ENTOMOLOGY

Bio efficacy of Cinnamaldehyde from *Cinnamomum verum* essential oil against *Culex quinquefasciatus* (Diptera: Culicidae)

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Abstract

This research aims to study the efficacy of *Cinnamomum verum* (Cv) extracts for ovicidal, larvicidal, and repellent activities against *Culex quinquefasciatus* mosquito vectors. The active components of *C. verum* or cinnamon oil by Gas Chromatography (GC) analysis showed the highest cinnamaldehyde at 83.53%. For ovicidal assay, *C. verum* essential oil at concentrations 12.5, 25 and 50 ppm at 72 h had 100% egg hatch inhibition and had a significant difference when compared to the control group ($p<0.05$). Larvicidal activity showed that concentrations of 25 and 50 ppm were highly effective in killing 100% mosquito larvae. Morphological changes in egg raft showed a faded color and eggs that seemed to have split from their raft while the larvae changed to a pale white wrinkled body with a destroyed inner tube of the body and were motionless. A Scanning electron microscope study showed that the eggshell and micropyle were wrinkled with the chitin peeled out. After treatment with cinnamon oil, larvae appeared to have a wrinkled body, the thorax and abdominal cuticle were also destroyed with high density of oil particles observed on mouth brushes and obstructing the spiracle. The repellent assay showed that cinnamon oil could repel both male and female mosquitos for up to 180 min. From the results, it was concluded that cinnamon oil had highly effective repellency against *Culex quinquefasciatus* adults and insecticidal activity on eggs and larvae stages evidenced by LC_{50} at 6.59±0.54, 9.07±0.67 and 36.91±7.56 ppm and its morphological changes indicated how the mosquito could not survive after cinnamon oil treatment hence this may be a useful alternative method that is green friendly for controlling mosquitoes in endemic areas.

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Introduction

Mosquito-borne diseases is a public health problem in many countries especially in the tropical areas such as India, and some parts of Thailand (WHO, 2014). Since 2017, Sakon Nakhon, located in Northeastern part of Thailand, has had higher cases from mosquito vectors after floods (reported from Sakon Nakhon provincial health office). *Culex* species, are highly prevalent in both urban and rural areas and are responsible for the transmission of Lymphatic filariasis; a disease which targets lymphatic system (WHO, 1982). The WHO's recommendation for prevention of vector borne diseases largely depends on vector control, which relies heavily on the use of synthetic chemical insecticides, including Dichlorodiphenyl-trichloroethane, Dieldrin and Malathion (WHO, 2014). The use of chemicals is advantageous because it is

a fast solution with rapid rate of knockdown and the strong mosquito excito-repellency is convenient but has an impact on the ecosystem such as water sources, housing and air pollution. Currently, there are many kinds of herbs used for preventing mosquitoes or insects such as *Cymbopogon citratus*, *Eucalyptus citriodora* oil, *Syzygium aromaticum*, *Curcuma longa* and *Zingiber officinale* (Phasomkusolsil & Soonwera, 2011; Kalaivani *et al*., 2012; Madreseh-Ghahfarokhi *et al*., 2018; Soonwera & Sittichok, 2020). The effects of plant compounds usually exert toxicity by multiple mechanisms of action such as ovicidals, larvicidals, pupicidals, adulticidals, oviposition deterrents, repellents, reproduction inhibitors (Amer & Mehlhorn, 2006).

Thai cuisine has many herbs and some of the herbs are widely used to prevent insect infestation. *Cinnamomum verum* or Cinnamon; a medicinal plant belonging to the family Lauraceae, is a small tropical tree that originated in Sri Lanka, East and Middle Asia (Shu *et al*., 2008). *Cinnamomum verum* is generally used in cooking many dishes. In Thailand, *C. verum* aromatic oils from the roots, bark, leaves, twigs and flowers are used in several aspects including pharmaceutical, food flavoring, cosmetics and repellent manufacturing industries (Ranasinghe *et al*., 2017; Hamidpour *et al*., 2015; Wijesekera, 1978; Nabavi *et al*., 2015). *C. verum* has various active compounds such as Cinnamaldehyde, cinnamate, cinnamic acid transcinnamaldehyde, cinnamyl acetate and eugenol (Singh *et al*., 2007; Senanayake *et al*., 1987). Moreover, traditionally cinnamon has been medically used for antitussive, anti-arthritis, anti-oxidant, anti-inflammatory antimicrobial, antifungal activities and reported on as repellent for insects such as *Lucilia sericata*, *Paederus fuscipes*, *Bemisia tabaci*, *Megalurothrips sjostedti* (Khater & Geden, 2018; Zhang *et al*., 2016; Abtew *et al*., 2015; Emilie *et al*., 2015), and including mosquitoes such as *Anopheles gambiae* (Deletre *et al*, 2015; Thomas *et al*, 2017) *Aedes aegypti*, *Anopheles stephensi*, *Culex quinquefasciatus*, (Amer *et al*, 2006). However, there are a few reports on *C. verum* effects on each life cycle stage of mosquitoes thus the present study aims to investigate the effects of essential oil from *C. verum* on all the stages of a mosquito; on the larvicidal, ovicidal, and repellent activities and morphology changes of *C. quinquefasciatus.*

Figure 1. *Cinnamomum verum* barks.

Materials and Methods

Study area

This study was carried out at Department of Thai Traditional Medicine, Microbiology and Parasitology Laboratories for mosquito's cultivation and Plant distillation at Faculty of Industry and Technology, Rajamangala University of Technology Isan, Sakon Nakhon campus from October 2018 – December 2019.

