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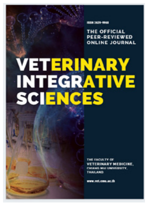

Research article

Physical characteristics, chemical compositions, and insecticidal activity of plant essential oils against chicken lice (*Menopon gallinae*) and mites (*Ornithonyssus bursa*)

Nattha Vigad[1], Wattana Pelyuntha[1], Prapakorn Tarachai[1], Sunee Chansakaow[2] and Kridda Chukiatsiri[1,*]

[1]Faculty of Animal Science and Technology, Maejo University, Chiang Mai 50290, Thailand
[2] Department of Pharmaceutical Sciences, Faculty of Pharmacy, Chiang Mai University, Chiang Mai 50200, Thailand

Abstract

A preparation of essential oils to control chicken lice (*Menopon gallinae*) and mites (*Ornithonyssus bursa*) was developed. Each essential oil was effective against lice and mite in vitro. Citronella oil at the lowest concentration of 0.208 µg/cm2 resulted in a mortality rate of 100% in chicken lice, whereas a higher concentration of cloves, lemongrass, ginger, Makwan oil (0.416 µg/cm2), and Litsea oil (0.832 µg/cm2) was also found to be effective. A 100% rate of mortality for mites was observed using citronella and ginger oil at a concentration of 0.416 µg/cm2. At the same concentration, cloves, lemongrass, Litsea, and Makwan oil exhibited mortality rates in mites of 77.96%, 93.33%, 87.30%, and 93.49%, respectively. The efficacy of citronella oil and ginger oil against lice and mites was further examined in vivo. Citronella and ginger oil affected the rate of decline in lice from day 1 to day 14, whereas the number of mites living in nests declined from day 1 to day 7. The reduction of these parasitic insects may be correlated with the chemical constituents present in each essential oil. The active ingredients likely acted insecticidal agents against both parasitic insects. Moreover, the preparation developed here did not cause any side effects, such as dermatitis and respiratory disorders, during animal trials. Hence, preparations comprised of the essential oils of citronella and ginger can be further developed and used as insecticidal agents to control and/or eliminate chicken lice and mites on commercial farms.

Keywords: Contact toxicity assay, Insecticides, *Menopon gallinae*; *Ornithonyssus bursa*; Phytochemicals, Plant essential oil

Corresponding author: Kridda Chukiatsiri,Faculty of Animal Science and Technology, Maejo University, Chiang Mai 50290, Thailand.
Tel: 66 875432 Fax: 66 53353830 Email: kridda003@hotmail.com.

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INTRODUCTION

External parasites in chickens, such as lice (*Menopon gallinae*), mites (*Ornithonyssus bursa*), fleas, bedbugs, ticks, and flies, are currently having a huge impact on the poultry industry (Anshnafi and Shetu, 2004; Buranapim et al., 2018). The problems caused by these external parasites represent an important risk factor for the health of farm animals. They can result in reduced productivity levels on chicken farms leading to significant economic losses (Arends, 2003; Sofunmade, 2003; Maina, 2005). Importantly, infestations with these parasites do not immediately cause death in chickens; however, the number of parasites on reluctant chicken hosts may cause other problems ranging from minor irritations, a reduction in egg-laying, and debilitation among the animals. In some of the more extreme cases, death can occur as an eventual and indirect consequence of these parasitic infestations (Hinkle and Corrigan, 2020).

Menopon gallinae is commonly referred to as the chicken body louse (shaft louse). This louse can be identified by their short antennae that are concealed in grooves behind their eyes (Clay, 1969). *Menopon gallinae* can infect a wide range of birds including domestic chickens, migratory birds, and guinea fowl. As it is more commonly known, the shaft louse mainly feeds on skin and feather debris. It prefers to remain on the chest, shoulders, and the backs of birds. In addition, it may also drain blood from the wounds it creates (Kumar et al., 2017). Adult females can typically glue a few hundred eggs to the shafts of the feather of host birds. Approximately 4 to 7 days after egg deposition, larvae will hatch and develop through several nymphal stages to ultimately emerge as adults. The life cycle of the shaft louse on the host takes place entirely within 3 to 5 weeks. Transmission from one animal to another can occur by direct contact with an animal infested with lice.

Ornithonyssus bursa, typically referred to as the tropical fowl mite, is a common parasite of domestic and wild birds that is entirely restricted to warm, tropical, and subtropical regions. This mite usually lives in the feathers of birds but will often lay its eggs in the birds' nests. *O. bursa* is known to have four pairs of legs, three of which are located on the sternal plate. It is also known to have a narrow-shaped body. The cycle of the *O. bursa* is comprised of 5 stages, which include the egg, larva, protonymph, deutonymph, and adult stages. The entire life cycle is estimated to last less than 1-2 weeks (Santillán et al., 2015; Lareschi et al., 2017; Bassini-Silva et al., 2019).

