. The information from these studies not only provides an insight on bioacaricidal properties of the selected plant species, but also exploites as an alternative way to control such pests in a safe and environmental friendly way and incorporate these acaricides rate for integrated pest management programs.

MATERIALS AND METHODS

Preparation of extracts

Duabanga grandiflora stem branches were collected from Kanchanaburi province located in western Thailand in January 2007. The plant material was identified by Mr. Pranai Penchit and the herbarium specimen (CHKU 00010) was deposited at the Bangkok Herbarium Botanical Research Unit, Plant Variety Protection Division, Department of Agriculture, Bangkok, Thailand. On the other hand, roots of *D. cauliflora* were collected in Trang province, southern Thailand in March 2007. The plant material was identified by Mr. Chamlong Phengklai and a voucher specimen (BKF 143220) was deposited at the Royal Forest Department, Phaholyothin Road, Bangkok, Thailand.

The stem branches of *D. grandiflora* were machine-cut into small pieces and dried at 40°C in a hot air oven before grinding into powder. Dried

powder (5 kg) was extracted with methanol 3×20 L at room temperature. The methanolic solution was filtered with a Whatman #1 filter paper and concentrated by a rotatory evaporator under reduced pressure to give the crude methanol extract (190 g) which was used for bioassay.

Dried powdered root of *D. cauliflora* (4 kg) was extracted with hexane (3 x10 L) at room temperature. The hexane solution was filtered with filter paper and the organic phase was evaporated under reduced pressure to give 23 g of crude hexane extract. The residue was then macerated in MeOH (3x10 L) at room temperature for 7 days and then filtered. The methanolic solutions were combined and evaporated under reduced pressure to give dark brown syrupy mass of crude methanol extract (130 g). The crude methanol extract was then dissolved, with sonication, in $CHCl_3$ (3x1 L) and filtered. The solutions were combined and evaporated under reduced pressure to give crude chloroform extract (26 g) which was used for bioassay.

Test mites

The stock colonies of *T. urticae* used in this study were obtained from Department of Agriculture, Ministry of Agriculture and Cooperatives, Bangkok, Thailand. They were cultured on the lower surface of mulberry leaf (*Morus alba* L.) placed on moistened cotton pads resting on sponges in the plastic box. The colonies were maintained at room temperature under laboratory conditions. The mulberry leaves were examined every few days and replaced with fresh ones when over-crowding of mites and yellow leaves were observed. All bioassays were conducted and carried out under the same environmental conditions as the culture.

The effect of extract residues on T. urticae

Leaf disc no choice test

Toxicity, repellency and egg-laying was evaluated in *T. urticae* females (3 days old) on leaves exposed to crude methanol extract of *D. grandiflora* and crude chloroform extract of *D. cauliflora*. Mulberry leaf discs (2 cm diam) were punched with a cork borer. The lower surface of each whole leaf disc was treated with 50 µl of crude extract solutions at different concentrations (0.5, 1, 2, 4 and 8% (w/v)). Mulberry leaf discs treated with ethanol 70 and 95% were used as the controls for crude methanol extract of *D. grandiflora* and crude chloroform extract of *D. cauliflora*, respectively. Dried leaf discs were then placed on moistened cotton pads in glass Petri dishes (9 cm diam) (3 leaf discs/replication, 3 replications/ treatment). Ten *T. urticae* females were transferred from the culture with a fine artist's brush into the center of each leaf disc. The rearing units were kept at room temperature where mite survival, mortality, repellency (number of mites off the discs) and number of eggs were recorded daily for 3 consecutive days.

Leaf disc choice test

The extracts residuals of the crude methanol extract of *D. grandiflora* and crude chloroform extract of *D. cauliflora* on the leaf were also used against *T. urticae* females for repellency and oviposition deterrent index by using leaf disc choice test. Each mulberry leaf disc (2 cm diam) was cut with a cork borer and divided into two equal parts by midrib of the leaf disc. One-half of leaf disc was treated with 25 µl of tested material at the concentrations of 0.5, 1, 2, 4 or 8% (w/v) whereas the other half was treated with its solvent (ethanol 70 or 95%). Leaf discs were allowed to completely dry at room temperature and placed on the moistened cotton pad in 9 cm diam glass Petri dishes (3 leaf discs/ replication, 3 replications/ treatment). Ten females (3 days old) were placed on the center of each leaf disc. Treated mites were maintained at room temperature. Percentage of *T. urticae* females alive and percentage of eggs laid on treated side of leaf discs were recorded on 1, 2 and 3 days after exposure. Oviposition deterrent index (ODI) was calculated as $\{(C-T)/(C+T)\} \times 100$ where C was the number of eggs laid on the control side and T was the number of eggs laid on the treated side (Dimetry *et al.*, 1993).

