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EFFECTS OFZINGIBER OFFICINALE, CURCUMA LONGA AND ALPINIA GALANGA ESSENTIAL OILS ON THE MORPHOLOGICAL CHARACTERISTIC OF COCOA POD BORER, CONOPOMORPHA CRAMERELLA

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ABSTRACT

The cocoa pod borer, Conopomorphacramerella, is a major threat to cocoa plantation in the South-East Asia region. Heavy reliance and prolonged use of synthetic chemical insecticide in managing this pest may lead to resistance mechanism. Therefore, plant natural product is an alternative for controlling C. cramerella, by using three Zingiberaceae species, Zingiberofficinale, Curcuma longa and Alpiniagalanga. The effectsof Zingiberaceae essential oils were evaluated in laboratory bioassays against C. cramerella. Z. officinale shows promising results with percentages of length reduction and deformities were significantly different compared to control. Overall results showed that Zingiberaceae essential oils have great potentials for C. cramerella management in the future.

Keywords: Theobroma cacao; cocoa pod borer; Conopomorphacramerella; Zingiberaceae.

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1. INTRODUCTION

Similar from other commodity and tropical crop that have been planted all over the world, cocoa, Theobroma cacao Linnaeus (Malvales: Sterculiaceae) is subjected to be attacked by diseases and insect pests [1]. Cocoa pod borer (CPB), ConopomorphacramerellaSnellen (Lepidoptera: Gracillariidae) is one of the most important biotic factors and has become the major threat to cocoa growing countries in the South-East Asia region. A female moth has the potential to lay egg singly or in groups of two or three on the pod surface. The egg stage lasts for 2 to7 days [2]. The first instar larvae usually tunnel through the egg shells after hatching. After that, the larvae bore into the pod surface until reaching the sclerotic layer of the husk. The entire larval stage takes 14 to 20 days to complete and tunnel out through the pod wall. Pupation occurs outside the pod within the oval shaped silken cocoon. Completion of pupation stage usually takes 6 to 8 days and followed by the emergence of adult moths. The adult usually rests transversely underneath the jorquette branches, especially in shady areas. Adult longevity is 1 to 30 days and generally lives for about a week.Even the life cycle of C. cramerella is relatively short; its economic impact to cocoa pod production is very significant. Crop loss to farmers can be substantial and continuous infestations may cause unacceptable level of damage, influenced by the feeding and oviposition preferences for pods.

As C. cramerella persisted, it was proven that no unilateral approach was successful in managing the C. cramerella. A combination of several management approaches and control techniques such as proper agronomic practices, biological control, chemical control, resistant planting materials and Integrated Pest Management (IPM) were highly recommended to reduce the infestation [3]. Even IPM and other control approaches were suggested, in most cases, growers prefer only to implement chemical control as a single technique in managing C. cramerellainfestation. Biweekly prophylactic treatment with chemical insecticides was considered as one of the most effective approaches.However, the cost is expensive [4]. The effectiveness of insecticide control depends on the appropriate timing of spraying, which usually relies on cocoa cropping calendars. However, prolonged use of chemical insecticides may produce the risk of insecticide resistance due to excessive use and increase production cost. Chemical insecticides must be suggested as a last resort, therefore prudent management effort is necessary to suppress the infestation of C. cramerella.

As an alternative to chemical insecticides, an effort is warranted to find unconventional methods to currently used insecticides for controlling C. cramerella. One of the approaches that should be taken into consideration is the use of natural products produced by plants. Many plants produced chemicals that may play roles as oviposition deterrent, insect repellent and antifeedant either as larvicidal, pupicidal or adulticidal agents. For ideal plants, in addition to their inherent pesticide effectiveness must be rustic, perennial and easily cultivated. Some plant families with the most promising effects as botanical insecticides were derived from Meliaceae, Rutaceae, Annonaceae and Piperaceae [5]. Over the years, botanical pesticides derived from whole plants or some essential parts of plants have been used in managing insect pests. Water extract of plants, plant powder products, mixed of several plants and essential oils (EOs) had been technically used for controlling insect pests in many countries. EOs is basically volatile, natural and characterized with strong odor [6]. EOs is known for their roles as an antiseptic such as bactericidal, virucidal, fungicidal and other medicinal properties. Pure compounds or blends of several major chemical components in EOs found to be toxic to insects.