Numerous approaches have been developed for the scrutiny, control, and elimination of external parasites in poultry. Certain pesticides have been widely used on poultry farms including butenolides, ryanoids, neonicotinoids, organochlorine, organophosphates and carbamates, and pyrethroids (Sanbornn et al., 2004). However, long-term exposure to these pesticides can have toxic impacts on humans and animals. Moreover, the residues of these pesticides are often left behind in the environment and have been identified in the food production chain. Consequently, these residues can unintentionally harm or kill other living creatures (Aktar et al., 2009; Sharma et al., 2019).

Nowadays, natural ingredients are being used as substitutes for chemical or synthetic pesticides. Plant-derived extracts and/or oils have been determined to be safer, unassociated with residual toxicity, and capable of naturally degrading in the environment. Plant-derived essential oils are one of the most effective agents now being used as insecticides and insect repellent agents against a wide range of insect species especially ectoparasitic insects (Lachance and Grange, 2014). For example, lemongrass oil is considered an effective miticidal and ovicidal agent. It was found to be able to kill the Sarcoptes scabiei collected from naturally infected rabbits in China (Li et al., 2020). The insecticidal properties of this essential oil have also been observed in the treatment of lice (*Lipeurus caponis*) on infected chickens. A reduction of 67.3% of lice was observed in birds that had been treated with lemongrass oil (Pumnuan et al., 2020). Moreover, lemongrass oil at 1% (v/v) concentration significantly reduced the reproductive efficacy of the cattle tick Rhipicephalus microplus by 100% . The same study also revealed that ginger oil (10% v/v) could provide more than 94% effectiveness in insecticidal protection. (Pazinato etal., 2016). Ginger oil has been used as an acaricidal and repellent agent against Rhipicephalus bursa in herds of infested sheep. Notably, this oil exhibited 39.1%acaricidalefficacyanda62.2%degreeofrepellence(Madreseh-Ghahfarokhi et al., 2019). Eugenol, the main component extracted from the dried flower buds of cloves exhibited acaricidal activity against the scabies mites that had infected humans. Clove essential oil exhibited a highly toxic effect by killing this mite within 15 min of contact (Pasay et al., 2010). Citronella oil obtained from the leaves and stems of Cymbopogon nardus has generally been used as a mosquito repellent against Aedes aegypti, a known vector of dengue fever (Songkro et al., 2018). It is commonly used to treat head lice in children and chicken lice (L. caponis) in poultry (Mumcuoglu et al., 2004; Pumnuan et al., 2020). House dust mites (*Dermatophagoides farinae* and *D. pteronyssinus*) and stored food mites (*Tyrophagus putrescentiae*) can be killed by Litsea oil at LC50 values of 1.54, 1.83 and 3.90 μg/cm² (Jeon and Lee, 2016). This oil also exhibited contact toxicity against two insect pests, including the cigarette beetle (*Lasioderma serricorne*) and the booklouse (Liposcelis bostrychophila), with LC_{50} values of 27.33 and 71.56 μg/cm^2, respectively (Yang et al., 2014). Makwan oil extracted from the fruit of Zanthoxylum myriacanthum also resulted in the 65% mortality of Rhipicephalus microplus (Nogueira et al., 2014). The above referenced evidence can be informative and beneficial in the development of essential oil preparations to control chicken mites and lice. Accordingly, it is worth noting that the plants included in this study are readily available from a number of different places in the world. These plants could be a potential high-value source in the development of natural insecticidal and repellent products due to their easy access and broad availability. For this reason, current applications of these essential oils in the poultry industry for the purposes of insect pest control has become of significant interest.

Therefore, the aims of this study were (i) to study the physical characteristics and chemical compositions of six essential oils including clove oil, citronella oil, lemongrass oil, Litsea oil, ginger oil, and Makwan oil; (ii) to study the insecticidal activity and efficacy of the selected essential oils against chicken lice and mites under laboratory conditions and in practical applications in animals; and (iii) to observe the toxicity of these substances to the health of animals after exposure to preparations of selected essential oils. The findings of this study can serve as preliminary information in the preparation of animal health products that can then be used in the control and/or elimination of chicken lice and mites on commercial chicken farms.

MATERIALS and METHODS

Plant essential oils

Six medicinal plants, including cloves (*Syzygium aromaticum* (L.) Merr. & LMPerry), citronella (*Cymbopogon nardus* Rendle.), lemongrass (*Cymbopogon citratus* (De ex Nees) Stapf.), Litsea (*Litsea cubeba* (Lour.). Pers.), ginger (*Zingiber officinale* (Roscoeand.)), and Makwan (*Zanthoxylum myriacanthum* Wallich ex Hook.f.), were included in this study. The raw plant materials (Table 1) used for essential oil distillation were collected from the Medicinal Plant Garden located at the Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand. Voucher specimens were deposited at the same location. All essential oils were distilled by in-house procedures. Briefly, all raw plant materials were freshly cut, air-dried in an oven at 70 ºC for 24 h, and milled into powder using an herb grinding machine. Powders were screened through an 80-mesh sieve and directly kept in zip-lock plastic bags at 4 ºC for further use. A measured amount of 150 g of dried milled powder was immersed in a 1-L clean tank. Then, 70% ethanol solvent was poured into the tank at a ratio of 1:7 and allowed to stand for 48 h. The extracts were decanted into another container, filtered through filter paper, and evaporated over a water bath at 60 ºC to obtain the essential oil.