Plant essential oils preparation

Cinnamomum verum bark (50 Kg) were bought from Traditional shop, Yasothon Province), botanically identified by Botanist of Thai Traditional pharmaceutical unit (Figure 1). *C. verum* bark was extracted by Thermosyphon Distillation (Heat pipe heat exchanger).The machine was set to 100°C for 12 h and then solutions were filtered with 0.45 um of filter paper, after that cinnamon essential oil was kept and protected from light until use.

Chemical composition of *Cinnamomum verum* by GC-MS analysis

Gas chromatography-mass spectrometry (GC-MS) analysis was carried out to identify the components in cinnamon essential oil extracts using Shimadzu QP2020 NX, Japan with a HP-5MS column (30 m × 0.25 mm, film thickness of 0.25 mm) and GC-MS solution software. He (99.99%, 6 ml/min) was used as the carrier gas , the column temperature was retained at 60°C for 5 min and programmed to 280°C with a rate of 5 °C/min, the injector and ion source interface temperatures were 230 and 280°C, respectively, and injection volume 1ul in split mode (1:10). After that, the *C. verum* oil were identified through comparing mass spectrum and retention indices with those given in the library database by Scan mode.

Selective ion monitoring (SIM) was set for quantification and was suitable for identifying the sample components using a mass spectrum for cinnamaldehyde (Sigma®). The sample preparation for evaluating the linearity of the calibration curves in the cinnamaldehyde (Sigma®) solution ranged from 31.25, 62.5, 125, 250 and 500 ppm to confirm the recovery and amounts of compound of samples (*C. verum* oil 50 ppm). The area percentages were calculated by electronic integration of FID peak areas without the use of response factors correction.

Mosquito test populations

The *C. quinquefasciatus* strain was obtained from Parasitology and Entomology laboratory in the Faculty of Natural Resources from October, 2018 to January, 2019 and the ethics were approved by Rajamangala University of Technology Isan; ID 52/2561. Next to the water, a straw and a basin were set up at night to catch spawning mosquitoes. This population was brought to the laboratory along with its source water and shifted to a plastic container. The freshly emerged adults were shifted to a plastic cage (30×30×30 cm). After confirmation of the morphological characteristics, the sexes were separated within 24 h of emergence to avoid any chance of mating. All the experiments were conducted in standard laboratory conditions at 25±2°C and 70±5% RH with the 12/12 h (L/D). Adult females were fed on pig blood and adult males were fed on syrup. For mosquito's population management, other female mosquitoes (3-5 day old) were permitted to mate to obtain the egg and larvae for the insecticidal tests. The adults (both males and females) are then kept in the plastic cage for 4-5 days and maintained with the same condition, females were fed on blood to lay egg. The females will lay eggs two days after they feed

on blood, egg rafts on the surface of a suitable body of water, methods were adapted from previous study (Richards *et al*, 2012; Das, Garver and Dimopoulos, 2007; Laurence, 1985). Normally, one day after the female of eggs raft (~100-200 eggs/raft); the egg raft had light white color and was oval shaped; after 2-3 days, the color of eggs raft changed to dark-brown and floated above the water. The 3rd instar larvae was a clear brown long piped body with the stomach having 8-9 segments and it was constantly moving. Larvae were kept in plastic trays (25×35×5 cm) containing 0.5 g of sterilized diet (fish food powder).

Insecticidal activity of cinnamon oils against egg and larvae stages

The concentrations of the oils were determined according to a preliminary test and used in the range of 3.125 to 50 ppm and 6.25 to 50 ppm (diluted with 1%acetone in distilled water) for the ovicidal and larvicidal tests. The experimental conditions at 25±2°C and 70±5% RH with the 12/12 h (L/D). Samples were divided into 6 groups: 1 egg raft/group, after incubation and 10 of the 3rd stage larvae /group exposed with 5 concentrations 3.125 to 50 ppm and 6.25 to 50 ppm of cinnamon oil and diluents control were determined, observed and recorded larvae death at 24 h and 24, 48 and 72h for the and egg hatching testing. The criteria of larval death: loss of tufts of bristles of segments; reduced thickness of the exoskeleton and loss of integrity of the peritrophic membrane, indicated by shrinkage, and loss of definition observed in the internal organs of the larva (Pratti *et al*, 2015).

All experiments were done in triplicates. The percentage of hatchability and percentage of larva mortality after treatment were calculated, as the formula below (Botas *et al*., 2017):

Ovicidal activity

% of mortality = (Number of hatched larvae/ Total number of eggs) × 100

Larvicidal activity

% of mortality = (Number of dead larvae/ Number of larvae in treatment) × 100

Repellent test

The repellent activity of the cinnamon oil was performed according to the methodology described by a previous study (Govindarajan, 2011). One hundred of *C. quinquefasciatus* male and female adults, 4–5 days old, were kept in a net cage (30×80×30 cm^3). The repellent assay was divided into 4 groups: i) positive control pyrethrin the standard repellent (10% pyrethrins, KAYARI®,Thaporn marketing co., LTD, Thailand), ii) negative control group, cottons were soaked with syrup for male adult and animal blood for female adult at same concentrations, iii) cinnamon essential oil group at concentration 50 ppm, and iv) olive oil (diluents control) and all groups, cottons were soaked with 1,000 µl of solutions.