Based on the phytochemical compounds, the plants widely planted in Malaysia that may lead to pesticidal properties should be investigated. Zingiberaceae (Order: Zingiberales) is one of the largest families in the plant kingdom and recognized as the most important herbaceous species in the tropics [7]. Zingiberaceae produced a fleshy complex rhizome with the base of each aerial stem consisting of an erect, ovoid or ellipsoid structure (primary tuber). Many species from Zingiberaceae was commercially planted and used as spices, medicines, flavoring agents, food preservatives, medicinal uses, chemical compositions and their interactions with insect species.However, until to date, there is no report in Malaysia on the potential use of Zingiberaceae plants in managing cocoa pests, particularly the C. cramerella. Three species of Zingiberaceae plants that widely planted in Malaysia; Alpiniagalanga (galanga), Curcuma longa (turmeric) and Zingiberofficinale (ginger) were reported to have effects on insect pests via oviposition deterrent, insect repellent and antifeedant activities [8]. Being an environmentally benign approach, their effectiveness was demonstrated on several insect pests belong to orders Diptera, Lepidopteraand Coleoptera in several forms such as raw plantmaterials, plant extract and EOs. Insidious characteristics of C. cramerellalarvae prevents

them from any means of physical and chemical controls, thereby leaving only egg, pupae and adult stages in their life cycle that can be targeted for control. Pupae can be relatively easily found on the pod surface, green or dry leaf litters on the ground than other stages. Therefore,this study has been focused on the effects of Z. officinale, C. longa and A. galangaEOs on the pupa morphological characteristics of C. cramerella.

2. EXPERIMENTAL

Laboratory observation of the ZingiberaceaeEOs was performed at the Entomology Laboratory at the Cocoa Research and Development Center (CRDC) BaganDatuk (3.906 N, 100.866 E) of the Malaysian Cocoa Board (MCB). EOs of Z. officinale, C. longa and A. galanga were purchased from an authorized dealer in Malaysia. Effectiveness of EOs was tested for bioassay tests using four different concentrations; 100ppm, 200ppm, 400ppm and 800ppm with additional of 0.5ml of Tween 80 as a surfactant. Water with additional of 0.5ml of Tween 80 were served as a control. Concentrations were agitated, and left for 24 hours prior to the experiment.

Approximately, 200 to 300 ripe cocoa pods were harvested and placed in a large container as pupation sites, and covered with dry cocoa leaves. Numbers of pupa pupates on the dry leaves were recorded after 24 hours until there was no pupa emerged from cocoa pods. Pupa was kept individually in the petri dish and labeled as Days 1, 2, 3, 4 and 5 regarding to the day after emergence. Pupawere sprayed with different EOs using a hand sprayer at a range of 15cm, left air-dried for an hour before kept individually in close lid petri dish. The number of successful emerging adult, deformed adult, deformed pupa and pupa mortality were recorded before 24hours, 48 hours and 72 hours prior to spraying occasions. Three pupa of Days 1, 2, 3, 4 and 5 for each treatment were selected for pictorial assessment. The length of pupa was recorded and the picture was captured using Dino-Lite Premier Handheld Digital Microscope at magnification of 30x (Dino Capture 2.0, model AM7013MZT). Morphological characteristics of the pupa were recorded after 24 hours, 48 hours and 72 hours spraying with EOs. The experiment was repeated five times for each EOs.

Data obtained from these bioassay tests were arranged separately based on the treatments and replicates in Microsoft® Excel 2007. Exploratory Data Analysis (EDA) was used to test the

normality of selected data and transformation to normalize the variance. Data were subjected to statistical analysis using one-way Analysis of Variance (ANOVA) and PROC GLM, SAS software from SAS® Version 8.

