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Acaricidal Activities of Plant Essential Oils from Three Plants on the Mushroom Mite, *Luciaphorus perniciosus* Rack (Acari: Pygmephoridae)

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Abstract.- Essential oils of *Litsea cubeba, Litsea salicifolia* and *Melaleuca cajuputi* were tested against *Luciaphorus perniciosus* by contact and fumigation methods. Contact toxicity bioassay was made by dropping essential oils at rates of 0, 0.06, 0.33, 0.66, 3.3, 6.6, 33, 66 and 99 μl/cm^2 into special mite cages. Fumigation was done in a knockdown chamber of 2.5x10^4 cm^3 in size at the concentrations of 0, 0.0012, 0.006, 0.012, 0.06, 0.12, 0.6 and 1.2 μg/cm^3. The essential oil of *L. cubeba* was found to be the most toxic to *L. perniciosus* by both contact and fumigation methods with LD_{50} values equaling to 0.932 and 0.166 μg/cm^3, respectively, followed by essential oil of *L. salicifolia* whose LD_{50} values of 2.793 and 0.410 μg/cm^3 were recorded. Essential oil of *M. cajuputi* demonstrated a low effect on *L. perniciosus* as compared to those of *L. cubeba* and *L. salicifolia*. Essential oil formulations were delivered by using citronella grass or black pepper oils as major components and *L. cubeba* as minor component. Mite mortality rate increased when 0.01% *L. cubeba* oil was added to either citronella grass or black pepper oil at all treatments by contact method.

Keywords: Essential oil, wild plant, fumigant, mushroom mite, *Luciaphorus perniciosus*

INTRODUCTION

Several mites are known to attack cultivated mushrooms in Thailand. *Luciaphorus perniciosus* Rack (Family Pygmephoridae) is considered to be the most important mite causing yield losses in mushroom production. They often cause 10-20% yield loss and occasionally total crop loss. Control of mite populations in mushroom farms is remarkably limited and chemical substances are sometimes used during the beginning of growing period. Therefore, only sanitary measures are to be appropriately applied in the mushroom house.

Plant essential oils may provide an alternative means of controlling mushroom mites since they contain a rich array of bioactive chemicals that can be used to control several agricultural pests. To date, there have been many reports regarding the use of essential oils or crude extracts from plants for controlling several, especially phytophagous and parasitic mites.

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The essential oil of *Chenopodium ambrosioides* L. is known to increase the mortality of adults and eggs of two-spotted spider mite, *Tetranychus urticae* Koch (Chiasson *et al.*, 2004). Chang *et al.* (2001) reported that the essential oil of *Taiwania cryptomerioides* Hayata at the rate of 12.6 μg/cm^2 caused 67% and 36.7% mortalities of the house dust mite, *Dermatophagoides pteronyssinus* (Trouessart) and *D. farinae* Hughes, respectively. Kim *et al.* (2003) found that eugenol and its derivatives, acetyleugenol, isoeugenol and methyleugenol showed much lower LD_{50} values of 0.67, 1.55, 3.71 and 5.41 μg/cm^2, respectively, in controlling *D. pteronyssinus*. In 2008, Insung and Pumnuan reported that fumigation by clove and cinnamon oils at a concentration of 1.2 μg/cm^3 completely killed *D. pteronyssinus*. These two oils demonstrated the LD_{50} values of 0.092 and 0.232 μg/cm^3, respectively.

Raynaud *et al.* (2000) stated that the acaricidal activity of a dichloromethane extract of *Uvaria pauciovulata* Hook. F. & Thoms against *D. pteronyssinus* which had an EC_{50} of 0.028 g/m^2. Akendengue *et al.* (2003) found that methanol and hexane extracts of *U. versicolor* Pierre ex Engl. &

Diels gave EC_{50} values of 0.095 and 0.12 g/m^2, respectively, and dichloromethane extract of *U. klaineana* Engler & Diels yielded EC_{50} value of 0.85 g/m^2 in controlling *D. pteronyssinus*.

