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Fumigant Toxicity of Lemon Grass, Citronella Grass and Black Pepper Essential Oils against Mushroom Mite (*Dolichocybe Indica* Mahunka)

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Acaricidal activitity of essential oils from lemon grass (*Cymbopogon citratus* (Dc.ex.Nees)), citronella grass (*Cymbopogon nardus* Rendle.) and black pepper (*Piper nigrum* Linn.) against mushroom mite, *Dolichocybe indica* Mahunka was investigated by using the fumigation method. The essential oil treatments were applied in knockdown chambers sized 25 L at the concentrations of 0 (5% Tween-20 in water), 0.003, 0.006, 0.03, 0.06, 0.3, 0.6 and 3.0 µg/L air, for 1 hr. The mite mortality was observed at 6 hr after each treatment. The results presented that the lemon grass essential oil at 3 µg/L air was highly toxic to the mushroom mite with 100% mortality, and the LC_{50} was obtained at 0.179 µg/ L air. In addition, mortality of the mite treated by essential oils of citronella grass and black pepper at 3 µg/L air were at 82.5 and 78.8% with the LC_{50} at 0.842 and 1.163 µg/L air, respectively.

Keywords: fumigation, acaricidal, knockdown chamber

Introduction

The mushroom mite (*Dolichocybe indica* Mahunka) is generally considered as the major pest causing extensive yield losses in mushroom production, particularly in Tree Ear (*Auricularia auricular* Judae), Split Gill (*Schizophylum commune* Fr), Yanagi Matsutake (*Agrocybe cylindracea* (Dc.Ex.Fr.) Maire.) and Golden Needle (*Flammulina velutipes* Karst.) (Grudloyma *et al.*, 2010). Unfortunately, the management of mite infestation has generally been limited to the application of chemical substances, and are usually restricted to problems of application methods limited, timing and residue. Consequently, plant essential oils and crude extracts have recently been considered a potential alternative acaricide and have become a rich source of

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pesticide studies. For example, essential oils of *Mentha longifolia* (L.) Huds., *Salvia officinalis* L. and *Myrtus communis* L. against *Tetranychus urticae* Koch. were recently studied and the results were considerably resourceful (Motazedian *et al.*, 2012). Moreover, extracts from clove and cinnamon were also found highly toxic against a mushroom mite (*Luciaphorus perniciosus* Rack) (Pumnuan *et al.*, 2008). In addition, acaricidal activity of some wild plants in Thailand (*Justicia adhatoda* L. and *Litsea cubeba* (Lour.) Pers.) were also observed presenting high potency in controlling *L. perniciosus* and *Formicomotes heteromorphus* Magowski (Insung *et al.*, 2008). Pumnuan *et al.* (2010) reported the toxicity of *L. cubeba* essential oil against *L. perniciosus* by contact and fumigation methods. Besides, Rongpol *et al.* (2009) reported the use of lemon grass, citronella grass and black pepper essential oils in controlling *L. perniciosus*. Furthermore, lemon grass and citronella grass essential oils were also reported as having inhibition effect on the pregnant female *L. perniciosus* (Pumnuan *et al.*, 2009). More importantly, although plant essential oils normally present some side effects against microorganism (Cheng *et al.*, 2008; Matan *et al.*, 2008; Velluti *et al.*, 2003), these essential oils were found having minimal toxicity on mushroom (*Lentinus polychrous* Le'v and *Pleurotus ostreatus* (Jacq. Fr.) Kummer) (Youngnit *et al.*, 2010).

This study investigated the acaricidal activities of lemon grass, citronella grass and black pepper essential oils in controlling an economic mushroom mite, *D. indica* under laboratory condition by fumigation method.

Materials and methods

Stock culture of mushroom mite

Colonies of *D. indica* were obtained from a mushroom farm in Phetchaburi province, Thailand. The mites were reared on sorghum grain infested with mycelia of *Schizophylum commune* Fr. under the temperature of 25±2°C.

Extraction of essential oils

The essential oils were obtained by water-distillation using a Clevenger-type apparatus for 6 hr from leaves of lemon grass (*Cymbopogon citratus* (Dc.ex.Nees)), leaves of citronella grass (*Cymbopogon nardus* Rendle.) and seeds of black pepper (*Piper nigrum* Linn.). The extracted oils were collected and dehydrated over anhydrous sodium sulphate and stored in amber-colored vials at 10-12 ℃ for further experiments.

Fumigation bioassay

This study applied the fumigation bioassay method modified from Pumnuan *et al.* (2010). Samples of 20 non-physogastric *D. indica* mites were transferred to the mite cage made of acrylic sheets (3x5x0.45 cm) perforated into frustum of cone. The base was covered with red filter paper and the top was covered with a cover glass. Then, the mite cages were placed into the 25 L knockdown chambers (Burkard Co., England). Prior to the treatments, 5% of Tween-20 was used as the essential oil emulsifier in distilled water. Then, essential oils at concentrations of 0 (5% Tween-20 in distilled water), 0.005, 0.01, 0.05, 0.1, 0.5, 1.0 and 5.0% with volume of 1.5 ml (or 0.003, 0.06, 0.03, 0.06, 0.3, 0.6 and 3.0 µg/ L air, respectively) were sprayed into the chamber. The mite cages were left in the chambers for 1 hr after each treatment and mortality of the mushroom mites were observed 6 hr thereafter. In particular, the treated mites were considered dead when no appendage moves were observed as probed with a small hair brush.

Statistical analysis

The experiment was a completely randomized design with five replicates. The actual death rates were calculated using Abbot's formula (Abbott, 1987). The data were processed using the logarithmic transformation before applying analysis of variance (ANOVA) and Duncan's multiple range tests (DMRT) in SAS. Then, the statistical outputs were presented. The median little concentration (LC_{50}) was calculated by the probit analysis in SPSS.