3. RESULTS AND DISCUSSION

The length of pupa at 24hours, 48 hours and 72 hours after treated with different concentrations of Z. officinale EO was shown in Fig. 1; C. longa (Fig. 2) and A. galanga (Fig. 3). There was no significant difference ($p>0.05$) in length recorded for Z. officinale at all exposure periods. Similar results were obtained for different concentration and pupa age. In contrast to Z. officinale fluctuated results were recorded from C. longa and A. galanga. For Day-1 and Day-3 pupa that treated with A. galanga, there was no significant difference ($p>0.05$) recorded between exposure period and concentrations. However, Day-2 and Day-4 pupa show differences of pupa length between concentrations after 72 hours of exposure (between 100ppm and 400ppm; and 200 and 400ppm). While for Day-5, insufficient data were obtained due to pupa successful emerges as an adult. Similar observation was obtained when the pupa were emerged as an adult after Day-5 pupa treated with C. longa. Day-1 and Day-4 pupa showed no differences in pupa length after 72 hours treated with C. longa. However, on the Day-2 pupa, the length was differed significantly ($p<0.05$) at concentration of 200ppm compared with other treatments and Day-3 was differed at 400ppm compared with 100ppm. In general, the results suggest that the different concentrations of EOs did not influence the length of pupa at different age.