Lee (2004) compared the components of oils derived from *Foeniculum vulgare* fruit oil with benzyl benzoate against *D. farinae* and *D. pteronyssinus*. He found that most toxic compound to *D. farinae* was p-anisaldehyde (11.3 mg/m^2) while p-anisaldehyde (10.1 mg/m^2) was much more effective against *D. pteronyssinus* than benzyl benzoate (67.5 mg/m^2).

To date, several crude extracts of many plant species were tested against stored product mites, *Tyrophagus putrescentiae* Schrank (Kwon *et al*., 2003; Kim *et al.*, 2003), pig mange mites (Mägi *et al.*, 2006) as well as house dust mites (Kim *et al.*, 2006; Rim and Jee, 2006; Saad *et al.*, 2006). As for the mushroom mite, Pumnuan *et al.* (2008) reported that clove and cinnamon extracts induced 88.7% mortality of *L. perniciosus* when applied at the rate of 125 µg/cm^2. Moreover, dichloromethane extracts of clove and cinnamon showed the highest toxicity against *L. perniciosus* with the LD_{50} values of 34.97 and 35.57 µg/cm^2, respectively.

The purpose of this study was to investigate the acaricidal activities of some plant essential oils and their formulations on controlling the mushroom mite, *L. perniciosus* under the laboratory conditions.

MATERIALS AND METHODS

Stock culture of mushroom mites

Colonies of *L. perniciosus* were obtained from the Department of Agriculture, Ministry of Agriculture and Co-operatives, Bangkok. They were reared on sorghum grain infested with mycelia of *Lentinus polychrous* Le'v and kept at 27±2°C.

Plant species and essential oils extraction

Mature fruits of *L. cubeba* and *L. salicifolia* were collected at Doi Ang-khang (19º54'N, 99º2'E), Fang district, Chiang Mai province in June 2007. Fresh leaves of *M. cajuputi* were collected at Kasetsart University, Bangkhen campus, (13º98'N, 48º18'E) in June 2007. The voucher specimens (#CHKU 00022, #CHKU 00023 and #CHKU 00028) were deposited at the Bangkok Herbarium, Botanical Research Unit, Department of Agriculture, Bangkok, Thailand.

The essential oil was extracted by water-distillation using a Clevenger-type apparatus for 6 h. The superior phase was collected from the condenser, dried over anhydrous sodium sulphate and stored in amber-colored vials at 10-12ºC for further experiments.

Experimental treatments

Contact toxicity bioassay

A glass tube, 0.4 cm in diameter and 3 cm long with fine nylon mesh on both ends, was used to confine the mite samples. Each glass tube was treated internally with 25 µl of essential oil at various concentrations (0, 0.001, 0.005, 0.01, 0.05, 0.1, 0.5, 1.0 and 1.5% equaling to 0, 0.066, 0.33 0.66, 3.3, 6.6, 33, 66 and 99 µl/cm^2, respectively) and 95% ethanol was used as the control. The solution was then distributed evenly around the inner wall of the test tube and allowed to air dry before 10 non-physogastric mites were introduced into each glass tube. Observations were made 12 and 24 h after treatment and the number of dead mites was recorded.

Fumigation

Samples of 10 non-physogastric mites were transferred to the mite cage made out of an acrylic sheet (3x5x0.45 cm) perforated into frustum of cone. The base of cone was 0.25 cm in diameter and covered with a filter paper; the top was 0.5 cm in diameter and covered with a cover glass (1 x 1 cm). All mite cages were placed into the 2.5x10^4 cm^3 knockdown chamber (Burkard Co., England). Essential oils at concentrations of 0 (10% tween-20 in distilled water), 0.002, 0.01, 0.02, 0.1, 0.2, 1.0 and 2.0% with volume of 1.5 ml (or 0, 0.0012, 0.006, 0.012, 0.06, 0.12, 0.6 and 1.2 µg/cm^3, respectively) were sprayed into the chamber. The mite cages were left in the chamber for 1 h after the treatment, and mortality of mushroom mites were observed at 12 and 24 h thereafter.

