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Original Article

Effects of insecticidal essential oil fumigations on physiological changes in cut *Dendrobium* Sonia orchid flower

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Abstract

This study investigated essential oil (EO) formulas with high insecticidal properties, but low physiological impacts on cut *Dendrobium* Sonia orchid flower. Fumigation toxicities of EOs from 18 medicinal plants at 2.0 and 3.0 µl/L air were examined against adults of thrips (*Frankliniella schultzei*) and larvae of mealybug (*Pseudococcus jackbeardsleyi*). The effective EO mixtures, optimal concentrations fumigation and air circulation periods were investigated. Then, field experiments were conducted, and changes in L*, a* and b* values, percentages of weight loss and anthocyanin contents of the EO-fumigated flower were observed and compared to the methyl bromide and control fumigations. The results showed that clove and cinnamon demonstrated high insecticidal properties against the insects (>85% mortality) and low physiological changes in the flower. In particular, fumigations with 2.0 µl/L air of a mixture between clove and cinnamon EOs (1:3) for 3 hr with 15-min air circulation demonstrated the highest thrips and mealybug mortalities (92.2 and 74.6%, respectively). The EO fumigation formula presented less impact on color change and anthocyanin content than methyl bromide fumigation which showed higher reduction of anthocyanin content (22.9 mg/100g FW) when compared to the control (13.6 mg/100g FW). The percentages of weight loss in the flower fumigated with EO, control and methyl bromide were about 10.4, 7.9 and 14.8%, respectively. In general, applications of EO at higher concentrations resulted in higher insect mortalities and more impacts on physiological changes which involved anthocyanin degradation and higher percentages of weight loss. Further studies might consider applications of clove and cinnamon EO formulas via other methods. In addition, revisions of the EO mixture can also be examined in order to obtain the most effective and environment friendly insect management approach.

Keywords: fumigation, methyl bromide, phytotoxicity, insecticidal

1. Introduction

Cut orchid flower is considered as one of the most important ornamental plants in Thailand. (Office of Agricultural Economic, 2013). Particularly, *Dendrobium* Sonia is a popular hybrid with its fast growing, high productivity, bright red–purple color with shades of whites in the center and longer vase life (Piluek and Wongpiyasatid, 2010). However, the problems of product impurity and insect contamination reducing the productivity have frequently been reported. In particular, thrips and mealybug are among the most frequently detected insect pests (Piluek and Wongpiyasatid, 2010; Williams, 2004). Conventionally, the insect management relies mainly on the applications of methyl bromide and phosphine. Unfortunately, many studies have reported the adverse effect of methyl bromide on ozone layer depletion (MBTOC, 2010), while phosphine has been reported as being resisted by insects and causing physiological impacts on many produces (Athie and Mills, 2005; Liu, 2011; Pimentel *et al.*, 2008). Therefore, modern insect pest management study has been geared toward the development of high potential bio-insecticides. Particularly, plant essential oils (EOs) have been extensively studied and proposed as being another high potential option (Ayvaz *et al.*, 2008;

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Benelli *et al.*, 2012; Pumnuan and Insung, 2012). For example, extracts of *Momordica charantia*, *Melia azadarach* and *Azadirachta indica* were reported to be effective in controlling thrips population (Fiaz *et al.,* 2012). In addition, oil extracts from eucalyptus, cotton seed, cinnamon, rosemary, soybean, rosemary and lavender were reported as being toxic to nymphs and adults of mealybug (Cloyd *et al.*, 2009; Govindaiah *et al.*, 2006).