Results and Discussions

Fumigant toxicity of lemon grass, citronella grass and black pepper essential oils against *D. indica* are presented in Table 1. In general, lemon grass essential oil presented significantly higher mortality of *D. indica*, particularly at the concentrations of 0.6 and 3.0 µg/L air (86.1 and 100.0%, respectively), when comparing to the other treatments. The non-significant different mortality percentages were observed in the citronella grass and black pepper essential oils treatment groups at the same concentrations (68.0 and 82.5%, and 59.7 and 78.8% mortality, respectively). The results found in this study were generally in congruence with Veeraphant *et al.* (2011) in which lemon grass essential oil was reported presenting 100% mortality of *D. pteronyssinus* at 0.1% concentration by residue contact method. In addition, Pumnuan *et al.* (2009) reported higher inhibition rate of hatching in *L. perniciosus* treated by lemon grass essential oil than in the citronella essential oil treatment group, with the

EC_{50} at $1.81x10^4$ and $1.97x10^4$ µg/L air, respectively. Jaenson *et al.* (2006) reported repellent activity of lemon grass essential oil against *Ixodes ricinus* (Acari: Ixodidae). Besides, results of the LC_{50} examination also showed significantly higher toxicity of lemon grass essential oil against the mite when comparing to the citronella grass and black pepper essential oils (the LC_{50} at 0.179, 0.842 and 1.163 µg/L air, respectively) (Table 2). There were some previous reports about the effect of these essential oils fumigation on mushroom mites, *F. heteromorphus* (Pumnuan *et al.*, 2009), and *L. perniciosus* (Rongpol *et al.*, 2009) which presented the LC_{50} of 11-89 µg/L air and 20-135 µg/L air, respectively. Interestingly, when comparing the LC_{50} of these essential oils against *D. indica*, *F. heteromorphus* and *L. perniciosus*, it can be seen that LC_{50} observed from *D. indica* was considerably lower. This indicates that effective essential oil fumigation treatments against *F. heteromorphus* and *L. perniciosus* can also be potentially applicable in the management of *D. indica*.

Table 1. Percentage of mortality of mushroom mite (*Dolichocybe indica* Mahunka) after treated with essential oils from lemon grass, citronella grass and black pepper by fumigation method at 6 hr.

Essential oils of Plants	Percentage of mortality [1, 2/] (Average ±SD)							
	Concentration (µg/L air)							
	0	0.003	0.006	0.03	0.06	0.3	0.6	3
Lemon grass	0.0 ±6.4	19.3 ±7.7^{a}	26.9 ±7.7^{a}	47.3 ±9.7^{a}	58.6 ±4.8^{a}	72.1 ±1.9^{a}	86.1 ±5.9^{a}	100.0 ±0.0^{a}
Citronella grass	0.0 ±6.4	13.9 ±3.9^{a}	19.0 ±4.5^{ab}	43.2 ±7.5^{a}	48.7 ±6.9^{b}	59.7 ±6.2^{b}	68.0 ±7.3^{b}	82.5 ±5.8^{b}
Black pepper	0.0 ±6.4	11.3 ±7.7^{a}	16.3 ±4.8^{b}	32.2 ±2.9^{b}	38.3 ±3.9^{c}	50.6 ±2.5^{c}	59.7 ±4.9^{c}	78.8 ±8.8^{b}

[1/]Means in column followed by the same common letter were not significantly different ($P<0.05$) according to DMRT.
[2/]The data were processed using the logarithmic transformation before the statistical analysis. Then, the statistical outputs were presented.

Table 2. The median lethal concentration (LC_{50}) of mushroom mite (*Dolichocybe indica* Mahunka) after treated with essential oils from lemon grass, citronella grass and black pepper by fumigation method at 6 hr.

Essential oils of Plants	LC_{50} (µg/ L air)	Slope	SE
Lemon grass	0.179	3.245	0.28
Citronella grass	0.842	0.558	0.056
Black pepper	1.163	0.556	0.054

It was found in the current study that the mortality percentage obtained from the citronella grass essential oil treatment group was higher than in the group treated by black pepper essential oil. Similarly, Mägi *et al.* (2006) reported a higher percentage (92.2%) of mortality in swine sarcoptic mange mites treated by citronella grass essential oil in 4 weeks after treatments when

comparing to the group treated by black pepper essential oil (88.4%), when using in 1% emulsion. Although the results in this study showed lower mortality percentages in *D. indica* treated by the black pepper essential oil, the percentage was considerably high when comparing to the control. Pumnuan and Insung (2012) reported high performance of black pepper essential oil against *L. perniciosus* on day 7 after fumigation at 10^4 µg/L air.

In addition, the results in this study also showed that essential oils of plants in the same genus (*Cymbopogon*) namely *C. citratus* (lemon grass) and *C. nardus* (citronella grass) could possibly yield in significant different mortal potentiality. In general, the similar finding was also found in many studies For example, Boonyuan *et al.* (2014) reported significantly higher toxicity of lemon grass essential oil against *Aedes aegypti* population when comparing to citronella grass essential oil.

In Table 2, nearly ten times differences in the mortalities of *D. indica* treated by lemon grass and black pepper essential oils were observed. This difference could possibly be a result of different secondary metabolite contents of the essential oil samples (Yanar *et al.*, 2011). Among the tested essential oils, lemon grass essential oil had the steeper slope, while black pepper essential oil had shallower slope. These indicated that the mite did not homogenously respond to the tested essential oils. Similarly, the same variations were observed with plant extracts tested on insects (Gökçe *et al.*, 2006).

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