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## Fumigation Test of Water Based Essential Oils against House Dust Mite [*Dermatophagoides pteronyssinus* (Trouessart)]

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Wipawee Meepoomru, Jarongsak Pumnuan and Ammorn Insung (2017). Fumigation Test of Water Based Essential Oils against House Dust Mite [*Dermatophagoides pteronyssinus* (Trouessart)]. International Journal of Agricultural Technology 13(7.1): 1355-1360.

Acaricidal property of essential oils from clove (*Syzygium aromaticum*), Cinnamon (*Cinnamomum bejolghota* Sweet and standard eugenol against the house dust mite, (*Dermatophagoides pteronyssinus* (Trouessart)) was performed by using fumigation method. The essential oils were applied within 25 L knockdown chamber. The essential oils of clove, cinnamon and standard eugenol diluted in ethanol and water with different ratios as 95:5, 80:20, 70:30, 60:40, 50:50, and 40:60 at various concentrations of 0, (95% ethanol), 0.3, 0.6, 0.9, 1.2 and 1.5 µl/L air against the mite were evaluated within the fumigation period of 1 hr, then the mortality of mite was observed at 24 hr after fumigation. The result showed that clove essential oil with ethanol and water ratio of 95:5 had a high fumigation effect when 100% mortality of mite at 0.6 µl/L air was obtained with the LC<sub>50</sub> value at 0.36 µl/L air. It showed no significant difference when compared to standard eugenol at 0.6 µl/L air which gave the LC<sub>50</sub> values at 0.59. Followed by cinnamon essential oil, at 1.2 µl/L air, it showed the LC<sub>50</sub> value at 0.45 µl/L air. Remarkably result was obtained when clove essential oil with ethanol and water at the ratio 40:60 gave only 17.4% mortality at 0.6 µl/L air. The much more water contained the lower acaricidal activity was appeared.

**Keywords:** fumigation, house dust mite, ethanol, water, essential oil

### Introduction

Dust mite is a tiny animal associated with ticks and spiders. Their life closely relates to human and eating man and animal skills as food. (Tilak, 1994) Dust mites are spreading around the world and are often found in homes. (Heimerdinge *et al.*, 2006) Dust mite, remarkably is a source of allergens, which are harmful to human health, such as respiratory disease. (Kondreddi *et al.*, 2006) Recent studies indicate that at least 45 percent of young people with asthma suffer from allergic dermatitis. (Potter, 2017) For those who are allergic, the acute effects of bronchial asthma and shortness of breath may be fatal. Currently, dust mite control is available in a variety of ways, such as washing

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sheets, pillows and blankets in hot water to prevent dust mites. Keep relative humidity in the room below 50% and vacuum the carpet regularly are also appropriate. (Jeong *et al.* 2006; Vyszynski-Moher *et al.*, 2002). Dust mite has a habit of staying in the bedding, it is difficult to use direct spraying methods. If the herbal oil is effective in killing dust mites, It can be an interesting alternative. Like the research report of Insung and Pumnuan (2008) reported that clove and cinnamon essential oils were successful in killing *D. pteronyssinus* with 100% mortality at concentration of 1.2  $\mu\text{g}/\text{cm}^3$  and  $\text{LD}_{50}$  at 0.092 and 0.232  $\mu\text{g}/\text{cm}^3$ , respectively.

## **Materials and methods**

### ***Dust Mite Culture***

House dust mite, used in experiments was maintained in a mite bottle kept in mite chamber at  $25 \pm 1$  °C with relative humidity of  $86 \pm 1$  % RH, Rat feed, wheat germ and yeast at the proposal by weight of 4:4:1, respectively were mixed as mite food. (adapted from Insung and Boczek, 1995)

### ***Essential oil preparation***

The essential oils tested in this study from 2 plants including clove (*Syzygium aromaticum* (L.) (Merr. and Perry) and cinnamon (*Cinnamomum zeylanicum* Blume.) as well as standard eugenol. were purchased from Thai-China Flavours and Fragrances Industry Co., Ltd., Thailand.