In general, EOs are applied in insect pest management by various methods, such as direct spray, residue contact, and fumigation (Amiri *et al.*, 2013; Ateyyat *et al.*, 2009; Choi *et al.*, 2003; Iþýk and Görür, 2009; Koul *et al.*, 2008; Muzemu *et al.*, 2011; Pinheiro *et al.*, 2013). In particular, fumigation method has been considered one of the most effective approaches for closed system application. Many reports presented the effectiveness of plant EO fumigations against stored product pests (Chu *et al.*, 2013; Isman, 2000; Mahfuz and Khalequzzaman, 2007; Pumnuan *et al.*, 2012; Yi *et al.*, 2006). However, the application of EOs in insect pest management can also have side effects on the treated produces. Despite their potential insecticidal properties, botanical EO applications can also cause physiological changes on the treated plants (De Almeida *et al.*, 2010; Gao *et al.*, 2014). In general, the effects of plant EOs on agricultural products can be observed in various damages such as degermination, defective seed growth and defective radical elongation (De Almeida *et al.*, 2010). For example, Kobaisy *et al.* (2001) reported 80-100% growth reduction on lettuce seed treated with kenaf EO at 0.3 mg/ml. Additionally, Pumnuan *et al.* (2005) reported burning symptom in kale leaves contacted with hexanolic extract of *Gloriosa superba*, *Acacia catechu* and *Archidendron jiringa*. In cut flowers, changes in color, percentage of weight loss, vase life, anthocyanin contents, and browning symptom were normally observed (Almasi *et al.*, 2012; Dahal, 2013; Sapers, 1993).

In general, plant EOs are recently considered a prospective source of potential and environmental friendly insecticides. Nonetheless, drawbacks of inaccurate applications were also reported. Thus, this study presented effects of insecticidal EO fumigations on physiological changes in cut *Dendrobium* Sonia orchid flower.

2. Materials and Methods

This study investigated ascendant insecticidal EO fumigation formulas against insect pests of cut *Dendrobium* Sonia orchid flower. In addition, physiological changes were also examined after the fumigations.

2.1 Samples preparations

2.1.1 Insects preparation

The insects used in this study were adults of thrips (*Frankliniella schultzei* (Trybom) and larvae of mealybug (*Pseudococcus jackbeardsleyi* Gimpel & Miller). The thrips were collected from natural sources in Bangkok province, Thailand, and cultured on lotus in insectary. Besides, the mealybug was collected from natural sources in Chachengsao province, Thailand, and cultured on pumpkin fruits in laboratory. The insects were cultured in insecticide free at the Department of Plant Production Technology, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang (KMITL), Bangkok, Thailand, until used. Prior to the fumigations, samples of 10-15 adults of thrips were transferred to a petal of uninfected lotus placed in a plastic box (5×7×3 cm) with a netted cap. In addition, samples of 10-15 larvae of mealybug were transferred into an insect cage made of an acrylic sheet (3×5×0.5 cm) perforated into frustum of cone with 0.25 cm-diameter base covered with a filter paper (Whatman® No.1), and 0.5 cm-diameter top covered with a cover glass (2×2 cm).

2.1.2 Cut orchid flower preparation

In this study, cut *Dendrobium* Sonia orchid flower was collected from an orchid farm in Nakorn Pathom province, Thailand. The selected orchid flowers inflorescences were particularly with 18 cm long stalks (from the cut to the first lower flower) and with 4 blooming and 4 budding flowers. The inflorescences were weighed for initial fresh weights. Subsequently, the cut ends of the inflorescences were covered with soaked cotton in plastic tubes filled with 8 ml of 5% glucose. Totally, 9 inflorescences of the orchid were prepared for each of the 3-replicated fumigation. Then, the inflorescences were stored at 25±1°C until used.

2.1.3 EOs preparation

The EOs in this study were extracted from eighteen species of medicinal plants belonging to eight different families (Table 1). EOs of the plants were extracted by using water-distillation method with a Clevenger-type apparatus, for the period of 6 hr. Subsequently, the extracted oils were collected and dehydrated over anhydrous sodium sulfate and stored in amber-colored vials at 10°C until used.

2.2 Examination of ascendant insecticidal EO formulas with low physiological impacts on cut orchid flower

In this experiment, insecticidal properties of EOs from the different medicinal plants and mixtures of the EOs were examined. In addition, physiological changes on the treated flower were examined and applied as measures for the EOs and formula selections.

2.2.1 Examination of ascendant insecticidal EOs

This experiment examined fumigation toxicity of different EOs against adults of thrips and larvae of mealybug. The fumigations were conducted in a 25 L glass cylinder knockdown fumigation chamber (Burkard Co., England).

