Fumigation and residual contact toxicity of lemon grass, betel vine, myrtle grass and clove essential oils against stored product mite, *Tyrophagus* sp.

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Abstract

Fumigation and residual contact toxicity of lemon grass (*Cymbopogon citratus* (Dc.ex.Nees)), betel vine (*Piper betle* Linn.), myrtle grass (*Acorus calamus* Linn.) and clove (*Syzygium aromaticum* (Linn.)) essential oils against adult stored product mite (*Tyrophagus* sp.) was investigated. The fumigation bioassay was performed with 0 (95% ethanol), 0.3, 0.6, 0.9, 1.2 and 1.5 µl/L air essential oils in 25 L fumigation chamber for 1 hr. The mortalities were observed at 24 hr after the treatments. The residual contact bioassay was carried out with the essential oils at 0 (95% ethanol), 0.02, 0.04, 0.06, 0.08 and 0.10 µl/cm^2 in a glass tube, sized 0.4 cm in diameter and 3 cm in length covered with filter paper on both ends. The mortalities were observed at 12 hr after the treatments. The results of fumigation bioassay showed that clove essential oil at 1.5 µl/L air showed 95.6% mortality with LC_{50} of 0.64 µl/L air, followed by lemon grass essential oil with LC_{50} values of 0.96 µl/L air. In addition, residual contact of clove and lemon grass essential oils at 0.10 µl/cm^2 showed 100% mortality with LC_{50} value of 0.041 µl/cm^2 and 71.9% mortality with LC_{50} value of 0.061 µl/cm^2, respectively. However, essential oils of betel vine and myrtle grass presented low toxicity in both fumigation and residue methods.

Keywords: mold mite, fumigation chamber, clove, lemon grass

1. Introduction

Stored product mites or stored food mites are considered an important pest infesting crops and commodities in warehouses and barns. These infestations usually causes product disqualification, loss of nutrition, and seed germination inhibition (Chao-peng, 1983). The mites found in Thailand in 2000-2003 included *Lardoglyphus* sp., *Tyrophagus* sp., *Suidasia* sp., *Sancassania* sp., *Rhizoglyphus* sp., *Aleuroglyphus* sp., *Aceria* sp. and *Histiostoma* sp. (Charanasri et al., 2003). Particularly, mold mite, *Tyrophagus* sp. is the most commonly found across the country. This polyphagous pest normally spreads over post-harvested grains, animal feeds, or dried crops. Moreover, the mite has recently been considered a major pest in mycological laboratories, since it generally feeds on fungus, and causes contamination in the samples (Mullen and O'Connor, 2009). In addition, the outbreaks of mold mite can intensify the epidemic of microorganism diseases through feeding behavior and fecal contamination (Curtis et al., 1981).

The management of stored product mites usually involves an integration of different pest control methods (Insung, 2000), such as sanitation management, physical management, pesticide control, and biological control. Nonetheless, stored product mites are highly resistant to pesticides, thus the management of these pests normally requires a great deal of highly toxic chemicals. Consequently, issues regarding chemical residues and contaminations in the treated products have become a major concern among scientists. Weinzierl and Higgins (2008) reported that deltamethrin plus chlorpyrifos-methyl residues could remain effective for

1 year or longer in dry grain. Recently, biological approaches have been introduced, and management of the pest has become a subject of interest in many biological pesticide studies. Insung (1995) studied the effect of extracts from *Piper retrosractum* and *Artemisia dracunculus*, and reported that at the concentration of 1% the extracts could control *Tyrophagus putrescentrae* at 95.7% and 68.7%, respectively. Kim et al. (2003) reported the contact toxicity of *Eugenia caryophyllata* against *T. putrescentiae*. In addition, Tak et al. (2006) studied the effect of chemical components from root of *Paeonia suffruticosa* against *T. putrescentiae* and reported that paeonol and benzoic acid extracted from the root resulted in the LD_{50} at 5.29 and 4.80 µg/cm^2, respectively. Moreover, essential oils form betel vine, myrtle grass and clove were also found effective in controlling stored product mite, *Suidasia pontifica* with the LD_{50} at 41.79, 28.34 and 24.28 µg/cm^2, respectively (Pumnuan and Insung, 2011). In addition, many studies reported that lemon grass essential oil showed the most toxicity effect against mushroom mites, *Luciaphorus perniciosus* (Pumnuan and Insung, 2012), and *Dolichocybe indica* (Pumnuan et al., 2014). In general, it was found in many studies that plant extracts showed high potentialnorn management of insect pests.