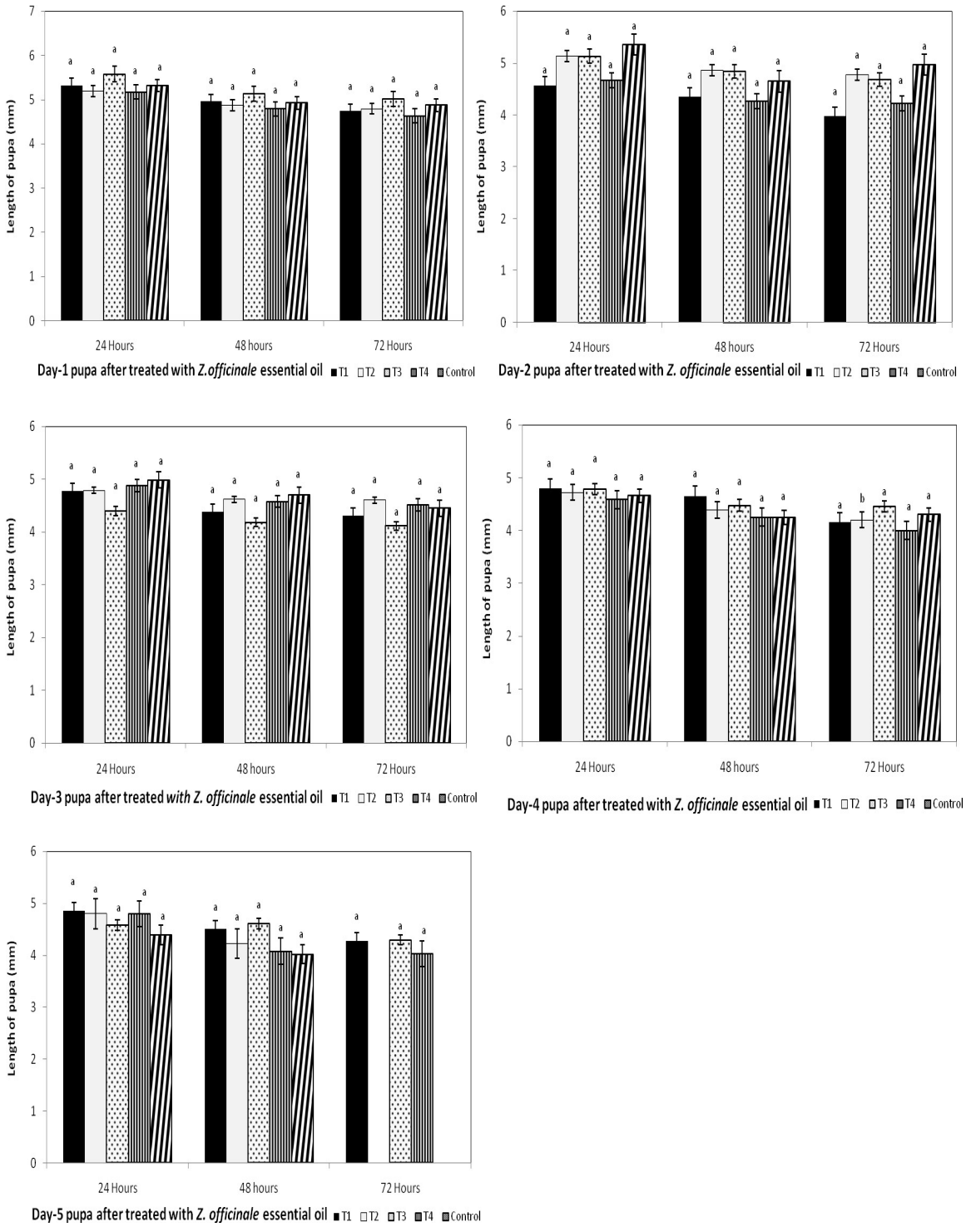

Fig.1. The length of the pupa (Days 1, 2, 3, 4 and 5) after treating with ginger, Z. officinaleEOs at 100ppm (T1), 200ppm (T2), 400ppm (T3), 800ppm (T4) and control