Initially, the insect samples were simultaneously placed into the chamber. EOs at concentrations of 0 (95% ethanol), 3.3 and 5.0% with volume of 1.5 ml (or 0, 2 and 3 µl/L air, respectively) were sprayed into the chamber. The insects were left in the chamber for 1 hr after the treatment, mortalities were observed at 24 hr thereafter. The insects were considered lifeless when no appendage motions were observed as probed with a small brush. In particular, the actual death rates were calculated via Abbot's formula (Abbott, 1987). The experiment was conducted in a completely 3-randomized replication design. Then, the EOs which caused more than 80% mortality were selected for further experiments.

2.2.2 Examination of EO formulas with low physiological impacts on cut orchid flower

In this experiment, the results from 2.2.1 were applied. In particular, physiological changes of cut orchid flower caused by different high insecticidal EO mixture fumigations were examined. The experiment was divided into 3 consistent assays. All fumigations were conducted in 1 cubic meter glass chamber equipped with 7 inch-diameter air circulator (25 watt). In each of the fumigations, a total of 9 inflorescences were placed at the chamber center and injected with each EO mixture by 10 pound per square inch (GAST® Model 1031-102A) atomizer air pump at 1 ml/7 sec. In addition, air circulation for 1 hr with no EO injections was set up as control treatment. Then, changes in L*, a* and b* values and percentages of weight loss of the treated flower were examined every 3-day interval until the complete senescence was observed. EO fumigation formulas which caused no significant physiological changes on the flower when compared to the controls were applied in the next assays. Firstly, physiological changes of the orchid flower against mixtures of the selected ascendant insecticidal EOs from 2.2.1 were examined. Secondly, physiological changes of the orchid flower against various fumigation periods (1, 2, 3 and 4 hr) with the selected EO formulas were examined. Thirdly, physiological changes of the orchid flower against different air circulation periods (all-time and 15-min) were examined. In addition, air circulation for 15 min and 1 hr with no EO injections were set up as the control treatments. In this assay, anthocyanin contents of the fumigated flower and the control were also examined.

2.3 Reverse experiment

In this experiment, insecticidal properties of the EO fumigation formulas against the insects were re-examined. The fumigations were conducted in DOA (Department of Agriculture) fumigation chamber using the same observation procedures as in 2.2.1. In addition, fumigation with methyl bromide at 28 mg/m^3 (recommended rate) was comparably conducted. The insect mortalities, changes in L*, a* and b* values, percentage of weight loss and anthocyanin contents were examined and compared.

2.4 Tests of physiological change on cut orchid flower

2.4.1 Percentage of weight loss

Percentages of weight loss of the treated orchid cut flowers at each particular observation periods were calculated as following; %Weight loss = [(initial weight – observed weight) / initial weight]*100.

2.4.2 Color changes

Color changes in the treated orchid cut flower were measured using CIE L* a* b* color space system by Color Flex spectrophotometer. Totally, 3 opened orchid cut flowers from each treatment were with 3 petal each were measured for L*, a* and b* values. In general, L* value indicates lightness ranged from 0 for the levels of darkness to 100 for the levels of lightness. The a* value indicates shades of red-green, colors ranged from positive value (+a*) for redness to negative value (-a*) for the levels of greenness. Besides, b* value indicates shades of yellow-blue color ranged from positive value (+b*) for the levels of yellowness to negative value (-b*) for the levels of blueness.

2.4.3 Anthocyanin content

Anthocyanin content in the petal of treated orchid cut flowers was measured with spectrophotometer, using the methods proposed by Ragana (1997). In this test, 0.5 g of small pieces flower petals were mixed with 10 ml of 85% ethanolic HCl (95% ethanol : 1.5 N HCl = 85:15), then homogenized and kept at 4°C for 24 hr. The extractant was then filtered with filter paper. Subsequently, 300 µl of the obtained extract was mixed with 3 ml of 85% ethanolic HCl and measured for the absorbance at wavelength 535 nm. Next, the anthocyanin content was calculated as following; Total anthocyanin = Total A / 98.2; Total A = (A_{535} * final volume (ml) * 100) / weight (g); A_{535} = absorbent at 535 nm]. The data were reported in units of mg/100 g fresh weight.

