EFFECTS OF ZINGIBERACEAE BASED BOTANICAL PESTICIDES BIOPESTICIDES AND DELTAMETHRIN INSECTICIDE TO THE ACTIVITIES OF COCOA BLACK ANTS, *Dolichoderus thoracicus* (SMITH)

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ABSTRACT

Cocoa pod borer, *Conopomorpha cramerella* is is the most devastating pest responsible for the decline of cocoa production in Southeast Asia. Several control methods have been implemented to reduce the impact of *C. cramerella* including application of the botanical pesticides. Hence, very few reports on the botanical pesticide and insecticide effect on the beneficial insect associated with cocoa ecosystems had been reported in Malaysia. This study investigates the effect of botanical pesticide and insecticide on the cocoa black ants (CBA), *Dolichoderus thoracicus*, a biological control agent against the *C. cramerella.* Four Zingiberaceae based botanical pesticides formulations were applied: *Alpinia galanga* F1, *A. galanga* F2, *Zingiber officinale* F1, *Z. officinale* F2 with insecticide (a.i deltamethrin), and water as control treatment. The introduction of artificial ant nests stuffed with dry cocoa leaves was carried out with two different fields observation with six replicates. The acoustic strength in decibel (dB) of *D. thoracicus* population was observed for 60-days at three intervals; natural sound, 5-seconds, and 30-seconds after minor disturbance of the nests. Results denoted that *D. thoracicus* actively moves into the nests started to increase from day-10. The highest activity was observed in day-60 (60.139 a) and significantly different ($p < 0.05$) from the first 30 days of observation. *Zingiber officinale* and *A. galanga* formulations performed better than deltamethrin, regarding the *D. thoracicus* activities. The decibel derived from ecoacoustic sound showed almost similar results between treatments and suggested the stability of *D. thoracicus* population between treated and non-treated nests. The results suggest the artificial ant nests constructed with treated dry cocoa leaves either with Zingiberaceae formulations or insecticide may have less effect to the activity of *D. thoracicus.* The Zingiberaceae based botanical pesticide is safe for beneficial insects of cocoa at a lower dose, and can be applied for *D. thoracicus* population built-up strategies in the future.

Keywords: Botanical pesticides, cocoa, cocoa black ants, deltamethrin, *Dolichoderus thoracicus*, Zingiberaceae.

ABSTRAK

Ulat pengorek buah koko, *Conopomorpha cramerella* merupakan serangga perosak utama yang menyebabkan pengurangan pengeluaran koko di rantau Asia Tenggara. Beberapa langkah kawalan telah diimplementasi bagi mengurangkan serangan, termasuk melibatkan penggunaan pestisid botani. Bagaimanapun, hanya sedikit kajian telah dijalankan yang berkaitan kesan pestisid botani terhadap serangga berguna di tanaman koko dari Malaysia. Kawasan Kawasan yang sama. Oleh itu, kajian ini dijalankan untuk melihat kesan pestisid botani terhadap semut hitam koko, *Dolichoderus thoracicus*, yang merupakan agen kawalan biologi terhadap *C. cramerella*. Empat formulasi dikaji iaitu *Alpinia galanga* F1, *A. galanga* F2, *Zingiber officinale* F1 dan *Z. officinale* F2 serta racun serangga (bahan perawis aktif deltamethrin), dan air sebagai kawalan. Dua kajian lapangan dijalankan, dengan melibatkan pengenalan sarang semut buatan yang diisi dengan daun koko kering yang telah dirawat. Aktiviti *D. thoracicus* direkodkan selama lima minit pada hari pertama, 3, 7, 10, 15, 30, 45, dan 60. Kekuatan bunyi akustik dalam unit desibel (dB) yang menggambarkan populasi *D. thoracicus* di dalam sarang direkodkan selama 60 hari pada tiga selang masa, iaitu: keadaan semula jadi, 5 saat dan 30 saat selepas gangguan minimum dilakukan. Keputusan menunjukkan aktiviti *D. thoracicus* masuk dan keluar daripada sarang buatan meningkat bermula dari hari ke-10. Aktiviti tertinggi adalah pada hari ke-60 (60.139 a), dan berbeza secara signifikan ($p < 0.05$) dari 30 hari yang pertama. Penggunaan formulasi ke atas sarang semut *Z. officinale* dan *A. galanga* menunjukkan keputusan aktiviti semut yang lebih baik jika dibandingkan dengan penggunaan deltamethrin. Kadar desibel yang terbit dari bunyi ekoakustik menunjukkan tiada perbezaan signifikan dan menyarankan kestabilan populasi semut di antara sarang dirawat dan sarang yang tidak dirawat. Keputusan ini mencadangkan formulasi Zingiberaceae atau racun serangga mempunyai kesan minimum terhadap aktiviti *D. thoracicus*. Pestisid botani Zingiberaceae mungkin selamat terhadap serangga berguna koko jika digunakan pada dos yang rendah dan boleh digunakan dalam strategi peningkatan populasi *D. thoracicus* di masa hadapan.

Kata kunci: Pestisid botani, koko, semut hitam koko, deltamethrin, *Dolichoderus thoracicus*, Zingiberaceae.

INTRODUCTION

Cocoa, *Theobroma cacao* (Linnaeus) (Malvales: Sterculiaceae) is an important crop that is widely planted in humid tropical regions. The diversity of insects was recorded in the cocoa field, where they can be classified due to their roles in the ecosystem, either as beneficial insects, pollinators, neutral insects, and insect pests, which can contribute risk to the yield of cocoa. Like other commodity and tropical crop that has been planted all over the world, cocoa tree is vulnerable to be attacked by diseases and insect pests (Bateman 2015). More than 1,400 insect species recorded feeding on cocoa (Entwistle 1972) and approximately 200 species were documented in Malaysia (Lee et al. 2013, 2014). Among listed insects, only a few may contribute to significant losses, and the most important is the *Conopomorpha cramerella* Snellen (Lepidoptera: Gracillariidae) and the mirid, *Helopeltis* spp. (Hemiptera: Miridae) which has become a major nuisance to the Southeast Asia region (Saripah et al. 2017). Many factors contributed to the decline of cocoa plantation, for example Indonesia and Malaysia are suffered with the infestation of *C. cramerella* and *Helopeltis* spp. attack

(McMahon et al. 2015). These pest problems incur significant losses which contributed to the yield loss and driving farmers to replace the cocoa with other crops.