Landing counts were recorded at 0, ½, 1, 1½, 2, 2½, 3, 3½, 4, 4½, 5, 5½ and 6 h (s). The petri dishes with soaked cotton were removed and rotated between the exposure intervals to avoid position bias. The mean of percentage repellency for each group were calculated based on the data of the three replicates, following the formula (Govindarajan and Sivakumar, 2012) (2012).

$$\% \text{ Repellency} = [(T_a - T_b)/T_a] \times 100$$

Where T_a is the number of mosquito landings in the negative control group; T_b is the number of mosquito landings in the treated group.

Statistical analysis

All data were expressed as means ± standard error of triplicate measurements. Standard Deviation (SD) did not exceed 5% for the majority of the values obtained. The treatment means were subjected to a one-way ANOVA and Bonferroni correction test by SPSS V.16.0 and lethal Concentration 50% (LC_{50}) was subjected to Probit analysis.

Results

Identification active compounds by GC-MS analysis

Essential oils extracts of *C. verum* were analyzed by GC-MS and their quantitative compositions were determined (Table 1). The main components of essential oils were Cinnamaldehyde, (E)-, trans-.beta.-Ocimene, alpha.-Terpineol, Bicyclo[2.2.1]heptan-2-ol and Benzene, 1-(1-butenyl)-4-methoxy-, trans-, respectively (as shown in Table 1). The standard curve of cinnamaldehyde containing data and the results were represented in linear with a multiple R^2=0.996. Quantitative analysis of standard cinnamaldehyde compared to cinnamon oil extracts, 50 ppm, were shown area by area. The % area and retention times (RT) of sample cinnamon oil were cinnamaldehyde, (E)-42585548 (RT 2.759 min), Benzene 6.37 (RT 3.43 min) and Acetic acid 5.30 (RT 3.80 min) while standard Cinnamaldehyde, (E)- at 62.5, 125, 250 and 500 ppm % area were

Table 1. The major compounds present in *Cinnamomum verum* (cinnamon) oil by GC-MS.

Retention time (min)	Compound name	Area (%)
10.557	Cinnamaldehyde, (E)-	83.53
5.362	trans-.beta.-Ocimene	2.01
6.934	Eucalyptol	0.50
8.862	Benzenepropanal	0.77
9.080	endo-Borneol	0.66
9.381	alpha.-Terpineol	1.49
10.688	Bicyclo[2.2.1]heptan-2-ol,	1.29
12.054	Benzene, 1-(1-butenyl)-4-methoxy-, trans-	4.72
12.804	Acetic acid, cinnamyl este	2.67
29.049	Unknown	0.68
	Total	100

568957, 1434372, 5194439 and 9343177, respectively, at the same retention time of 2.75 min as cinnamon oil had area 42585548 (Figure 2).

The effect of cinnamon essential oil on the hatching of *C. quinquefasciatus* eggs

Cinnamon essential oil considerably influenced the viability of *C. quinquefasciatus* eggs in laboratory condition. The cinnamon essential oil at concentrations ranging from 0, 3.125, 6.25, 12.5, 25 and 50 ppm for 0-72 h showed different effects for percentage of egg hatching as 93.13±2.07, 53.84±6.32, 17.05±17.05, 0.00±0, 0.00±0 and 0.00±0%, respectively. The LC_{50} values of ovicidal assay were 3.31±0.31 ppm (Figure 3) and color changes showed the eggs of *C. quinquefasciatus* after cinnamon essential oil treatment were faded in color and seemed to have split from their raft (Figure 4).