2.5 Statistical analysis

The experiments in this study were completely randomized design (CRD) with 3 replications. The data were statistically analyzed by analysis of variance (ANOVA) and mean comparison by Duncan's multiple range test (DMRT).

3. Results

3.1 Examination of ascendant insecticidal EO formulas with low physiological impacts on cut orchid flower

3.1.1 Examination of ascendant insecticidal EOs

Overall, from the 18 selected medicinal plants, 2 EOs including clove (*Syzygium aromaticum*) and cinnamon

(*Cinnamomum bejolghota*) demonstrated remarkably high mortalities in both insects. The EO fumigations at 2.0 µl/L air resulted in more than 82% mortalities, while 3.0 µl/L air fumigations resulted in more than 86% mortalities. In particular, fumigations with clove and cinnamon EOs at 3.0 µl/L air resulted in more than 86% mortalities in mealybug and more than 95% mortalities in thrips (Table 1).

3.1.2 Examination of EO formulas with low physiological impacts on cut orchid flower

Firstly, physiological changes (L*, a*, b* and percentages of weight loss) of the cut orchid flowers fumigated with different mixtures between different ratios of clove and cinnamon EOs (4:0, 3:1, 2:2, 1:3 and 0:4 represented by Cl4Ci0, Cl3Ci1, Cl2Ci2, Cl1Ci3 and Cl0Ci4, respectively) at 3.0 µl/L air were examined (Table 2). On day 3 after fumigation, L* and b* values from all formulas presented no significant differences when compared to the control. No significant differences in a* value were observed from formulas Cl4Ci0 (36.9) and Cl1Ci3 (38.0) when compared to the control (39.0). In addition, formula Cl4Ci0 showed no significant differences in percentage of weight loss (6.4%) when compared to the control (5.4%). In general, the flower fumigated with Cl4Ci0 and Cl1Ci3 stayed undamaged to day 6 after fumigation. Particularly, L* values from Cl4Ci0 (24.7) and Cl1Ci3 (23.1) were not significantly different from that of the control treatment (22.5). In addition, despite the significant differences in a*, b* and percentages of weight loss when compared to the control, the values from Cl4Ci0 and Cl1Ci3

Table 1. Mortality percentages (Means) of the adults of thrips, *Frankliniella schultzei* and larvae of mealybug, *Pseudococcus jackbeardsleyi* at 24 h after fumigations with plant essential oils at the concentrations of 2 and 3 ml/L air.

Family / Scientific name	Common name	Plant part	%Mortality			
			2 ml/L air		3 ml/L air	
			Thrips	Mealybug	Thrips	Mealybug
MYRTACEAE						
1. *Syzygium aromaticum*	Clove	Dried bud	83.4	82.7	95.0	87.3
2. *Eucalyptus globulus*	Blue gum	Fresh leaf	<50	58.2	54.9	71.8
LAURACEAE						
3. *Cinnamomum bejolghota*	Cinnamon	Dried bark	84.1	82.7	95.6	86.4
PIPERACEAE						
4. *Piper nigrum*	Black pepper	Dried seed	79.3	<50	88.6	53.8
5. *Piper betle*	Betel vine	Fresh leaf	<50	<50	66.4	60.6
ZINGIBERACEAE						
6. *Zingiber cassumunar*	Cassumunar ginger	Fresh rhizome	81.4	<50	88.0	<50
7. *Curcuma longa*	Turmeric	Fresh rhizome	<50	<50	<50	<50
8. *Alpinia nigra*	Galanga	Fresh rhizome	<50	<50	63.8	50.3
9. *Zingiber officinale*	Ginger	Fresh rhizome	<50	<50	57.1	<50
10. *Amomum krervanh*	Cardamom	Dried seed	78.5	63.3	84.7	70.9
GRAMINEAE						
11. *Cymbopogon nardus*	Citronella grass	Fresh leaf	60.7	77.5	72.9	81.9
12. *Cymbopogon citratus*	Lemon grass	Fresh leaf	72.7	70.7	85.2	81.5
RUTACEAE						
13. *Citrus aurantifolia*	Lemon	Fresh peel	<50	<50	56.6	<50
14. *Citrus maxima*	Pummelo	Fresh peel	<50	<50	55.3	<50
15. *Citrus reticulate*	Tangerine	Fresh peel	<50	<50	<50	<50
16. *Citrus hystrix*	Kaffir lime	Fresh leaf	<50	<50	60.0	50.8
LABIATE						
17. *Ocimum basilicum*	Sweet basil	Fresh leaf	<50	<50	65.3	53.0
COMPOSITAE						
18. *Eupatorium odoratum*	Bitter bush	Fresh leaf	<50	<50	<50	<50
Control (95% ethanol)			0	0	0	0