Statistical analyses

All data were analysed using analysis of variance (ANOVA) and Least Significant Difference Test (LSD) was employed to compare the treatment means ($P = 0.05$) using the SAS program (SAS Institute Inc., Cary, NC).

RESULTS

Leaf disc no choice test

Leaf disc no choice test was used to investigate the toxicity, repellency of residuals of crude methanol extract of *D. grandiflora* and crude chloroform extract of *D. cauliflora* on female *T. urticae* (Table I). At day 1 after exposure, 8% *D. grandiflora* methanol extract residual significantly decreased the survival rate of *T. urticae* population (38.9%) due to 27.8% mortality and 33.3% repellency activities compared to the control. At the end of experiment, 4 and 8% crude methanol extracts of *D. grandiflora* decreased the population of *T. urticae* 24 and 81%, respectively while 0.5, 1 and 2% crude extracts reduced the *T. urticae* population by 8-12% when compared to 11% of the control.

Survival rates of mites on leaf discs with 2, 4 and 8% crude chloroform extracts of *D. cauliflora* were significantly lower than that of the control at day 1 after treatment (Table I). The lowest survival rate (51.1%) was found at 8% crude extract where 11.1% of mites died on the leaf disc and over 37.8% fell off, indicating repellency activity. At day 3 after exposure, 4 and 8% crude extracts of *D. cauliflora* decreased the population of *T. urticae* 49 and 72%, respectively while 0.5, 1 and 2% crude extracts reduced the *T. urticae* population by 26-40% when compared to 9% of the control. From this study, it seems to be that crude chloroform extract of *D. cauliflora* is more effective in reducing the population of *T. urticae* than the crude methanol extract of *D. grandiflora* under the leaf disc no choice test.

The fecundity rate of *T. urticae* that stayed on leaf discs treated with *D. grandiflora* crude methanol extract and *D. cauliflora* crude chloroform extract is presented in Table II. At day 1 after treatment, the results indicated that the number of egg/female was significantly reduced by all test concentrations except at 0.5% of crude methanol extract of *D. grandiflora* when compared to the control. The lowest daily egg production (0.4 eggs/female) was noted when 8% crude extract was applied. Number of daily egg production tended to increase at day 2 after exposure and slightly decreased or remained constant at day 3 after

Table I.- Accumulated mortality and repellency rates of *Tetranychus urticae* females at 1, 2 and 3 days after staying on mulberry leaf discs treated with different concentrations of plant crude extracts.

Plant crude extracts	Treatments	Day after treatment								
		1			2			3		
		% of Mite (mean ± S.E.) [1/]								
		Live	Dead	Repelled	Live	Dead	Repelled	Live	Dead	Repelled
Methanol extract of *D. grandiflora*	0.5%	97.8±2.2 aA	2.2±2.2 bA	0.0±0.0 bA	94.4±2.9 aA	4.4±2.9 bA	1.1±1.1 bA	92.2±2.2 aA	6.7±1.9 bA	1.1±1.1 bA
	1%	97.8±2.2 aA	1.1±1.1 bB	1.1±1.1 bA	93.3±1.9 aA	5.6±1.1bAB	1.1±1.1 bA	91.1±2.9 aA	6.7±1.9 bA	2.2±1.1 bA
	2%	95.6±2.9 aA	3.3±1.9 bA	1.1±1.1 bA	91.1±2.2 aA	6.7±0.0 bA	2.2±2.2 bA	87.8±2.9abA	8.9±2.2 bA	3.3±1.9 bA
	4%	86.7±3.3 aA	3.3±1.9 bA	10.0±3.9bA	85.6±4.4 aA	3.3±1.9 bA	11.1±4.8bA	75.6±5.9 bA	12.2±4.8bA	12.2±4.0 bA
	8%	38.9±8.0bA	27.8±5.6aA	33.3±8.4aA	24.4±6.2 bA	31.1±5.6 aA	44.4±9.7aA	18.9±4.0 cA	35.6±4.8 aA	45.6±8.7aA
	Control	97.8±1.1 aA	2.2±1.1 bB	0.0±0.0 bA	95.6±1.1aAB	4.4±1.1 bAB	0.0±0.0 bA	88.9±4.0 aB	11.1±4.0 bA	0.0±0.0 bA
Chloroform extract of *D. cauliflora*	0.5%	98.9±1.1abA	0.0±0.0 cB	1.1±1.1 cB	81.1±2.9 abB	15.6±2.9 abA	3.3±0.0 cA	74.4±6.8 abB	22.2±6.8 aA	3.3±0.0 cA
	1%	94.4±1.1abA	3.3±1.9 bcA	2.2±2.2 cB	78.9±9.9abA	15.6±9.1 abA	5.6±1.1cAB	65.6±16.4abA	26.7±15.3aA	7.8±1.1 cA
	2%	85.6±4.0bcA	8.9±4.0abcB	5.6±4.0bcA	73.3±5.8bAB	18.9±2.9abAB	7.8±4.4bcA	60.0±6.9 bB	28.9±1.1 aA	11.1±6.2bcA
	4%	72.2±4.0 cA	13.3±5.1 aA	14.4±1.1bA	63.3±6.7bAB	20.0±8.4 aA	16.7±1.9bA	51.1±4.0 bcB	25.6±8.0 aA	23.3±5.1 bA
	8%	51.1±9.1 dA	11.1±2.2abC	37.8±7.8aA	38.9±6.2 cA	18.9±1.1 abB	42.2±5.9aA	27.8±6.8 cA	26.7±0.0 aA	45.6±6.8 aA
	Control	100.0±0.0aA	0.0±0.0 cB	0.0±0.0 cA	97.8±2.2aAB	2.2±2.2 bAB	0.0±0.0 cA	91.1±2.9 aB	7.8±2.2 aA	1.1±1.1 cA