As for commercial use, various essential oil formulations were tested by using essential oils of citronella grass (*Cymbopogon nardus* (Linn.) Rendle) or black pepper (*Piper nigrum* Linn.) as a major component and 0.01% *L. cubeba* as a minor component. As described earlier, the contact

toxicity bioassy was used to determine percentage of mite mortality.

Mites were considered dead if their appendages did not move when probed with a small hair brush. Abbot's formula (Abbott, 1925) was used to calculate the actual death rates. The experiment was designed in three completely randomized replicates. The data obtained were statistically analyzed by applying analysis of variance (ANOVA) and Duncan's multiple range tests (DMRT). The LD_{50} was calculated by the probit method.

RESULTS

The essential oils obtained from three plant species could be used to reduce *L. perniciosus* numbers under laboratory conditions. Using the contact method, the essential oil of *L. cubeba* at the rate of 99 μg/cm^2 showed the highest toxicity to *L. perniciosus*, causing 97.5±4.1% mortality at 12 h, while 97.0±4.7 and 91.9±8.9% mortality induced by *L. salicifolia* and *M. cajuputi* were recorded at the same rate (Table I). At this rate, the essential oils of *L. cubeba* and *L. salicifolia* caused significantly higher mortality rates as compared to those induced by *M. cajuputi* oil. Furthermore, *L. cubeba* and *L. salicifolia* showed LD_{50} values of 0.932 and 2.793 μg/cm^2 where up to 19.705 μg/cm^2 was recorded for *M. cajuputi* oil.

As the time passed, mortality rates of *L. perniciosus* increased in all treatments. *L. cubeba* and *L. salicifolia* essential oils still induced significantly higher mortality rate at the concentration of 33 μg/cm^2 (96-97%) as compared to *M. cajuputi* oil which caused its highest mortality rate (99%) at the concentration of 66 μg/cm^2. However, the activity of all essential oils at the rate of 33 μg/cm^2 did not differed significantly (Table I).

Using the fumigation method, the highest mortality rate (78-91%) of *L. perniciosus* occurred when 1.2 μg/cm^3 of *L. cubeba*, *L. salicifolia* and *M. cajuputi* was applied (Table II). In addition, *L. cubeba* caused significantly higher mortality of *L. perniciosus* as compared to those mites treated with *L. salicifolia* and *M. cajuputi* oils. The LD_{50} values confirmed this result where 0.16, 0.41 and 0.47 μg/cm^3 were recorded for *L. cubeba*, *L. salicifolia* and *M. cajuputi* oils, respectively. At 24 h post treatment, mortality rates of *L. perniciosus* increased tremendously at all lower concentrations. However, *L. cubeba* and *L. salicifolia* oils caused 93-95% mite mortality at the rate of 0.12 μg/cm^3 where the higher dose (0.6 μg/cm^3) was required for *M. cajuputi* oil to induced 93% mortality.

Essential oils of either *P. nigrum* or *C. nardus* alone could cause death of *L. perniciosus* adult within 12 h by contact method. However, the mortality rates of *L. perniciosus* caused by *P. nigrum* and *C. nardus* at the rate of 3.3 μg/cm^3 was relatively low comparing to those of higher concentrations (Table III). Adding 0.01% *L. cubeba* oil to *P. nigrum* and *C. nardus* oils could increase the mortality rate of the mushroom mite in all treatments where 16% and 45% increases in mortality rates were observed in the formulations of *P. nigrum*+0.01% *L. cubeba* oil and *C. nardus*+0.01% *L. cubeba* oil at the rate of 3.3 μg/cm^2, respectively (Table III).

DISCUSSION

Our results indicated that essential oils of *L. cubeba*, *L. salicifolia* and *M. cajuputi* showed contact and fumigation toxicities against *L. perniciosus* adults. Similar results were found by Ko Ko (2009) and Ko Ko *et al.* (2009) where fumigant and contact toxicity effects of *L. cubeba*, *L. salicifolia* and *M. cajuputi* against *Sitophilus zeamais* Motschulsky and *Tribolium castaneum* (Herbst) were shown. Noosidum *et al.* (2008) reported that *Aedes aegypti* L. demonstrated clear behavioral escape responses to these three essential oils in both contact and non-contact trials in excito-repellency test chamber.