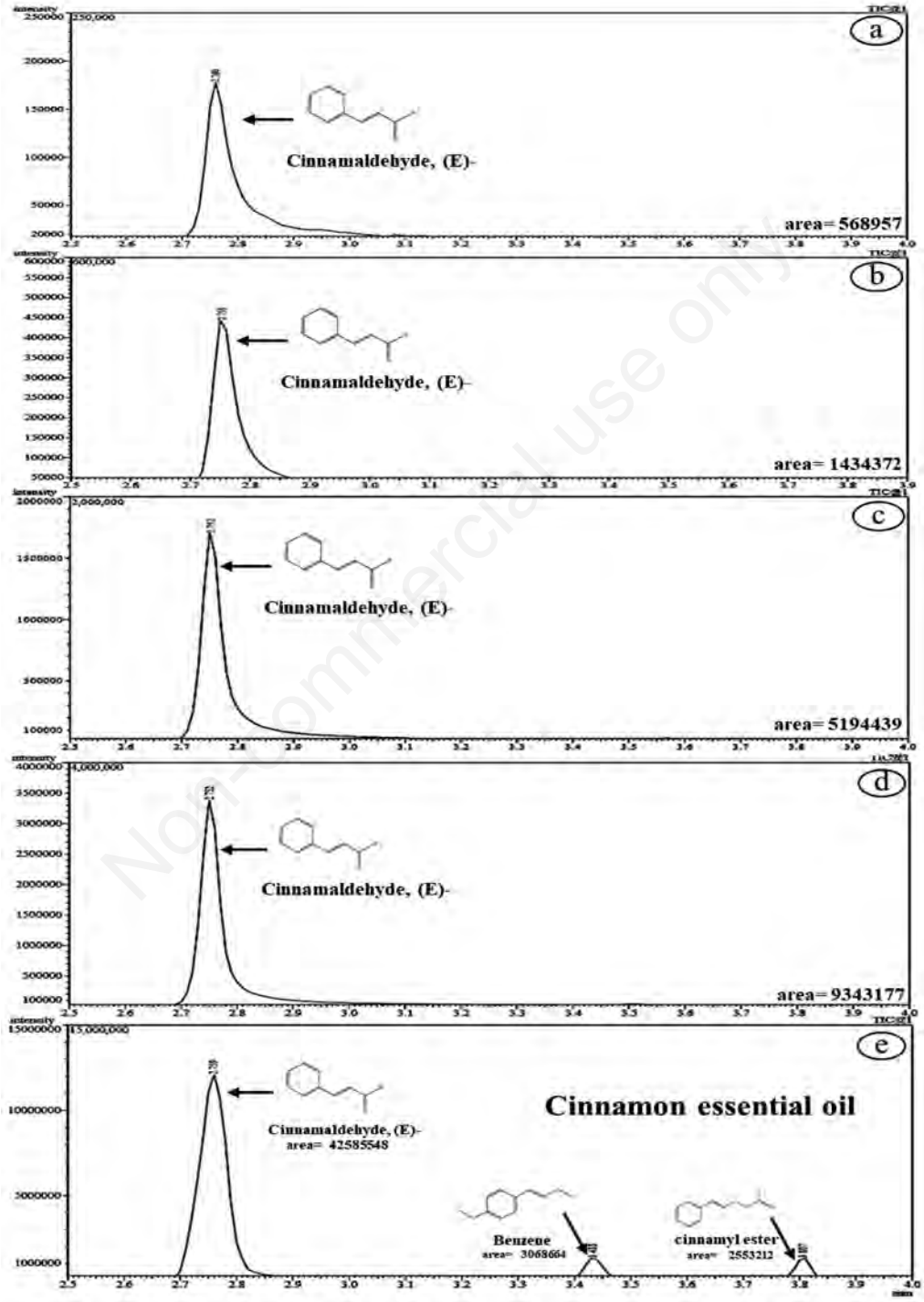

Figure 2. GC-MS SIM chromatogram analysis; standard Cinnamaldehyde, (E)- at concentrations of 62.5, 125, 250, and 500 ppm (a, b, c and d, respectively), in 50 ppm of *Cinnamomum verum* essential oil (e).

The effect of cinnamon essential oil on the survival of *C. quinquefasciatus* larvae

Cinnamon essential oil concentrations at 50% lethal concentration (LC_{50} values) for larvicidal test were 13.45±0.82 ppm and with 25 and 50 ppm of cinnamon oil extracts having the ability to kill 100% of larvae (Figure 5). Observation of morphological changes after treatment showed significant difference in the appearance from control group.In the control group; third instar larvae had clear brown body with the three regions of head, thorax and abdomen compact. The inner respiratory tube inside the abdominal were long; starting from the thorax to the end of the abdominal segment with the normal 9 segments in the abdomen and siphon is terminal outer tube are a respiratory organ and the surviving larvae had constantly moving. After cinnamon essential oil (treatment group), the larvae were characterized by a pale white wrinkled body with the inner respiratory tube of the body lost as a part of the body and it had decreased movement at 25 and 50 ppm no movements (Figure 6).

Repellent activity of cinnamon essential oil against *C. quinquefasciatus* male and female adults

The repellency test was divided into 4 groups: distilled water (control), olive oil (diluent control), cinnamon oil concentration of 50 ppm and 10% pyrethrin (positive control). Repellency for treatment groups on *C. quinquefasciatus* male and female were observed for up to 180 minutes. Results of repellency bioassay on male *C. quinquefasciatus*, observed at 180 min, of distilled water (control), olive oil (diluent control), 10% pyrethrin (positive control) and cinnamon oil concentration of 50 ppm groups were 0±0.00, 19.57±6.25, 89.66±5.80 and 95.18±4.58, respectively while those of female *C. quinquefasciatus* showed 0±0.00, 13.93±3.74, 86.78±10.36 and 86.07±3.75, respectively with significantly difference to control and olive oil groups. While cinnamon oil had similar effects with pyrethrin, both groups could provide 100% protection up to 0, 30, 60, 90, 120 and 150 min against both *C. quinquefasciatus* male and female (Figure 7).

The Scanning electron microscope study of *C. quinquefasciatus* eggs and larvae

Comparative morphometric and morphological studies of eggs and larvae under scanning electron microscope (SEM) were undertaken, in control and treatment groups (cinnamon oil). The morphology in eggs and larvae of *C. quinquefasciatus* after treatment differed from the egg's control group which showed a cylindrical shape in raft, rounded head-end and the egg raft was tidily arranged with a slightly wrinkled micropyle (Figure 8a-d). After cinnamon essential oils treatment at a concentration of 50 ppm, the eggs were found to have a wrinkled eggshell, dark skin, chitin peeling out and with withered characteristics, the eggs broke out from the egg terminals of the egg raft and a shrunk micropyle was also observed (Figure 8e-h).

Under scanning electron microscope, *C. quinquefasciatus* larvae control group, the head (Figure 8i,j), thorax (Figure 8s), abdomen (Figure 8t) and spiracle (Figure 8k,l) appeared normal: thick mouth brushes (Figure 8i), the antennae are normally developed (Figure 8j) and siphon and spiracle can pass air in and out (Figure 8k,l). After cinnamon essential oils treatment at a concentration of 50 ppm, the larvae was observed by SEM it was found that the body was wrinkled (Figure 8u-x). In addition, the thorax and abdominal cuticles were also wrinkled (Figure 8w,x), high density of oil particles was observed on mouth brushes (Figure 8m), the antenna were wrinkled (Figure 8n), particles found in cinnamon essential oils obstructed in the respiration on siphon and spiracle (Figure 8o,p).

