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Insecticidal activity of essential oil formulas and their physiological effects on eggplant

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Abstract

This study examined fumigation toxicity of 18 medicinal plant essential oils (EOs) against adult of aphid (*Aphid gossypii*) and whitefly (*Bemisia tabaci*). Then, non-target effects of the EO mixtures on physiological changes of eggplant (*Solanum melongena*) were tested. The insecticidal property and physiological toxicity of the fumigation formulas were also examined and compared to methyl bromide (MB) fumigation. The results showed that the eggplant fumigated with clove (*Syzygium aromaticum*) and lemon grass (*Cymbopogon citratus*) EOs mixture at the ratio of 1:3 (Cl1Le3) showed no significant physiological changes when compared to the control treatment. The formula resulted in similarly high mortalities (82-100%) of both insects when compared to MB. However, MB fumigation caused complete senescence appeared before day 3 observations. On the contrary, the eggplant fumigated with Cl1Le3 at 3 µL/L air showed no differences in the physiological changes when compared to the control throughout the 9-day examinations.

Key words: Pytotoxicity, methyl bromide, fumigation, clove, lemon grass

Introduction

Vegetables are important export crops of Thailand (OAE, 2013a; 2013b). However, problems involving insecticide residues and insect contaminations have been threatening the credibility of the produces in many countries. Aphid, whitefly, thrips and mealybug are major insects found contaminating export vegetable, particularly eggplant which has been mentioned as a special contamination observation list (OCA, 2013).

In Thailand and other developing countries, insect pest management in agricultural industry relies largely on different applications of synthesized insecticides, such as methyl bromide and phosphine fumigations, which are among the most popular postharvest insect control management methods (Misumi *et al.,* 2009). In Thailand, chemical insecticide fumigation is widely accepted for its considerably high performances and time saving advantages. However, the applications of these insecticides have recently been questioned on their impacts on environment and fresh produce degradation. Particularly, methyl bromide is prohibited worldwide for its effects against ozone-depleting in the atmosphere (MBTOC, 2010). Similarly, phosphine has been reported for causing insect resistance and damages on produces and the issues are controversially discussed worldwide (Daglish, 2004). It is generally accepted that continuing use of a particular chemical pesticide results resistance and higher application volume (Limam and Jemaa, 2014). Current researches have, therefore, highlighted the development of high performance and environmental friendly insect control agents or "green pesticides".

Basically, green pesticides are plant derived insecticidal products such as plant extracts and essential oils. These products are considered environmental friendly as they are highly biodegradable and nontoxic to mammals, birds, and fish (Koul *et al.,* 2008; Misra *et al.*, 1996; Pavela *et al.*, 2013; Pavela, 2014; Stroh *et al.*, 1998). In addition, essential oils are highly economical in application and show considerable commercial significance when used as fumigant in storage containers (Solgi and Ghorbanpour, 2014). Therefore, many medicinal plant essential oils and their insecticidal organic compounds have been extensively studied in the recent years. In fact, essential oil compounds feature multiple pest control properties such as toxicity, repellency, feeding deterrence and oviposition deterrence (Koul *et al*., 2008; Pavelar *et al.*, 2009). Koschier and Sedy (2001) reported antifeedant effect of marjoram and rosemary essential oils against onion thrips (*Thrips tabaci*). Cloyd *et al.* (2009) reported that essential oils from cottonseed, cinnamon, rosemary, soybean and lavender caused more than 90% mortality of citrus mealybug (*Planococcus citri* (Risso)). Pumnuan *et al.* (2015) studied fumigation toxicities of essential oils from clove, cinnamon and lemon grass and reported that at 3.0 µL/L air, these essential oils caused more than 85% mortalities in thrips and mealybug. In addition, clove, cinnamon and lemon grass essential oils also showed high toxicity against many other insects and mites (Kheradmand *et al.,* 2015; Olianwuna and Umoru, 2010).

However, damages on produces treated with essential oil fumigation were also reported (De Almeida *et al.*, 2010; Gao *et al.*, 2014). Particularly, symptoms involving degermination, defective radical elongation (De Almeida *et al.*, 2010) and physical degradations (Cloyd *et al.,* 2009; Meyer *et al.*, 2008) are generally observed. Kobaisy *et al.* (2001) reported physical damages on lettuce and bentgrass treated with kenaf (*Hibiscus cannabinus*) essential oil. In addition, Cloyd *et al.* (2009) reported phytotoxic of plant essential oils on coleus (*Solenostemon scutellarioides*), transvaal daisy (*Gerbera jamesonii*) and poinsettia (*Euphorbia pulcherrima*) plants. Physiological damages which include changes in color, weight loss, fruit firmness and texture condense (Maqbool *et al.,* 2011) are generally dependent on application duration, plant species, temperature, and the types of applied essential oil (WSU, 2013). However, appropriate essential oils and application approaches can minimize phytotoxic effects on plants. Karamaouna *et al.* (2013) evaluated phytotoxicity severity of essential oils on grape leaves and reported low phytotoxic symptoms of lemon (*Citrus limon*), orange (*Citrus sinensis)* and thyme (*Satureja thymbra*) essential oils on the grape leaves, whereas the highest phytotoxicity was observed from basil (*Ocimum basilicum*) essential oil. Obviously, further experiments on essential oils are required to investigate appropriate applications to ensure the organoleptic characteristics, such as fruit color, aroma, or firmness of export produces.