Insung and Pumnuan (2008) stated that the essential oil of citronella grass (*C. nardus*) was remarkably toxic to *D. pteronyssinus* with LD_{50} value of 0.935 μg/cm^3 24 h after fumigation. Moreover, essential oil of *P. nigrum* showed acaricidal effect on the spider mite, *Eotetranychus cendanai* Rimando with LC_{50} of 23.6 ml/l (Sornlek, 2001). George *et al.* (2009) also reported that essential oil of black pepper at 0.14 mg/cm^3 could be used to repel the red mite *Dermanyssus gallinae* (De Geer) during the first 2 days. Insung *et al.*

Table I.- Effect of wild plant essential oils on the mortality of *Luciaphorus perniciosus* Rack by contact toxicity bioassay at 12 and 24 h.

Concentration ($\mu g/cm^2$)	12 h				24 h			
	M. cajuputi	*L. cubeba*	*L. salicifolia*	%CV	*M. cajuputi*	*L. cubeba*	*L. salicifolia*	%CV
0 (Control)	0.0±0.0 gA[1]	0.0±0.0 gA	0.0±0.0 hA	-	0.0±0.0 fA	0.0±0.0 dA	0.0±0.0 dA	-
0.066	30.5±12.4 fB	45.6±13.2fA	44.6±9.0 gA	28.2	89.9±9.1 eA	92.3±7.3 cA	87.9±8.5 cA	9.2
0.33	37.6±12.1 eB	52.3±10.1eA	50.7±11.2fA	23.7	92.6±6.4deA	91.9±7.6 cA	90.9±8.8 cA	8.4
0.66	44.9±12.0 dB	59.4±13.6dA	59.0±11.7eA	22.7	92.9±7.5deA	91.9±7.6 cA	90.9±8.0 cA	8.4
3.3	49.3±11.6cdC	70.8±14.8cA	63.4±11.0eB	20.4	93.9±6.2 cA	95.6±7.3 bA	94.6±6.3 bA	7.0
6.6	53.0±13.0 cB	75.2±15.3cA	74.2±14.8dA	21.3	96.3±5.6bcA	94.9±7.3bcA	95.3±7.3 bA	7.1
33	72.5±12.0 bB	86.6±11.5bA	83.6±10.7cA	14.1	96.6±4.8bcA	96.6±5.5abA	97.6±5.0abA	5.3
66	76.2±9.6 bB	95.5±5.1 aA	91.3±11.1bA	10.2	99.0±3.1abA	99.7±1.8 aA	99.0±3.1 aA	2.7
99	91.9±8.9 aB	97.5±4.1 aA	97.0±4.7aA	6.5	100.0±0.0aA	99.3±2.5 aA	99.7±1.8 aA	1.8
%CV	21.4	17.0	16.3		6.7	7.0	7.4	
LD_{50}	19.705	0.932	2.793		-	-	-	
Slope	0.019	0.027	0.023		-	-	-	
SE	0.002	0.003	0.002		-	-	-	

[1] Means in row with the same contact time followed by the same capital letters are not significantly different and means in column followed by the same common letters are not significantly different at the 5% level as determined by LSD (α=0.05)

Table II.- Effect of wild plant essential oils on the mortality of *Luciaphorus perniciosus* Rack by fumigation method at 12 and 24 h.