Table 2. The L*, a* and b* values and percentages of weight loss of cut orchid flowers fumigated with essential oil formulas at 3.0 µl/L air on days 3 and 6 after fumigations.

Formulas[2]	Means[1]							
	3 days				6 days			
	L* value	a* value	b* value	%Weight Loss	L* value	a* value	b* value	%Weight Loss
Control	21.5 a	39.0 a	-13.3 a	5.4 d	22.5 c	36.1 a	-13.7c	8.3 a
Cl4Ci0	23.3 a	36.9 abc	-11.8 a	6.4 cd	24.7 abc	31.3 b	-10.5 b	16.6 b
Cl0Ci4	25.1 a	36.0 bc	-14.2 a	8.7 b	26.4 a	30.0 b	-7..8 a	19.5 b
Cl1Ci3	22.6 a	38.0 ab	-12.4 a	6.8 c	23.1 bc	30.9 b	-10.6 b	17.5 b
Cl2Ci2	24.6 a	35.1 c	-12.1 a	10.4 a	26.3 a	29.7 b	-8.6 ab	20.6 b
Cl3Ci1	24.9 a	35.5 c	-12.7 a	9.3 ab	25.5 ab	28.9 b	-7.7 a	19.9 b
%CV	7.9	3.8	-9.4	8.9	5.6	6.5	-13.8	17.0

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

[2]Clove : cinnamon ratios at 4:0, 3:1, 2:2, 1:3 and 0:4 formulas represented by Cl4Ci0, Cl3Ci1, Cl2Ci2, Cl1Ci3 and Cl0Ci4, respectively.

showed the least changes among all formulas. Therefore, Cl4Ci0 and Cl1Ci3 were selected for the next experiment, with a reduced concentration from 3.0 to 2.0 µl/L air in order to lessen physiological changes on the flower.

Secondly, physiological changes of the orchid flower against 2.0 µl/L air of Cl4Ci0 and Cl1Ci3 at various fumigation periods (1-4 hr) were examined. In general, 1-4 hr Cl4Ci0 fumigations resulted in completely senescence after day 6 (Figure 1). Besides, 4 hr Cl1Ci3 fumigation resulted in completely senescence after day 9. Nonetheless, 1-3 hr Cl1Ci3 fumigations showed no severe senescence until day 12. The fumigated flower showed higher L* values (30.1-30.7), Lower a* values (28.9-32.4) and lower b* values between -11.5 and -2.5 than the control (22.6, 38.7 and -2.5, respectively). Besides, the percentages of weight loss in all fumigations ranged from 25.3 to 32.8%, or about 18% higher than the control (14%) (Figure 1D). In general, no significant differences in the changes of L*, a* and percentage of weight loss were observed among 1-3 hr fumigations with Cl1Ci3. Moreover, the results in 3.1.1 showed that 1 hr fumigations with 2.0 µl/L air resulted in less than 85% insect mortalities. Therefore, only 2-3 hr fumigations with 2.0 µl/L air Cl1Ci3 were selected for the next experiment.