Therefore, this study investigated high potential essential oil formulas against aphid (*Aphid gossypii* Glover) and whitefly (*Bemisia tabaci* Gennadius), while yielding low physical changes on eggplant (*Solanum melongena* L.) in order to solve insect contamination problem and secure postharvest quality of export vegetables.

Materials and methods

Sample preparation

Insect sample preparation: Adults of aphid (*A. gossypii*) and whitefly (*B. tabaci*) were collected from naturally infested sources in Bangkok, Thailand. The insects were cultured in insecticide free insectary at Department of Plant Production Technology, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang (KMITL), Bangkok, Thailand. Prior to the fumigation, the samples of 10-15 adults of aphid and whitefly were transferred onto a leaf of eggplant and a leaf of star gooseberry, respectively, and placed in a netted-cap plastic box (5×7×3 cm).

Eggplant preparation: Eggplant (Thai light round green; *S. melongena*) was cultivated at the vegetable farm in Nakhon Pathom province, Thailand. The fruit was collected after 45 days of cultivation. Only the fruits with approximately 5 g in weight and 4 cm in diameter were selected. Totally, 15 eggplant fruits were prepared for each 3-replicated fumigation. The samples were stored at 25±1°C until used.

Essential oil preparation: The essential oils in this study were extracted from 18 species of medicinal plants (Table 1), previously reported in many studies for having toxicity against insects and mites. The oils were extracted by using water-distillation method with a Clevenger-type apparatus for 6 h. The extracted oils were collected and dehydrated over anhydrous sodium sulfate and stored in amber-colored vials at 10 °C.

Essential oil formula with low impact on eggplant

Highly insecticidal essential oil selection: The essential oil fumigations were conducted in a 25 L glass cylinder fumigation chamber (Burkard Co., England). Initially, the insect samples were simultaneously placed into the chambers. Subsequently, each essential oil at the concentrations of 0.0 and 3.0 µL/L air were sprayed into the chambers. The insects were left in the chambers for 1 h, and mortalities were observed at 24 h thereafter. The insects were considered lifeless when no appendage motions were observed as probed with a small brush. The actual death rates were calculated via Abbot's formula (Abbott, 1987). The experiment was conducted in a completely 3-randomized replication design. Then, essential oils which resulted in remarkably high mortalities were selected. Insecticidal property of the selected essential oils were then examined again at various concentrations (0, 0.6, 1.2, 1.8, 2.4, 3.0 and 3.6 µL/L air) using the same procedures as in the earlier fumigation. Consequently, particular essential oils that were commonly highly toxic against all insects were selected by using LC_{50} and LC_{90} for further examination.

Essential oil formula with low impact on eggplant: Physiological changes on eggplant as caused by mixtures of the selected essential oils from the earlier experiment at the ratios of 4:0, 3:1, 2:2, 1:3 and 0:4 at 3.0 µL/L air were examined. The fumigations were conducted in 1 cubic meter glass chambers equipped with 7 inch-diameter air circulator (25 watt). A total of 15 eggplant fruits were placed in the center of the chamber for each fumigation, and then each essential oil mixture was injected by 10 lbf/in^2 (GAST® Model 1031-102A) atomizer air pump at 1 mL/7 sec. In addition, 15-min air circulation for 2 h with no essential oil injection was set up as the control treatment. Then, changes in L*, a* and b* values, percentages of weight loss and firmness were examined every 3-day interval until complete senescence. Essential oil fumigation formulas which caused no significant physiological changes when compared to the control were selected. Physiological toxicity of the selected essential oils formulas at different concentrations (2, 3, 4 and 5 µL/L air) were examined by using the same procedures as in the earlier experiment.

Percentage of weight loss was calculated as following; %Weight loss = [(initial weight - observed weight) / initial weight] x 100.