Several management practices and pest control tactics were implemented to ensure this commodity resulted in good profits with an acceptable level of pest infestation. However, heavy reliance on farm inputs and management practices might reduce insect biodiversity and increased pest problems (Mouysset et al. 2013; Stoate et al. 2001). The increment used of agricultural inputs has led to higher crop yields, unfortunately may accompanied by a decline in biodiversity. High input of agrochemicals such as herbicides, insecticides, and fungicides; and synthetic fertilizers are often considered to be detrimental to many biotic species. It was believed that pest management strategies highly influenced the biodiversity of insects due to their responses to landscape context. One pest population may benefit from techniques designed to limit another pest population (Mediene et al. 2011).

As an alternative to currently using insecticides to control the pest problem, plant products gain more attention from researchers all over the world. Employing botanical pesticides to reduce heavy reliance on chemical insecticides is an alternative strategy that gains more attention in recent years. In insect management, plant-based pesticides were formulated using substances derived from plant extracts or essential oils (Eos). Many plant products and chemicals may play a role as oviposition deterrent, insect repellent or antifeedant either for larvicidal or adulticidal activities. The EOs produces promising results with high effectiveness, various modes of action, and low toxicity to non-target organisms. With the high number of active substances in the EOs (20 to 60), they can be characterized by main compounds with high concentrations, usually ranging from 20 to 85%.

In addition to inherent pesticide effectiveness, such plants must be rustic, perennial, and easily cultivated. Parkash and Rao (1997) cited that more than 800 species pose insecticidal and repellent antifeeding effects in agriculture, which will offer more variety for plant selection in the future. There were common herbs with active ingredients containing insecticidal properties such as clove (eugenol), eucalyptus (1,8-cineole), lemongrass (citronellal), mentha species (menthol), and *Thymus vulgaris* (thymol and carvacrol). These species have fumigant and contact insecticidal property as reported by Koul et al. (2008). Meanwhile, allicin derived from *Allium sativum* (Prowse et al. 2006) was able to play a role as antifeedant, repellent, an inhibitor of molting, respiration, and the reduction in insect fertility. For example, the limonoid group, azadirachtin from neem (*Azadirachta indica*), was proven as a practical botanical component that plays a role as an antifeedant, growth inhibitory, oviposition deterrent, antifertility, and repellent properties (Sundaram et al. 1995). This component is also established as a pivotal insecticidal ingredient, which acts as repugnant agent and induces sterility in insects by preventing oviposition and interrupting sperm production in males. The use of neem botanical pesticides was studied on numerous organisms for different problems, such as controlling golden apple snail (Rosdiyani et al. 2016) and mosquitoes (Chaudhary et al. 2017).

Among diverse family Zingiberaceae (Order: Zingiberales) with more than 1,000 known species is recognized as the essential herbaceous species in the tropics, with approximately 50 genera (Sirirungsa 1998). Family Zingiberaceae can be characterized by a strong and pungent aroma, which has been used as traditional medicine and food condiments, especially in Asia (Sirirungsa et al. 2007). Turmeric or *Curcuma longa* recorded more than 80 species and is one of the largest genera in this family. *C. longa* was reported to have ar-turmerone and turmerone active ingredients, which are useful for antifeedant and inhibitory activity on the growth of insect (Tripathi et al. 2002). Other essential species of Zingiberaceae are ginger (*Zingiber officinale*), galangal or Thai ginger (*Alpinia galanga*), torch-ginger (*Etlingera elatior*), cekur (*Kaempferia galanga*) and cardamom (*Amomum* sp. *and Elettaria* sp). EOs of *Z. officinale, A. galanga*, and *C. longa* had been tested on pupation preferences, inhibitory effect and morphological characteristics of *C. cramerella* (Saripah et al. 2017, 2019a, 2019b).

The other control approach is implementing biological control by using insects in the managing of cocoa pests. *Dolichoderus thoracicus* usually thrive well in cocoa-coconut ecosystems, especially in coastal belt areas of Peninsular Malaysia. *Dolichoderus thoracicus* is mutually associated with the cocoa mealybug (CM), *Cataenococcus hispidus* Morrison (Homoptera: Pseudococcidae). Abundance of *D. thoracicus* was successful in reducing infestations of *C. cramerella* and rodent pests (Ahmad Saleh et al. 2020; Saripah & Azhar 2007; Saripah 2014a, 2014b).

The activities of ants were monitored based on the visual observation of ants moving into and out of the nests and the acoustic strength inside the artificial ant nests. Detection of insects by referring to the sound produces is a practical method and has been practiced in agricultural post-harvest commodities. For example, the creeping sound activity of post-harvest insects in grain storage was useful to estimate their population, as well as their species (Geng et al. 2017). The acoustic technology was also studied by Aflitto and Hofstetter (2013) that detect coleopteran bark beetles entering trees thus facilitate in protecting the wood and trees. The sound produced by the beetle can be an indicator of a management tool by disrupting their communication ability. Detection of the sound of insect activities to locate their habitat had been studied for red palm weevil (*Rhynchophorus ferrugineus*), coconut rhinoceros beetle (*Oryctes rhinoceros*) and *Sitophilus oryzae* and the stored-product insects (Mankin et al. 2011), *Leptogenys kitteli* ants (Chiu et al. 2011), larva of wood boring insects, *Hylotrupes bajulus* (Bilski et al. 2017) and female Mediterranean fruit flies, *Ceratitis capitata* (Mizrach et al. 2005)

Acoustic sound derived from certain insect species is also useful to evaluate biodiversity (Riede 1993; Sueur et al. 2008) due to their sound-producing capability. Acoustic insects are common, and some species are recognized as signaling, chorus, or singing insects. Monitoring insects using acoustic signals is a passive technique; however, able to detect organisms that produce signs, even there are relying on multiple factors, such as the amplitude and frequency of sounds emitted (Llusia et al. 2011). Hence, based on the literature, this study investigates the acoustic strength inside the artificial nests after the material for nest constructions were treated with *Z. officinale* and *A. galanga* formulations.