Thirdly, physiological changes of the orchid flower against 2-3 hr fumigations of 2.0 µl/L air Cl1Ci3 with different air circulation periods (all-time and 15-min) were examined (Figure 2). In general, the results showed that, L*, a* and b* values, anthocyanin content and percentages of weight loss from 15-min air-circulated fumigation presented no significantly different physiological changes on the treated flowers throughout the 12-day observations when compared to the control (22.6 to 24.1, 38.6 to 42.0, -18.7 to -14.7, 19.0 to 20.9 and 11.0 to 14.7, respectively). On the other hand, all-time air-circulated fumigation resulted in higher physiological changes. Thus, 2-3 hr fumigations of EO formula Cl1Ci3 at

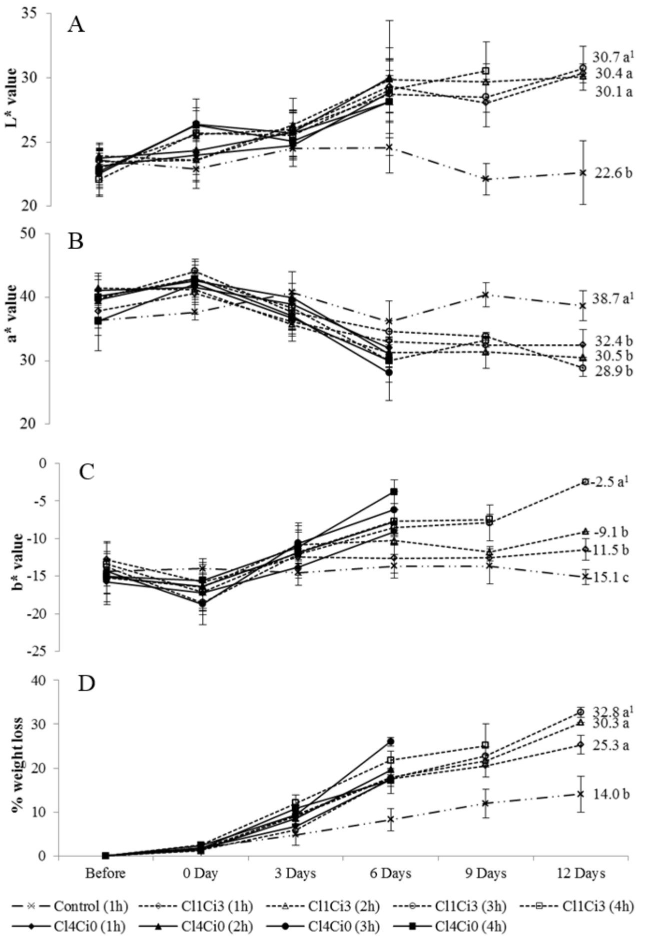

Figure 1. The L*, a* and b* values (A-C) and percentages of weight loss (D) of cut orchid flowers fumigated with different essential oil formulas at 2.0 µl/L air during the 12-day examination ([1]Means in the same common letter were not significantly different (P<0.05) according to DMRT).