Color changes were measured, using CIE L a b color space system by Color Flex spectrophotometer. Totally, 9 spots on 3 treated fruits (3 spots each) from each observation period were examined for L*, a* and b* values. The L* values indicated levels lightness ranged from 0 for darkness to 100 for lightness. The a* values indicated shades of red-green color ranged from positive values (+a*) for the levels of redness to negative values (-a*) for the levels of greenness. The b* values indicated shades of yellow-blue color ranged from positive values (+b*) for the levels of yellowness to negative values (-b*) for the levels of blueness.

Firmness of eggplant was measured with penetrometer. A plunger (1.11 cm in diameter) was pressed into the fruits, approximately 1 cm deep. The equipment was placed and pressed in 3 directions on each fruit and the results were reported in Newton (N).

Reverse experiment: Insecticidal properties of the essential oil formulas with minimal physiological toxicity on eggplant were re-examined. In addition, fumigation with methyl bromide at 20 mg/L air (recommended rate) was comparably conducted. The fumigations were conducted in DOA (Department of Agriculture) fumigation chamber. The insect mortalities, changes in L*, a* and b* values, percentage of weight loss and firmness were examined and compared, using the same observation procedures as in the earlier experiments.

Statistical analysis: The experiments 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). Lethal concentration of each essential oil needed in killing 50 and 90% of the insects (LC_{50} and LC_{90}, respectively) were calculated via probit analysis.

Results

Highly insecticidal essential oil selection: From the 18 selected medicinal plants, 4 essential oils including clove (*Syzygium aromaticum* (L.) Merr.&L.M. Perry), cinnamon (*Cinnamomum bejolghota* (Buch.-Ham.) Sweet), citronella grass (*Cymbopogon nardus* Rendle.) and lemon grass (*Cymbopogon citratus* (Dc.ex.Nees) Stapf) at 3.0 µL/L air demonstrated remarkably high mortalities (62.0-100.0% mortalities) in aphid (*A. gossypii*) and whitefly (*B. tabaci*) (Table 1). These essential oils were highly toxic against whitefly than aphid as evident from lower LC_{50} and LC_{90}. The essential oil of lemon grass presented the highest toxicity to aphid with LC_{50} at 1.70 µL/L air, followed by essential oil of clove, cinnamon, and citronella grass with LC_{50} at 2.07, 2.27 and 2.35 µL/L air, respectively. Essential oil of clove presented the highest toxicity to whitefly with LC_{50} at 1.36 µL/L air, followed by essential oil of lemon grass, cinnamon, and citronella grass with LC_{50} at 1.45, 1.52 and 1.60 µL/L air, respectively (Table 2). Therefore, the essential oils of clove and lemon grass were selected for the next experiment.

Essential oil formula with low impact on eggplant: Physiological changes on eggplant as caused by fumigations of mixtures between clove and lemon grass essential oils at different ratios (4:0, 3:1, 2:2, 1:3 and 0:4 represented by Cl4Le0, Cl3Le1, Cl2Le2, Cl1Le3 and Cl0Le4, respectively) at 3.0 µL/L air were examined (Table 3). On day 3 after fumigation, no significant differences in L* value, percentages of weight loss, and firmness from all formulas were observed when compared to the control. No significant differences in a* value were observed

Table 1. Mortality percentages (means) of the adults of aphid (*Aphid gossypii* Glover) and whitefly (*Bemisia tabaci* Gennadius) at 24 h after fumigations with plant essential oils at the concentrations of 3 µl/L air, 1 h fumigation

Family / Scientific name	Common name	Plant part	Mortality (%) *A. gossypii*	Mortality (%) *B. tabaci*
Myrtaceae				
1. *Syzygium aromaticum*	Clove	Dried bud	67.3	85.7
2. *Eucalyptus globulus*	Blue gum	Fresh leaf	57.5	60.2
Lauraceae				
3. *Cinnamomum bejolghota*	Cinnamon	Fresh leaf	66.5	83.2
Piperaceae				
4. *Piper nigrum*	Black pepper	Dried seed	<50	<50
5. *Piper betle*	Betel vine	Fresh leaf	<50	<50
Zingiberaceae				
6. *Zingiber cassumunar*	Cassumunar ginger	Fresh rhizome	62.0	78.8
7. *Curcuma longa*	Turmeric	Fresh rhizome	60.8	58.4
8. *Alpinia nigra*	Galanga	Fresh rhizome	<50	65.9
9. *Zingiber officinale*	Ginger	Fresh rhizome	<50	<50
10. *Amomum krervanh*	Cardamom	Dried seed	62.2	52.9
Gramineae				
11. *Cymbopogon nardus*	Citronella grass	Fresh leaf	63.1	80.9
12. *Cymbopogon citratus*	Lemon grass	Fresh leaf	75.8	83.1
Rutaceae				
13. *Citrus aurantifolia*	Lemon	Fresh peel	<50	56.1
14. *Citrus maxima*	Pummelo	Fresh peel	<50	<50
15. *Citrus reticulate*	Tangerine	Fresh peel	<50	<50
16. *Citrus hystrix*	Kaffir lime	Fresh leaf	50.4	60.9
Labiate				
17. *Ocimum basilicum*	Sweet basil	Fresh leaf	<50	57.2
Compositae				
18. *Eupatorium odoratum*	Bitter bush	Fresh leaf	50.4	56.3
Control (95% ethanol)			0	0