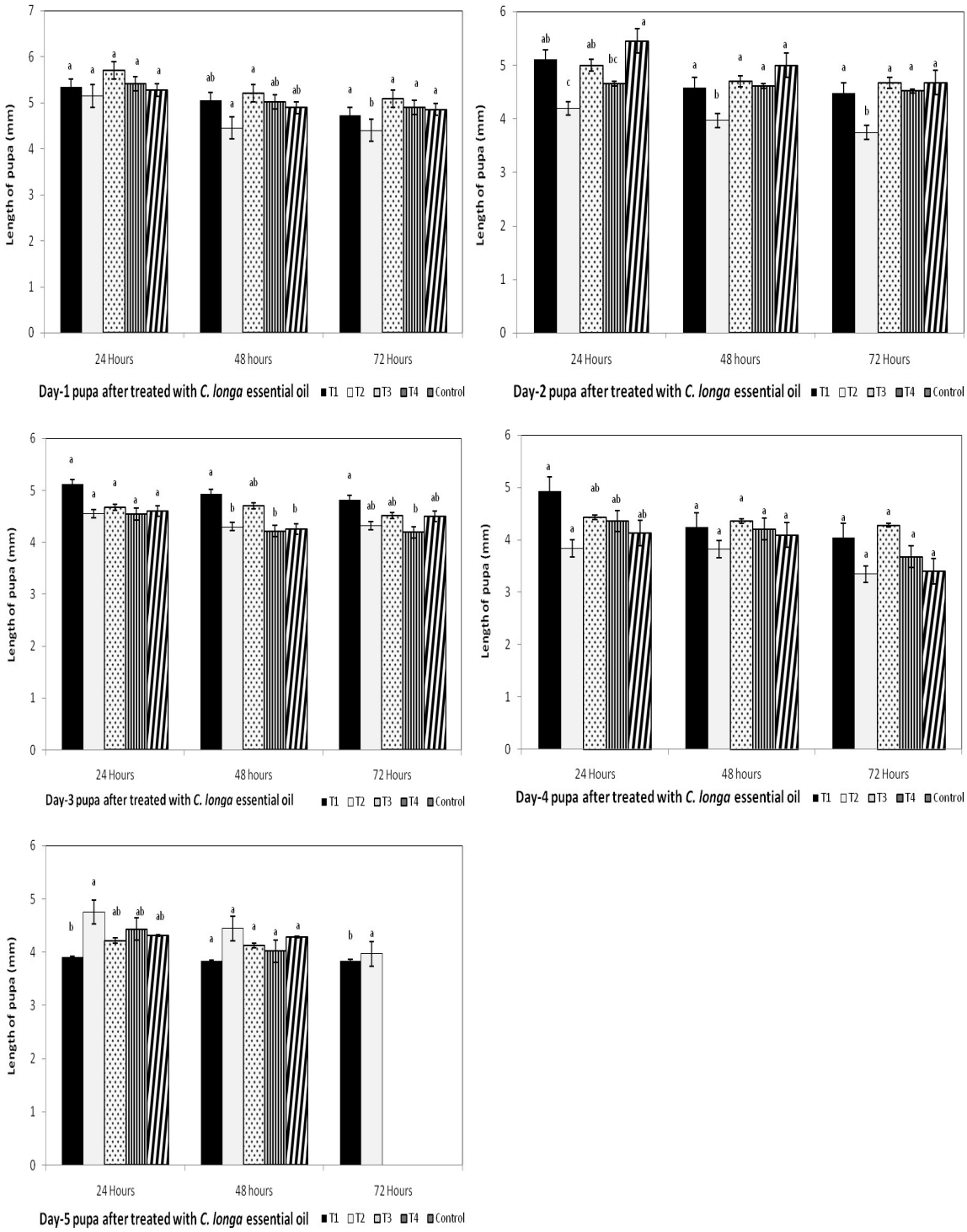

Fig.2. The length of the pupa (Days 1, 2, 3, 4 and 5) after treating with turmeric, C. longa essential oil at 100 ppm (T1), 200ppm (T2), 400ppm (T3), 800ppm (T4) and control