Table 1 Chemical composition present in each type of essential oil.

Essential oils	Part of use	%Yield	Chemical composition	% Peak area
Clove oil	Flower bud	4.96	Eugenol	88.9
Citronella oil	Leaf	4.40	Citral	34.23
			Neral	26.71
			Limonene	17.15
Lemongrass oil	Leaf	0.3	Citral	42.33
			Neral	31.44
Litsea oil	Fruit	3.73	Citronellal	44.11
			Geraniol	17.40
Ginger oil	Rhizome	1.38	α-Zingiberene	30.21
			β-Sesquiphellandrene	13.04
			ar-Curcumene	10.47
Makwan oil	Fruit	9.63	2-Undecanone	50.04
			α-Phellandrene	11.84
			Limonene	11.61

Physical parameters of essential oils

Specific gravity

The determination of the specific gravity of the liquid fats and oils in the essential oils was carried out according to the method described by ASTM (2006) and Hati et al. (2010). In this method, the ratio of the weight of a unit volume of the sample to the weight of water at 20 °C with 40 % relative humidity was calculated using a digital electronic balance.

Refractive index

The refractive index of each essential oil was determined with the use of a Refractometer. The analysis was conducted in triplicate and average refractive index values were then reported. (Mieso and Befa, 2020).

Optical rotation

Each essential oil was diluted with analytical grade chloroform (Merck, New Jersey, USA) to yield a final concentration of 40 g/L. The optical rotation value was then measured using a CETI Polaris polarimeter. The reading process was run in triplicate at 20 °C (Noudogbessi et al., 2012).

Chemical composition in essential oils using GC-MS analysis

Six essential oils were analyzed on an Agilent 7890A Gas chromatograph (Agilent Technology, Santa Clara, CA, USA) - JEOL AccuTOF-GCv Mass spectrophotometer (JEOL, Ltd, Tokyo, Japan) equipped with a DB5-MS column (30 m × 0.25 mm i.d., 0.25 um film thickness, J&W Scientific, Folsom, CA). The oven temperature was programmed as follows: isothermal temperature was set to 40 °C for 1 min; the temperature was then raised by 6 °C/min to reach 250°C and held there for 4 min; helium was used as the carrier gas at a rate of 1.5 ml/ min; the effluent of the GC column was introduced directly into the source of the MS via a transfer line (280 °C); ionization was then obtained by electron impact (70eV, source temperature 230 °C). The scan range was ultimately determined to be 25–800 amu. Compounds were tentatively identified by a comparison of the mass spectra values of each peak with those of the authentic samples in the NIST MS library (Park et al., 2007).

Contact toxicity test

Filter-paper contact bioassay was used to evaluate the toxicity and persistence of the toxic and repellent effects of these essential oils towards lice and mites. Essential oils were diluted using a mixture of distilled water, absolute ethanol, and tween 20 at a ratio of 50: 25: 25 as a diluent to obtain concentrations ranging from 0.052, 0.104, 0.208, 0.416, and 0.832 μg/cm^2 for our study involving lice. In cases involving mites, essential oils were diluted using the same diluent at a different ratio of 40: 25: 35 to yield concentrations of 0.416, 0.832, 1.664, 3.328 and 6.656 μg/cm^2. Concentrations of the essential oils were selected based on our preliminary study by screening through a wide range of concentrations. We then determined the most effective concentrations for our purposes. The same diluents without essential oils were used as the negative control.

Adult chicken lice and mites were collected from native infected chickens. The genus and species of the lice and mites collected were confirmed through observations of the body and appendages by trained veterinarians and entomologists. The identification of the lice and mites was further confirmed with other related forms of evidence including literature reviews, as well as determinations of skin lesions and diseases, host specificity, season and temperature, and the prevalence rate of infestations. Field trips and visits were also undertaken to chicken farms and free-range farms in several areas located in Chiang Mai, Thailand to confirm the presence or absence of both parasitic insects. All insects were kept in a conical tube at ambient temperatures and taken to a laboratory within 3 to 4 h of being collected.

For the toxicity test, filter papers (WhatmanTM, Sigma-Aldrich, Missouri, USA) were dipped into containers holding desired concentrations of the essential oils. They were then dried for 3 min under a fume hood and placed on petri-dishes. Subsequently, 10 lice or 20-30 mites were transferred onto the plates and they were sealed with thermoplastic film (Parafilm® M, Sigma-Aldrich, Missouri, USA). The mortality rates of the lice and mites were observed every 10 min for 1 h intervals and then every 1 h for 24 h intervals. Three-independent assays were performed. The mortality rate at 24 h was calculated using the following formula (1).