Table 2. Insecticidal activity (LC_{50} and LC_{90}) of some essential oils against adults of aphid (*Aphid gossypii* Glover) and whitefly (*Bemisia tabaci* Gennadius) at 24 h after fumigations at various concentrations, 1 h fumigation

Insects	Essential oils	Regression equation	Chi-square (DF)	LC_{50} (range) (µL/L air)	LC_{90} (range) (µL/L air)
A. gossypii	Clove	Y = -1.393+0.672x	24.282 (5)	2.07 (1.61-2.61)	3.98 (3.26-5.58)
	Cinnamon	Y = -1.624+0.714x	17.326 (5)	2.27 (1.91-2.71)	4.07 (3.45-5.25)
	Citronella grass	Y = -1.506+0.641x	23.713 (5)	2.35 (1.88-2.98)	4.35 (3.54-6.24)
	Lemon grass	Y = -1.334+0.786x	24.181 (5)	1.70 (1.26-2.16)	3.33 (2.77-4.42)
B. tabaci	Clove	Y = -1.088+0.800x	36.086 (5)	1.36 (0.72-1.86)	2.96 (2.37-4.34)
	Cinnamon	Y = -1.208+0.795x	37.716 (5)	1.52 (0.90-2.04)	3.13 (2.51-4.56)
	Citronella grass	Y = -1.221+0.762x	24.449 (5)	1.60 (1.12-2.03)	3.29 (2.72-4.42)
	Lemon grass	Y = -1.004+0.691x	34.438 (5)	1.46 (0.78-2.00)	3.32 (2.64-4.98)

Table 3. The L*, a* and b* values, percentage of weight loss, and firmness of eggplant on day 3 and 6 after fumigation with essential oil formulas at 3.0 µL/L air, 2 h fumigation

Formulas+	Means++									
	Day 3 after fumigation					Day 6 after fumigation				
	L* value	a* value	b* value	%WL+++	Firmness (N)	L* value	a* value	b* value	%WL	Firmness (N)
Control	73.82^{a}	-6.09^{a}	20.76^{b}	1.30^{a}	90.93^{a}	71.96^{a}	-5.60^{a}	22.62^{a}	1.59^{b}	89.88^{a}
Cl4Le0	74.40^{a}	-7.35^{bc}	22.53^{ab}	1.59^{a}	82.44^{a}	73.00^{a}	-7.04^{c}	22.63^{a}	1.92^{a}	73.13^{b}
Cl3Le1	71.68^{a}	-7.56^{c}	23.89^{a}	1.62^{a}	90.57^{a}	74.41^{a}	-6.41^{bc}	22.04^{a}	2.07^{a}	79.24^{ab}
Cl2Le2	74.43^{a}	-5.97^{a}	20.14^{b}	1.62^{a}	87.94^{a}	72.11^{a}	-6.41^{bc}	22.99^{a}	1.84^{ab}	78.68^{ab}
Cl1Le3	72.89^{a}	-6.25^{ab}	22.32^{ab}	1.42^{a}	87.06^{a}	71.68^{a}	-5.81^{ab}	23.56^{a}	1.79^{ab}	85.23^{a}
Cl0Le4	74.23^{a}	-6.50^{abc}	21.79^{ab}	1.75^{a}	86.18^{a}	73.34^{a}	-6.67^{c}	22.75^{a}	2.08^{a}	80.54^{ab}

+ Clove : Lemon grass ratio 4:0, 3:1, 2:2, 1:3 and 0:4 represented as formulas Cl4Le0, Cl3Le1, Cl2Le2, Cl1Le3 and Cl0Le4, respectively,
++ Means in the same column followed by the same common letter were not significantly different (P<0.05) according to DMRT,
+++ weight loss.

Table 4. Mortality percentages of adult aphid (*Aphid gossypii* Glover), whitefly (*Bemisia tabaci* Gennadius) at 24 h after fumigations in field experiment with essential oil formulas at 2.0 and 3.0 µL/L air and methyl bromide at 20 mg/L air in DOA (Department of Agriculture) fumigation chamber for 2 h fumigation

EOs formulas+ (concentration)	Mortality (%)++	
	Aphid sp. (adult)	*B. tabaci* (adult)
Control	0.0±5.8[c]	0.0±9.2[c]
Methyl bromide (20 mg/L air)	100.0±0.0[a]	100.0±0.0[a]
Cl1Le3 (2 µL/L air)	82.4±6.6[b]	88.5±6.1[b]
Cl1Le3 (3 µL/L air)	90.3±9.6[ab]	100.0±0.0[a]

+Clove : Lemon grass ratio 1:3 represented as formula Cl1Le3,
++ Means in the same column followed by the same common letter were not significantly different (P<0.05) according to DMRT.