MATERIALS AND METHODS

Experimental Design

The experiment was established at the Malaysian Cocoa Board, Cocoa Research and Development Center (CRDC) Bagan Datuk (3.906 N, 100.866 E), Perak, Malaysia. The studies were divided into two experiments, where the former was observed on the *D. thoracicus* activities, and the latter were on the acoustic sound of the artificial ant nests. The populations of *D. thoracicus* were monitored four months before the experiment date.

Formulation Preparation

The formulation of treatments were prepared in the laboratory, which were a) *Z. officinale* Formulation 1 (F1), *Z. officinale* Formulation 2 (F2), *A. galanga* Formulation 1 (F1), *A. galanga* Formulation 2 (F2), insecticide (a.i deltamethrin), and control (water). Botanical based pesticide formulations are a preliminary formulation developed from *Z. officinale* and *A. galanga,* and differed by the concentration of each essential oils (EOs) integrated in each formulation. F2 formulation was produced with a higher concentration of EOs (400 ppm) compared to F1 (200 ppm). Other ingredients added in the formulation was the stripped palm kernel methylesters (Edenor®, MEPK 12-18 MY, Emery Oleochemicals), non-ionic hydrophophilic surfactant polyoxyethylene sorbitan monooleate, (Tween® 80, Sigma-Aldrich) and lipophilic surfactant sorbitan monooleate (Span® 80, Sigma-Aldrich). The preliminary formulation was prepared using a homogenizer at a speed of 13,500 rpm for 5 minutes until complete solubilization. All treatments were diluted with water up to 500ml and prepared 24 hours prior to the experiment.

Artificial Ant Nests Construction

Dry cocoa leaves were collected from cocoa blocks that are free from insecticide spraying for more than six months. Dry cocoa leaves were brought back to the laboratory and left for one week before being treated. Each formulation was transferred to a 1L pressure sprayer bottle and was applied by spraying the leaves individually and air-dried for 24 hours. Approximately 100 dried leaves were sprayed for each treatment. The leaves were then stuffed into a 30 cm x 45 cm transparent plastic bag at a weight of approximately 250 gram per nest. Breathing holes (0.5 cm) were made to allow air ventilation and water discharge from the plastic.

***Dolichoderus thoracicus* Activities Observation**

Six mature cocoa trees with existing populations of *D. thoracicus* were selected as a replicate, and both experiments were conducted on the same trees. An artificial ant nests from each treatment were hung and altogether, each sampled tree was hung with six treated artificial ant nests. Evaluation on the number of *D. thoracicus* was counted based on 5-mins counting per tree. The number of ants walking through the ropes that were attached to the nest was divided into two observations. The former was the number of *D. thoracicus* walking into the nest, and the latter was the number of *D. thoracicus* walking out from the nest. Total estimated time spent per tree was 35-mins, and the total number of observations was 36 (6 treatments x 6 replicates). Observations on *D. thoracicus* activities were undertaken only between 8.00 am to 10.00 am by three well-trained staff. The first five data collections were recorded at day 1, 3, 7, 10, and 15. After that, data collections were undertaken every 15 days, at day 30, 45, and 60.

Acoustic of *Dolichoderus thoracicus* Observation

An acoustic device (Sound Level Meter Decibel Logger (KLPU Digital) for monitoring or detecting insects that is non-destructive, remote, automated and able to monitor hidden insect infestation was used. In the second observation, the acoustic strength, which was devoted to the population of *D. thoracicus* inside the nests, was recorded for 60-days using Digital Sound Level Meter Decibel Logger (KLPU Digital) to record the decibel between 30 to 130 dB. The three intervals recorded were the natural sound before the disturbance, 5-seconds, and 30-seconds after minor disturbance of the nests. Minor disturbance of the nests was carried out by gently pressing the nests, and the action was repeated three times. All data from both experiments were arranged separately in Microsoft® Excel 2007 and were subjected to statistical analysis using Analysis of Variance (ANOVA), correlation, regression, and GLM analyses using SAS software from the SAS® Software Version 8.

RESULTS AND DISCUSSION

A correlation analysis was performed to assess the association between the activity of *D. thoracicus*, involving 5-minutes observation of the ants moving into and out of the nests; and the nest's age. These bivariate analyses show a less significant correlation between *D. thoracicus* activities and nests' age for all treatments (Table 1 and Figure 1). Despite the lack of positive correlation for Zingiberaceae formulations and control treatment, the activities of *D. thoracicus* showed a strong negative relationship ($r = 0.061$, $p > 0.05$) between the nest's age treated with deltamethrin. Regression analysis was performed for each treatment between *D. thoracicus* activities against the artificial nest's age to obtain the least square regression. There was an increase of one unit in sampling occasions associated with an estimated increase of 0.919 *D. thoracicus* activities for control treatment. The increase of *D. thoracicus* activities were followed by *A. galanga* F1 (0.796), *Z. officinale* F1 (0.494), *A. galanga* F2 (0.414) and *Z. officinale* F2 (0.099). Whereas for treatment with deltamethrin, there was an increase of one unit in sampling occasions that was associated with an estimated decrease from 0.05 of the ant's activities. The highest variation in the *D. thoracicus* activities were explained by the variation of nest's age observed in *A. galanga* F1 (39.30%), followed by *Z. officinale* F1 (17.90%). In conclusion, there was a significant relationship between all treatments and variations of the nest's age applied at the 0.05 levels of significance, except for deltamethrin treatments.