Concentration ($\mu g/cm^3$)	12 h				24 h			
	M. cajuputi	*L. cubeba*	*L. salicifolia*	%CV	*M. cajuputi*	*L. cubeba*	*L. salicifolia*	%CV
0 (Control)	0.0±0.0 fA[1]	0.0±0.0 gA	0.0±0.0 gBA	-	0.0±0.0 eA	0.0±0.0 fA	0.0±0.0 eA	-
0.0012	22.4±9.1eB	35.4±8.2 fA	26.5±8.6 fB	29.2	68.3±12.0dA	76.8±10.8eA	71.1±7.0 dA	13.8
0.0060	27.2±7.4eC	43.5±5.2 eA	32.7±7.4 efB	18.8	78.2±10.3cA	85.9±7.2 dA	78.2±7.0 cA	10.2
0.012	35.4±7.2dB	53.7±9.2 dA	36.1±5.9 eB	17.6	81.7±8.8 cA	88.0±6.4cdA	82.4±7.2 cA	8.9
0.06	36.7±7.7dC	54.4±6.4 dA	43.5±10.6dB	18.3	88.7±8.8 bA	92.3±5.9bcA	90.1±8.8 bA	8.8
0.12	46.3±8.8cB	63.9±7.4cA	51.7±8.8 cB	15.3	89.4±7.bA	95.8±5.1abA	93.0±7.2abA	7.2
0.6	59.2±6.5bC	79.6±13.1bA	67.3±10.8bB	15.1	93.7±6.3abA	99.3±2.6 aA	97.2±4.6 aA	4.9
1.2	80.3±7.0aB	91.2±8.3 aA	78.2±11.9aB	11.1	98.6±3.5 aA	100.0±0.0aA	97.2±4.6 aA	3.4
%CV	18.4	15.0	20.4		10.6	7.4	8.3	
LD_{50}	0.476	0.166	0.410		-	-	-	
Slope	1.331	1.565	1.243		-	-	-	
SE	0.124	0.150	0.123		-	-	-	

[1] Means in row with the same contact time followed by the same capital letters are not significantly different and means in column followed by the same common letters are not significantly different at the 5% level as determined by LSD (α=0.05)

(2008) studied the acaricidal activities of eight plants leaf extracts against *L. perniciosus* by a filter paper contact toxicity bioassay and demonstrated that methanolic extract of *L. cubeba* caused the death of *L. perniciosus* with a LD_{50} value of 19.58 $\mu g/cm^2$. We also found that the essential oil of this plant was even more effective than its leaf extract by having the LD_{50} value of only 0.93 $\mu g/cm^2$ in the contact bioassay.

In order to promote the use of plant essential oils, a small amount of *L. cubeba* essential oil was added to black pepper or citronella grass oils and greatly improved their effectiveness, even though these two oils had their own contact toxicity against *L. perniciosus*. The results clearly demonstrated that formulations of either black pepper or citronella grass oils at the rate of 3.3 $\mu g/cm^2$ with 0.01% *L. cubeba* oil could enhance the mite mortality rate up

Table III.- Effect of various essential oil formulations on the mortality of *Luciaphorus perniciosus* Rack by contact toxicity bioassay at 12 h.

Concentration main component (μg/cm^2)	*P. nigrum*	*C. nardus*	*P. nigrum* + *L. cubeba* (0.01%)	*C. nardus* + *L. cubeba* (0.01%)	%CV
0 (Control)	0.0±0.0 dA[1]	0.0±0.0 dA	0.0±0.0 dA	0.0±0.0 cA	-
3.3	62.2±15.3 cC	59.5±10.0 cC	72.3±12.8 cB	85.8±9.1 bA	17.1
6.6	77.7±19.3 bB	83.8±14.0 bAB	90.5±8.0 bA	89.9±7.6 bA	15.4
33	90.5±7.0 aAB	85.1±11.3 bB	91.2±7.4 bAB	95.9±5.1 aA	8.8
66	95.3±5.2 aAB	92.6±5.9 aB	98.0±4.1 aA	97.3±4.6 aA	5.2
99	95.9±5.1 aA	94.6±5.2 aA	98.6±3.5 aA	98.0±4.1 aA	4.7
%CV	15.5	13.1	9.8	7.7	

[1] Means in row followed by the same capital letters are not significantly different and means in column followed by the same common letters are not significantly different at the 5% level as determined by LSD (α=0.05)

to 16% and 45%. The results of this study have indicated that these three plant essential oils and the essential oil formulations showed potential to be used as a botanical acaricide against the mushroom mite, *L. perniciosus.* However, studies on application under rearing conditions are still needed.

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