[1/] Means±SE followed by the same letters are not significantly different as determined by Least Significant Difference test (LSD) ($\alpha = 0.05$). Small case letters compared means in column and capital letters compared means in row.

Table II.- Daily egg production and total number of eggs produced by *Tetranychus urticae* females at 1, 2 and 3 days after staying on mulberry leaf discs treated with different concentrations of plant crude extracts.

Crude plant extracts	Treatments	Egg/female/day (Mean±SE) [1/]			Total egg production [1/]	% Reduction
		1 Day	2 Days	3 Days		
methanol extract of *D. grandiflora*	0.5%	5.2 ± 0.3 aB	6.9 ± 0.1 aA	6.8 ± 0.3 aA	551.3 ± 44.4 a	-
	1%	3.9 ± 0.3 bB	5.1 ± 0.4 bcA	5.1 ± 0.3 bA	411.0 ± 29.0 b	12.9
	2%	3.4 ± 0.1 bA	4.3 ± 0.2 cA	3.6 ± 0.5 cA	324.0 ± 23.1 c	31.4
	4%	2.6 ± 0.3 cA	3.1 ± 0.3 dA	2.7 ± 0.4 cA	228.0 ± 9.3 d	51.7
	8%	0.4 ± 0.2 dA	1.2 ± 0.5 eA	1.2 ± 0.3 dA	36.7 ± 4.7 e	92.2
	Control	5.0 ± 0.2 aA	5.9 ± 0.6 abA	5.2 ± 0.8 bA	472.0 ± 24.5 ab	
chloroform extract of *D. cauliflora*	0.5%	4.8 ± 0.1 aAB	5.6 ± 0.3 aA	4.4 ± 0.4 aB	417.3 ± 49.3 a	11.7
	1%	3.1 ± 0.6 bA	2.5 ± 0.7 bA	3.1 ± 0.2 bA	236.7 ± 21.2 b	49.9
	2%	2.7 ± 0.1 bcA	0.8 ± 0.2 cB	0.9 ± 0.3 cdB	120.0 ± 59.5 bc	74.6
	4%	1.8 ± 0.2 cA	1.1 ± 0.1 cA	1.6 ± 0.4 cA	108.3 ± 28.1 c	77.1
	8%	0.6 ± 0.1 dA	0.2 ± 0.1 cA	0.4 ± 0.1 dA	25.3 ± 12.4 c	94.6
	Control	4.9 ± 0.6 aA	6.4 ± 0.7 aA	4.6 ± 0.0 aA	472.7 ± 51.9 a	

[1/] Means ± SE followed by the same letters are not significantly different as determined by Least Significant Difference test (LSD) (α = 0.05). Small case letters compared means in column and capital letters compared means in row.

exposure. At the end of this experiment, total egg production on leaf discs with 2, 4 and 8% crude extracts were significantly lower than the control. The lowest egg production (36.7 eggs) was recorded for mites contacted with 8% crude extract. Maximum percentage of egg reduction (92.2%) was recorded at 8% concentration, followed by 51.7% at 4% crude extract.

