



Composition of Three Zingiberaceae Essential Oils and Their Efficacy Against the Survivability of Cocoa Pod Borer, *Conopomorpha cramerella* (Snellen) Eggs

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ABSTRACT

The use of botanical extracts derived from potential plants is promising due to their target-specific, biodegradable, and can be implemented in insect management programs. This study was conducted to observe the potential of three Zingiberaceae essential oils; Lesser galanga, *Alpinia galanga*, Turmeric, *Curcuma longa*; and ginger, *Zingiber officinale* against the Cocoa pod borer, *Conopomorpha cramerella* (Lepidoptera: Gracillariidae) - the most devastating pest of cocoa in the Southeast Asia region. Bioassay on the *C. cramerella* eggs were performed using four different concentrations of EOs at 100, 200, 400, and 800 ppm. It is found that the *A. galanga* performed as the best EOs that can disrupt egg hatchability ($0.025b \pm 0.158$), where only 0.03 eggs hatched and significantly different ($p < 0.05$) with control ($2.367a \pm 0.928$), where 2.37 eggs were successfully hatched. The concentration of EOs at 800 ppm was able to influence the penetration rate of pre-larva on the cocoa pod. During large cage observation, the mean of *C. cramerella* eggs were the highest at control ($0.900a \pm 1.029$) and significantly different ($p < 0.05$) with *C. longa* ($0.150b \pm 0.483$), *A. galanga* ($0.050b \pm 0.221$) and *Z. officinale* ($0.025b \pm 0.158$). Higher concentrations (400 and 800 ppm) able to hinder *C. cramerella* from depositing eggs after cocoa pods were treated with treatments. The effect of Zingiberaceae EOs towards the egg hatchability may provide a foundation for their potential in managing *C. cramerella* in the future.

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Introduction

The emergence of Cocoa pod borer (CPB), *Conopomorpha cramerella* (Lepidoptera: Gracillariidae) had become a serious threat to cocoa plantation in Malaysia, and in the Southeast Asia region. Previously, this pest was declared as an invasive alien insect species (IAS) to Malaysia, and never becomes a nuisance pest to the cocoa industry. The insect started attacking cocoa plantations during 1980 at Sabah state, 1983 at Sarawak state and during 1986 in greater Peninsula Malaysia. Since then, the cocoa planting was struggling with the effective management since its severe infestation may lead up to 100% yield loss if left untreated.

The life cycle of *C. cramerella* is relatively short, approximately 27 to 33 days. An adult female lays eggs

singly or in groups of two or three on the cocoa pod surface and freshly laid eggs are orange in color with a length less than 0.5mm. The *C. cramerella* is capable to lay up to 300 eggs during their maturity stage (Lee *et al.*, 2013), and continuous infestation may lead to significant yield loss (Azhar *et al.*, 2000). The egg stage lasts for '2-7 days'. First instar larvae usually tunnel through the eggshell and bore into the pod surface until reaching the sclerotic layer of the husk. The entire larval stage takes 14-18 days to complete, with 4-6 instars. Subsequently, the larvae tunnel out through the pod wall and leave a sign of exit holes. Pupation occurs outside the pod within the oval-shaped silken cocoon on another part of the canopy, on the furrow of the pod, green or dried leaves and other debris. Completion of the pupation stage usually takes 6 to 8 days (Saripah *et al.*, 2019). An adult

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emerged after completing the pupal stages and often rests transversely underneath the jorquette branches, especially in shady areas. Prominent symptoms of *C. cramerella* infestation can be observed on the cocoa pods, with uneven yellowing or premature ripening due to the presence of larval tunneling inside the pod. Cocoa beans were hardened and clumped together, that leads to difficulty in extracting from the pod husk and mucilage (Lee et al., 2013).