Table 5. The means of L*, a* and b* values, percentage of weight loss (WL) and firmness of eggplant on day 9 after fumigation with essential oil formula Cl1Le3 at 2.0-5.0 µL/L air for 2 h fumigation

Concentration (µL/L air)	Means+				
	L* value	a* value	b* value	WL (%)	Firmness (N)
0	74.71[a]	-5.42[bc]	21.48[a]	2.11[ab]	76.66[a]
2	74.60[a]	-5.76[c]	21.79[a]	1.74[c]	75.59[a]
3	72.00[ab]	-4.97[b]	20.84[a]	2.13[ab]	74.06[a]
4	69.91[bc]	-4.16[a]	18.57[b]	2.43[ab]	63.88[b]
5	68.67[c]	-4.00[a]	17.60[b]	2.61[a]	64.00[b]

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

Table 6. The means of L*, a* and b* values, percentage of weight loss (WL) and firmness of eggplant on day 9 after fumigation with essential oil formula[a] Cl1Le3 at 2.0 and 3.0 µL/L air, and methyl bromide at 20 mg/L air for 2 h fumigation

Treatments+	Concentration	Means++				
		L* value	a* value	b* value	% WL	Firmness (N)
Control	0 µL/L air	66.75[a]	-6.75[a]	26.62[a]	2.52[a]	76.61[a]
Cl1Le3	2 µL/L air	65.75[a]	-7.98[a]	25.94[a]	3.12[a]	76.51[a]
Cl1Le3	3 µL/L air	69.30[a]	-8.45[a]	24.23[a]	3.55[a]	75.49[a]
Methyl Bromide+++	20 mg/L air	-	-	-	-	-

+Clove : Lemon grass ratio 1:3 represented as formula Cl1Le3,
++Means in column followed by the same common letter were not significantly different (P<0.05) according to DMRT,
+++Means were not obtained because of a complete senescence appeared before day 3 observation.

from formulas Cl2Le2 (-5.97), Cl1Le3 (-6.25) and Cl0Le4 (-6.50) when compared to the control (-6.09). Formulas Cl4Le0, Cl2Le2, Cl1Le3 and Cl0Le4 showed no significant differences in b* value (20.14-22.53) when compared to the control (20.76). In summary, Cl1Le3 fumigation, showed no significant differences in all physical change parameters when compared to the control treatment. Therefore, Cl1Le3 were selected for the next experiment.

When varied the concentration it was found that Cl1Le3 fumigations at 2.0 and 3.0 µL/L air showed no severe senescence on day 9 observation. The fumigated eggplants showed lower L* values (72.00-74.06), higher a* values (between -5.76 and -4.95) and lower b* values (20.84-21.79) than the control (74.71, -5.42 and 21.48, respectively) (Table 5, Table 7). The percentages of weight loss (1.74 to 2.31%) were not significantly different when compared to control (2.11%) (Table 7). The fruit firmness (74.06-75.59 N) was slightly lower than the control (76.66 N) (Table 7). However, no significant differences in all physical parameters were observed when compared to the control. Therefore, Cl1Le3 at the concentrations of 2.0 and 3.0 µL/L air were selected for the reverse experiment.

Reverse experiment: The fumigations of Cl1Le3 at 2.0 and 3.0 µL/L air and methyl bromide resulted in 82.4, 90.3 and 100.0% mortalities, respectively in aphid, and 88.5, 100.0 and 100.0% mortalities, respectively in whitefly. In addition, the fumigation with Cl1Le3 formula at 3.0 µL/L air resulted in no significant mortalities in both insects when compared to methyl bromide fumigations (Table 4). However, methyl bromide fumigation caused severe physiological changes on the eggplant immediately after fumigation, and a complete senescence appeared before day 3 observation. Remarkably higher L*, b* and a* values were observed when compared to the control. On the contrary, Cl1Le3 formula at 3 µL/L air showed no differences in physiological changes when compared to the control throughout the 9-day observation (Table 6, Table 8).