% Mortality = (number of dead insects after being treated/number of insects treated) x 100 (1)

In addition, lethal concentrations (LC) of each essential oil used to kill insects at 50% (LC_{50}), 90% (LC_{90}), and 99% (LC_{99}) were formulated using the R Studio program of Windows.

In vivo assay

To study the effects of these essential oils on insects, citronella oil and ginger oil were selected to evaluate the lowest LC_{50}, LC_{90}, and LC_{99} values according to the R Studio calculation under in vitro investigation. This study was approved of by the Animal Ethics Committee, Maejo University, Chiang Mai, Thailand under approval number: MACUC 024A/2561.

Chickens infected with lice (n = 90) were obtained from the Royal Project Doi Kham Farm, Chiang Mai, Thailand. Chickens were randomly divided into 3 groups (10 chickens/group) and were provided with food and water ad libitum, while lighting was continuous. A three-independent replicate assay was performed. A baseline was established by counting the number of lice (t = 0) on each chicken at specific feathered areas of the back, chest, abdomen, both sides of the body, under the wings, at the vents, and around the cloaca (2.5 x 2.5 cm^2) of the birds. Then, the preparation of each essential oil (4% v/v, 5 mL/1 kg of chicken body weight) was sprayed on the same areas of each chicken recruited in group 1 (ginger oil) and group 2 (citronella oil), while chickens in group 3 were treated with 0.15% (v/v) trichlorfon at the same area. The number of lice was counted after the birds were treated for 1, 7, and 14 days.

In this study, to investigate the effects of the selected essential oils on mites, chicken nests carrying mites were surveyed on farms located at the area of Galyani Vadhana District, Chaing Mai, Thailand. Chicken nests and bedding materials that were confirmed to be infected with mites were divided into 3 groups with 3 replicates (n = 9). The first and second groups were treated by spraying a preparation of each essential oil (4% v/v, 5 mL) on the nests or bedding materials over an area of 20 x 20 cm^2, whereas 0.15% trichlorfon was used to treat chicken nests or bedding materials in the third group. Five sheets of the paper were placed into the nests or bedding materials for 5 min in order to collect the number of mites required to establish a baseline (t = 0) at 1, 7, and 14 days. Mites were counted by stunning them with 5 mL of absolute ethanol. The % incidence of lice and mites on day 0 was normalized to 100%, whereas the % incidence on the following days was calculated according the given formula (2).

Incidence (%) = (number of insects collected at Tx/number of insects collected at T0) x 100 (2)

Where T0 and Tx represented the initial number of insects on day 0 and on each sampling day (1, 7, and 14), respectively. Moreover, the toxicity of the selected essential oils on the health of the chickens was observed through visual inspections. The appearance of irritation on the skin of the chickens, along with certain respiratory related-symptoms, were indicative of a toxic reaction to the oils (Arthur and Axtell, 1982; Martin and Muliens, 2012).

Statistical analysis

Statistical analysis was performed using the R Studio program of Windows. Mean ± standard error values of triplicate experimental runs were reported. A statistical comparison was performed using the one-way analysis of variance (ANOVA) followed by Duncan's multiple-range test (DMRT). Furthermore, lethal concentrations of the essential oils needed to kill 50, 90, and 99% of the lice and mites (LC_{50}, LC_{90}, and LC_{99}, respectively) were determined using the R Studio program.

RESULTS

Physical parameters of essential oils

Essential oils, including those of cloves, citronella, lemongrass, litsea, ginger, and Makwan plants, were subjected to analysis for specific gravity, refractive index, and optical density. The specific gravity values of cloves, citronella, lemongrass, litsea, ginger, and makwan oil were 1.0547, 0.8874, 0.8911, 0.8762, 0.8742, and 0.8370, respectively, whereas the refractive index values were 1.5320, 1.4618, 1.4826, 1.4785, 1.4862, and 1.4479, respectively. The optical density values of each essential oil were -1.97, +0.34, -1.63, +6.83, -35.52, and + 9.89, respectively.

GC-MS analysis

The main composition of each essential oil was analyzed by GC-MS and is shown in Table 1. Eugenol represented the highest composition of clove oil, while citral, neral, and limonene were the main constituentss present in citronella oil. Citral and neral were also present as the main constituents in lemongrass oil, whereas citronellal and geraniol were found to be present in litsea oil. The α-Zingiberene was the major com ponent in ginger oil, followed by β-sesquiphellandrene, and ar-curcumene. Furthermore, 2-undecanone, α-phellandrene, and limonene were the main constituents in Makwan oil.

Contact toxicity test

The % mortality rate of lice after being treated with essential oils at 24 h is shown in Table 2. The mortality rate of lice rose significantly ($P < 0.05$) when the concentration of essential oil was increased. Interestingly, citronella oil completely killed lice by 100.0% at a concentration of 0.208 μg/cm^2, whereas the other essential oils were determined to be effective at a concentration of 0.416 μg/cm^2.