Various attempts have been undertaken to manage the infestation caused by this tiny moth. To check the yield loss, various management practices such as, schedule insecticide spraying, pruning, fertilization, and weekly pod harvesting were proven effective in reducing the infestation rate (Saripah and Alias, 2016). Although various mitigation techniques were promoted, the use of chemical insecticides is still the most adopted approach among the cocoa growers (Alias, 2011). Growers usually prefer a technique that is cheap, easy to handle, with long-lasting results, and give high returns. Depending on the pest and country, most the cocoa farmers believe in the effectiveness of chemical application and continue using it as a primary control approach (Bateman, 2008). Biweekly prophylactic treatments, or all year spraying with chemical insecticides are widely implemented among cocoa growers, giving a total of 24 rounds in a year (Lee et al., 2013). Insecticides that have been recommended for the suppression of *C. cramerella* population is mainly from the pyrethroid group, which have fast knockdown characteristics but their repeated applications may hasten the resistance build-up in the pest populations (Azhar et al., 2000). Pyrethroid group is the most common insecticide use in cocoa, and implemented worldwide, which accounting for more than 30% of global use (Shukla et al., 2002).

Apart from the effectiveness, excessive and prolonged use of chemical insecticides may risk of insecticide resistance, effects to the non-target organisms and beneficial insects, pollinators, and pose environmental problems and health risk of the grower. Goodel et al., 2001; Fishel, 2011; Saripah, 2014; Colosio and Moretto, 2008; Hemingway et al., 2002; Joshi et al., 2000; Li et al., 2007). To reduce the risk of pest resistance, the application of chemical insecticides must be limited in cocoa plantations.

Alternative control approach should be investigated, with minimal risk to the cocoa growers as well as the environment. Many plant products and chemicals may play a role as oviposition deterrent, insect repellent, antifeedant either as larvicidal, pupicidal or adulticidal activities. It has been proven effective against several agricultural pests in Africa (Agboka et al., 2009). This botanical insecticide is relatively target specific,

biodegradable, and can be used in insecticide resistance management programs. Parkash and Rao (1997) had listed more than 800 plant species having insecticidal and repellent antifeeding effects, and their potential to be implemented in agriculture sectors.

Zingiberaceae (Order: Zingiberales) is one of the largest families in the plant kingdom, with more than 1,000 species were documented (Wohlmuth, 2008). The importance of Zingiberaceae plants may be due to the presence of essential oils such as limonene, eugenol, pinene, and geraniol (Habsah et al., 2005). Several important Zingiberaceae plants such as *Kaempferia galanga* (galangal), *Curcuma longa* (turmeric), *Alpinia galanga* (lesser galanga) and *Zingiber officinale* (ginger) were widely studied for the food preservatives, medicinal uses, chemical compositions and their interaction with insect species. Three Zingiberaceae essential oils (*Zingiber officinale*, *Curcuma longa*, and *Alpinia galanga*) were also studied on their pesticidal activities (Saripah et al., 2017) and as adult emergence inhibition (Saripah et al., 2019) An integrated approach of managing *C. cramerella* using two plant extracts including *Z. officinale* suggested that this species could be used in managing the CPB pest using the push pull system in the trial at Papua New Guinea (Iamba and Masu, 2020). They were also found to be effective against Spotted Wing Drosophila (SWD), *Drosophila suzukii* (Diptera: Drosophilidae) and the Brown Marmorated Stink Bug (BMSB), *Halyomorpha halys* (Hemiptera: Pentatomidae) (Saripah and Zhang, 2018).

Based on the promising results in the previous reports, the present study was expedited with the objective to investigate the effect of three Zingiberaceae essential oils towards the egg survival of *C. cramerella* after treating with *Z. officinale*, *C. longa* and *A. galanga*.

Materials and Methods

Source of Zingiberaceae essential oils

The sources of pure essential oils (Galangal essential oil, ginger essential oil and turmeric essential oil), were purchased from Best Formula Industries Malaysia (BF1 Malaysia), a local authorized manufactured and supplier of essential oils in Malaysia.

Chromatography to extract Zingiberaceae essential oils

The analyses of volatile component for each EOs were extracted using Solid Phase Micro Extraction (SPME) using a Gas Chromatograph Mass Spectrometry (GC-MS) at Cocoa Innovative and Technology Center (CITC), Malaysian Cocoa Board, Nilai, Negeri Sembilan, Malaysia. The analysis of volatiles extracted by Solid Phase Micro Extraction (SPME) was carried out using an Agilent Gas Chromatograph Mass Spectrometry (GC-MS)