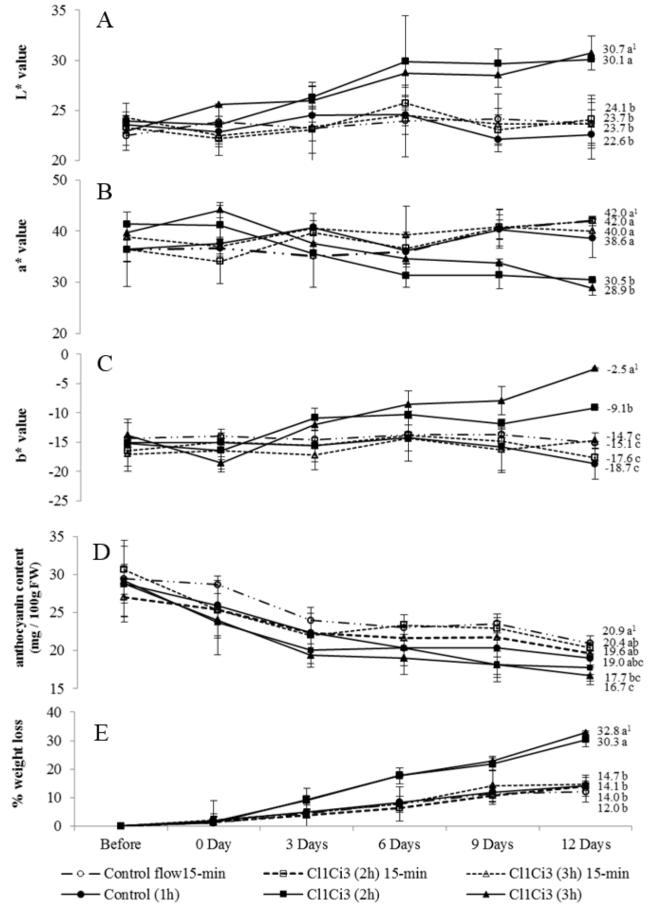

Figure 2. The L*, a* and b* values (A-C), anthocyanin content (D) and percentages of weight loss (E) of cut orchid flowers fumigated with 2.0 µl/L air Cl1Ci3 incorporated all-time and 15-min air circulation during the 12-day examination ([1]Means in the same common letter were not significantly different (P<0.05) according to DMRT).

2.0 µl/L air with 15-min air circulation were selected for the reverse experiment.

3.2 Reverse experiment

In general, no significant differences in mealybug mortalities were observed when compared to methyl bromide and 3-hr Cl1Ci3 fumigations (100 and 92.2%, respectively). In thrips, the EO fumigations at 2 and 3 hr and methyl bromide fumigation resulted in 66.1, 74.6 and 100% mortalities, respectively (Table 3). Despite its high performances, methyl bromide fumigation caused observable physiological changes on the flower immediately after the fumigation and then a complete senescence before day 3 observation. The flower fumigated with methyl bromide showed remarkably higher L*, b* and a* values when compared to the control. On the contrary, the flower fumigated with the Cl1Ci3 formula showed no differences in physiological changes when compared to the control throughout the 12-day examination. Besides, total anthocyanin content from the 2-hr fumigation with the Cl1Ci3 formula (20.0 mg/100 g fresh) were not significantly different when compared to the 3-hr fumigation (17.8 mg/100 g fresh) and the control (22.9 mg/100 g fresh) (Table 3).

4. Discussion

4.1 Examination of ascendant insecticidal EO formulas with low physiological impacts on cut orchid flower

4.1.1 Examination of ascendant insecticidal EOs

In general, from 18 medicinal plants in this study, clove and cinnamon presented the highest mortalities against thrips (*F. schultzei*) and mealybug (*P. jackbeardsleyi*). The results in Table 1 indicate that higher concentrations result in higher mortalities. In general, clove and cinnamon EOs

Table 3. The L*, a* and b* values, percentages of weight loss and anthocyanin contents of cut orchid flowers against 2-3 hr fumigations of 2 µl/L air Cl1Ci3 compared to methyl bromide (28 g/m^3) 15-min air circulation on day 3 after fumigations, and mortality percentages of the adults of thrips, *Frankliniella schultzei* and larvae of mealybug, *Pseudococcus jackbeardsleyi* at 24 h after fumigations.

Treatments	Means[1]						
	Physiological changes					%Mortality	
	L* value	a* value	b* value	% weight loss	anthocyanin content (mg/ 100g FW)	Thrips	Mealybug
Control	23.3 b	45.2 a	-15.5 c	7.9 b	22.9 a	0.0 c	0.0 c
Cl1Ci3[2] (2 hr fume)	21.1 b	42.8 ab	-15.4 c	8.5 b	20.2 ab	66.1 b	75.5 b
Cl1Ci3 (3 hr fume)	22.3 b	39.7 bc	-12.3 b	10.4 b	17.8 b	74.6 b	92.2 ab
Methyl bromide (28 g/m^3)	27.5 a	35.2 c	-9.0 a	14.8 a	13.6 c	100.0 a	100.0 a
%CV	9.5	6.6	12.2	13.6	10.4	17.1	14.3

[1]Means in the same column followed by the same common letter were not significantly different (P<0.05) according to DMRT.
[2]Clove : cinnamon ratio at 1:3 formulas represented by Cl1Ci3

have been found effective in controlling many insects and mites (Ahmed, 2010; Isman, 2000; Paranagama *et al.*, 2003; Papachristos and Stamopoulos, 2002; Prakash and Rao, 1997; Pumnuan and Insung, 2012, Pumnuan *et al.*, 2012; Toloza *et al.*, 2008).