In addition, citronella and ginger oil exhibited a 100% mortality rate for mites at the lowest concentration of 0.416 μg/cm^2, followed by 0.832 μg/cm^2 for litsea and makwan oils, and 1.664 μg/cm^2 for clove and lemongrass oils (Table 3). Subsequently, the negative control (diluents) was not found to have affected the mortality of both insects.

After calculation for LC at 50, 90, and 99%, citronella and ginger oil were the most effective candidates to combat these parasitic insects (Table 4). Therefore, these essential oils were selected for further investigation.

In vivo assay

To study the insecticidal effect of certain selected essential oils on chicken parasites, only ginger oil and citronella oil were used (Table 5). The average count of lice exposed to the ginger oil preparation at the initial date was 602.0±102.81 and was normalized at an incidence rate of 100%. Ginger oil could effectively eliminate the lice at an incidence rate of 48.59% (n = 293.33±63.37) on day 1 of the treatment. The significant incidence ($p < 0.05$) of lice treated with the ginger oil preparation was observed on day 7 (35.03%; n = 214.00±61.26) and day 14 (22.67%; n =134.67±15.53), respectively. The number of lice treated with the citronella oil preparation was also reduced by yielding the % incidence from 100% (n = 726.7±144.8) to 65.55% (n = 479.3±120.9) on day 1 of the treatment. When the insects were exposed to citronella oil on day 7 and day 14, the incidence was found to have continually decreased (44.68%, n = 328.7±101.5 and 32.63%, n = 240.3±73.6, respectively). In contrast to the control group (trichlorfon), the number of lice on the initial date was 755.7±207.4 (100%), whereas the % incidence at each sampling time (on days 1, 7, and 14) was lower for both essential oil treatments (n = 58.2±27.3, 38.0±7.0, and 31.3±3.2, respectively).

Table 2 Mortality rate (%) of chicken lice at 24 h via residue filter paper contact assay.

Essential oils	% Mortality at different concentration (μg/cm2)				
	0.052	0.104	0.208	0.416	0.832
Clove oil	50.00±11.55^{a*}	66.67±18.56^{ab*}	80.91±5.01^{ab*}	100.00^{c}	100.00^{c}
Citronella oil	80.00±5.77$^{a\#}$	96.97±3.03^{b*}	100.00^{b*}	100.00^{b}	100.00^{b}
Lemongrass oil	76.67±3.33$^{a\#}$	76.67±6.67^{a*}	80.00±5.77^{a*}	100.00^{b}	100.00^{b}
Litsea oil	78.15±6.74$^{a\#}$	79.63±9.82^{a*}	90.00±10.00^{a*}	96.67±3.33^{a}	100.00^{a}
Ginger oil	80.00±11.54$^{a\#}$	83.33±6.67^{a*}	96.67±3.33^{a*}	100.00^{a}	100.00^{a}
Makwan oil	72.22±4.01$^{a\#}$	80.00±5.77^{ab*}	93.33±6.67^{bc*}	100.00^{c}	100.00^{c}

All values are presented as mean ± standard error values of triplicate experimental runs. Lowercase letters in the same row and symbols in the same column, which are connected by different letters, are considered significantly different ($P < 0.05$).

For the reduction and elimination of mites, citronella oil exhibited the best effective performance as an insecticide. The average number of mites counted reduced from 104.67±10.50 (100%) to 22.67±10.12 (21.89%) on day 1 of the treatment. However, this mite was resilient and the incident rate of this mite after being treated with citronella oil returned to 25.96% (n = 27.33±5.51) and 68.07% (n = 72.33±32.15) on days 7 and 14, respectively. For ginger oil, the initial number of mites counted was 74.00±7.94 (100%). This number rapidly reduced to 32.67±21.5 (44.88%) and 5.67±1.03 (8.24%) on days 1 and 7, respectively. However, the mite population recovered to yields of 28.3 (40.97%) on day 14 of the treatment. Similar to the outcomes of the lice study, the use of trichlorfon resulted in the lowest incidence of mites during this investigation.

Moreover, the use of both essential oils did not cause any side effects or have any harmful effect on either the skin or respiratory tracts of animals during the course of this assay.

Table 3 Mortality rate (%) of chicken mites at 24 h via residue filter paper contact assay.

Essential oils	% Mortality at different concentration (μg/cm2)				
	0.416	0.832	1.664	3.328	6.656
Clove oil	77.96±12.68$^{a\#}$	93.12±6.08$^{b\#}$	100.00^{b}	100.00^{b}	100.00^{b}
Citronella oil	100.00^{a*}	100.00^{a*}	100.00^{a}	100.00^{a}	100.00^{a}
Lemongrass oil	93.33±3.33$^{a*\#}$	97.78±2.23$^{ab\#}$	100.00^{b}	100.00^{b}	100.00^{b}
Litsea oil	87.30±6.49$^{a\#}$	100.00^{b*}	100.00^{b}	100.00^{b}	100.00^{b}
Ginger oil	100.00^{a*}	100.00^{a*}	100.00^{a}	100.00^{a}	100.00^{a}
Makwan oil	93.49±0.42$^{a*\#}$	100.00^{b*}	100.00^{b}	100.00^{b}	100.00^{b}

All values are presented as mean ± standard error values of triplicate experimental runs. Lowercase letters in the same row and symbols in the same column, which are connected by different letters, are considered significantly different (P < 0.05).