Agilent 5975C equipped with a CTC PAL3 Autosampler. The injector port has a deactivated glass SPME liner 0.75mm I.D supplied by Supelco. The GC was fitted with a 30m capillary column HP-5MS 5% Phenyl Methyl polysiloxane with a 0.25 mm i.d. Volatile compounds were extracted using SPME fiber coated with polydimethylsiloxane / divinylbenzene (PDMS/DVB) 65 μ m. The liquid sample 2.5 μ L was placed in the SPME vial and conditioned for 30 min at the extraction temperature (140°C). The fiber was exposed for 10 min to the headspace of the vial for extraction purposes. The volatiles extracted by the fibers were thermally desorbed within 2 min and introduced in the capillary column. The GC was set up with a constant flow of 1.0 ml/min (helium), the oven temperature was programmed to start at 60 °C for 2 min, increase to 200°C in heating rate 10°C/min before further heated to 280°C at a heating rate 15°C/min and hold for 2 min. The MS was set up with the source at 280°C, where electronic ionization energy was -70 eV and with a 1200V in the detector. The compounds were identified by a combination of the US National Institute of Standards and Technology (NIST) 2011 library of mass spectra.

Bioassay

Treatments of *Z. officinale*, *C. longa* and *A. galanga* EOs were prepared at four different concentrations; 100 ppm, 200 ppm, 400 ppm and 800 ppm with the addition of a nonionic surfactant, Polyoxyethylene (20) Sorbitan monooleate (Tween 80), as per prior reports (Saripah *et al.*, 2017; 2018). Each concentration was vortexed for 1 to 2 minutes at 1800 rpm. Control was prepared using the same amount of Tween 80 with addition, water up to 100 ml. Concentrations were prepared 24 hours in advance, stored in dark amber glass bottle at 4°C prior to the experiment. The concentration of EOs was tested on the egg of *C. cramerella*. The observation was divided into two different phases, 1) Laboratory observation where direct spraying of treatments were conducted on the *C. cramerella* eggs 2) Large cage observation where the number of deposit eggs and entry holes was recorded. For laboratory observation, wild *C. cramerella* eggs on the developed cocoa pods were marked and brought back to the laboratory. Eggs were left remain undisturbed on the cocoa pods surface, and three eggs were used for each treatment. Eggs then were directly sprayed using a hand sprayer with different concentrations. The number of pod surface penetration for individual treated eggs was observed at Day-5. The experiment was repeated four times for each EOs and control; and the data collected was based on the number of entry holes, as a sign of successful egg hatching.

Observation of the large cage was started where adult *C. cramerella* (10 to 15 pairs) was allowed to mate in a

transparent container for 48 to 72 hours. Before the introduction of the adult, ten cocoa pods with no previous symptom of infestation were allocated for each treatment. Pods were sprayed with different treatment of a range of 15cm using a hand sprayer. Pods were air-dried for two hours, transferred to the large cage, and *C. cramerella* adults were then released into the cage. The number of eggs deposited on cocoa pods was recorded every 24 hours, until Day-3. The number of penetration entry on the epicarp was observed using the slicing technique, where a thin layer of pod surface was carefully peeled off using a sharp knife. Successful oviposition was found based on the number of deposit eggs after pods were treated with treatment and the number of penetration of eggs on the epicarp of the cocoa pod.

Statistical analysis

Data collected from these observations were arranged separately based on the treatments and replicates in Microsoft® Excel 2007. All data were subjected to statistical analysis and a Duncan's Multiple Range Test (DMRT) analyzed significant differences in SAS software from SAS® Version 8. The interpreted result was considered significant if $p < 0.05$ in One-way Analysis of Variance (ANOVA) and PROC GLM.

Results

Volatile compounds of Zingiberaceae essential oils

The analysis of volatiles extracted from SPME analysis for *Z. officinale*, *C. longa* and *A. galanga* are shown in Table 1. *Z. officinale* recorded the highest number of peaks throughout the study, as well as the number of volatile constituents in the essential oil. Some of the essential components observed were camphene, alpha-phellandrene, alpha-pinene, beta-pinene, citronellol, geraniol, eugenol, caryophyllene, terpineol and eucalyptus. There were 17 peaks and 35 volatiles observed from *C. longa* EO, with benzaldehyde, pentadecane, dodecanoic acid, isopropyl myristate, hexadecanoic acid, linoleic acid and isopropyl stearate found in oils. Meanwhile, 20 peaks and 33 volatiles observed in *A. galanga*, with several important volatiles; i.e. 1-propanol, 2-propanol, benzaldehyde, ar-turmerone.