### ***House dust mite bioassay method***

Amout 10 house dust mite aduts were transferred to mite cage, size  $3 \times 5 \times 0.45$  cm. Essential oils from clove and cinnamon as well as standard eugenol were diluted in ethanol mixed with water at different proportions of 95:5, 80:20, 70:30, 60:40, 50:50 and 40:60 were prepared. The mite bioassay was performed by spraying those essential oils of standard eugenol formulars into the knock down chamber, size  $25 \times 10^4$   $\text{cm}^3$  for amout of 1.5 ml, with different concentrations of 0 (95% ethanol), 0.3, 0.6, 0.9, 1.2 and 1.5  $\mu\text{l}/\text{L}$  air. The fumigation period was 1 hr, and mortalities of mite were observed at 24 hr after fumigation. The experiment was done in 5 replications.

### ***Statistical analysis***

The experiment was completely randomized design (CRD) when the percentage of mite mortality was calculated after Abbott's formula (Abbott's,

1987). Data was analyzed for variance and mean difference by DMRT (Duncan's new multiple range test) Probit analysis was used to obtain  $LC_{50}$  value.

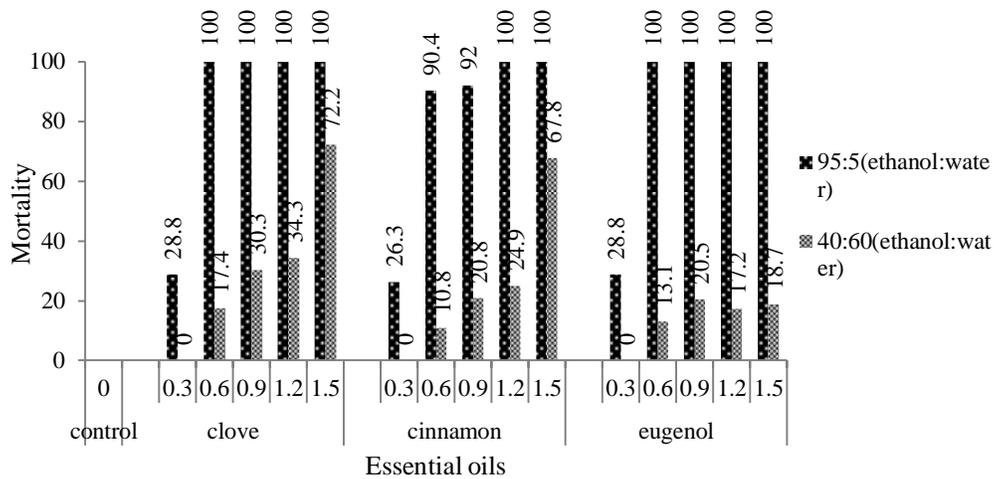
## Results and Discussion

The clove essential oil diluted in ethanol and water at the ratio of 95:5 had a high fumigation effect when 100% mortality of mite at 0.6  $\mu\text{L}$ /L air was obtained with the  $LC_{50}$  value at 0.36  $\mu\text{L}$ /L air. It showed no significant difference when compared to standard eugenol at 0.6  $\mu\text{L}$ /L air which gave the same  $LC_{50}$  value at 0.36  $\mu\text{L}$ /L air. Cinnamon essential oil at the same proportion also presented high acaricidal property but higher concentration as 1.2  $\mu\text{L}$ /L air showed completely control the mite with the  $LC_{50}$  value at 0.45  $\mu\text{L}$ /L air. It was found that both cinnamon essential oil and standard eugenol caused similar result to clove essential oil for all experiments (Table 1). Normally those essential oils as well as eugenol diluted in 95% ethanol presented extremely toxic effect to the house dust mite. Accordingly to Insung and Pumnuan (2008) reported that clove and cinnamon essential oils were successful in killing *D. pteronyssinus* with 100% mortality at concentration of 1.2  $\mu\text{g}/\text{cm}^3$  and LD50 at 0.092 and 0.232  $\mu\text{g}/\text{cm}^3$ , respectively. In addition, the essential oils of clove, cinnamon and eugenol which is the main active ingredient contains in both essential oils caused the mortality of mite *Suidasia pontifical* with  $LC_{50}$  values at 0.419, 0.467 and 0.378  $\mu\text{g}/\text{cm}^3$  respectively (Pumnuan and Insung, 2012). Other reports regarding acaricidal effect as the essential oil of clove was highly toxic to the mold mite, at the concentration of 1.5  $\mu\text{g}/\text{L}$  air gave 92% mortality with the  $LC_{50}$  value at 0.625  $\mu\text{g}/\text{L}$  air when the essential oil of cinnamon showed the  $LC_{50}$  value at 0.707  $\mu\text{g}/\text{L}$  air (Nuchpo *et al.*, 2013). In this study, essential oils diluted in ethanol and mixed with water with different proportions were prepared to get some water based essential oil formulars and then their acaricidal properties were evaluated. It was found that water based essential oils of clove and cinnamon and eugenol gave rather low effect in killing the mite. Remarkably result was obtained when clove essential oil with ethanol and water at the ratio 40:60 gave only 17.4% mortality at 0.6  $\mu\text{L}$ /L air compared with 100% mortality at 0.6  $\mu\text{L}$ /L air of the essential oil with 95% ethanol. Clearly result appeared when compared the mortality of mite caused by the essential oils and eugenol diluted in ethanol and water at the ratios of 95:5 and 40:60 with different concentrations presented their much big different mortality. Water based essential oils were inactive at at 0.3  $\mu\text{L}$ /L air. The much more water contained the lower acaricidal activity was appeared (Table 1 and Figure 1).