Table 4 Lethal dose of essential oils on chicken lice and mites via contact toxicity assay.

Essential oils	Lethal concentration (%)					
	Lice			Mite		
	LC_{50}	LC_{90}	LC_{99}	LC_{50}	LC_{90}	LC_{99}
Clove oil	0.46	9.06	19.45	1.44	85.66	177.57
Citronella oil	-	-	-	-	-	-
Lemongrass oil	0.79	32.6	69.06	2.90	95.95	197.50
Litsea oil	0.65	25.81	54.69	1.45	39.76	81.56
Ginger oil	-	-	-	-	-	-
Makwan oil	6.47	93.08	187.59	2.75	123.07	254.37

DISCUSSION

Plant-derived essential oils have been widely used as natural insecticides in various human and animal care products. Essential oils are known to be relatively non-toxic to humans and animals, and can be rapidly self-degraded in the environment. In this study, the physical parameters, including specific gravity, refractive index, and optical density of six essential oils, were evaluated. These physical parameters are concordant with those used in other reports. The physical parameters of ginger oil obtained from China were reported by Nandi et al. (2013). This oil exhibited specific gravity, refractive index, and optical rotation values of 0.9033, 1.4870, and -39.41, respectively, whereas Bangladeshi ginger oil exhibited values of 0.9176, 1.4877, and -38.24, respectively. With citronella oil, the values of specific gravity and refractive index were 0.9005 and 1.487, whereas the optical density value was recorded at -4.00 (Timung et al., 2016). An understanding of the physical parameters of each essential oil is necessary to define their desired specifications and to confirm the conformity of each oil. The refractive index provided a qualitative test of the purity of the essential oil but did not indicate the % purity; whereas, optical rotation can be used to analyze the purity of the oils. Specific gravity can be used as an indication of the density of the oil in relation to that of water. Notably, essential oils are lighter than water and immiscible with water to form a separate upper layer (Mestri, 2016).

In addition, the chemical compositions of each of the essential oils included in this study were also investigated. litsea oil extracted from L. cubeba is widely used as an aromatic refresher as it can promote an energetic feeling. Listea oil is known to contain geranial, neral, and limonene as its main constituents. These constituents are known to exhibit antimicrobial, antioxidant, anti-cancer, and anti-inflammatory activities (Kamle et al., 2019). Clove oil is produced by distilling the dried flower buds of the clove plant. It has been traditionally used as an antimicrobial agent and as a pain reliever

Table 5 Effect of essential oil preparations on incidences of infection involving chicken lice and mites.

Insects	Days	% Incidence[1]		
		Ginger oil	Citronella oil	Trichlorfon
Lice	0	100.00$^{a\#}$	100.00$^{a\#}$	100.00$^{a\#}$
	1	48.59±2.99^{b*}	65.55±3.00^{a*}	8.61±2.91^{c*}
	7	35.03±2.65^{b*}	44.68±4.07^{a*}	5.28±0.97^{c*}
	14	22.67±1.87^{b*}	32.63±2.07^{a*}	4.35±0.67^{c*}
Mites	0	100.00$^{a\#}$	100.00$^{a\#}$	100.00$^{a\#}$
	1	44.88±16.54^{a*}	21.89±6.01^{b*}	15.40±3.85^{c*}
	7	8.24±5.42^{b*}	25.96±1.87^{a*}	6.19±3.12^{b*}
	14	40.97±21.64^{b*}	68.07±14.14^{a*}	3.33±3.33^{c*}

[1]The % incidence of lice and mites at day 0 were normalized as 100%. All values provided as mean ± standard error values of triplicate experimental runs. Lowercase letters in the same row and symbols in the same column, which are connected with different letters, indicate significant differences (P < 0.05).