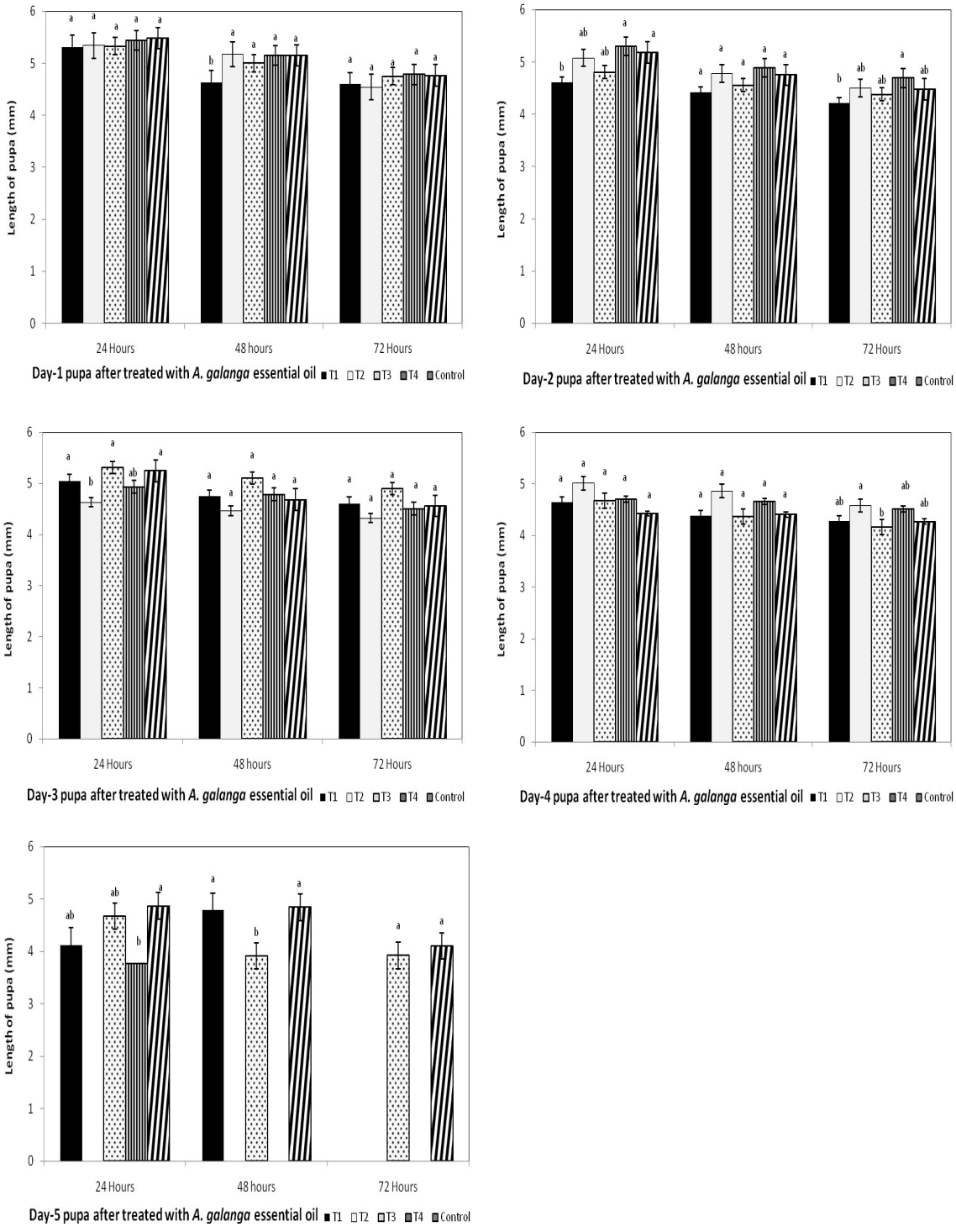

Fig.3. The length of the pupa (Days 1, 2, 3, 4 and 5) after treating with lesser galanga, A. galanga essential oil at 100 ppm (T1), 200ppm (T2), 400ppm (T3), 800ppm (T4) and control

Significant reduction of pupa length after 72 hours treated with Z. officinale is shown in Table 1. Similar results denoted for C. longa, where 400ppm and control was significantly different

with 100ppm and 200ppm. However, no significant difference ($p>0.05$) was observed between treatments for A. galanga. The control treatment shows the least length reduction for both C. longa and A. galanga. In general, C. longa and A. galanga shows significant difference ($p<0.05$) in pupa length compared with control after 24 hours (Table 2). No significant difference was recorded after 48 hours between treatment ($p> 0.05$) and only A. galangal produce the difference after 72 hours. Percentage of length reduction after 72 hours shows significant differences between Z. officinale and C. longa compared to control. Example of length measurement of pupa is shown in Fig. 4.

Table 1. Percentage of pupa length reduction after 72 hours treated with Zingiberaceae EO

Treatment	Percentage of Length Reduction After 72 Hours		
	Z. officinale	**C. longa**	**A. galanga**
T1 (100 ppm)	15.720 a	13.736 ab	14.250 a
T2 (200 ppm)	14.993 a	17.406 a	13.052 a
T3 (400 ppm)	10.147 b	8.995 c	13.133 a
T4 (800 ppm)	14.993 a	12.444 bc	13.187 a
T5 (Control)	13.105 ab	8.553 c	12.901 a

Means followed by the same letter at vertical rows shows no significant different at $p\leq0.05$.

Table 2. Pupa length and length reduction after treated with ZingiberaceaeEOs

Essential Oils	Pupa Length After Treated With Zingiberaceae EOs (Hours)			Length Reduction (%)
	24	**48**	**72**	
Z. officinale	4.905 ab	4.579 a	4.445 a	13.966 a
C. longa	4.790 b	4.535 a	4.450 a	13.051 a
A. galanga	4.825 b	4.639 a	4.239 b	13.408 ab
Control	5.143 a	4.660 a	4.561 a	11.422 b

Means followed by the same letter at vertical rows shows no significant different at $p\leq0.05$.