Table 1. Activities of *D. thoracicus* in the artificial ant nests

Treatment	**Correlation (r)**	**Regression (Y)**	**Co. of correlation (R^2)**	**F Statistic**	**95% Conf. Limits**	**Mean ($\bar{x}$±s.d)**
A. galanga F1	0.637 ($p < 0.05$)*	9.201+0.796x	0.393	60.72*	0.593-0.999	34.625±21.375
A. galanga F2	0.429 ($p < 0.05$)*	18.745+0.414x	0.080	8.13*	0.125-0.703	27.604±29.784
Z. officinale F1	0.423 ($p < 0.05$)*	10.92+0.494x	0.179	20.45*	0.277-0.710	21.479±23.690
Z. officinale F2	0.261 ($p < 0.05$)*	26.993+0.099	0.004	0.40*	-0.212-0.411	29.115±30.898
Deltamethrin	0.061 ($p > 0.05$)	18.024-0.05x	0.038	0.36	-0.224-0.124	16.917±17.087
Control	0.469 ($p < 0.05$)*	29.317+0.919x	0.221	26.59*	0.565-1.273	48.958±39.671

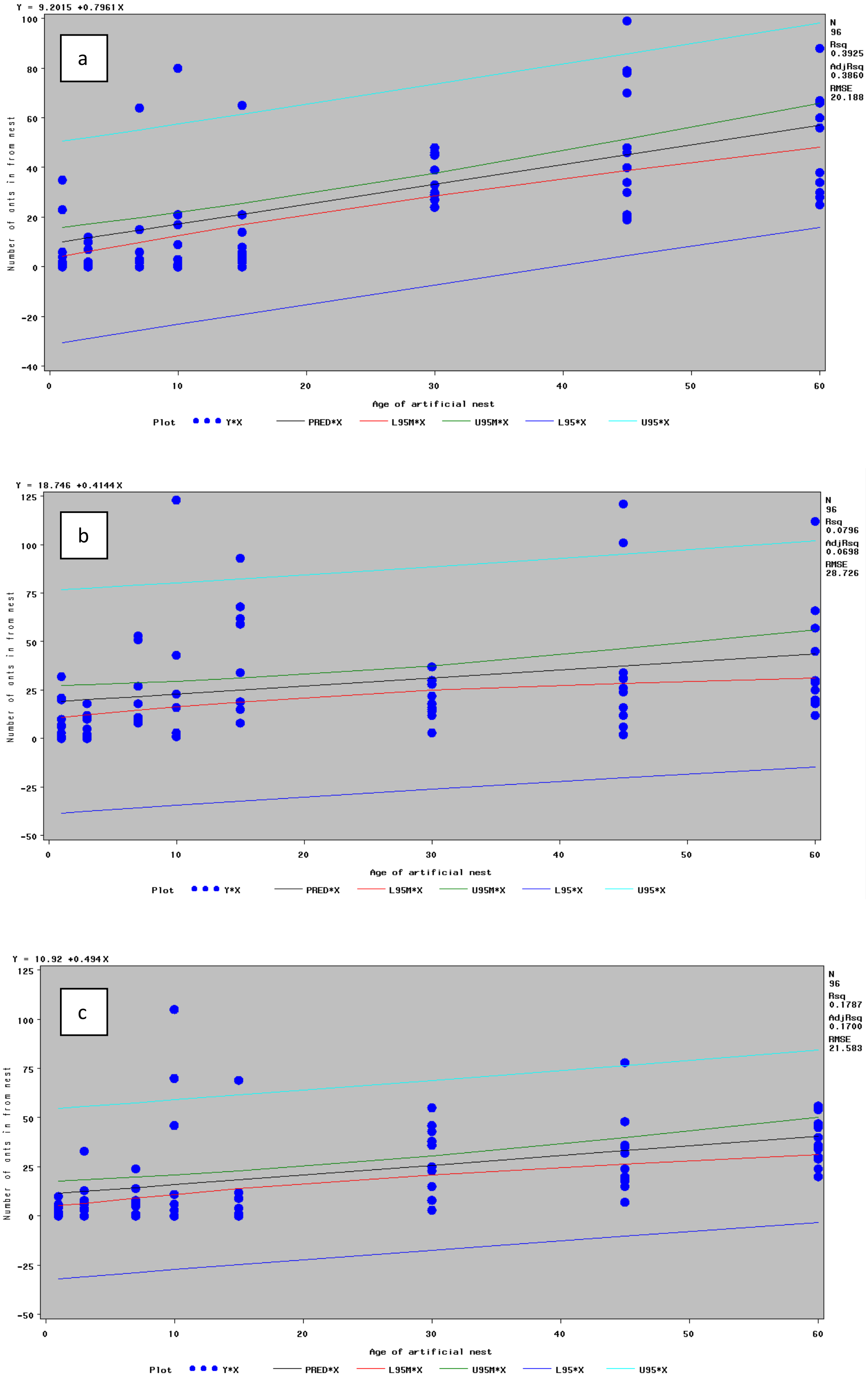

Figure 1a. Regression analysis for the number of ants in the nests against the age of artificial nests at a) *A. galanga* F1, b) *A. galanga* F2 and c) *Z. officinale* F1

