

Effects of Seven Plant Essential Oils on Mortalities of Chicken Lice (*Lipeurus caponis* L.) Adult

Jarongsak Pumnuan*, Ammorn Insung and Ampon Klompanya

Faculty of Agricultural Technology, King Mongkut's Institute of Technology
Ladkrabang, Bangkok, Thailand

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Abstract

Chicken lice (*Lipeurus caponis* L.) are external parasite of chicken and farmers often use malathion and carbaryl insecticides to control this parasite. However, the use of chemical insecticides may result in direct toxicity to chicken or contamination in chicken meats. Thus, this study investigated the effect of seven plant essential oils (EOs) against adult chicken lice. Contact bioassay was performed by laying 10 adult chicken lice on treated filter paper added with EOs at concentrations of 0 (95% ethanol as a control), 0.079, 0.157, 0.236 and 0.314 $\mu\text{l}\cdot\text{cm}^{-2}$. Mortality percentages were observed at 3, 6 and 12 h intervals after the treatments. Clove, cinnamon, turmeric and star anise EOs were able to eliminate the lice completely (100% mortalities) at 12 h exposure which were higher than lemon grass, citronella grass and piper EOs. Clove EO was the most effective oil with LC_{50} of 0.132, 0.085 and 0.039 $\mu\text{l}\cdot\text{cm}^{-2}$ at 3, 6 and 12 h, respectively. Furthermore, clove EO at the 0.157 $\mu\text{l}\cdot\text{cm}^{-2}$ showed the highest mortalities with LT_{50} at 1.438 h. Our study suggests that clove EO could be used as an alternative medicinal insecticide to control chicken lice in farms.

Keywords: clove, cinnamon, star anise, contact method, insecticide, chicken lice
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1. Introduction

Poultry lice are important external parasite of poultry [1] that consume host tissues, secretions of quill feathers, etc. Their biting is usually irritating and painful and poultry will become restless resulting in decrease of feed intake [2]. Chicken lice (*Lipeurus caponis* L.), parasitic wing lice of chicken, may pierce the pulp of feathers or the skin which may be extremely dangerous especially to young poultry, even if they only feed by nibbling along the feather surface and/or eat epidermal debris [3]. Invasion of these lice are often found in many countries, including Bangladesh [4], Ethiopia [5], Malawi [6], Turkey [7], Philippines [8], Nigeria [9], California [10] and Thailand [11].

In Thailand, the initial survey found that farmers often used malathion and carbaryl insecticides to control this parasite. Report from Turkey revealed that synthetic pyrethroid insecticides were applied for controlling chicken lice [12]. The use of organophosphate, pyrethroid and spinosyns insecticides to prevent lice and mite of poultry was recommended [13]. However, louse control can cause an intense growth of resistances against long-used insecticides [14]. The use of chemical pesticides may cause contamination in the chicken meats because animals intended for

* Corresponding author: Tel.: +66 02-329-8499 Fax: +66 02-329-8499
E-mail: jarongsak.pu@kmitl.ac.th

human food may absorb residual pesticides in their feed, water or during direct/indirect exposure in the course of pest control [15] and may cause direct toxic effect to chicken.

Nowadays, none of these methods are efficient for complete protection. Consequently, control method of high effectiveness against insects with non-toxic effect, non-residue toxicity in meat, environmentally friendly has focused on alternative bio-pesticides which specifically entails the property of natural degradability. Especially, essential oils (EOs) are good candidates for safer control agents that may provide good anti-lice activity and low levels of evolved resistance [16]. Essential oils were extensively studied and used against some insects and mites [17-20]. Plant EOs of clove, cinnamon, turmeric, star anise, black piper, citronella grass and lemon grass were previously reported as high potential against insect and mite pests [21].

The objective of this study was to evaluate the effectiveness of seven plant essential oils, namely clove (*Syzygium aromaticum*), cinnamon (*Cinnamomum bejolghota*), turmeric (*Curcuma longa*), star anise (*Illicium verum*), black piper (*Piper nigrum*), citronella grass (*Cymbopogon nardus*) and lemon grass (*Cymbopogon citratus*) against adult of chicken lice (*L. caponis*) by residue filter paper contact method in laboratory conditions.