Discussion

In this study, we investigated the effect of cinnamon essential oil extracts for the elimination of eggs, larvae, pupae and adults of *C. quinquefasciatus*. The essential oil isolated from the bark of *C. verum* was analyzed using GC-MS. The cinnamon essential oil was yellow-light in color and had an odor of cinnamon. Nine compounds accounting for 99.32% of all essential oil were identified. The main active compound in cinnamon essential oil extracts was cinnamaldehyde, (E)- 83.53% and it has the same structure and retention time with cinnamaldehyde standard (Figure. 2). According to a previous study on its chemical compositions, *Cinnamomum* genus, *C. zeylanicum*, bark essential oil had the

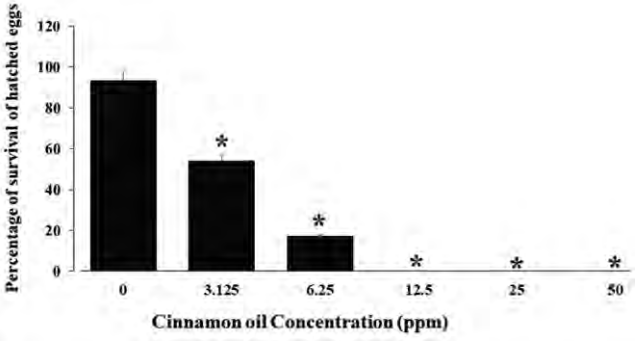

Figure 3. The percentage of survival of *C. quinquefasciatus* eggs after cinnamon oil treatment. *significantly different $p<0.05$ of control *vs* cinnamon oil groups.

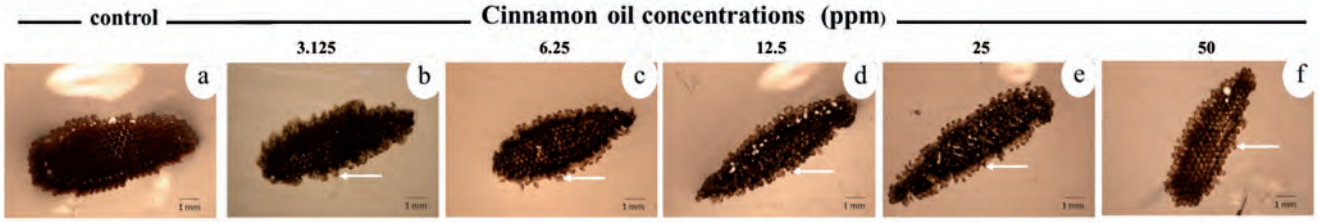

Figure 4. The morphological changes in egg raft, *C. quinquefasciatus*: (a) control (b-f) cinnamon essential oil extracts at 3.125, 6.25, 12.5, 25 and 50 ppm, respectively.

highest content of cinnamaldehyde at 77.34%, *C. verum* was found (E)-cinnamaldehyde at 52.87%, *C. cassia* was found (E)-cinnamaldehyde at 62.96 % and others (Batiha *et al*., 2020; Kallel *et al*., 2019; Liang *et al*, 2019)

The results of the test of *C. quinquefasciatus* eggs showed that the percentage of hatching from eggs to larvae was 0 ± 0.00 at a concentration 12.5, 25 and 50 ppm at 72 h (Figures 3 and 4). In consistent with the study, Veni *et al*. (2017) which reported that *Terminalia chebula* could kill adults of mosquito and inhibit the egg hatching (100% mortality) at 150 µg /ml *Ae. aegypti*, *An. gambiae* and *C. quinquefasciatus*. Similar report of Govindarajan *et al*. (2011b) showed that the crude extract of *Cardiospermum halicacabum* extracts of methanol and benzene exerted 100% mortality at 300 ppm against *C. quinquefasciatus* and *Ae. aegypti* attained the complete ovicidal activity at 400 ppm. The crude extract of *C. pulcherrima* exerted 0 hatchability on *C. quinquefasciatus*, *Ae. aegypti* and *An. Stephensi* at 375, 300 and 225 ppm, respectively. In agreement with this, Reegan *et al*. (2015) also reported that hexane extract of *Limonia acidissima* had high ovicidal activity at 79.2% and 60% on *C. quinquefasciatus* and *Ae. Aegypti* eggs, respectively.

Apart from this, the result of external surface changes by SEM study on *C. quinquefasciatus* egg hatching after exposure to cinnamon essential oils (Figure 8) showed that chitin wall may have been destroyed by the oils entering via an eggshell pore leading to embryo toxicity thus altering the egg hatching process (Ramkumar *et al*., 2019).