**Table1.** Percentage mortality adults of *Dermatophagoides pteronyssinus* (Trouessart) adult caused by essential oils of clove and cinnamon and eugenol diluted in ethanol and water at different ratios.

Ratio of ethanol : water	Mortality (%)						LC <sub>50</sub>	Slope ± S.E.
	Concentration (µl/L air)							
	Control	0.3	0.6	0.9	1.2	1.5		
Clove 95:5	0.0±0.0 <sup>A</sup>	28.8±6.44 <sup>A</sup>	100.0±0.0 <sup>A</sup>	100.0±0.0 <sup>A</sup>	100.0±0.0 <sup>A</sup>	100.0±0.0 <sup>A</sup>	0.36	7.85±1.37
Clove 80:20	0.0±0.0 <sup>A</sup>	21.1±5.39 <sup>B</sup>	66.7±2.47 <sup>B</sup>	94.6±7.4 <sup>A</sup>	100.0±0.0 <sup>A</sup>	100.0±0.0 <sup>A</sup>	0.50	4.31±0.42
Clove 70:30	0.0±0.0 <sup>A</sup>	10.5±3.64 <sup>C</sup>	56.4±2.3 <sup>C</sup>	70.2±7.2 <sup>B</sup>	95.4±4.0 <sup>A</sup>	100.0±0.0 <sup>A</sup>	0.67	3.21±0.29
Clove 60:40	0.0±0.0 <sup>A</sup>	0.0±0.0 <sup>D</sup>	45.9±0.4 <sup>D</sup>	56.8±7.1 <sup>C</sup>	52.8±6.2 <sup>B</sup>	93.1±7.2 <sup>A</sup>	1.01	3.04±0.32
Clove 50:50	0.0±0.0 <sup>A</sup>	0.0±0.0 <sup>D</sup>	42.8±2.7 <sup>D</sup>	40.6±10.0 <sup>D</sup>	47.2±2.45 <sup>B</sup>	79.2±6.1 <sup>B</sup>	1.06	1.81±0.22
Clove 40:60	0.0±0.0 <sup>A</sup>	0.0±0.0 <sup>D</sup>	17.4±1.2 <sup>E</sup>	30.3±8.93 <sup>E</sup>	34.3±8.14 <sup>C</sup>	72.2±18.3 <sup>B</sup>	1.16	2.16±0.25
%CV	-	37.12	2.85	11.40	6.38	9.18		
Cinnamon 95:5	0.0±0.0 <sup>A</sup>	26.3±11.31 <sup>A</sup>	90.4±2.7 <sup>A</sup>	92.0±7.8 <sup>A</sup>	100.0±0.0 <sup>A</sup>	100.0±0.0 <sup>A</sup>	0.45	4.28±0.43
Cinnamon 80:20	0.0±0.0 <sup>A</sup>	17.8±7.87 <sup>B</sup>	81.8±4.9 <sup>AB</sup>	86.7±6.4 <sup>A</sup>	100.0±0.0 <sup>A</sup>	100.0±0.0 <sup>A</sup>	0.52	3.92±0.38
Cinnamon 70:30	0.0±0.0 <sup>A</sup>	11.9±3.84 <sup>B</sup>	62.2±3.9 <sup>B</sup>	57.6±9.3 <sup>B</sup>	97.0±5.3 <sup>A</sup>	97.1±4.0 <sup>A</sup>	0.71	2.65±0.23
Cinnamon 60:40	0.0±0.0 <sup>A</sup>	0.0±0.0 <sup>C</sup>	32.6±5.2 <sup>C</sup>	38.2±9.4 <sup>C</sup>	42.4±8.4 <sup>B</sup>	95.4±4.2 <sup>A</sup>	1.02	2.41±0.25
Cinnamon 50:50	0.0±0.0 <sup>A</sup>	0.0±0.0 <sup>C</sup>	21.1±5.5 <sup>C</sup>	36.1±11.9 <sup>C</sup>	40.5±13.11 <sup>B</sup>	83.1±5.6 <sup>B</sup>	1.11	2.10±0.24
Cinnamon 40:60	0.0±0.0 <sup>A</sup>	0.0±0.0 <sup>C</sup>	10.8±5.2 <sup>D</sup>	20.8±8.6 <sup>D</sup>	24.9±7.34 <sup>C</sup>	67.8±14.25 <sup>C</sup>	1.91	2.15±0.28
%CV	-	62.57	10.15	16.40	10.88	7.36		
Eugenol 95:5	0.0±0.0 <sup>A</sup>	28.8±8.8 <sup>A</sup>	100.0±0.0 <sup>A</sup>	100.0±0.0 <sup>A</sup>	100.0±0.0 <sup>A</sup>	100.0±0.0 <sup>A</sup>	0.36	6.83±1.80
Eugenol 80:20	0.0±0.0 <sup>A</sup>	15.0±3.9 <sup>B</sup>	39.6±9.9 <sup>B</sup>	100.0±0.0 <sup>A</sup>	100.0±0.0 <sup>A</sup>	100.0±0.0 <sup>A</sup>	0.96	2.96±0.32
Eugenol 70:30	0.0±0.0 <sup>A</sup>	8.3±8.2 <sup>B</sup>	32.7±12.9 <sup>BC</sup>	58.2±2.9 <sup>B</sup>	61.4±9.8 <sup>B</sup>	94.7±5.3 <sup>A</sup>	1.49	1.30±0.11
Eugenol 60:40	0.0±0.0 <sup>A</sup>	0.0±0.0 <sup>C</sup>	28.8±5.2 <sup>BC</sup>	35.0±5.9 <sup>C</sup>	33.6±6.9 <sup>C</sup>	54.0±16.4 <sup>B</sup>	2.30	0.81±0.10
Eugenol 50:50	0.0±0.0 <sup>A</sup>	0.0±0.0 <sup>C</sup>	25.0±8.6 <sup>C</sup>	21.7±5.6 <sup>D</sup>	21.9±5.4 <sup>D</sup>	34.6±7.7 <sup>C</sup>	3.00	0.62±0.11
Eugenol 40:60	0.0±0.0 <sup>A</sup>	0.0±0.0 <sup>C</sup>	13.1±4.6 <sup>D</sup>	20.5±5.3 <sup>D</sup>	17.2±6.4 <sup>D</sup>	18.7±7.2 <sup>D</sup>	3.73	0.53±0.12
%CV	-	60.03	20.12	7.40	10.71	12.30		