Mites on leaf discs treated with 1, 2, 4 and 8% crude chloroform extracts of *D. cauliflor*a produced significantly less number of egg/female/day than the control at 1, 2 and 3 days after treatment (Table II). Daily egg production seem to be decreased with increasing dosage. Moreover, the number of egg produced daily tended to decrease at day 2 after exposure. The lowest daily egg production (0.6, 0.2 and 0.4 eggs/female/day) was found at the highest concentration during the experiment. The total egg production decreased when doses of extract increased. The minimum total egg production was 25.3 eggs at 8% crude extract. The highest percentage of egg reduction was 94.6 at 8% crude extract where those on leaf discs treated with 2-4% crude extracts showed 75-77% egg reduction. Moreover, egg production on leaf discs with 1% crude extract was reduced by 50%.

Leaf disc choice test

Repellent and oviposition deterrent activities of *T. urticae* female after released on the leaf discs having one side treated with *D. grandiflora* crude methanol extract are shown in Table III. At day 1 after exposure, the percentages of live mites on the treated side in all treatments were lower than the control side (< 50%). Hence, it could be concluded that *D. grandiflora* crude methanol extract repelled *T. urticae*. The strongest repellency activity was found on the leaf discs having one side treated with 8% crude extract where no live mites were found on this side (0.0%). In addition, the leaf discs that one side was treated with 4% crude extract also showed strong repellency effect since only 4.4% of live mites were recorded on the treated side. The repellency activity of crude extract decreased when time progressed. At the end of experiment, the repellency effect decreased in most concentrations except at 8% crude extract. The number of live mites on treated side with 8% crude extract was only 9.5% which was significantly lower than the other treatments (50.0-75.4%).

Percentage of eggs on the treated side of leaf discs (treated with crude methanol extract of *D. grandiflora*) are presented in Table III. At day 1 after exposure, percentages of eggs laid on the treated side were lower than the control side in all treatments (< 50%). The highest percentage of eggs on the treated side (11.0%) was found at 0.5% crude extract whereas no eggs were found on the 8% concentration. As time progressed, the oviposition

Table III.- Percentage of *Tetranychus urticae* females alive and percentage of eggs laid on the treated side of leaf discs at days 1, 2 and 3 after painting with 25 µl of *Duabanga grandiflora* crude methanol extract.

Treatments	Mean % of live mite [1/]			Mean % of egg laid [1/]		
	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3
0.5%	23.3 **aB	60.7 *aA	50.0 ^{ns}bA	11.0 **aB	51.7 *aA	66.9 *aA
	(7.0/30.0)	(18.0/29.7)	(12.3/24.7)	(22.7/206.3)	(127.3/246.0)	(111.7/167.0)
1%	10.0 ***abcC	41.6 **abB	75.4 ^{ns}aA	2.0 **bC	40.1 *aB	85.4 *aA
	(3.0/30.0)	(12.3/29.7)	(14.3/19.0)	(3.7/184.7)	(75.7/188.7)	(93.7/109.7)
2%	15.6 **abB	56.8 *abA	54.2 **bA	5.4 ***abC	46.6 *aB	76.2 ^{ns}aA
	(4.7/30.0)	(16.7/29.3)	(13.0/24.0)	(10.3/191.0)	(99.7/214.0)	(91.7/120.3)
4%	4.4 **bcC	34.8 *bB	65.4 **abA	2.7 **abB	13.3 *bB	63.6 *aA
	(1.3/30.0)	(10.3/29.7)	(17.7/27.0)	(5.3/200.3)	(29.3/221.0)	(103.0/162.0)
8%	0.0 ***cA	3.3 ***cA	9.5 **cA	0.0 ***bA	0.3 **bA	3.4 **bA
	(0.0/30.0)	(1.0/30.0)	(2.7/28.3)	(0.0/229.0)	(0.7/239.3)	(4.7/139.0)

[1/] Means ± SE followed by the same letters are not significantly different as determined by Least Significant Difference test (LSD) (α = 0.05) (mites on treated side/total mites). Small case letters compared means in column and capital letters compared means in row.

* Asterisks indicated that the percentage was significantly different from 50%: *P < 0.05, **P < 0.01, ***P < 0.001.

Table IV.- Percentage of *Tetranychus urticae* females alive and percentage of eggs laid on the treated side of leaf discs at days 1, 2 and 3 after painting with 25 µl of *Diospyros cauliflora* crude chloroform extract.