Laboratory bioassay

Laboratory observation on the direct spray of treatments on the *C. cramerella* eggs are shown in Table 2 and Figure 1. The results denoted that the number of entry holes at different concentration of treatments was low throughout the data collection (Table 2). *A. galanga* performed as the best EOs (Figure 1a) that are able to disrupt egg hatchability ($0.025b \pm 0.158$) and significantly different ($p < 0.05$) with control ($2.367a \pm 0.928$).

Regardless of disparate treatment, the concentration of Zingiberaceae EOs at 800 ppm able to influence the penetration rate of pre-larva of *C. cramerella* (Figure 1b) on the cocoa pods. All Zingiberaceae performed better than control throughout this laboratory observation.

Caged bioassay

In the caged bioassay, the amount of eggs deposited after pods were sprayed with different treatments was counted every 24 hours for three days and the results are presented in Table 3. Mean of *C. cramerella* eggs and entry holes were illustrated at Figure 2. The mean of eggs were the highest at control ($0.900a \pm 1.029$) and significantly different ($p < 0.05$) with *C. longa* ($0.150b \pm 0.483$), *A. galanga* ($0.050b \pm 0.221$) and *Z. officinale*

($0.025b \pm 0.158$). The results denoted that *C. longa* able to reduce the number of entry holes ($0.125b \pm 0.335$), followed by *A. galanga* ($0.200b \pm 0.516$) and *Z. officinale* ($0.525b \pm 0.960$). Control treatment recorded the highest number of entry holes ($2.100a \pm 1.954$), and successfully penetrated the pick-up more than all Zingiberaceae EOs.

Higher concentration (400 and 800 ppm) ability to hinder *C. cramerella* from depositing eggs after cocoa pods was treated with treatments (Figure 3a). No eggs were observed from these high concentrations, compared to 100 and 200 ppm. Similar observations were observed where no visible entry holes were recorded at the concentration of 800 ppm for all Zingiberaceae EOs (Figure 3b).

Table 1. Important components derived from SPME analysis

Essential oils	No. of peak	Retention time	No. of volatiles	Important components
<i>Z. officinale</i>	45	5.458-22.603	94	Camphene, alpha-phellandrene, alpha-pinene, beta-pinene, citronellol, geraniol, eugenol, caryophyllene, terpineol and eucalyptus
<i>C. longa</i>	17	7.186-22.618	35	Benzaldehyde, pentadecane, dodecanoic acid, isopropyl myristate, hexadecanoic acid, linoleic acid, isopropyl stearate
<i>A. galanga</i>	20	8.199-22.608	33	1-propanol, 2-propanol, benzaldehyde, ar-turmerone

Table 2. Mean of *C. cramerella* entry holes after direct spray of treatments on the eggs

Essential oils	n	Treatment	Mean of <i>C. cramerella</i> entry holes \pm sd
<i>Z. officinale</i>	10	100 ppm	0.100 b \pm 0.316
	10	200 ppm	0.100 b \pm 0.316
	10	400 ppm	0.000 b \pm 0.000
	10	800 ppm	0.000 b \pm 0.000
<i>C. longa</i>	10	100 ppm	0.400 b \pm 0.699
	10	200 ppm	0.200 b \pm 0.422
	10	400 ppm	0.100 b \pm 0.316
	10	800 ppm	0.000 b \pm 0.000
<i>A. galanga</i>	10	100 ppm	0.100 b \pm 0.316
	10	200 ppm	0.000 b \pm 0.000
	10	400 ppm	0.000 b \pm 0.000
	10	800 ppm	0.000 b \pm 0.000
Control	30	Water	2.367 a \pm 0.928

Discussion

Z. officinale, *C. longa*, and *A. galanga* species were selected due to the presence of chemical components such as camphene, camphor, 1-8 cineole and α -humulene in their rhizome, and these chemicals are effective against *Sitophilus zeamais* and *Tribolium castaneum* (Suthisut et al., 2011). Throughout 10-day observations on the effects of Zingiberaceae EOs towards the Brown marmorated Stink Bug, BMSB (*Halyomorpha halys*) and Spotted Wing Drosophila, SWD (*Drosophila suzukii*), a promising result was obtained where *A. galanga* performed as the most potential as egg hatchability deterrence of BMSB and able managed to reduce life stage emergence of SWD (Saripah and Zhang, 2018).