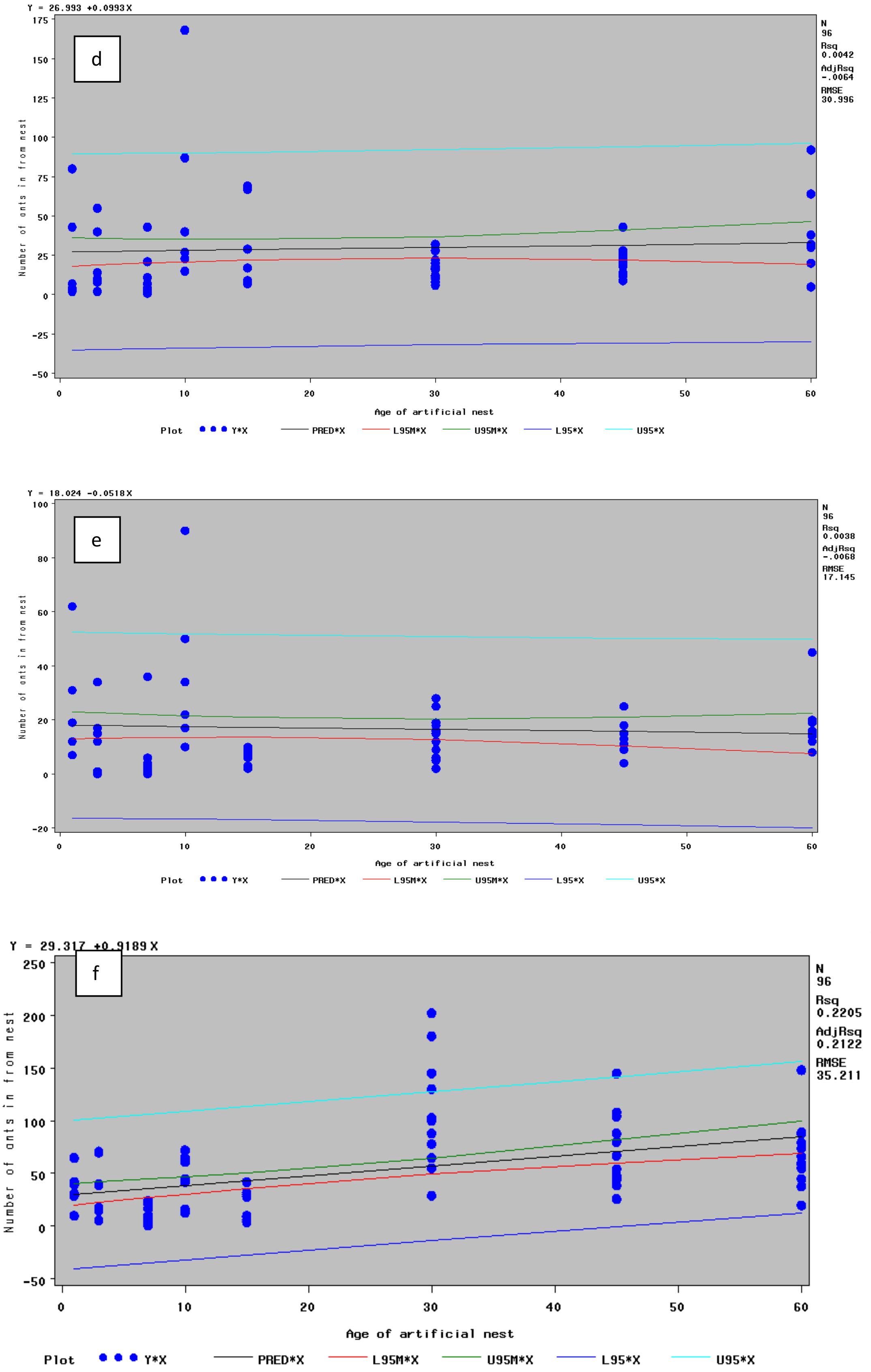

Figure 1b. Regression analysis for the number of ants in the nests against the age of artificial nests at d) *Z. officinale* F2, e) deltamethrin and f) control

Mean of *D. thoracicus* moving into the nests shows the highest at day-60 (60.139±30.742a) and followed by day-10 (59.722±44.582a) and day-45 (49.389±35.478ab). These days were significantly different (Table 2) compared with day-15 (37.683±28.471 c), day-1 (24.306±24.061d), day 3 (21.889±22.637d), and day-7 (19.611±18.361d). A similar observation was denoted for the mean of ants moving out of the nest, where the means for days-30, 45, and 60 were significantly different compared with the first 15 days of observation.

Among all treatments, control recorded the highest number of ants both for moving into (67.083±41.966a) and moving out 30.833±27.366a of the treated nest (Table 3), and significantly different ($p < 0.05$) compared with other treatments. The least number of ants' activities was recorded in treatment with deltamethrin where approximately 23 ants (23.042±20.826d) were observed moving into the treated nest and approximately ten ants (10.792±8.930c) were moving of from the treated nests.

Table 2. Mean of *D. thoracicus* moving into and out of the different age of the artificial ant nests

Days of observation	**Moving into the nest**	**Moving out of the nest**
1	24.306±24.061d	12.611±13.968c
3	21.889±22.637d	9.278±9.254cd
7	19.611±18.361d	6.333±6.000cd
10	59.722±44.582a	18.556±12.891b
15	37.683±28.471c	9.083±8.466d
30	45.500±45.179bc	28.028±27.891a
45	49.389±35.478ab	23.528±18.381ab
60	60.139±30.742a	28.556±15.722a

Table 3. Mean of *D. thoracicus* moving in and out of the artificial ant nest at different treatments

Treatment	**Moving into the nest**	**Moving out of the nest**
A. galanga F1	34.625±29.968bc	17.813±17.299b
A. galanga F2	42.542±35.438b	12.667±9.026bc
Z. officinale F1	28.771±27.987cd	14.188±15.574bc
Z. officinale F2	42.542±37.742c	15.688±11.707bc
Deltamethrin	23.042±20.826d	10.792±8.930c
Control	67.083±41.966a	30.833±27.366a

The acoustic sound strength inside the nests might devote to the population of ants inside the nest. The louder the decibel sound recorded indicates the higher number of ants, and the lower the decibel sound indicates the lower number of ants inside the nest (Table 4). After the first disturbance, there was a significant difference ($p < 0.05$) in term of decibel sound observed in 45-day old (13.070±7.231a) and 60-day old (11.020±7.894a) artificial nests, as compared to 30-day old (5.668±3.120b) and 15-day old artificial nest (1.563±1.437c). Whereas, after the second disturbance, the loudest decibel was observed on in 60-day old nests (23.658±10.509a), followed by 75-day old nests (12.023±7.848b), 30-day old nests (4.609±3.634c), and 15-day old nests (0.758±0.939d).