Treatments	Mean % of live mite [1/]			Mean % of egg laid [1/]		
	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3
0.5%	43.3 **aA	43.3 *bA	44.2 *aA	38.1 *aA	48.6 **aA	49.6 *abA
	(13/30)	(13/30)	(11.3/25.7)	(76.7/201.3)	(123.7/254.3)	(90/181.3)
1%	25.6 *bC	72.7 ^{ns}aA	48.1 **aB	11.6 **bB	51.2 **aA	59.0 ^{ns}aA
	(7.7/30)	(21.3/29.3)	(13/27)	(21.3/183.7)	(116/226.7)	(88.3/149.7)
2%	4.4 **cB	37.6 *bcA	24.0 **bA	2.4 *bcB	20.1 *bAB	41.5 ^{ns}abA
	(1.3/30)	(10.7/28.3)	(6/25)	(4/169)	(38/189.3)	(39.3/94.7)
4%	8.9 ***cA	15.5 **cdA	23.0 *bA	9.3 *bcB	8.0 *bcB	27.4 ^{ns}bcA
	(2.7/30)	(4.3/28)	(4.7/20.3)	(12.3/132)	(11/136.7)	(28.7/104.7)
8%	2.2 ***cB	9.2 **dAB	18.7 **bA	0.5 ***cA	2.2 **cA	6.6 *cA
	(0.7/30)	(2.7/29)	(4.7/25)	(1/192.7)	(4/179.3)	(6.7/101.3)

[1/] Means ± SE followed by the same letters are not significantly different as determined by Least Significant Difference test (LSD) (α = 0.05) (mites on treated side/total mites). Small case letters compared means in column and capital letters compared means in row.

* Asterisks indicated that the percentage was significantly different from 50%: *P < 0.05, **P < 0.01, ***P < 0.001.

deterrent effect on the treated side decreased in all concentrations. At day 3 after exposure, eggs laid on the leaf discs treated with 8% crude extract increased to 3.4% which was still significantly lower than the other concentrations.

Percentages of alive *T. urticae* females and percentages of egg laid on treated side of leaf discs treated with crude chloroform extract of *D. cauliflora* are shown in Table IV. At day 1 after application, all concentrations of crude extract significantly repelled mites from the treated sides. The live mites on treated sides were less than 50%. The 8% crude extract showed best repellency activity since only 2.2% of live mites were recorded on the treated side which was not significantly different from those at 2 and 4%. Repellency activity of crude extract tended to decrease in all treatments at 2 days after exposure. At 3 days after exposure, only 24.0, 23.0 and 18.7% live mites were found on sides treated with 2, 4 and 8% crude extracts.

All concentrations of *D. cauliflora* crude

chloroform extract inhibited egg laying of *T. urticae* on treated sides at day 1 after exposure (Table IV). The mites laid significantly lower number of eggs on the treated side as compared to the control side. Only 0.5% eggs were laid on the side treated with 8% crude extract. However, this result was not significantly different as compared to percentages of eggs laid by mites on side treated with 2 and 4% crude extracts. The oviposition deterrent activity tended to decrease as time progressed. Only 6.6% of eggs was found on sides treated with 8% crude extract at day 3 after exposure.

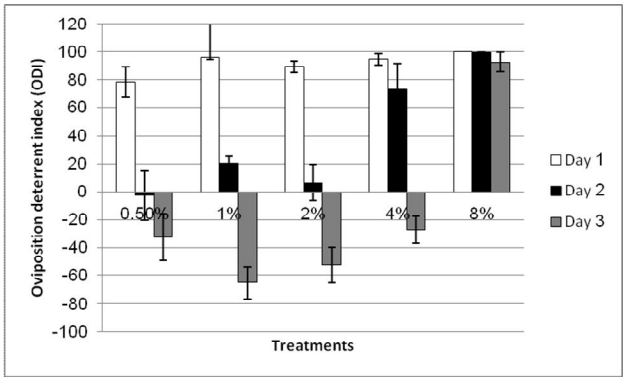

Fig. 1. Oviposition deterrent index (ODI) of *Tetranychus urticae* females at days 1, 2 and 3 on treated leaf discs half-painted with 25 µl of *Duabanga grandiflora* crude methanol extract.

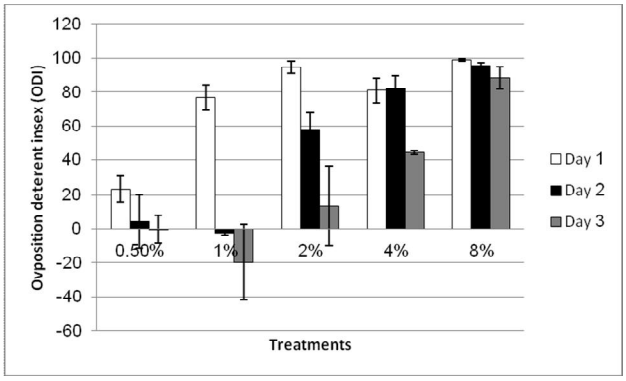

Fig. 2. Oviposition deterrent index (ODI) of *Tetranychus urticae* females at days 1, 2 and 3 on treated leaf discs half-painted with 25 µl of *Diospyros cauliflora* crude chloroform extract.