The results of larvae survival of *C. quinquefasciatus* after exposure to cinnamon oil at concentration 50 ppm for 24 h showed that 100% of larvae were killed (Figure 5). The death larvae were counted characterized by a pale white wrinkled body with the inner respiratory tube of the body lost as a part of the body and doesn't movements at all (Figure 6). The results of this study were similar to previous studies where *An. gambiae* larvae in the control group did not show any damage to their body parts; digestive and respiratory tracts were whole and well (Kringer, 2010). Pratti *et al*. (2015) reported larvae exposed to essential oil showed a toxic effectcs of the head, loss of tufts of bristles of segments; reduced thickness of the exoskeleton and loss of integrity of the peritrophic membrane, indicated by shrinkage, and loss of definition observed in the internal organs of the larva, reduced thickness of the

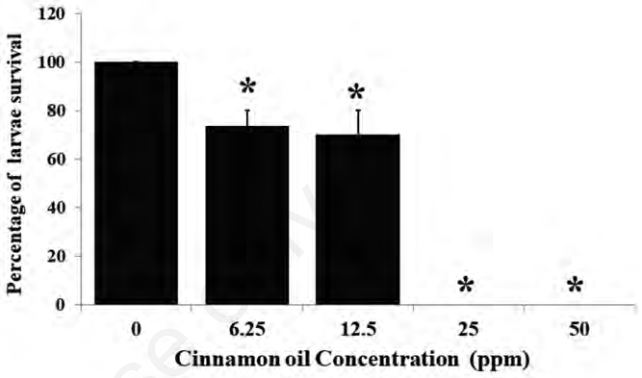

Figure 5. The percentage of survival larvae of *C. quinquefasciatus* after treatments. *significantly different $p<0.05$ of control vs cinnamon oil groups.

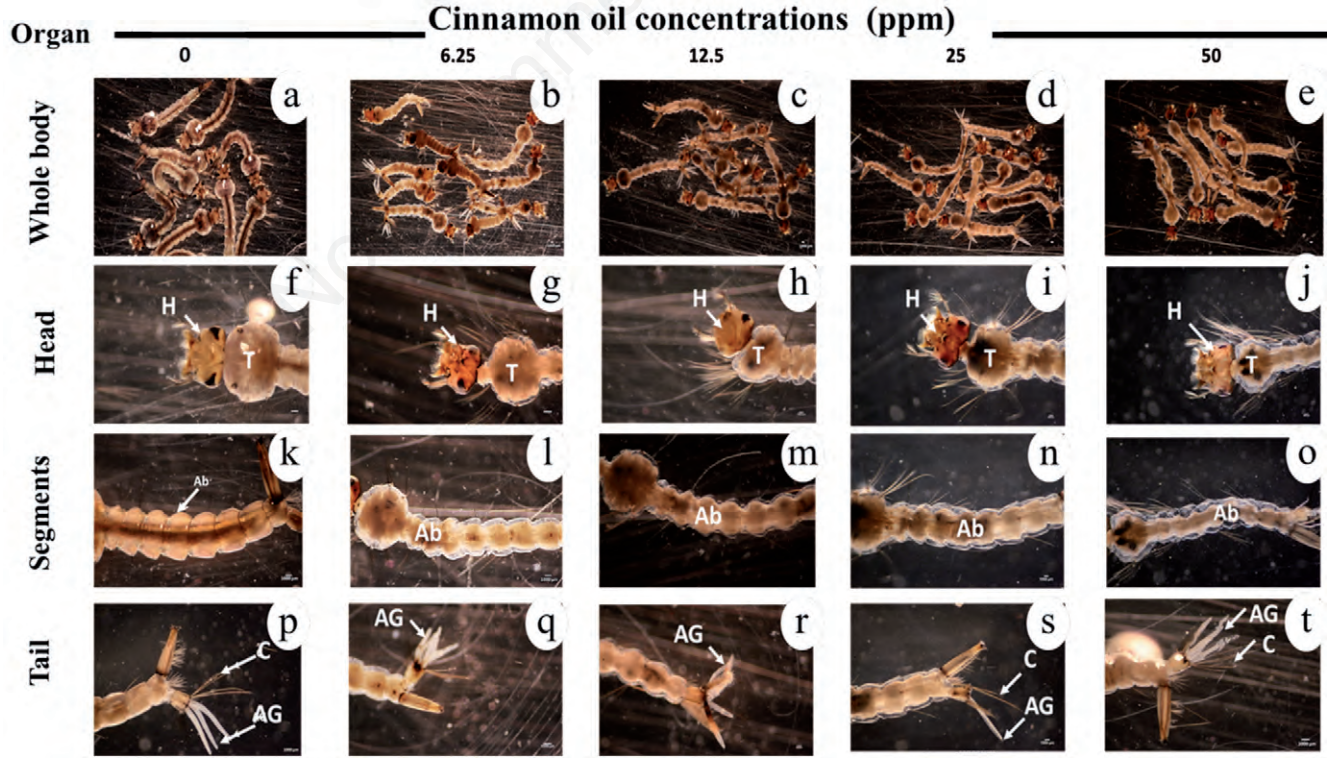

Figure 6. The morphological changes in *C. quinquefasciatus* larvae, (a, f, k, p) Control, (b, g, l, q) cinnamon essential oil 6.25 ppm, (c, h, m, r) cinnamon essential oil 12.5 ppm, (d, i, n, s) cinnamon essential oil 25 ppm, and (e, j, o, t) cinnamon essential oil 50 ppm, (Ab: Abdomen, C: Caudal hair, T: Thorax, H: Head, AG: Anal Gill).

exoskeleton and structural damage in the air siphon, Malpighian tubules and intestine. Similarly, Sutiningsih *et al*. (2019) found that the respiratory tube of *C. quinquefasciatus* larvae was folded, narrowed and the inside of the siphon damaged as well as blackened body and head. Tissues of the larvae lacked oxygen causing destruction of the nervous system and the damage to the siphon caused the larvae to have irregular breathing and eventually die. Essential oil damaged and its toxic compound could essential oil can increase the level of both cAMP and calcium in nervous cells (Jankowska *et al*, 2018). Similar observations on hindgut were also reported by Chaithong *et al*. (2006) in *Ae. aegypti* larvae when after treatment with pepper, damages to the respiratory and GI tracts or digestive tract (composed of gastric caeca, anterior midgut, posterior midgut and hindgut) physiological functions such as protein, lipid and carbohydrate metabolism, food absorptionand ionic balance (Marco *et al*, 2008). Essential oil caused disruption of the metabolism and respiration of the larvae so that it died. Among them, acetylcholine esterase enzyme breaks down the neurotransmitter acetylcholine which causes paralysis on the larvae (Fouad *et al*., 2018).