Table 4. The percentage of *D. thoracicus* decibel sound increments inside the artificial ant nests after first and second disturbances

Days of observation	**First disturbance**	**Second disturbance**
15	1.563±1.437c	0.758±0.939d
30	5.668±3.120b	4.609±3.634c
45	13.070±7.231a	12.023±7.848b
60	11.020±7.894a	23.658±10.509a

Under natural conditions, the decibel acoustic sound was recorded between 48.950 to 46.547 dB (Table 5). The increment in decibel was observed with initial disturbance in all treatments, where the loudest sound was recorded inside the control treatment (52.946±4.186a), and the lowest audio was in *Z. officinale* F2 treatment (49.967±3.360b). However, after the second disturbance, there was no significant difference observed between treatments. The results might indicate the number of *D. thoracicus* in the nests in all of the different treatments, were almost similar. Similar results were recorded for the percentage of sound increment after the first disturbance, where no significant difference ($p > 0.05$) was observed. However, after the second disturbance, the highest percentage of sound increment was recorded in the deltamethrin (12.087±11.200a), and the lowest percentage of sound increment recorded in was observed at *Z. officinale* F2 treated nests (7.235±7.772b).

Table 5. The decibel sound of *D. thoracicus* inside the artificial ant nest before, after first and second disturbances

Treatment	**Decibel acoustic sound (dB)**			**Percentage of the sound increment (%) after disturbance**	
	Before	**First**	**Second**	**First**	**Second**
A. galanga F1	48.243±1.390a	52.192±3.324ab	57.558±8.922a	8.239±7.115a	9.744±10.532ab
A. galanga F2	46.767±2.447b	51.367±5.919ab	57.179±9.736a	9.733±10.177a	11.390±15.834ab
Z. officinale F1	47.258±2.174b	50.417±3.991ab	55.750±8.678a	6.606±5.667a	10.061±10.177ab
Z. officinale F2	46.547±1.322b	49.967±3.360b	53.775±7.096a	7.304±5.519a	7.235±7.772b
Deltamethrin	48.733±1.548a	52.113±3.081ab	58.671±8.939a	6.928±5.246a	12.087±11.200a
Control	48.950±0.576a	52.946±4.186a	59.013±5.780a	8.151±8.303a	11.054±9.693ab

The results denoted that *D. thoracicus* actively moves into the nests starting from day-10, as in the previous observation by Saripah (2014a). The ants started to be active in roaming and foraging for food outside the nests after two weeks of the introduction. Later activity of of *D. thoracicus* moving into and out of the nest recorded the highest on day-60, and was significantly different as compared to the first 15 days. This might be due to the food hunting process, lack of food source, and population increment inside the nests. This behavior encouraged more ant activities outside the nest, as observed in the second month after the first augmentation date. This result may suggest that the population of ants became more stable with the age of nests (Saripah 2014a). The study denoted that a month after the first nest augmentation, intense ground trails of *D. thoracicus* were observed at the sampled blocks, and heavy ground trails were observed in the first two months after nests augmentation.

The highest increment of *D. thoracicus* activities for the control treatment between each sampling session might suggest that untreated, dry leaves positively influenced the ant population inside the nest and their daily activities. Meanwhile, the low concentration of EOs integrated into the formulation (F1) influenced more active activities of *D. thoracicus* for both *A. galanga* (0.796) and *Z. officinale* (0.494) compared to higher concentrations (F2). This might suggest that at higher concentrations, this botanical formulation might affect negatively the population and their activities. High dosage of EOS may increase non-targeted arthropods' risk (Pavela & Benelli 2016).

The result also denoted the significant relationship for all treatments and variation of the nest's age with ant activities, except for deltamethrin treatment. The decreasing activities of ants from the deltamethrin-treated nest contradicted all Zingiberaceae treatments, and this negative impact may affect the population of ants in the nest itself. The positive impact of Zingiberaceae on the activity of *D. thoracicus* might be due to the low concentration of EOs integrated into the formulation, thus was safe to this biological control agent. The contradicted result may occur if the concentration of EOS appears at high dosages, with the application of undiluted concentrates, or in the long term of exposure. Degradation processes of the formulation after exposure to the environment may also decrease residue on the treated leaves, therefore giving less impact to the population of *D. thoracicus* in the constructed nest. Degradation process especially sunlight photodegradation is one of the trigger factors for pesticides drift away after their release into the environment, especially if spraying were undertaken on the leaf surfaces (Katagi 2004). Besides, no direct spraying that was targeted to the ants, therefore, all treatments did not affect the activities and population built-up inside the nest.

Among the artificial nests, control recorded the highest number of ants, and the least number of ant's activities was recorded in nests treated with deltamethrin. It was believed that *D. thoracicus* might have shifted or roamed for a more convenient nest, and resulted in the highest activities at control, compared to the deltamethrin-treated and Zingiberaceae-treated nests. Similar trends were observed on the nest preference between a high-quality and low-quality nest by the rock ants, *Temnothorax albipennis.* That showed the ant successfully emigrated to the better quality nest regardless of the location (Franks et al. 2008). Colonies migrated to the better nest, even up to nine times further away than the collinear poorer one. Their observation denoted that *T. albipennis* started emigrating simultaneously to any nests, begin to look elsewhere, and then redirected all traffic to a more distant nest, exclusively to the high-quality nests. Ants might compensate for distance to find and allocate better nest during the decision-making and emigration process. In our study, the non-treated nest is believed to have the best quality nest material, which the dry cocoa leaves free of any treatment residue.

An insect can generate sounds in several ways; stridulation, percussion, vibration, tymbal mechanism, and air expulsion (Ganchev et al. 1988). The insect can be identified based on their ability to generate sound for communication purposes or as a by-product of eating, flying, or locomotion (Ganchev et al. 1988). The use of bioacoustics signal, especially of loudest insects such as crickets, cicadas, grasshoppers, and katydids, is useful for monitoring their population, species identification, habitat health assessment, and inventory of the biological diversity. The use of acoustic space and species richness could lead to more effective biodiversity monitoring tools (Aide et al. 2017). Therefore, the evaluation of acoustic strength inside the artificial ant nest might be used as a contrivance to estimate the population of *D. thoracicus* in this human-made habitat and compare the effect of treatment to the ants' population.