The oviposition-deterrent index (ODI) of *T. urticae* on leaf disc after half of the disc was treated with crude methanol extract of *D. grandiflora* are presented in Figure 1. At day 1 after exposure, leaf discs treated with 1, 2, 4 and 8% crude extracts strongly deterred oviposition with the ODI of 89.3-100.0%. Two days after exposure, the ODI of 73.3 and 99.5% were recorded at 4 and 8% crude extracts, respectively. At day 3 after exposure, ODI could not be detected at 0.5, 1, 2 and 4% crude extracts. only the 8% crude extract demonstrated 92.8% ODI.

ODI of *T. urticae* on leaf disc after half of disc was treated with crude chloroform extract of *D. cauliflora* are shown in Figure 2. At day 1 after treatment, the ODI of mites treated with 2, 4 and 8% crude extracts were significantly higher than at 0.5% crude extract. The ODI reduced as time passed, especially at low concentrations. At day 2 after exposure, the ODI of 8% crude extract was 95.7% while 82.3% was recorded for ODI at 4% crude extract. At day 3 after treatment, crude extracts at 0.5 and 1% showed no ODI while 4 and 8% crude extracts showed ODI of 44.5 and 88.3%, respectively.

DISCUSSION

The results of bioassays demonstrate that two plant extracts in this study showed different activities against females *T. urticae*. Their biological activities are probably caused by bioactive compounds in the extracts. Phytochemical study of the chloroform extract of *D. grandiflora* stem branches, obtained from the crude methanol extract, furnished betulinic acid, oleanolic acid, arjunolic acid, the flavonoids (acacetin, apigenin and acacetin 7-*O*-glucoside) as well as *p*-hydroxybenzaldehyde, vanillic acid, 6H-dibenzo[*b*,*d*]pyran-3,9-dihydroxy-6-one, 4-*O*-α-L-rhamnopyranosyl-3'-methoxyellagic acid in addition to 3-glycosyl-β-sitosterol (Auamcharoen *et al*., 2009a). On the other hand, the chloroform extract of roots of *D. cauliflora* furnished in addition to 3, 4-dihydro-4β,6-dihydroxy-5-methoxy-2α-methyl-1(2*H*)-naphthalenone), lupeol, betulinic acid, 7-hydroxy-4'-methoxyflavone (pratol), vanillic acid, 2,5-dimethyl-7-hydroxychromone and nicotinamide (Auamcharoen *et al*., 2009b). The active chemicals that occurred in the extracts depended on the plant species, plant parts and the

method of extraction. So, this is one reason why some plants showed high activity whereas others appeared low or no activity on pest.

The lowest survival rate of *T. urticae* was observed when the mites contacted with 8% crude methanol extract of *D. grandiflora* and crude chloroform extract of *D. cauliflora* due to maximum mortality and repellency (Table I). Results also indicated that both plant extracts exhibited slightly higher repellency effect than toxicity to *T. urticae*. All concentrations of both crude extracts repelled *T. urticae* from the treated leaf while control show no or low activities during the experiment. However, only 8% concentration of crude methanol extract of *D. grandiflora* and 4 and 8% of crude chloroform extract of *D. cauliflora* repelled mites at the rates which are significantly different from control during the experiment. This result was quite different from those of Chermenskaya *et al*. (2010) who reported the feeding behavior and oviposition of *T. urticae* after being treated with 1% of 96% ethanol extract of plant materials at 24 h after exposure. Only 0.1-0.6 female mites were observed on the leaves treated with nine plant extracts [*Plantago major* L. aerial parts (Plantaginaceae), *Anabasis aphylla* L. seeds (Chenopodiaceae), *Stachys tschatkalensis* Knorr. aerial parts (Lamiaceae), *Convolvulus krauseanus* Regel. and Schmalh. roots (Convolvulaceae), *Prangos lipskyi* Korov. aerial parts and roots (Umbelliferae), *Hedysarum daraut-kurganicum* Sultanova-endemic aerial parts (Fabaceae), *Senecio saposhnikovii* Krasch et. Schipcz. aerial parts (Compositae), *Mediasia macrophylla* (Ragel. and Schmalh.) M. Pimen. aerial parts (Umbelliferae). These plant extracts significantly deterred spider mite females with deterrent indices (DI) ranging from 60-95%. In tests with extracts of *Ailanthus altissima* L. leaves (Simarubaceae), *Vinca erecta* Ragel. and Schmalh. aerial part (Apocynaceae) and *Allium obliquum* (L.) whole plants (Liliaceae), mites were absent on the treated leaves giving deterrent index of 100%. Eggs were absent on the treated leaves with all tested extracts except the *M. macrophylla* where 0.4 eggs were found on treated leaves.