The results of volatile components obtained from SPME analysis were in agreement with Saripah and Zhang (2018) where GC-MS analysis denoted that Zingiberaceae EOs consists of several chemical elements that may be useful for pest control. In their observation, *Z. officinale* contains camphene, myrcene (sedative), limonene (botanical insecticide), linalool, 2-undecanone (insect repellent), elemenes (pheromones), farnesene, camphene, butylated hydroxytoluene (pesticide ingredient) and naphthalene. Alpha-pinene, camphene, phellandrene, limonene, 1,8-cineole, benzene, farnesene and phenol were found in *C. longa*. Meanwhile, alpha-pinene, limonene, 1,8-cineole, benzene, phenol and cinnamic aldehyde that used in fungicide ingredients were obtained *A. galanga*.

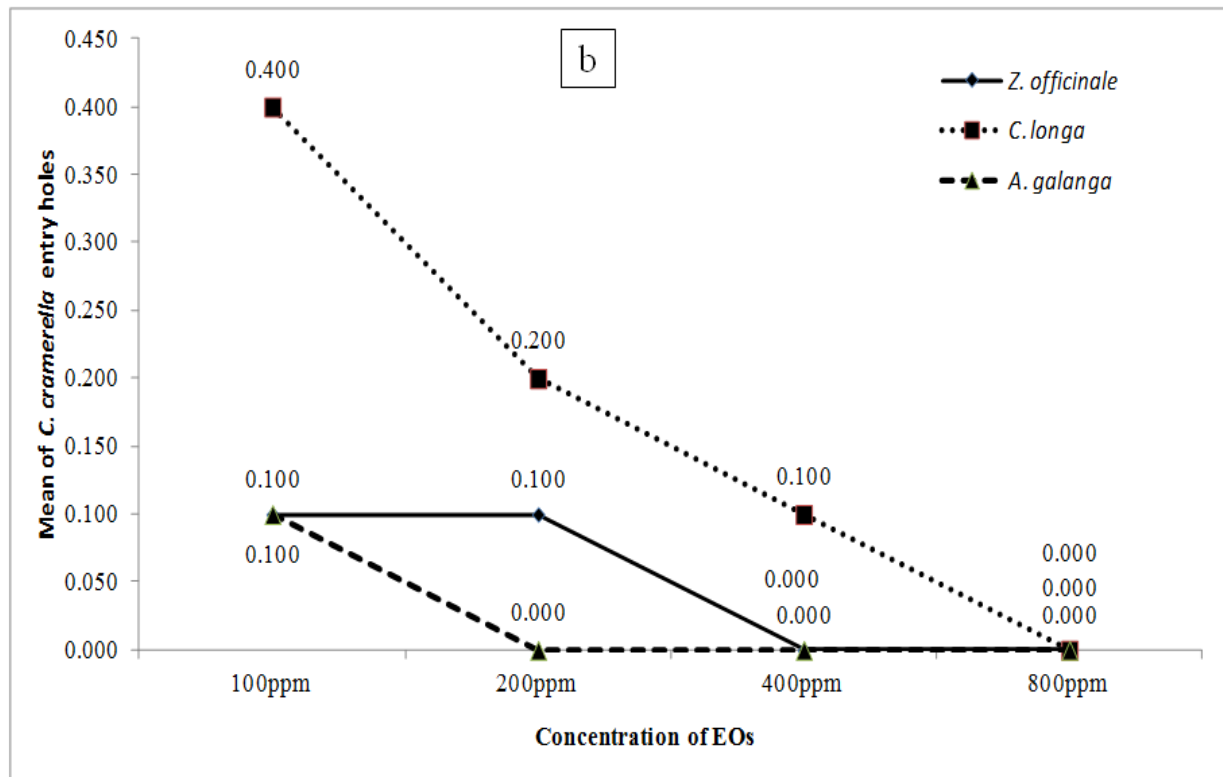
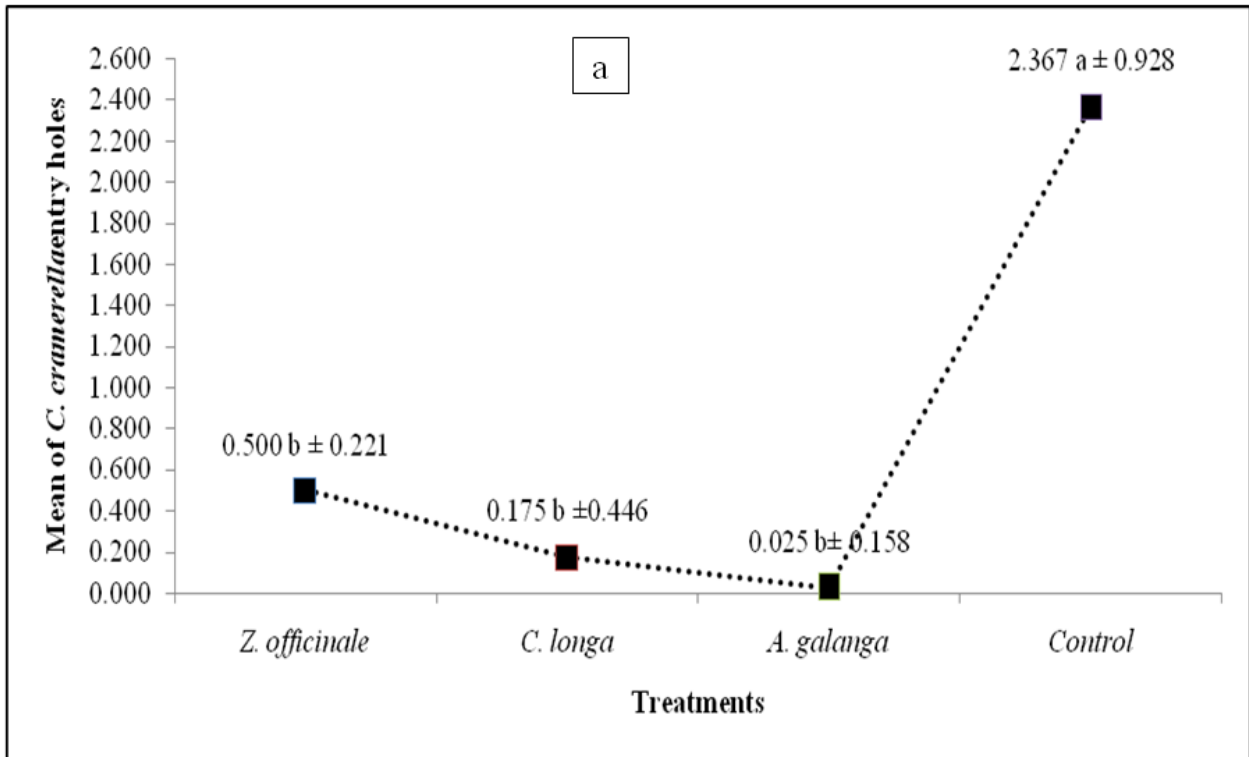


Figure 1. Mean of *C. cramerella* entry holes at different (a) Treatment (b) Concentration of treatment. Means within bars followed by the same letters are not significantly different at the 5 % level according to Duncan's Multiple Range Test.