2. Materials and Methods

2.1 Essential oil preparation

Essential oils from dried bud of clove (*S. aromaticum*), dried flower of star anise (*I. verum*), dried seed of black piper (*P. nigrum*), fresh rhizome of turmeric (*C. longa*), and fresh leaf of cinnamon (*C. bejolghota*), citronella grass (*C. nardus*) and lemon grass (*C. citratus*) which has been reported to contain insecticidal properties against adult of chicken lice (*L. caponis*) [22] are used in this study. All EOs were purchased from Thai-China Flavours and Fragrances Industry Co., Ltd., Bangkok, Thailand. The concentration of EOs at 0.1, 0.15 and 0.2% as well as the control group with the application of 0.2% of Tween-20 in water were applied.

2.2 Insect samples

An adult colony of chicken lice (*L. caponis*) was collected from native chickens at Learning Center and Management System Integrated with Urban Livestock Farm Learning, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang (KMUTL), Thailand.

2.3 Experimental treatments

The insecticidal activity test of the plant EOs against chicken lice was evaluated by using residue filter paper contact method. One ml of each plant EOs at concentrations of 0.2% in 95% ethanol was separately dropped on each filter paper (Whatman® No. 1) and placed onto Petri dish (with size of 90 mm in dia). By this application, the EO concentration was equal to $0.314 \mu\text{l}\cdot\text{cm}^{-2}$. The treated filter papers were air-dried for 5 min and 10 chicken lice adults were put into the petri dish. Mortality observations were recorded at 6 and 12 h after treatment. The plant EOs showing high effectiveness against chicken lice were selected for further experiments.

For further insecticidal activity test, those EOs at the concentrations of 0.05, 0.10, 0.15, 0.20 and 0.25% in 95% ethanol or 0.079, 0.157, 0.236, 0.314 and $0.393 \mu\text{l}\cdot\text{cm}^{-2}$, respectively were applied by using as the same method as described previously. Mortality observations were recorded at 2, 4, 6, 8, 10 and 12 h after treatment.

The experiment was replicated three times and statistically designed by completely randomized replication design (CRD). The actual death rates were calculated via Abbot's formula [23]. The data obtained were statistically analyzed by applying analysis of variance (ANOVA) and Duncan's multiple range tests (DMRT). Lethal concentrations of EOs needed to kill 50 and 90% of the insects (LC_{50} and LC_{90} , respectively) and lethal time of EOs needed to kill 50 of the insects (LT_{50}) were calculated via probit analysis.

3. Results and Discussion

The efficacy in terms of insecticidal properties of seven plant EOs against the adult of chicken lice (*L. caponis*) conducted by residue filter paper contact method in laboratory conditions showed that plant EOs of clove, turmeric, cinnamon and star anise were able to eliminate the lice completely (100% mortalities) at 12 h exposure which were higher than those of lemon grass, citronella grass and black piper EOs, respectively (Figure 1). At 6 h after treatment, the plant EO of clove showed to be the most effective insecticidal activity against the lice with completely 100% mortalities followed by turmeric and cinnamon EOs with mortality rate of 76.7 and 73.9%, respectively. The star anise EO caused only 39.5% mortality whereas plant EOs of black piper, lemon grass and citronella grass were the lowest group to control chicken lice with 1.7%, 6.1% and 11.9% mortality. Thus, the plant EOs with high effectiveness against the lice, including clove, turmeric and cinnamon were selected for further experiments to obtain the toxicity level. The insecticidal activity test by residue filter paper contact bioassay showed that clove EO was the most effective candidate with LC_{50} (at 3, 6 and 12 h) of 0.132, 0.085 and 0.039 $\mu\text{l}\cdot\text{cm}^{-2}$, respectively and LC_{90} of 0.168, 0.112 and 0.108 $\mu\text{l}\cdot\text{cm}^{-2}$ followed by EOs of cinnamon and turmeric with LC_{50} of 0.220, 0.090 and 0.038 $\mu\text{l}\cdot\text{cm}^{-2}$ and 0.256, 0.148 and 0.106 $\mu\text{l}\cdot\text{cm}^{-2}$, respectively (Table 1). Furthermore, clove EO at 0.157 $\mu\text{l}\cdot\text{cm}^{-2}$ showed the highest mortalities with LT_{50} at 1.438 h and was able to eliminate the lice completely (100% mortalities) at 4 h exposure (Table 2).