The results of the repellency test showed that cinnamon essential oil was repellent at concentration of 50 ppm providing 100% protection for up to 180 min against both *C. quinquefasciatus* males and females (Figure 7). In agreement with this, the essential oil of *S. guianensis* repellent values of 0.450 to 0.550 µg/cm^2 obtained 100% repellency against *Ae. aegypti* and *C. quinquefasciatus* for up to 120 min (*Aguiar et al*., 2015). It was also proved by Sritabutra *et al*. (2011) that lemon grass oil exhibited a high protection time of 98.66 and 98.00 min against *Ae. aegypti* and *An. dirus*, respectively, and the oil was also effective against *Ae. aegypti*, *An. dirus* and *C. quinquefasciatus* in which the protection time were 72, 132 and 84 min, respectively (Phasomkusolsil & Soonwera, 2011). Cinnamon is one of the essential oils with a pungent unique aroma which when detected through the antenna, mosquitoes will want to avoid the smell when they fly or touch the surface.

Normally, five types of stimuli are used by mosquitoes to locate hosts, namely:visual cues, water vapours, heat, CO_2 and body odor (McIver, 1978). The respective sensilla responding to these stimuli would be the compound eyes, grooved pegs, sensilla coeloconica, capitate pegs and sensilla trichoidea (Seenivasagan *et al*., 2009). Similarly, DEET action is also known to inhibit olfactory neuron receptors and masking attractive odors in *An. gambiae* (DeGennaro *et al*., 2013). DEET was identified as an acetylcholinesterase inhibitor when tested on neurons by (Corbel e*t al*., 2009). Abdelgaleil *et al*. (2009) suggested that these natural compounds monoterpenes from *Sitophilus oryzae* and *Tribolium castaneum* could act on several targets in the central nervous system of insects which is also an inhibitor of AChE, acetylcholinesterase (AChE) degrades the neurotransmitter at the cholinergic nerve synapse. When inhibited, acetylcholine accumulates and the receptors remain open, inducing paralysis and death; a mechanism similar that of organophosphorus and carbamate (Fukuto, 1990). Further, the repellent effect of cinnamaldehyde causes a possible interaction with specific odor receptors and/or Nav channels (Deletre *et al*., 2015).

As mention previously, cinnamon oil has shown numerous beneficial effects and had been a few reported on the toxic effect of cinnamon oil. Administration of cinnamon oil in vivo study could induce a significant increase in sperm motility, sperm count and reproductive organ weights in ameliorate sucrose-induced blood pressure mice (Preuss *et al*, 2006). For clinical trial demonstrated involving *C. zeylanicum* on oral treatment as regards symptoms, only one patient reported burning of mild intensity of the oral mucosa (Oliveira *et al*, 2014).

The use of cinnamon essential oils in pest control had been reported. The effective components of *Cinnamomum* species are cinnamaldehyde, benzaldehyde, and eugenol (Yang et al, 2005). Cinnamon oil from *C. zeylanicum* barks or leafs inhibited *P. humanus capitis* both eggs and adults, highest repellent activities to against *Lucilia sericata* (Khater and Geden, 2018), *A. stephensi*, *A. aegypti* and *C. quinquefasciatus* as well as leaf oil showed Lethal dose50 were 1.03 and 2.1 µg/mL against *A. tessellatus* and *C. quinquefasciatus* (Prajapati *et al*, 2005; Samarasekera, 2005). For presenting efficiencies of *C. verum* against on oviposition, larvae and pupae stages of *C. quinquefasciatus* (Andrade-Ochoa *et al*, 2018). In addition, species of *Cinnamomum* can against may kind of insects such head lice, *Musca domestica* and *Plodia interpunctella* (Benelli *et al*, 2008; Yang *et al*, 2005; Vel, 1996).