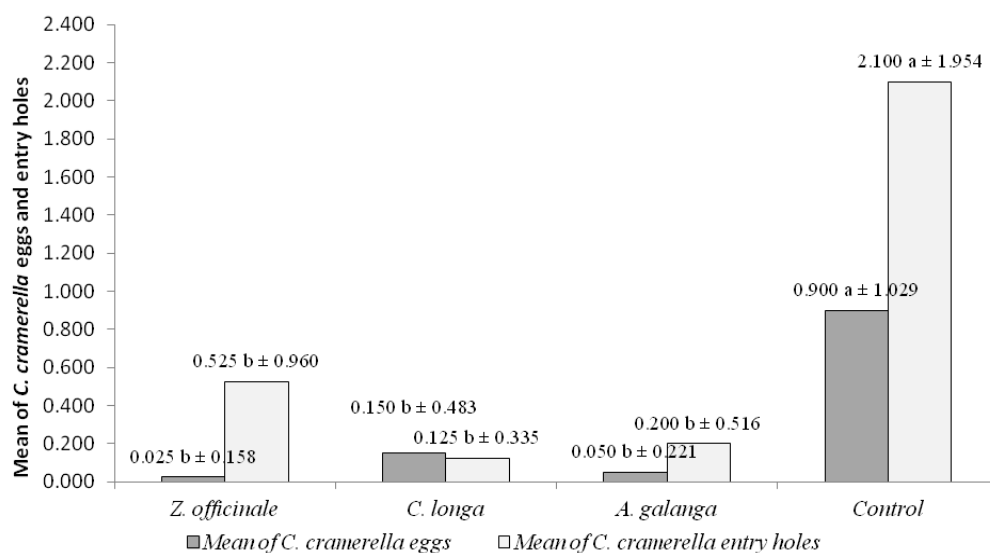


Figure 2. Mean of *C. cramerella* eggs at different treatment. Means within bars followed by the same letters are not significantly different at the 5 % level according to Duncan's Multiple Range Test.

The potential of Zingiberaceae essential oils was tested based on their effect on the eggs shows different results, where *A. galanga* performed as the best EOs under laboratory observation, *Z. officinale* recorded lowest mean of eggs under large cage observation, and *C. longa* performed the best for observation on the entry holes. The results might suggest that each EOs have similar potential which may be able to disrupt the life cycle of *C. cramerella* either by hindering the adult from depositing their eggs, or influenced successful rate of egg hatchability. Control recorded the highest number of eggs and entry holes either in the laboratory or large cage observations. The finding was in agreement with Saripah *et al.* (2019), where the highest healthy adult emergence was recorded under the control treatment, and significantly different with all Zingiberaceae EOs. The effects of Zingiberaceae EOs were also evaluated in Saripah *et al.* (2017) wherein the laboratory bioassays, *Z. officinale* shows promising results, with percentages of length reduction and deformities, were significantly different compared to control. The overall results also in agreement with the previous observation on deformities, where pupa deformities were the highest at higher concentrations of Zingiberaceae EOs (400 and 800 ppm). Saripah *et al.* (2019) discussed about the potential use of Zingiberaceae EOs as an adult emergence inhibition from a pupa to an adult based on the deformities and adult emergence inhibition observations. They strongly suggested that the mode of action of EOs could be through contact as an application of EOs when treatment was undertaken at pupa stage.

The use of eggs in this study was significant due to the estimation of recent population of *C. cramerella* can be

based on the distribution of eggs in the cocoa field. Egg distribution, later on, will help in the management decision; therefore, sampling of *C. cramerella* egg based on implementing management is crucial (Azhar and Long, 1991). Understanding the relationship between egg density and damage is essential as a tool for the successful control approach (Albert and Azhar, 2010). The information on the egg distribution pattern will be an indicator of the seriousness of the infestation level. Based on the egg distribution and infestation level, the selection of appropriate control approaches will be carried out to reduce the yield loss caused by this pest. There was no *C. cramerella* eggs were recorded on older pods more than 17 weeks before ripening (Azhar, 1992). Therefore, spraying of agricultural pesticides or pesticides must be carried out when the pod age is between 12 to 16 weeks. As in this study, only pod 4 to 4.5 months old were selected as a sampled pod to allow natural egg deposition on the cocoa pods. Even a high number of *C. cramerella* adults were introduced in the large cage experiment, the low amount of eggs deposited and low entry holes were recorded throughout the observation. It might be due to the presence of Zingiberaceae EOs residue on the treated pods may reduce the tendency of the adult to deposit their eggs on the pod surface. The oily coating on the pod surfaces might become an abiotic factor that influences low egg deposition of *C. cramerella*. Volatile components from Zingiberaceae treatments might influence the number of deposit eggs on control pods, due to the range between sampled pods was only 15cm. The volatile components with pungent odor might influence visitation rates of *C. cramerella* as observed in this study.