The study investigated the fumigation toxicity of medicinal plant essential oils including lemon grass, betel vine, myrtle grass and clove against *Tyrophagus* sp. with an objective of finding a potential alternative methods for pest management.

2. Materials and Methods

2.1. Mite stock

The stored product mite, *Tyrophagus* sp. was raised in mite bottles kept in a chamber at 25±1°C and 86±1% r.h. Saturated KCl was used to control humidity. The mite was fed by a mixture of rice, rat food, wheat germ and yeast at the proportion of 6:4:4:1 g, respectively (applied from Insung and Boczek, 1995).

2.2. Plant species and essential oils extraction

In this study, the essential oils from fresh leaf of lemon grass (*Cymbopogon citratus*) and betel vine (*Piper betle*), dried rhizome of myrtle grass (*Acorus calamus*), and dried flower bud from clove (*Syzygium aromaticum*) were extracted by using water-distillation method with a Clevenger-type apparatus, for the period of 6 hr. The extracted oils were collected and dehydrated over anhydrous sodium sulphate and stored in amber-colored vials at 10-12°C for further experimental phases.

2.3. Experimental treatments

2.3.1. Fumigation toxicity

This study used the fumigation method presented by Pumnuan et al. (2010) with some modification. Initially, the samples of 10-12 non-physogastric mites were transferred to a mite cage made out of an acrylic sheet (3 x 5 x 0.45 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 in the 25 L knockdown chamber (Burkard Co., England). Essential oils at the concentrations of 0 (95% ethanol), 0.5, 1.0, 1.5, 2.0 and 2.5% with the volume of 1.5 ml were sprayed into the chamber (or 0, 0.3, 0.6, 1.2 and 1.5 µl/L, respectively). The mite cages were left in the chamber for 1 h after the treatments, and mortalities of the mites were observed at 24 h thereafter. The experiment was completely randomized design (CRD) with 3 replications (3 mite cages per replication).

2.3.2. Contact toxicity bioassay

The contact treatment method applied in this study was modified from Pumnuan et al. (2010).

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. In general, 15 µl of the essential oil at various concentrations (0, 0.5, 1.0, 1.5, 2.0 and 2.5% equaling to 0, 0.02, 0.04, 0.06, 0.08 and 0.10 µl/cm^2, respectively) and 95% ethanol was used as the control. The solution was distributed evenly around the inner wall of the test tube and allowed to air dry, before 10-12 non-physogastric mites were introduced into each glass tube. Observations were made 24 h after treatment and the number of dead mites was recorded. The experiment was a CRD with 3 replications (3 mite cages per replication).

In general, mites were considered dead when the appendages did not move when probed with a small hair brush. Abbot's formula (Abbott, 1987) was used to calculate the actual death rates. Then, the data obtained were statistically analyzed by applying analysis of variance (ANOVA) and Duncan's multiple range tests (DMRT) in SAS. The LD_{50} was calculated by the probit analysis in SPSS.

3. Results and Discussion

The results of fumigation treatment showed that clove essential oil at 1.2 and 1.5 µl/L air was highly toxic to the *Tyrophagus* sp. with 85.0 and 95.6% mortality, respectively (LC_{50} at 0.64 µl/L air). The significant differences in mortality percentages were observed in lemon grass, betel vine and myrtle grass essential oils group at the same concentrations with 71.4, 48.0 and 28.6% mortalities, respectively (at 1.2 µl/L air), and 95.6, 80.0, 57.3 and 36.8% mortality, respectively (at 1.5 µl/L air). The LC_{50} were 0.96, 1.30 and 1.74 µl/L air, respectively (Table 1). Similar results were also reported by Insung and Pumnuan (2009) that clove essential oil completely killed house dust mite (*Dermatophagoides pteronyssinus*) at 1.2 µl/L air. In addition, Veeraphant et al. (2011) reported the acaricidal activity of clove essential oil that at the concentration 0.1% v/v the essential oil killed house dust mite (*D. pteronyssinus*) within 10 min, while lemon grass and betel vine essential oils killed within 15 and >20 min, respectively. The active essential oils of clove, lemon grass and betel vine showed LC_{50} values (24 h) at 0.0026, 0.0091 and 0.0091 ml/ml, respectively.

Table 1 Mortality percentages of *Tyrophagus* sp. after fumigation with medicinal essential oils at various concentrations (24 hours).