Leaf disc no choice test also showed that the crude methanol extract of *D. grandiflora* and crude chloroform extract of *D. cauliflora* tended to induce higher mortality and repellency of *T. urticae* with increasing doses and time (Table I). This finding was similar to the results of Gencsoylu (2007) who reported 46.6, 53.3 and 57.1% mortalities for *Tetranychus cinnabarinus* Boisd. females (Acari: Tetranychidae) exposed to residues of 250 gL/0.5L *Asphedolus aestivus* Brot. root extract for 24, 48 and 72 h, respectively. The crude methanol extract of *D. grandiflora* and crude chloroform extract of *D. cauliflora* were less effective with less than 50% mortality of *T. urticae* at the highest concentration (8%) over 3 days. In addition, low concentrations of both crude extracts caused low mortality rate of *T. urticae* which was not significantly different from the control. This result was similar to that of Sakunwarin *et al*. (2004). They revealed that the crude hexane and ethanol extracts of neem seeds (*Azadirachta indica* A. Juss), citronella grass stems and leaves (*Cymbopogen nardus* Rendle.), longan seeds (*Dimocarpus longan* Lour.), cube roots (*Derris elliptica* (Roxb.) Benth.), chinaberry leaves (*Melia azedarach* L.) and sweet oleander leaves (*Nerium indicum* Mill.) at 1% showed low mortality rate of *Tetranychus truncatus* Ehara female (Acari: Tetranychidae) which were not significantly different from the control at day 3 after exposure. On the other hand, this result differed from the results of Tewary *et al*. (2005) who reported that the petroleum ether extract of *Hedera nepalensis* L. (Araliaceae) leaves induced 35 and 38% mortalities of *T. urticae* at 0.5 and 1%, respectively, 48 h after exposure. Moreover, the petroleum ether extract of *H. nepalensis* fruits revealed 36% mortality of *T. urticae* at 1%. The aqueous methanol extract of *H. nepalensis* fruits exhibited 32 and 65% mortalities of *T. urticae* at 0.5 and 1%, respectively. The petroleum ether and aqueous methanol extracts of root of *Berberis lycium* L. (Berberidaceae) demonstrated 26 and 43% mortalities at 1%, 48 h after exposure.

Although, the crude methanol extracts of *D. grandiflora* and crude chloroform extract of *D. cauliflora* were slightly toxic to *T. urticae* female, both crude extracts showed moderate repellency and also inhibited egg production in this mite species (Table II) under leaf disc no choice bioassay and this also occured for the 70% methanol extracts of *Allium sativum* L. fruits (Liliaceae); *Capparis*

spinosa L. aboveground parts (Capparaceae); *Cyperus rotundus* L. roots (Capparaceae); *Cupressus sempervirens* L. leaves, fruits (Cupressaceae); *Euphorbia hierosolymitana* Boiss. milks (Euphorbiaceae); *Lupinus pilosus* L. leaves, fruits (Papilionaceae); *Melia azadirachta* L. fruits (Meliaceae); *Prosopis farcta* (Banks and Sol.) aboveground parts (Fabaceae); *Punica granatum* leaves, fruits (Lythraceae); *Rhus coriaria* L. leaves (Anacardiaceae); *Ruta chalepensis* L. aboveground parts (Rutaceae); *Salvia fruticosa* Mill. leaves (Lamiaceae) and *Tamarix aphylla* (L.) Karst. leaves, young branches, fruits (Tamaricaceae) against *T. cinnabarinus* (Mansour *et al*., 2004).