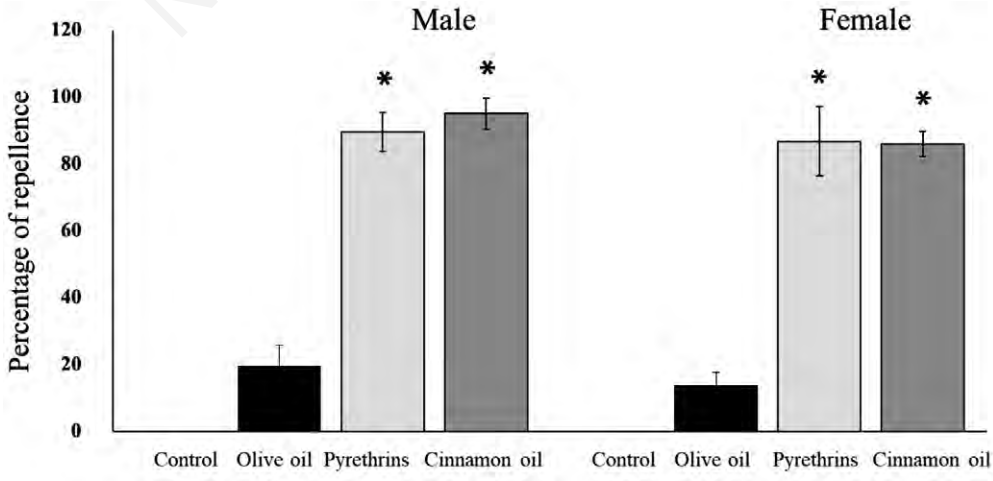

Figure 7. Repellency test of experimental group on adult males and females *C. quinquefasciatus*. *significantly different p <0.05 of control *vs* control groups.

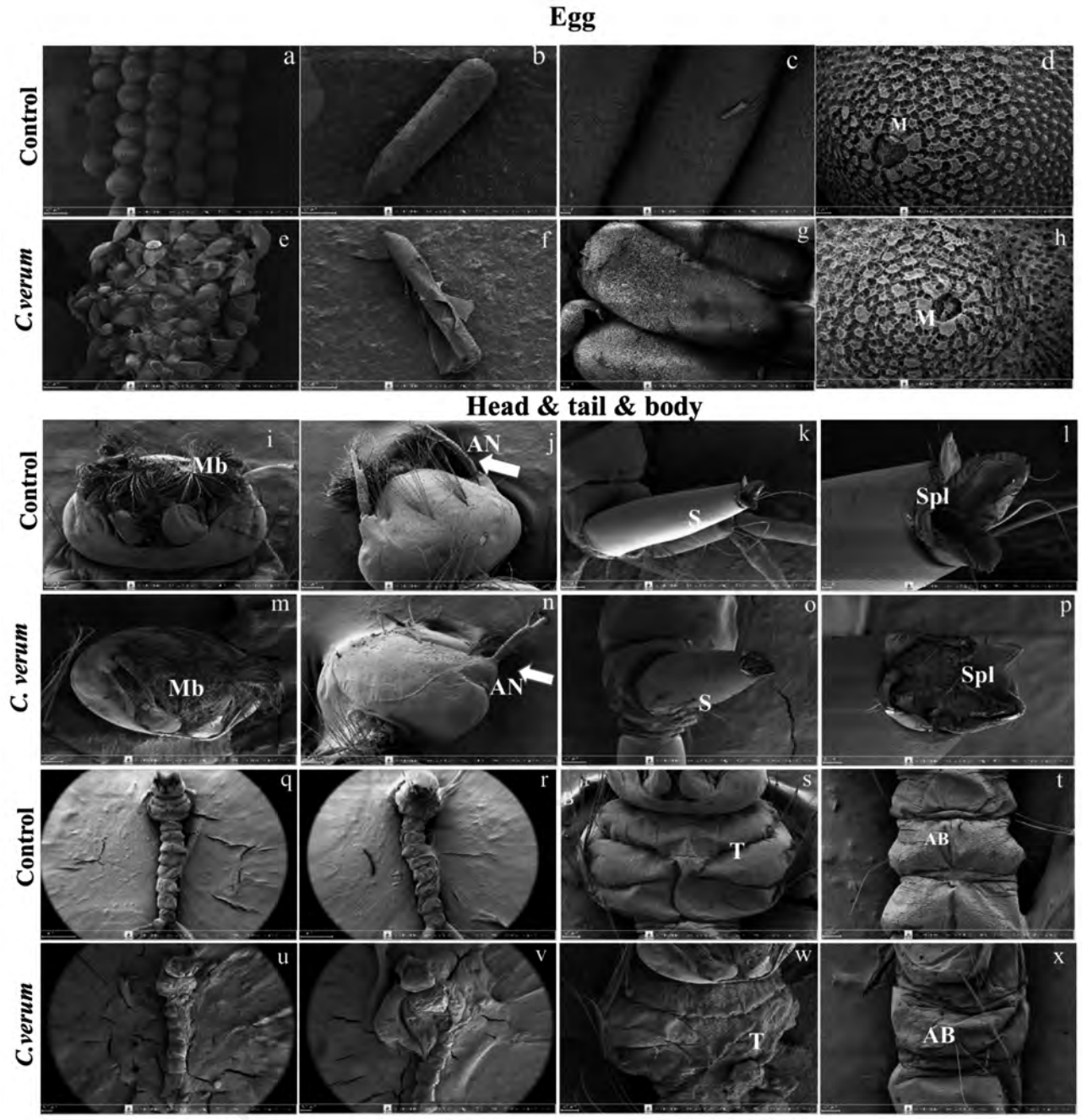

Figure 8. Scanning electron microscope of eggs surface of *C. quinquefasciatus* of control and after cinnamon oil treatment groups. (AB: abdominal part; An: antenna; Mb: mouth brush; M: Micropyle; S: siphon: Spl: spiracle: T: thorax).

Conclusions

This study concluded that cinnamon oil had high cinnamaldehyde ovicidal properties but their efficacies depend on concentrations and mosquito species. Egg hatching inhibition was dose-dependent manner. Overall, cinnamon oil showed a potential on larvicidal and repellent activities on *C. quinquefasciatus*. This study suggests further investigations to study other mosquito species to elucidate species-specific action of these compounds and conduct field trials.

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