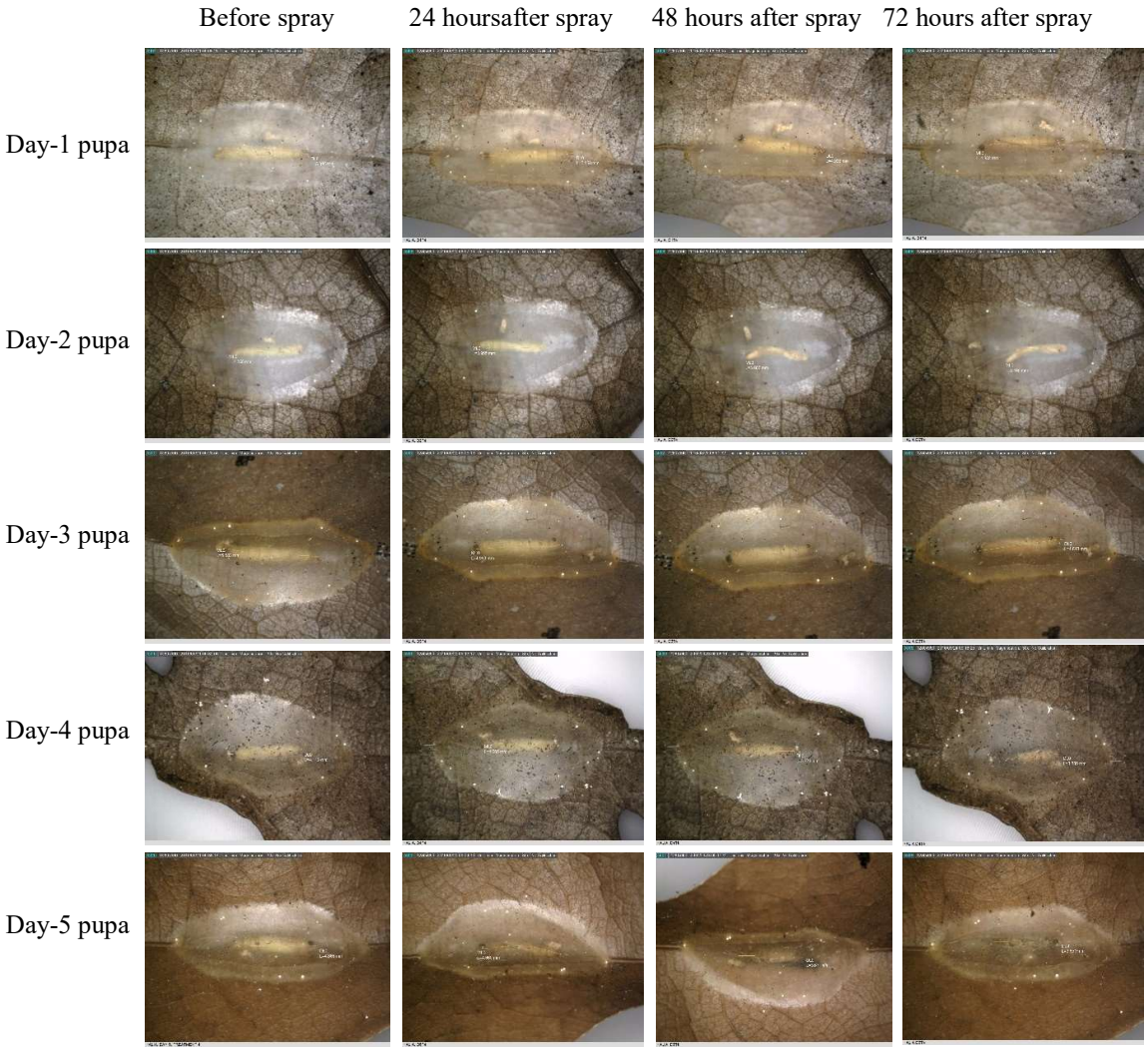

Fig.4. Length measurement of pupa after treated with 800ppm (T4) Z. officinale Eos

Regardless of the age of pupa and concentration of EOs, only Z. officinale shows significant difference with control (Fig. 5) in term of pupa length. The overall results showed that there is inconsistency effect of C. longa and A. galanga on pupa length. Therefore, the length of pupa may not be suggested as an important parameter for determining the effectiveness of EOs on C. cramerella. Nevertheless, several pupa treated with EOs shows deformities symptoms even though they managed to reach adult stages. The study also showed that two types of deformities were recorded at pupa and adult stages. Deformed pupa was occurring when the pupa unmanaged to survive in pupal stages. Meanwhile, deformed adult was occurring when the pupa successful turn as an adult, however later dead due to several types of morphological deformities. Deformities were the highest at A. galanga (0.750a) and Z. officinale (0.670a).

Control recorded lowest deformities with 0.147c.