Repellent activities of *D. grandiflora* crude methanol extract and *D. cauliflora* crude chloroform extract at high dosages (4-8%) tended to decrease when time progressed under choice leaf disc test (Tables III, IV). This result was similar to the results of Jones *et al*. (1996) who reported that the colupulone from hop extracts had no longer any repellent effect on *T. urticae* 64 and 88 h after treatment. In addition, both crude extracts at all concentrations tended to decrease egg-laying on treated side as time progressed (Tables III, IV). Kongkathip *et al*. (2004) also revealed that repellency and oviposition deterring activities decreased as time progressed in most treatments of *Melaleuca leucadendron* L. leaf crude extracts against *T. urticae* females. Kim *et al*. (2005) reported the repellent index of methanol extract of plant materials at 1% against *T. urticae* 1, 2 and 3 days after exposure. These plant extracts [*Machilus thunbergii* Siebold and Zucc. leaf (Lauraceae), *Albizzia coreana* Nakai twig (Leguminosae), *A. coreana* leaf, *Ficus erecta* Thunb. leaf (Moraceae), *Ligustrum japonicum* Thunb leaf (Oleaceae), *Pyracantha angustifolia* Franch. C.K. Schneid leaf (Rosaceae)] showed high repellency activity at 1 day after treatment before declining on 2 and day 3 after exposure. This finding was similar to the results of crude methanol extract of *D. grandiflora* at 1% where repellency rate decreased as time progressed. On the other hand, *N. indicum* leaf, *Dendropanax morbifera* Leveille leaf (Araliaceae), *Farfugium japonicum* (L.) Kitam leaf (Araliaceae), *Ranunculus japonicus* Thunb. leaf and root (Ranunculaceae) showed the highest repellency activity 1 day after treatment before decreasing at day 2 and slightly increasing again at day 3 after exposure. This finding was similar to the results of crude chloroform extract of *D. cauliflora* at 1% where 25.6, 72.7 and 48.1% live mites on treated side were recorded at day 1, 2 and 3 after exposure, respectively.

The plant extracts naturally degraded within a few hours or days after being exposured (Food and Agriculture Organization [FAO], 2000; Ghaderi *et al*., 2013). In contrast, sugar apple extracts applied on the leaf surface showed strong repellent effects as the dose increased and time progressed (Sakunwarin *et al*., 2004). The neemgard at 1% induced high repellency effect on *T. cinnabarinus* after four days, but showed no longer effect on mortality (Mansour *et al*., 1997). The neem seed hexane extract strongly affected the oviposition site of *Panonychus citri* (McGregor) since mites only laid eggs on untreated leaves which differed from this study (Jacobson *et al*., 1978). Jones *et al*. (1996) revealed that hop beta-acid fraction, naturally occurring beta-triketones found in several plants, could repel *T. urticae* females from the treated side for 18 h and reduced this activity afterwards. Moreover, this fraction at 10 and 100 g/L inhibited egg-laying on treated side as long as 88 h. These observations agreed with the results from this study wherein live mites and eggs were found on the side treated with crude extracts less than the control side. Only 8% crude extracts used in this study showed high oviposition deterrence until 72 h.

The results showed that time had an effect on oviposition deterrent activity of *T. urticae*. High oviposition-deterrent index was found shortly after exposure. Low concentrations (0.5-2%) of crude extracts induced no or low oviposition-deterrent index at day 3 after exposure. The oviposition deterrent indices (ODI) of *T. urticae* decreased with the decreasing concentration of *D. grandiflora* crude methanol extract and *D. cauliflora* crude chloroform extract. This finding was similar to Sakunwarin *et al*. (2004) who revealed that the higher concentration, up to 10% hexane and ethanol extracts of sugar apple, showed 100% ODI of *T. truncatus* and the ODI decreased as the concentration decreased. Dimetry *et al*. (1993) also

reported that neem azal-S showed 100% ODI of *T. urticae* at the concentration up to 0.1% and ODI decreased as the concentration decreased. At the highest concentration (8%), *D. grandiflora* crude methanol extract showed slightly higher oviposition deterrent index (ODI) of *T. urticae* than *D. cauliflora* crude chloroform extract during this experiment. The crude extract of *D. grandiflora* showed ODI ranging from 92.8-100.0% while 88.3-99.0% of ODI was recorded for crude extract of *D. cauliflora*. In contrast, the crude chloroform extract of *D. cauliflora* at 0.5-4% showed a higher ODI than the crude extract of *D. grandiflora* at the end of experiment.

Hence, the residual effects of crude methanol extract of *D. grandiflora* and crude chloroform extract of *D. cauliflora* might be used to protect the host from mite infestations by reducing total egg production and repelling mites from the plants. The toxicity, repellency and oviposition deterrent activities of *D. grandiflora* crude methanol extract and *D. cauliflora* crude chloroform extract against *T. urticae* were possibly caused by several chemical compounds in the extracts. Therefore, future investigations are needed to be carried out on the activity of the various active compounds in the extracts.

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