4.1.2 Examination of EO formulas with low physiological impacts on cut orchid flower

In Table 2, no significant changes in the color (lightness, redness and blueness) in Cl4Ci0, Cl1Ci3 and the control are observed on day 3 examinations when compared to the control. Besides, no significant weight loss difference is observed in Cl4Ci0, when compared to the control. In general, fumigations with plant EOs can increase loss of weight in plants. For example, Batish *et al.* (2006) and Kohli *et al.* (1998) reported that fumigations with eucalyptus EO resulted in losses of weight and water content in weed plants and increasing exposure period resulted in higher effects. However, the present study indicates that fumigation with particular EO formulas might present no weight changes effect on plants. In general, the flower fumigated with Cl4Ci0 and Cl1Ci3 stayed undamaged to day 6 after fumigation. However, the color lightness becomes higher, not significantly different when compared to the control. On the other hand, significantly different levels of redness, blueness and weight decreases are observed. It can be seen here that different ratios of even the same EOs can result in different effects. Miresmailli *et al.* (2006), Tripathi *et al.* (2009) and Kim *et al.* (2012) found that different combinations of selected constituents could potentially result in different synergistic effects.

Secondly, the results indicate a positive relationship between fumigation periods and degrees of discoloration (Figure 1A-1C). The longer fumigation periods result in lighter color and higher water loss. The changes in L*, a* and b* values indicate discoloration in the flower, particularly reduces in redness and blueness from the deep red-purple pre-fumigation color to paler and yellowish color. Marshall *et al.* (2010) mentioned that flowers normally faded during senescence, and the degree of this physiological change was normally dependent on factors such as temperature, light, nutrients, water availability and other surrounding conditions. In this study, Cl1Ci3 demonstrates changes in less color and water content than Cl4Ci0. Particularly, 1-3 hr Cl1Ci3 fumigations demonstrate minimum physical changes, with no significant differences in the lightness, redness and water content among the different fumigation periods

Thirdly, the findings prove that air circulation period was another factor influencing physiological changes of the fumigated flower. Particularly, changes in the percentages of weight loss are highly noticeable. Figure 2D demonstrates a significant difference in percentages of weight loss between the fumigations with all-time and 15 min air circulation, while the same air circulation periods show non-significantly different results even between different fumigation periods. Van Meeteren and Van Gelder (1999) reported that loss of plant weight after harvest usually caused by the rate of transpiration and the in-vase rehydration capacity. However, the results show that 15 min air circulation in Cl1Ci3 fumigations (both 2 and 3 hr) demonstrate no significant differences when compared to the control.

4.2 Reverse experiment

In general, the results indicate that although methyl bromide fumigation demonstrates high insect mortalities, the chemical causes serious physiological changes on the flower (Table 3). On the contrary, 2-3 hr fumigations of EO formula Cl1Ci3 at 2.0 µl/L air with 15-min air circulation show no significant physiological changes on flower despite considerably high insecticidal properties. In addition, the results demonstrate no significant differences in the changes of anthocyanin content of 2 hr Cl1Ci3 fumigations with 15 min air circulation when compared to the control, while methyl bromide fumigation shows significantly lower anthocyanin content. This finding indicates trivial impacts of the EO fumigation formula on the flower pigment deterioration. Many studies reported no impacts of EOs on plants pigments. On the contrary, applications of methyl bromide have been reported on serious damages on plants physiology.

5. Conclusions

From 18 plant EOs examined in this study, 2 and 3-hr fumigations of Cl1Ci3 at 2.0 µl/L air with 15-min air circulation show maximum insecticidal against the insects, while causing minimum impacts on physiological changes of the fumigated flower. In fact, complete mortalities of the insects would be preferable, and higher EO concentrations might yield higher mortalities. However, physiological effects on the fumigated plants are another crucial issue. Therefore, further studies might consider the application of the EO formula via other application methods. In addition, revisions of the EO mixture can also be examined in order to obtain the most effective and environment-friendly insect management approach.

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