There were many reports regarding the effectiveness of extract and EO of plants against insect and mite pests of poultry. Aquatic and ethanolic leaf extracts of *Conocarpus erectus* had some toxic effects (acaricidal and repellent properties) on poultry red mite, *Dermanyssus gallinae* in Iran [24]. *Thuja occidentalis* arborvitae and *Juniper* spp. (*Juniperus*) leaf EOs were also found to be effective against the poultry red mite (*D. gallinae*) [25]. Lans and Turner [26] reported nineteen species of plants conducted for parasite control in poultry farm of British Columbia, Canada. Our study showed that EO of clove was effective to control chicken lice and this EO has been reported for insecticidal property against insect and mites pests, including fruit fly (*Ceratitis capitata*) [27], head louse (*Pediculus humanus capitis*) [28], maize weed (*Sitophilus zeamais*) [29], thrips (*Frankliniella schultzei*) [20], mealybug (*Pseudococcus jackbeardsleyi*) [20], rice weevil (*Sitophilus oryzae*) [30], pear psyllid (*Cacopsylla chinensis*) [31], aphid (*Aphis gossypii*), whitefly (*Bemisia tabaci*) [32], red spider mite (*Oligonychus coffeae*) [33], house dust mite (*Dermatophagoides farina* and *Dermatophagoides pteronyssinus*) [34]. Our study suggests that clove EO or maybe the combination of clove EO with cinnamon and turmeric EOs as mixture could be used as a new alternative medicinal insecticide to control chicken lice. Combination of EOs are a combination of chemical compounds in these EOs together, which will encourage the EOs mixture to be more effective in pest eradication. The combination of chemical compounds from plant were also reported to enhance EO efficacy. Koul *et al.* [35] reported that the combination of anethole and 1,8-cineole demonstrated reduction in the population of red flour beetle (*Tribolium castaneum*). Synergism or additive effects of monoterpenoid binary mixtures against tobacco cutworm larvae (*Spodoptera litura*) was also reported [36].

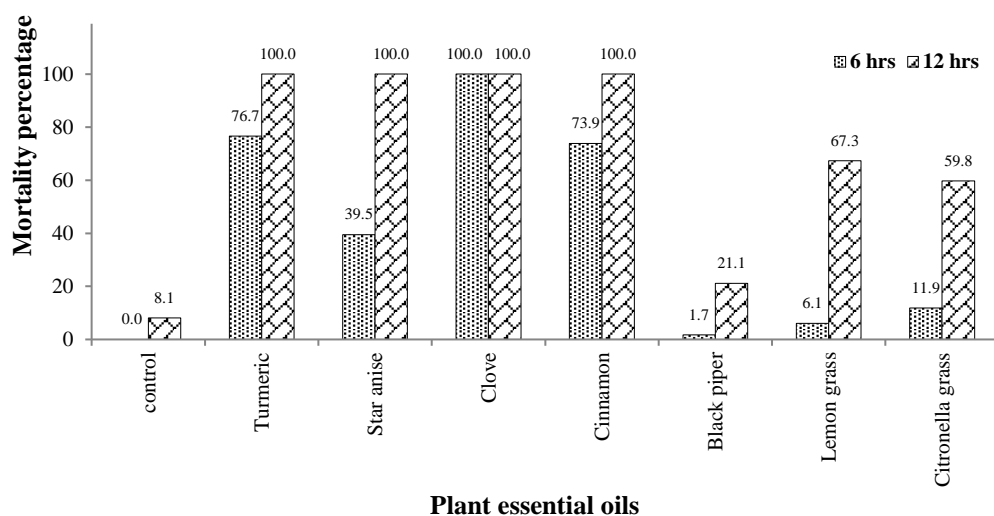


Figure 1. Mortality percentage of the adults of chicken lice (*Lipeurus caponis* L.) at 6 and 12 h after residue filter paper contact test with plant essential oils at the concentration of $0.314 \mu\text{l}\cdot\text{cm}^{-2}$