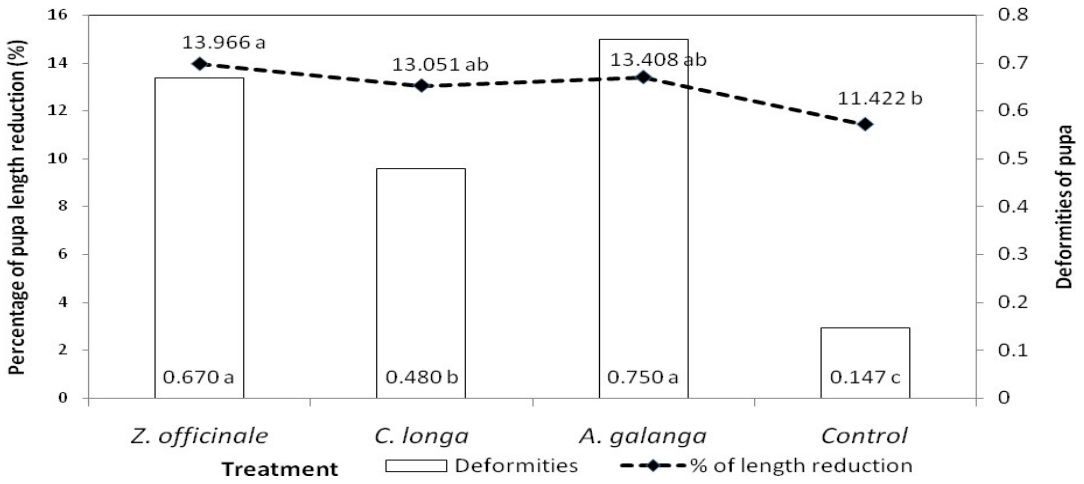

Fig.5. Percentage of pupa length reduction and deformities

Plant extracts and EOs of Zingiberaceae contain monoterpenoids, diterpenoids, sesquiterpenoids and other essential components that might be beneficial for pest control [9-10]. Some Zingiberaceae species have potential against insect pest, due to the presence of essential oils such as limonine, eugenol, pinene and geraniol [11]. The rhizome of Alphinia, Zingiber and Curcuma contains major compounds of camphene, camphor, 1-8 cineole and α-humulene. These compounds were leading as fumigant to Sitophiluszeamais and Triboliumcastaneum, serious pests of stored products in Thailand [8]. Direct toxicity and fumigant activity of monoterpenoids found in several Zingiberaceae plants such as terpinen-4-ol, 1,8-cineole, linalool, R-(+)-limonene and geraniol was effective against different life stages of Triboliumconfusum [12]. Zingiberaceae species can be implemented either in a form of aqueous extracts or EOs, as in this study.

Almost all EOs regardless to different concentrations have little or no effect on the length of pupa. The average length (n=75) of the Day-1 pupa was 5.786-5.992 mm (Figs. 1-3), Day-2 (5.134-5.223 mm), Day-3 (5.049-5.090 mm), Day-4 (4.090-4.818 mm) and 4.575-4.924 mm for Day-5 old pupa. Clearly, the length was gradually decreased with the age of pupa, where older pupa tends to be shorter.

Pupa is the stage where C. cramerella is more likely to be transported.Therefore, control of this pupal stage must be taken into consideration as part of the complete management

approach. The pupa occupies three quarters of the entire brown-silk cocoon, which constructed with an initial layer of silk attached to the surface over and covers the pupa [13]. Due to low or no food intake during C. cramerella pupation stage, therefore it was believed that the mode of action as a direct contact where penetration through cocoon walls is occurring. There were several white bubbles-like spheres are scattered over the edge of the cocoon with unknown function [13]. These white bubble-like spheres may function as breathing holes and allows air ventilation inside the cocoon. If this assumption is true, the bubble may have potential as a liquid pathway for EOs to penetrate inside the cocoon. As an agreement with this assumption, further investigation must be carried out to confirm the function of these bubble-like spheres.

4. CONCLUSION

The study has demonstrated the potential use of ZingiberaceaeEOs as biopesticide for controlling C. cramerella. Disrupting pupal development has a very important economic bearing because it will interrupt theadultemerging from pupae, and later will reduce pod damage. The spread of pupa through movement of infested pods is limited if the pod breaking is conducted in the field. However, proper practices must be conducted due to remaining larva inside the pod husk or pupa on the husk may success to complete their life cycle. Failure in controlling pupa may proliferate C. cramerella population and later will expand into the surrounding cocoa plantations. Selection of plant species especially those who associated with direct contact must be carried out properly to reduce any antagonistic effect to the farmers. The selected Zingiberace species, Zingiberofficinale, Curcuma longa and Alpiniagalanga are safer for both human and environmental health. Deformities at pupa stages have strongly suggested that the mode of action of EOs could be through contact as application of EOs was carried out at pupa stages. Thus, spraying with ZingiberaceaeEOs could help in reducing the population of C. cramerellasoon either as single tool or as a part of integrated pest management strategy.

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