Discussion

Highly insecticidal essential oil selection: In the study, clove, cinnamon, citronella grass and lemon grass essential oils caused the highest mortality of aphid (*A. gossypii*) and whitefly (*B. tabaci*), and Table 1 shows that higher concentrations resulted in higher mortalities. In this study, clove essential oil presented the highest toxicity against whitefly, while lemon grass essential oil showed the highest toxicity against aphid. Pumnuan *et al.* (2015) found that clove, cinnamon, citronella grass and lemon grass essential oils were highly effective in controlling thrips (>72.9% mortality) and mealybug (>81.5% mortality). In addition, each essential oil showed particular insecticidal property against many other insects. For example, the essential oil of clove showed insecticidal property against fruit fly (*Ceratitis capitata* (Wiedemann)) (Arancibia *et al.,* 2013), head louse (*Pediculus humanus capitis* (De Geer)) (Choi *et al.,* 2010) and pear psyllid (*Cacopsylla chinensis* (Yang and Li)) (Tian *et al.,* 2015). The essential oil of citronella grass was found toxic against Mediterranean fruit fly (*Ceratitis capitata* (Wiedemann)) (Arancibia *et al.,* 2013), thrips (*Frankliniella schultzei* (Trybom) and green peach aphid, *Myzus persicae* (Sulzer) (Pinheiro *et al.,* 2013). The essential oil of lemon grass showed insecticidal property against larger grain borer (*Prostephanus truncates* (Horn)) (Masamba *et al.*, 2003), and house fly (*Musca domestica* L.) (Pinto *et al.*, 2015) when the essential oil of cinnamon showed toxicity against rice weevil (*Sitophilus oryzae* (L.)) and cowpea weevil (*Callosobruchus maculatus* (F.)) (Ahmed and Salam, 2010).

The essential oils of clove and lemon grass were in general more toxic against aphid (*A. gossypii*) and whitefly (*B. tabaci*) when compared to essential oils of cinnamon and citronella grass. Higher toxicity of clove and lemon grass essential oils were also found in the study of essential oil fumigation against maize weevil (*Sitophilus zeamais* Motsch.) (Pumnuan *et al.,* 2012). These essential oils showed the highest toxicity against many other insects and mites (Akhtar *et al.,* 2008; Hanifah *et al.,* 2011; Kim *et al.,* 2003; Kim *et al.,* 2006; Masamba *et al.*, 2003; Pinto *et al.*, 2015). Therefore, the results in this study suggested that clove and lemon grass essential oils can possibly be used as the main components in formulating insecticidal essential oil formulas, as these essential oils showed acceptable performances in many studies.

Table 7. The L*, a* and b* values, percentage of weight loss, and firmness of eggplant on day 0, 3, 6 and 9 after fumigation with essential oil formula[+] Cl1Le3 at 2.0-5.0 µL/L air for 2 h fumigation

Items	Concentration (µL/L air)	Means[++]				
		Before	0 Day	3 Days	6 Days	9 Days
L* value	0 (Control)	76.41^{a}	76.00^{a}	73.82^{a}	71.37^{a}	74.71^{a}
	2	75.19^{a}	75.05^{a}	72.89^{a}	71.01^{a}	74.06^{a}
	3	75.96^{a}	74.78^{a}	71.68^{a}	70.33^{ab}	72.00^{ab}
	4	74.73^{a}	73.99^{a}	70.49^{a}	67.33^{b}	69.91^{bc}
	5	75.19^{a}	73.13^{a}	69.85^{a}	67.03^{b}	68.67^{c}
a* value	0 (Control)	-5.43^{a}	-5.27^{a}	-6.39^{a}	-5.63^{b}	-5.42^{bc}
	2	-4.77^{a}	-4.96^{a}	-6.25^{a}	-5.81^{b}	-5.76^{c}
	3	-5.57^{a}	-4.83^{a}	-6.09^{a}	-4.98^{ab}	-4.95^{b}
	4	-5.18^{a}	-4.64^{a}	-5.38^{a}	-4.39^{a}	-4.16^{a}
	5	-5.52^{a}	-4.80^{a}	-5.12^{a}	-4.29^{a}	-4.00^{a}
b* value	0 (Control)	19.56^{a}	19.71^{a}	20.76^{a}	22.62^{a}	21.48^{a}
	2	20.00^{a}	20.43^{a}	21.14^{a}	24.23^{a}	21.79^{a}
	3	20.09^{a}	19.99^{a}	19.64^{a}	22.38^{a}	20.84^{a}
	4	18.88^{a}	19.00^{a}	18.67^{a}	19.00^{b}	18.57^{b}
	5	20.50^{a}	18.76^{a}	18.99^{a}	18.33^{b}	17.60^{b}
Weight loss (%)	0 (Control)	0.00	0.80^{b}	1.30^{a}	1.59^{c}	2.11^{ac}
	2	0.00	1.07^{a}	1.42^{a}	1.56^{c}	1.74^{b}
	3	0.00	1.12^{a}	1.55^{a}	1.97^{b}	2.13^{ab}
	4	0.00	1.18^{a}	1.63^{a}	2.23^{a}	2.43^{ab}
	5	0.00	1.18^{a}	1.78^{a}	2.35^{a}	2.61^{a}
Firmness (N)	0 (Control)	102.61^{a}	94.54^{a}	90.93^{a}	89.88^{a}	76.66^{a}
	2	96.92^{a}	93.24^{a}	87.06^{a}	85.23^{ab}	75.59^{a}
	3	99.91^{a}	92.39^{a}	91.87^{a}	89.39^{a}	74.06^{a}
	4	104.60^{a}	95.41^{a}	90.09^{a}	79.00^{bc}	63.88^{b}
	5	97.85^{a}	92.40^{a}	89.30^{a}	74.52^{c}	64.00^{b}