The behavior of *D. thoracicus*, which provides acoustic sounds, might be due to the vibration of body parts as can be recognized for some hymenopteran species. The insect's behavioral sound modes were classified into the congregational songs, the calling songs, the courtship songs, and the protest squawks. The protest squawks are generated when the insect is captured or disturbed. This warning sound can be recognized as intimidation or fight and were produced as a sign to warn other insects of danger. The idea of this protest squawks that are usually produced by singing insects of cicadas, crickets, and katydids may be applied to explain the sound increments after the nest was disturbed, and it might be a sign to warn other ants about the possibility of danger outside the nest. The louder decibel recorded inside the nest is believed to represent the higher number of *D. thoracicus* individuals, compared to lower decibel data.

The percentage of *D. thoracicus* decibel sound increment inside the artificial ant nest after first and second disturbances shows days-45 and 60 recorded higher decibels compare to day-15 and 30. A similar method was introduced by placing the apparatus inside the nest (approximately 10cm inside and between the depth layer of dry leaves) as soon as possible to ensure the sound is generated inside the nest to standardize data collection. Placing the apparatus in the depth of the nests was implemented to reduce the presence of geophony (e.g., wind, rain) and anthophony (e.g., footsteps, vehicle) elements. The strength might be occur due to the high population of ants inside the nest, compared to the first 30 days of nest augmentation. In general, all treatments recorded the decibel increment after the first and second disturbances, compared to the natural sound before any interference. The percentage of decibel sound increment showed no significant difference after the first disturbance was undertaken. Among treatment, *Z. officinale* F2 recorded a lower variation of sounds after the second disturbance. Without any interference, the decibel sounds were high in control, deltamethrin, and *A. galanga* F1 nests and significantly different when compared to *Z. offinale* F1 and F2, and *A. galanga* F2 nests.

In constructing the nest, dry cocoa leaves are the most important element for population built-up inside the nest later on. Based on the results, Zingiberaceae formulations and insecticide may have fewer limitations to the activity of *D. thoracicus*, if the treatment is only applied on the dry leaves that are stuffed inside the nest. The ants started to enter the nest as soon as the nest hangs on the cocoa trees and gradually increased after ten days. This situation might be due to the degradation process of treatment residue on the leaves, leaving a convenient situation for ants to accommodate the nest after that. Monitoring using acoustic sound, which is not an invasive method, non-intrusive, and requires a less intensive workforce, is essential; compare to conventional destructive sampling for the visual count to estimate the population of *D. thoracicus* inside the nest. The device used in this study is reliable and easy to use with minimal cost; therefore, the acoustic device has been considered as a technique for monitoring the ant population inside the treated nest. However, visual counting to monitor the cocoa trees' ant activities, especially moving into and out of the nests, is still crucial to estimate their daily activities after the artificial ant nests were treated.

CONCLUSIONS

As a part of the biological control agent, *D. thoracicus* may indirectly reduce the infestation of cocoa pests, particularly *C. cramerella* and *Helopeltis* spp. Therefore, the management approach in combating major cocoa pests must be assured having less or no negative impact on this biological control agent and other cocoa beneficial insect species. The results might suggest that Zingiberaceae formulations (*A. galang*a and *Z. officinale*) may have fewer limitations to the activity of *D. thoracicus,* and thus may be safe to this beneficial insect of cocoa. Consideration of either control approach that might affect beneficial insects' population is essential; therefore, it should be added as one of the pest decision program criteria. Selective and safe control might ensure the cocoa environment is resilient for beneficial organisms. Nevertheless, it can still maintain good production reward profits for the cocoa grower.

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REFERENCES

Aflitto, N.C. & Hofstetter, R.W. 2013. Use of acoustics to deter bark beetles from entering tree materials. *Pest Management Science* 70: 1808-1814.

Ahmad Saleh, Wan Mohd Hafezul Wan Abdul Ghani, Abu Hassan Ahmad, Che Salmah Md Rawi and Hasber Salim, 2020. Boosting cocoa blackant (*Dolichoderus thoracicus*) (Smith) (hymenoptera:formicidae) population using artificial nests. *Journal of Entomology* 17: 110-116.

Aide, T.M., Hernandez-Serna, A., Campos-Cerqueira, M., Acevedo-Charry, O. & Deichmann, J.L. 2017. Species richness (of insects) drives the use of acoustic space in the tropics. *Remote Sensing* 9 (1096): 1-12.

Bakkali, F., Averbeck, S., Averbeck, D. & Idaomar, M. 2008. Biological effects of essential oils – a review. *Food and Chemical Toxicology* 46(2): 446-475.

Bateman, R. 2015. *Pesticide Use in Cocoa. A Guide for Training Administrative and Research Staff*. 3rd Edition. London: International Cocoa Organization (ICCO).

Bilski, P., Bobiński, P., Krajewski, A. & Witomski, P. 2017. Detection of wood boring insects' larvae based on the acoustic signal analysis and the artificial intelligence algorithm. *Archives of Acoustics 42*(1): 61–70.

Chiu, Y.K., Mankin, R.W. & Lin, C.C. 2011. Context dependent stridulatory responses of Leptogenys kitteli (Hymenoptera: Formicidae) to social, prey, and disturbance stimuli. *Annals of the Entomological Society of America* 104:1012-1020.

Entwistle, P.F. 1972. *Pests of Cocoa*. UK: Longman.

Franks, N.R., Hardcastle, K.A., Collins, S., Smith, F.D., Sullivan, M.E., Robinson, E.J.H. & Sendova-Franks, A.B. 2008. Can ant colonies choose a far-and-away better nest over an in-the way poor one? *Animal Behavior* 7(2): 323-334.