<sup>1/</sup>Means in row followed by the same capital letter and column followed by the same common letter are not significantly different at the 5% level as determined by DMRT (P < 0.05).



**Figure 1.** Comparison of the motality of *Dermatophagoides pteronyssinus* (Trouessart) caused by clove and cinnamon essential oils and eugenol diluted in ethanol and water at the ratios of 95:5 and 40:60 with different concentrations.

### Conclusion

Fumigant toxicity of essential oils from clove (*Syzygium aromaticum*), cinnamon (*Cinnamon bejolghota* Sweet and standard eugenol against the house dust mite, (*Dermatophagoides pteronyssinus* (Trouessart)) presented that the clove essential oil and standard eugenol diluted in ethanol and water at the ratio of 95:5 had a highest fumigation effect gave 100% mortality of mite at 0.6 μL/L air with the LC<sub>50</sub> value at 0.36 μL/L air. Essential oils of clove and cinnamon as well as eugenol diluted in 95% ethanol presented extremely toxic effect to the house dust mite. But water based essential oils of those and eugenol gave rather low effect in killing the mite. The higher water contained, the lower acaricidal activity was obtained.

### Acknowledgement

This research was supported by government budget for the fiscal year 2017 via Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang under the research project of natural products for pest control research center.

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(Received: 23 October 2017; accepted: 25 November 2017)