[+] Clove: Lemon grass ratio 1:3 represented as formula Cl1Le3,
[++] Means in same common letter were not significantly different ($P<0.05$) according to DMRT

Essential oil formula with low impact on eggplant: Many papers have been published and confirmed excellent insecticidal efficacy of essential oil. However, research interests have been moving toward essential oil phytotoxicity on germination and weed control whereas, very few studies investigated toxicity of the insecticidal essential oils on the treated commercial plants, particularly fresh produces such as fresh flower, fruit and vegetable. In fruits and vegetables, deterioration is normally indicated by weight loss, softening and color changes which are largely dependent on dehydration and fumigations with plant essential oils can increase loss of weight (Batish *et al.*, 2006 and Kohli *et al.*, 1998), and change of colors in plants (Castillo *et al.,* 2010). However, there are also cases that essential oil treatments helped preserve quality of fresh produces (Gao *et al.*, 2014; Solgi *et al.*, 2014; Castillo *et al.*, 2010).

In this study, Cl1Le3 showed no significant physiological changes in all parameters on day 3 and 6 observations when compared to the control (Table 3). On the other hand, Cl4Le0, Cl3Le1 and Cl0Le4 fumigations presented significant impact on colors of eggplant, while no weight loss difference were observed when compared to the control. These particular results are in some aspects dependent on the fact that many compounds in essential oils are particularly unique in terms of their structures and biological activities. Combinations of aromatic compounds

Table 8. The L*, a* and b* values, percentage of weight loss, and firmness of eggplant on day 0, 3, 6 and 9 after fumigation with essential oil formula+ Cl1Le3 at 2.0 and 3.0 µL/L air, and methyl bromide (MB) at 20 mg/L air for 2 h fumigation

Items	Concentrations (µL/L air)	Means[++]				
		Before	0 Day	3 Days	6 Days	9 Days
L* value	0 (Control)	71.68^{a}	67.55^{a}	66.15^{a}	63.86^{a}	66.75^{a}
	2	72.71^{a}	64.72^{a}	67.26^{a}	67.69^{a}	65.62^{a}
	3	69.97^{a}	70.31^{a}	66.71^{a}	68.68^{a}	68.30^{a}
	MB (20 mg/L air)	71.68^{a}	61.21^{a}	47.93^{b}	-	-
a* value	0 (Control)	-8.21^{a}	-7.09^{b}	-8.13^{b}	-7.78^{a}	-8.45^{a}
	2	-8.07^{a}	-7.28^{b}	-8.01^{b}	-7.26^{a}	-7.98^{a}
	3	-8.43^{a}	-8.51^{c}	-7.47^{b}	-6.95^{a}	-6.75^{a}
	MB (20 mg/L air)	-8.23^{a}	-5.14^{a}	6.10^{a}	-	-
b* value	0 (Control)	22.22^{a}	23.40^{b}	24.86^{a}	23.93^{a}	26.62^{a}
	2	21.94^{a}	25.92^{a}	25.66^{a}	24.74^{a}	25.94^{a}
	3	21.41^{a}	22.68^{b}	23.94^{a}	23.23^{a}	24.23^{a}
	MB (20 mg/L air)	22.55^{a}	24.76^{ab}	20.02^{b}	-	-
Weight loss (%)	0 (Control)	0.00	0.95^{b}	1.87^{b}	2.49^{a}	2.52^{a}
	2	0.00	1.69^{a}	2.65^{a}	3.17^{a}	3.12^{a}
	3	0.00	1.72^{a}	2.18^{b}	3.10^{a}	3.55^{a}
	MB (20 mg/L air)	0.00	1.74^{a}	3.02^{a}	-	-
Firmness (N)	0 (Control)	88.69^{a}	90.68^{a}	82.82^{a}	79.03^{a}	76.51^{a}
	2	88.60^{a}	83.44^{a}	81.34^{a}	78.21^{a}	76.61^{a}
	3	93.46^{a}	87.68^{a}	80.10^{a}	79.07^{a}	75.49^{a}
	MB (20 mg/L air)	88.52^{a}	80.86^{a}	72.02^{b}	-	-