Table 1. Mortality percentage of the adults of chicken lice (*Lipeurus caponis* L.) at 6 and 12 h after residue filter paper contact test with plant essential oils at various concentrations

Plant essential oils	% Mortality ^{1/} (Means±S.D.)						LC ₅₀ (μl·cm ⁻²)	LC ₉₀ (μl·cm ⁻²)
	Concentration (μl·cm ⁻²)							
	control	0.079	0.157	0.236	0.314	0.393		
3 h								
Turmeric	0.0±0.0 ^{Db}	0.0±0.0 ^{Dd}	0.0±0.0 ^{Dd}	52.2±13.5 ^{Cb}	74.3±14.8 ^{Bb}	100.0±0.0 ^{Aa}	0.256	0.332
Cinnamon	0.0±0.0 ^{Db}	5.1±8.9 ^{CDd}	12.5±6.5 ^{Cd}	48.8±5.8 ^{Bb}	100.0±0.0 ^{Aa}	100.0±0.0 ^{Aa}	0.220	0.299
Clove	0.0±0.0 ^{Cb}	3.3±5.8 ^{Cd}	81.2±17.1 ^{Bb}	100.0±0.0 ^{Aa}	100.0±0.0 ^{Aa}	100.0±0.0 ^{Aa}	0.132	0.168
6 h								
Turmeric	0.0±0.0 ^{Cb}	8.3±9.2 ^{Cd}	51.6±13.2 ^{Bc}	100.0±0.0 ^{Aa}	100.0±0.0 ^{Aa}	100.0±0.0 ^{Aa}	0.148	0.204
Cinnamon	0.0±0.0 ^{Db}	59.9±16.3 ^{Cb}	77.9±11.4 ^{Bb}	100.0±0.0 ^{Aa}	100.0±0.0 ^{Aa}	100.0±0.0 ^{Aa}	0.090	0.166
Clove	0.0±0.0 ^{Cb}	38.8±10.2 ^{Bc}	100.0±0.0 ^{Aa}	100.0±0.0 ^{Aa}	100.0±0.0 ^{Aa}	100.0±0.0 ^{Aa}	0.085	0.112
12 h								
Turmeric	8.6±2.4 ^{Da}	29.3±6.1 ^{Cc}	75.7±10.5 ^{Bb}	100.0±0.0 ^{Aa}	100.0±0.0 ^{Aa}	100.0±0.0 ^{Aa}	0.106	0.189
Cinnamon	8.6±2.4 ^{Ba}	97.4±4.4 ^{Aa}	100.0±0.0 ^{Aa}	100.0±0.0 ^{Aa}	100.0±0.0 ^{Aa}	100.0±0.0 ^{Aa}	0.038	0.107
Clove	8.6±2.4 ^{Ba}	97.0±5.2 ^{Aa}	100.0±0.0 ^{Aa}	100.0±0.0 ^{Aa}	100.0±0.0 ^{Aa}	100.0±0.0 ^{Aa}	0.039	0.108

^{1/} Means in each column followed by the same common letter and means in row followed by the same capital letter were not significantly different ($P < 0.05$) according to DMRT.