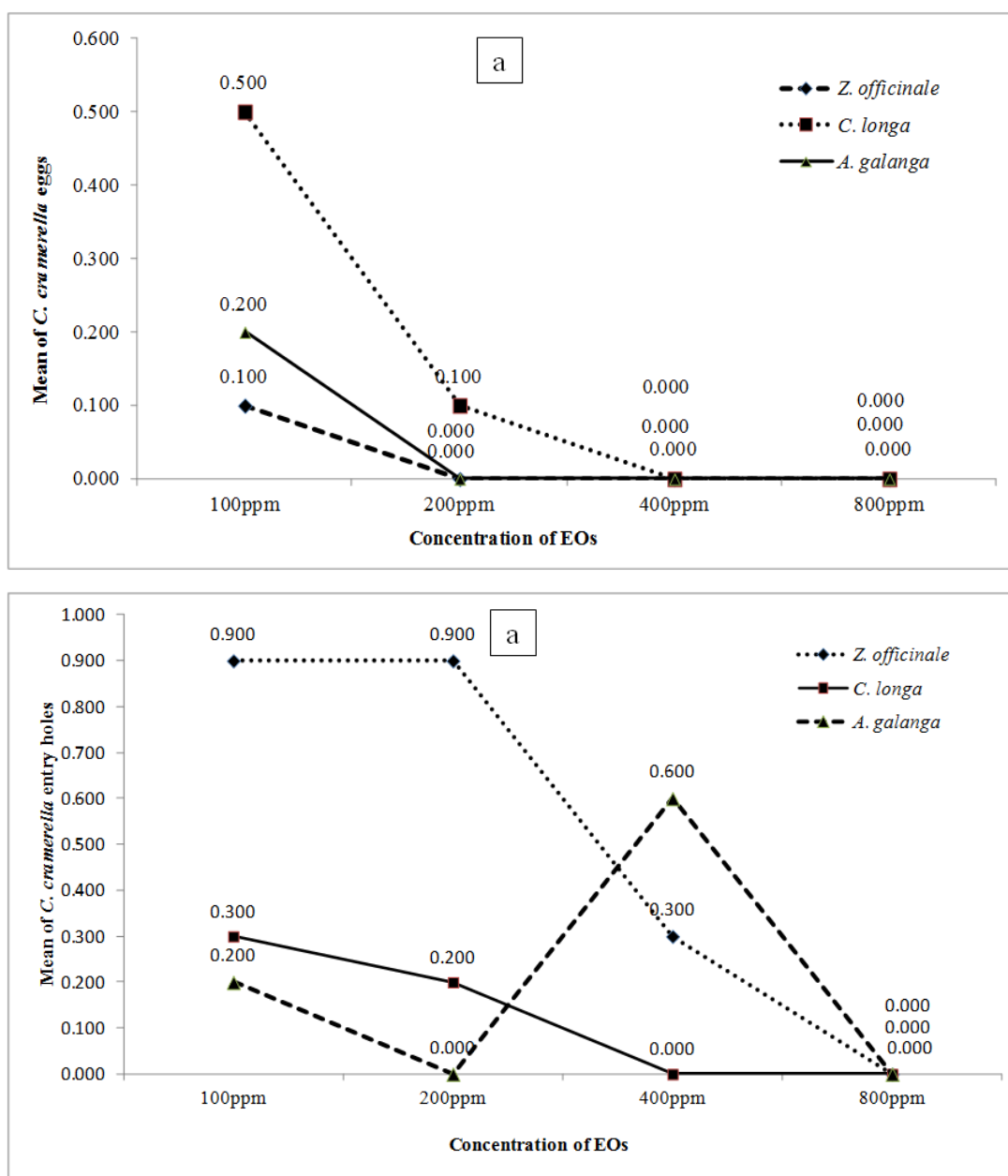


Figure 3. Mean of (a) *C. cramerella* eggs and (b) entry holes at different concentration of treatment.

Conclusion

All Zingiberaceae EOs (*Z. officinale*, *C. longa*, and *A. galanga*) were able to interrupt egg hatchability and reduced the percentage of successful penetration on the cocoa pod surface compared to the control treatment. Overall results might suggest that each EOs are capable to disrupt the life cycle of *C. cramerella* either by hindering the adult from depositing their eggs, or influenced successful rate of egg hatchability. These findings may provide a foundation for further observation on the efficacy of Zingiberaceae EOs against the survivability of *C. cramerella* eggs.

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Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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