for toothaches, muscle pains, and respiratory illnesses. In addition, clove oil has been widely acknowledged as an insect repellant and has exhibited an anti-parasitic effect as a result of the eugenol contained within (Tian et al., 2015; Jairoce et al., 2016). Citronella oil and lemongrass oil can be obtained from a wide range of Cymbopogon species. These oils exhibit certain beneficial properties and are probably best known as natural mosquito repellants (Maia et al., 2011). They also possess antibacterial, antifungal, and anti-parasitic activities. These oils have relaxing effects on humans when delivered through inhalation, resulting in a sense of relief from stress and a stimulation in brain function (Li et al., 2013; Wei and Wee, 2013; Dawood et al., 2021). The major constituents of citronella oil (C. nardus) are geraniol (35.7% of total volatiles), trans-citral (22.7%), cis-citral (14.2%), geranyl acetate (9.7%), citronellal (5.8%), and citronellol (4.6%) (Nakahara et al., 2013), whereas geranial (31.5 to 39.9%), neral (30.1% to 34.5%), and myrcene (14.5% to 16.6%) are the major components of the essential oil of lemongrass (*C. citratus*) (Hanaa et al., 2012). In this study, Makwan oil was obtained from *Z. myriacanthum* through the distillation process. This oil is known to exhibit antimicrobial and anti-inflammatory activities (Ji et al., 2016). The fruits of *Z. myriacanthum* are usually used as a flavoring agent in food preparation processes such as in barbecuing and stewing, and in the roasting of meat and the boiling of fish. It has also been used for the treatment of insect bites and intestinal disorders. In addition, Makwan oil has also shown a protective effect against diabetes and oxidative stress (Dahab et al., 2019). The essential oil derived from the Makwan plant is rich in limonene, sabinene, α-pinene, and β-phellandrene (Li et al., 2014; Sriwichai et al., 2019). Ginger oil has been used in traditional medicine for long periods of time because of its potential health benefits and its safety. It has also been touted for its effectiveness in aromatherapy and in various topical applications. Ginger oil is extracted from the ginger root of *Z. officinale*. It has exhibited impressive antimicrobial, anti-viral, anti-inflammatory, antinociceptive, and antioxidant activities (Jeena et al., 2013; da Silva et al., 2018; Sririwichitchai et al., 2018; Wang et al., 2020). The essential oil of ginger has been characterized by a high percentage of sesquiterpenes (66.7%), monoterpenes (17.3%), and aliphatic compounds (13.6%). The predominant sesquiterpenes are zingiberene (46.7%), valencene (7.6%), β-funebrene (3.1%), and selina-4(14),7(11)-diene (1.0%). The major monoterpenes have been characterized as citronellyl n-butyrate (19.3%), β-phellandrene (3.7%), camphene (2.6%), and α-pinene (1.1%) (Sharma et al., 2016). However, the physical parameters and the chemical composition of each essential oil presented here differ slightly from those of the above reports. These differences might be due to the different sources of each collected plant, the cultivation area of each plant, the preparation process for each essential oil, as well as the extraction and distillation methods used to obtain each oil. Each step of the process could then have affected the physical parameters and chemical composition of each essential oil included in this study (Ncube et al., 2012; Liu et al., 2015; Liu et al., 2016; Ghosh, 2018). However, some chemical compositions of the essential oils obtained in this study were concordant to those of previous reports. This could indicate the effective bioactivity of these plants when they are used to develop insecticides.

The effects of several essential oils on insects have been reported. Essential oils, such as lemongrass oil, have exhibited promise as miticidal and ovicidal agents against mites (*Sarcoptes scabiei*) (Li et al., 2020). Ten essential oils, including lavender, bitter orange, geranium, tea tree, clove, eucalyptus, Manuka cade, Japanese cedar, and palmarosa oil, have exhibited potential efficacy against mites *S. scabiei* (Fang et al., 2016). Moreover, some other commercially available plant-derived essential oil products have also been found to be active against other insects. This is true of the citrus mealybug (*Planococcus citri* (Risso)), western flower thrips (*F. occidentalis* (Pergande)), the two-spotted spider mite (Tetranychus urticae Koch), the sweet potato whitefly B-biotype (*Bemisia tabaci* (Gennadius)), and the green peach aphid (*M. persicae* (Sulzer)) (Cloyd et al., 2009). This determination will ultimately serve as evidence to support the outcomes of this study.

In this study, the effects of six essential oils were examined in the in vitro assay. The results indicated that citronella and ginger essential oils were quite effective at lower concentrations when compared to the other oils. These results were concordant with the findings of other reports. Citronella oil was reported to be effective against thrips (*Frankliniella schultzei*) and the green peach aphid (Myzus persicae), which are transmitters of phytoviruses (Pinheiro et al., 2013), while ginger oil was effective against the red flour beetle (*Tribolium castaneum* Herbst) and the cotton leafworm (*Spodoptera littoralis* (Boisd.)) (Chaubey, 2011; Hamada et al., 2018). However, other oils have been found to be effective but at increased concentrations. Hence, citronella and ginger oils were selected to be developed as essential oil preparations of plants and were assessed for further in vivo analysis.