Table 2. Mortality percentage of the adults of chicken lice (*Lipeurus caponis* L.) at the various concentrations after residue filter paper contact test with plant essential oils at the 2-12 h

Plant essential oils	% Mortality ^{1/} (Means±S.D.)						LT ₅₀ (h)	LT ₉₀ (h)
	Times (h)							
	2	4	6	8	10	12		
<i>0.157 μl·cm⁻²</i>								
Turmeric	0.0±0.0 ^{De}	3.3±5.8 ^{Dd}	51.6±13.2 ^{Cc}	57.4±10.8 ^{BCc}	72.5±10.5 ^{ABb}	75.5±10.5 ^{Ab}	7.284	10.526
Cinnamon	2.8±4.8 ^{Dde}	21.8±4.8 ^{Cc}	77.9±11.4 ^{Bb}	80.9±10.5 ^{Bb}	100.0±0.0 ^{Aa}	100.0±0.0 ^{Aa}	5.368	7.933
Clove	72.7±15.2 ^{Bb}	100.0±0.0 ^{Aa}	100.0±0.0 ^{Aa}	100.0±0.0 ^{Aa}	100.0±0.0 ^{Aa}	100.0±0.0 ^{Aa}	1.438	2.663
<i>0.236 μl·cm⁻²</i>								
Turmeric	12.2±6.7 ^{Cd}	72.8±16.7 ^{Bb}	100.0±0.0 ^{Aa}	100.0±0.0 ^{Aa}	100.0±0.0 ^{Aa}	100.0±0.0 ^{Aa}	3.290	4.658
Cinnamon	0.0±0.0 ^{Ce}	67.2±19.5 ^{Bb}	100.0±0.0 ^{Aa}	100.0±0.0 ^{Aa}	100.0±0.0 ^{Aa}	100.0±0.0 ^{Aa}	3.745	4.438
Clove	100.0±0.0 ^{Aa}	100.0±0.0 ^{Aa}	100.0±0.0 ^{Aa}	100.0±0.0 ^{Aa}	100.0±0.0 ^{Aa}	100.0±0.0 ^{Aa}	-	-
<i>0.314 μl·cm⁻²</i>								
Turmeric	26.9±3.3 ^{Bc}	100.0±0.0 ^{Aa}	100.0±0.0 ^{Aa}	100.0±0.0 ^{Aa}	100.0±0.0 ^{Aa}	100.0±0.0 ^{Aa}	2.337	3.036
Cinnamon	90.3±0.5 ^{Ba}	100.0±0.0 ^{Aa}	100.0±0.0 ^{Aa}	100.0±0.0 ^{Aa}	100.0±0.0 ^{Aa}	100.0±0.0 ^{Aa}	0.661	1.986
Clove	100.0±0.0 ^{Aa}	100.0±0.0 ^{Aa}	100.0±0.0 ^{Aa}	100.0±0.0 ^{Aa}	100.0±0.0 ^{Aa}	100.0±0.0 ^{Aa}	-	-

^{1/} Means in each column concentration followed by the same common letter and means in row followed by the same capital letter were not significantly different ($P < 0.05$) according to DMRT.

Non-toxic alternative options are hence needed for control of insect pest and natural products from plants especially EOs are good candidates for safer control agents that may provide good insecticidal activity and low levels of evolved resistance [15]. Our study suggests that clove EO could be used as an alternative medicinal insecticide to control chicken lice in the farm.

4. Conclusions

Insecticidal properties of plant EOs against the adult of chicken lice (*L. caponis*) by residue filter paper contact method in laboratory conditions revealed that clove EO was the most effective candidate with the LC₅₀ at 3, 6 and 12 h of 0.132, 0.085 and 0.039 $\mu\text{l}\cdot\text{cm}^{-2}$, respectively. Furthermore, the clove EO at 0.157 $\mu\text{l}\cdot\text{cm}^{-2}$ showed the highest mortalities of chicken lice with LT₅₀ at 1.438 h, and this clove EO was able to eliminate the lice completely (100% mortalities) within 4 h exposure while the clove EO at the concentration of 0.236 $\mu\text{l}\cdot\text{cm}^{-2}$ was effective to kill the lice completely at 2 h. Our study suggests that clove EO or maybe the combination with cinnamon and turmeric EOs as the mixture could be used as an alternative medicinal insecticide to control chicken lice in farm.

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