[+]Clove: Lemon grass ratio 1:3 represented as formula Cl1Le3, [++]Means in same common letter were not significantly different ($P<0.05$) according to DMRT.

in essential oils did not only feature synergistic effects. Some combinations may also yield antagonistic results. In other words, substances that caused very high toxicity in some combinations may present low or no impact when the ratios of their contents were changed (Pavela, 2015). As a result, combinations of essential oils at different ratios also yield different results. Some studies have recently highlighted the investigation of binary mixtures of essential oils and their insecticidal and phytotoxic activities (Choi *et al.,* 2010; Kim *et al.*, 2012; Miresmailli *et al.,* 2006; Tripathi *et al.*, 2009; Hummelbrunner and Isman 2001). In our previous paper, we studied the effects of essential oil combinations at different ratios and found that different ratios of combinations between clove and cinnamon resulted in significant toxicity against cut orchid flower (Pumnuan *et al.* (2015). In addition, the same ratios at different fumigation periods or even air circulation periods during the fumigation also yielded different results. Many studies (Pavela *et al.,* 2009; Barbosa *et al.,* 2012) concluded that toxic potential of essential oils and their compounds can vary significantly due to many causes. Particularly, those intrinsic and extrinsic factors including plant species, plant ages, parts, chemotypes, harvest conditions, application methods, essential oil concentrations and volumes, all induce in different responses and phytotoxicity (Boyd and Brennan, 2006).

Reverse experiment: Although methyl bromide fumigation demonstrated high insect control performance, the chemical caused serious physiological changes on the eggplant (Table 4). Normally, uses of methyl bromide as a fumigant can reduce quality of raw produces (Hansen *et al.*, 2000; Akagawa *et al.* 1997). On the contrary, the fumigation of essential oil formula of Cl1Ci3 at 3.0 μL/L air for 2 h showed significantly lower physiological damages (as discussed in the previous section) while yielding similarly high insect control performance. In this study, single essential oils (Cl4Le0 and Cl0Le4) demonstrated relatively low insect mortality. The combination formulas (Cl3Le1, Cl2Le2 and Cl1Le3), on the other hand, resulted in relatively high insect mortality. The findings in Pavela (2014) indicated that it is not necessary that insecticidal activities of essential oils are dependent on their major components. The biological efficacies can be significantly influenced by minor substances which show no or minimal toxicities when used individually. In principle, many action mechanisms of individual substances in essential oils still remain unknown, and both mutual mixing ratios and the molecular structures of the compounds play an important role indicating their biological activities. Koul *et al.* (2013) reported synergism activities of aromatic compounds in plant essential oils with more than 20 other substances. Eugenol as major compound found in clove (Stokłosa *et al.*, 2012; Bainard *et al.*, 2006; Shahi *et al.*, 2007), which in this study was a major essential oil used in the formula combination, is reported as having synergism with Isoeugenol, 1,8-Cineole, Linalool, L-carvol, (R)-(+)-limonene, β-Citronellol, Carvacol, Thymol, etc. (Pavela, 2015).

In this study, it is highly possible that eugenol or other substances in clove essential oil synergized some active compounds in lemongrass essential oil and generated high insecticidal activities against aphid (*A. gossypii*) and whitefly (*B. tabaci*). However, further studies should deliberately investigate synergistic mechanisms of compounds in clove and lemongrass essential oils. However, the use of plant essential oil to control insect pests plays relatively high cost when comparing with chemical use. Then we have to use small amount of essential oil by using some further modified application methods. Certainly, it is safe to human and environment.

Problems involving insect contamination and effects of chemical insecticide toxicity on export agricultural produces have highlighted needs for the development of alternative post-harvest insecticides and treatments, particularly ones that yield high performances but low toxicity on plants. In other words, although higher insect mortality is more preferable, physiological changes on fresh produces as caused by insecticidal treatments is another crucial issue. In this study, 2 h fumigations with mixture of clove and lemon grass essential oil at the ratio of 1:3 (Cl1Le3) at 3.0 μL/L air showed maximum insecticidal property against aphid (*A. gossypii*) and whitefly (*B. tabaci*), while causing minimum physiological changes on the fumigated eggplant. The results present an effective alternative of "green pesticide" which results high insect control alongwith safe product. However, it seems that synergistic mechanism of the essential oils should be examined as well as their application techniques and revisions of the essential oil mixture can also be considered in order to fulfill the most beneficial insect pest management program.

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