Essential oils	Mortality (%) Concentrations (µl/L air)						LC_{50}	LC_{90}	Slope	SE
	0	0.3	0.6	0.9	1.2	1.5				
Lemon grass	0.0±0.0^a	10.9±1.0^b	33.3±13.1^b	43.9±7.9^b	71.4±4.1^b	80.0±6.1^b	0.96	1.64	1.879	0.139
Betel vine	0.0±0.0^a	8.4±4.8^b	17.4±5.7^c	26.5±4.6^c	48.0±5.5^c	57.3±4.3^c	1.30	2.16	1.489	0.139
Myrtle grass	0.0±0.0^a	9.3±1.6^b	13.7±3.3^c	22.2±7.1^c	28.0±7.4^d	36.8±4.6^d	1.74	2.98	1.032	0.137
Clove	0.0±0.0^a	34.3±8.5^a	50.8±7.8^a	69.8±8.7^a	85.0±7.1^a	95.6±6.1^a	0.64	1.26	2.096	0.146
% cv	-	34.4	28.8	17.9	10.6	7.9				

[1]Means in column followed by the same superscript letter were not significantly different (P<0.05) according to DMRT.

The residual contact toxicity of clove and lemon grass essential oils at 0.10 µl/cm^2 showed 100 and 71.9% mortalities with LC_{50} value at 0.041 and 0.061 µl/cm^2, respectively. However, essential oils of betel vine and myrtle grass presented low toxicity (54.9-59.6% mortality with LC_{50} value of 0.081-0.085 µl/cm^2) (Table 2). The results found in this study were generally in congruence with Pumnuan and Insung (2011) in which clove essential oil was reported presenting 98.5% mortality of *S. pontifica* at 0.080 µl/cm^2 with LC_{50} at 0.024 µl/cm^2, and essential oils of myrtle grass and betel vine showed LC_{50} at 0.028 and 0.042 µl/cm^2, respectively by dry film method. Saad et al. (2006) reported that clove essential oil was the more effective against house dust mites (*D. pteronyssinus*) (LC_{50} = 29.78×10^{-6} g/ 0.5 g of

dust) more than lemon grass essential oil (LC_{50} = 300.66×10^{-6} g/ 0.5 g of dust). In addition, dichloromethane extracts of clove was also reported presenting the highest contact toxicity against mushroom mites (*Luciaphorus perniciosus* and *Formicomotes heteromorphus*) (Pumnuan et al., 2008).

Table 2 Mortality percentages of *Tyrophagus* sp. caused by medicinal essential oils at various concentrations by contact method (24 hours).

Essential oils	Mortality (%) Concentrations (µl/cm^2)						LC_{50}	LC_{90}	Slope	SE
	0.00	0.02	0.04	0.06	0.08	0.10				
Lemon grass	0.0±0.0^a	37.6±4.6^{ab}	44.0±5.5^b	49.1±4.4^b	59.6±8.2^b	71.9±7.3^b	0.061	0.134	17.541	1.717
Betel vine	0.0±0.0^a	30.7±6.0^{bc}	33.2±3.2^c	38.2±8.6^c	42.6±6.3^c	55.6±6.9^c	0.085	0.178	13.722	1.710
Myrtle grass	0.0±0.0^a	25.4±4.5^c	33.2±7.3^c	40.3±3.8^{bc}	50.2±4.2^d	54.9±8.9^d	0.081	0.163	15.488	1.741
Clove	0.0±0.0^a	41.4±7.5^{ab}	56.7±4.8^a	66.1±8.4^a	81.1±8.0^a	100.0±0.0^a	0.041	0.082	30.744	2.201
% cv	-	17.1	12.9	13.7	11.8	9.5				

[1] Means in column followed by the same superscript letter were not significantly different (P<0.05) according to DMRT.

4. Conclusions

This study showed that fumigation and residual contact treatment of clove and lemon grass essential oils presented highly toxic effects to the stored product mite. In particular, these essential oils at 1.5 µl/L air showed more than 80.0% mortality by a fumigation method. In addition, residual contact of these essential oils at 0.10 µl/cm^2 showed 100% mortality. The results revealed that essential oils of lemon grass and clove can be potential alternative ingredients in producing environmental friendly pesticides. However, further studies should be performed on the application of these essential oils in field conditions. In addition, the effects of the essential oils on the quality of agricultural products is also needed study in order to reduces possible losses and enhance the performance of the biopesticides.

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