Essential oils exhibit specific modes of action on certain insects according to their chemical components. These essential oils may inhibit the activity of acetylcholinesterase, while their components can serve as antagonists of octopamine, a neurotransmitter in insect-receptors. Monoterpenes that are present in a variety of plant essential oils are neurotoxic to insects. For example, eugenol may act through the octpaminergic system by activating receptors for octopamine, which is known to be a neuromodulator. Eugenol mimics the action of octopamine and can increase the intracellular calcium levels of the insect body (Regnault-Roger, 1997; Enan, 2005; Regnault-Roger et al., 2012; Gonzalez et al., 2014). Moreover, the action of essential oils for cuticular penetration has also been reported (Tong and Bloomquist, 2013; Tak and Isman, 2015). The outer part of the insect exoskeleton, known as the epicuticle, consists of a wax layer. This layer limits water loss and serves as a kind of cement layer that protects the cuticle of the plant from external abrasions (Pritchard et al., 2015; Flochlay et al., 2017). The hydrophobic nature of oils can cause mechanical effects on parasites by disrupting the cuticular waxes and blocking the spiracles, leading to death by water stress or suffocation (Burgess, 2009). The mode of action of chemical acaricides against poultry red mites has been previously reported. These acaricides mostly target neurotransmitters and the synapses between neurons along with the synganglion tissue. These substances attack the voltage-gated Na^+ channels of pre-synaptic axons, propagating a continually depolarized membrane and leading to a loss of action potential and eventually mite paralysis. After paralysis, poultry red mites are unable to escape from the risk environment and become incapable of finding food, eventually resulting in death (Pritchard et al., 2015).

The results of the efficacy in vivo test revealed that citronella and ginger oil could affect the rate of lice decline from day 1 to day 14, whereas the number of mites in nests was found to have declined from day 1 to day 7. This may have been due to differences in the life cycles of lice and mites. The general life cycle of mites is approximately 1 week from the time they are hatched until they reach the mature adult stage (Sparagano et al., 2014), whereas lice are known to have a longer life cycle (3-5 weeks) (McCrea et al., 2005). This is why the recovery of mites was observed at a high rate on day 14. The volatility of the chemical components that are present in the two different essential oils tested could be a significant factor. The volatility could affect the mortality of both of the parasitic insects in this study. In our study, the preparation of ginger oil was found to be more effective in the control of chicken lice and mites than the preparation of citronella oil. The incidences of lice and mites treated with ginger oil were lower than those of the citronella oil. This means that ginger oil could effectively kill these insects more successfully than citronella oil. However, other reports have found that the use of a mixture of several plant leaves along with citronella can be very effective in the control of external parasites among egg-laying chicken operations. The leaves of the citronella plant did not affect the reduction of the lice population; however, mites were clearly affected. Citronella oil can exhibit an insect repellent activity due to the fact that it contains citronellal as the main active constituent, which has been classified as an acyclic monoterpene. This compound has been applied instead of N, N-diethyl-mtoluamide (DEET) in repelling and killing mosquitoes and insects. Furthermore, Limonene has been found to be able to control flea and mite infestations (Mekvichitsaeng et al., 1999). The diterpenoids in this essential oil, including columbin, solidagenone, limonene, nomilin, and helvolic acid, could achieve anti-growth and anti-feedant activities in insects (Singh & Upadhyay, 1993). In addition, limonene at 194.2 μg/cm^2 could affect the death of lice by 100% (Lee et al., 2019). Notably, ginger oil is rich in sequesterpene groups (major as α-zingiberene and ar-curcumene) and these molecules seem to be more slowly volatile than the chemical constituents that are present in citronella oil. This is why other authors of published studies have declared that citronella oil is considerably more effective than ginger oil. However, the use of ginger oil and its derivatives have had beneficial effects on poultry nutrition. Ginger oil can inhibit the growth of dangerous bacteria in the intestinal tracts of chickens. It can also improve blood and serum parameters, increase reproductive activities and muscle mass, and can increase body weight and laying rates, etc. (El-Hack et al., 2020). Hence, the use of certain essential oils, either citronella oil or ginger oil, may also confer a variety of other health benefits on chickens.

CONCLUSIONS

This study reported on the use of essential oils to eliminate lice and mites on commercially raised chickens. Ginger oil and citronella oil have exhibited the best degree of effectiveness at the lowest concentration of 0.208 μg/cm2 for lice and 0.416 μg/cm2 for mites in the in vitro assay. The chemical constituents present in these essential oils could be the active molecules that are related to their insecticidal effects on these parasitic insects. Both essential oils have been developed in preparations as insecticides. They have been found to be very effective and were able to control these parasites in vivo. Furthermore, chickens did not develop any harmful side effects after receiving treatments with these oils. The preparation developed here can be used to control lice and mites, but its use is still limited by the stability of its preparation, the formula optimization, and the duration of the essential oil action in vivo. There has also been a lack of studies involving mixtures of these essential oils. It is our contention that the findings of this study can be beneficially corroborated in future research.

CONFLICT of INTEREST

The authors declare that this research study was conducted without the influence of any commercial or financial relationships that could possibly be construed as a potential conflict of interest.

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AUTHOR CONTRIBUTIONS

NV, PT, SC, and KC conceived and designed the experiments. NV executed the lab experiments and analyzed the data. NV and WP prepared the first draft of manuscript. KC is the principle investigator of the project who was responsible for preparation of project proposal, procure funding, resource allocation, and manage human resource and along with PT and SC. All authors approved the final draft of manuscript.

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