Ganchev, T., Potamitis, I. & Fakotakis, N. 1988. Acoustic monitoring of singing insects.In Proceedings of Acaustics, Speech and Signal Processing (ICASSP-88).

Geng, S., Zhang, X. & Zhao, W. 2017. Detection and analysis of the stored grain insect creeping sound. *IOP Conference series: Materials Science and Engineering* 2422: 1-6.

Katagi, T. 2004. Photodegradation of pesticides on plant and soil surfaces. *Reviews of Environmental Contamination and Toxicology* 182: 1-78.

Koul, O., Walia, S. & Dhaliwal, G. 2008. Essential oils as green pesticides: Potential and constraints. *Biopesticide International* 4: 63-84.

Lee, C.H., Kelvin, L., Haya, R., Navies, M. & Saripah, B. 2013. *Cocoa Planting Manual. Sustainable Cocoa*. Kota Kinabalu: Malaysian Cocoa Board.

Lee, C.H., Albert, L.S.C., Soetikno, S.S., Jeremy, N.C.K., Ramle, K., Sabariah, S. & Haya, R. 2014. *Manual Latihan Keselamatan Koko: Pembangunan Modal Insan dan Perkongsian Pengetahuan dalam Piawaian Sanitari dan Fitosanitari (SPS) Koko di Asia Tenggara*. Sabah: Malaysian Cocoa Board, Kota Kinabalu.

Llusia, D., Marquez, R. & Bowker, R. 2011. Terrestrial sound monitoring systems, a methodology for quantitative calibration. *Bioacoustics* 20: 277-286.

Mankin, R.W., Hagstrum, D.W., Smith, M.T., Roda, A L. & Kairo, M.T.K. 2011. Perspective and promise: A century of insect acoustic detection and monitoring. *American Entomologist* 57: 30-44.

McMahon, P., Husing, P., Lambert, S., Mulia, S., Nurlaila., Susilo, A.W., Sulistyowati, E., Sukamto, S., Israel, M., Saftar, A., Amir, A., Purwantara, A., Iswanto, A., Guest, D. & Keane, P. 2015. Testing local cocoa selections in three provinces in Sulawesi: (i) Productivity and resistance to cocoa pod borer and *Phytophtora* pod rot (black pod). *Crop Protection* 70: 28-39.

Mediene, S., Valantin-Morison, M., Sarthou, J.P., de Tourdonnet, S., Gosme, M., Bertrand, M., Roger-Estrade, J., Aubertot, J.N., Rusch, A., Motisi, N., Pelosi, C. & Dore, T. 2011. Agroecosystem management and biotic interactions: A review. *Agronomy Sustainable Development* 31:491–514.

Mizrach, A., Hetzroni, A., Mazor, M., Mankin, R.W., Ignat, T., Grinshpun, J., Epsky, N.D., Shuman, D. & Heath, R. R. 2005. Acoustic trap for female mediterranean fruit flies. *Transactions of the American Society of Agricultural Engineers* 48(5): 2017–2022.

Mouysset, L., Doyen, L. & Jiguet, F. 2013. How does economic risk aversion affect biodiversity. *Ecological Applications* 23(1): 96-109.

Parkash, A. & Rao, J. 1997. *Botanical Pesticides In Agriculture.* Boca Raton: CRC Luis Publication.

Pavela, R. & Benelli, G. 2016. Essential oils as ecofriendly biopesticides? Challenges and constraints. *Trends in Plant Science* 21 (12): 1000-1007.

Prowse, G.M., Galloway, T.S., & Foggo, A. 2006. Insecticidal activity of garlic juice in two dipteran pests. *Agricultural and Forest Entomology* 8: 1-6.

Riede, K. 1993. Monitoring biodiversity: Analysis of Amazonian rainforest sounds. *Ambio* 22: 546-548.

Rosdiyani, M., Ismail, A.R. & Siti Noor Hajjar, M.L. 2016. Efficacy and persistence of neem in emulsion-in-water (EW) formulation against the golden apple snail. *International Journal of Scientific Engineering and Applied Science (IJSEAS)* 2(9): 99-107.

Saripah, B., Noor Hajjar, M.L.S., Alias, A. & Zhang, A. 2019a. Pupation preference of *Conopomorpha Cramerella* (Snellen) after treated with *Zingiber officinale*, *Curcuma longa* and *Alpinia galanga* essential oils. *Journal of Engineering and Applied Sciences* 14: 6150-6155.

Saripah, B., Noor Hajjar, M.L.S., Alias, A. & Zhang, A. 2019b. Inhibitory effect of Zingiberaceae essential oils against *Conopomorpha cramerella* (Snellen) Lepidoptera: Gracillariidae. *Journal of Bangladesh Agricultural University* 17(3): 349-354.

Saripah Bakar, M.L.S. Noor Hajjar, Alias, A. & Zhang, A. 2017. Effects of *Zingiber officinale*, *Curcuma longa* and *Alpinia galanga* essential oils on the morphological characteristic of Cocoa pod borer, *Conopomorpha cramerella*. *Journal of Fundamental and Applied Sciences* 9 (6S): 25-38.

Saripah, B., Alias, A. & Azhar. I. 2017. Mosquito bug in *Technical guidelines for the safe movement of cacao germplasm.* Revised from the FAO/IPGRI Technical Guidelines No. 20 (Third Update, October 2017). In End, M.J., Daymond, A.J. & Hadley, P. (eds.). *Global Cacao Genetic Resources Network* (*CacaoNet*), pp. 73-76. Rome, Italy: Bioversity International.

Saripah, B. 2014a. Sustaining cocoa black ant *Dolichoderus thoracicus* (Smith) using artificial nest in the cocoa ecosystem. *Malaysian Cocoa Journal* 8: 23-30.

Saripah, B. 2014b. Control of CPB using insecticides and Cocoa black ants. *Malaysian Cocoa Journal* 8: 14-22.

Saripah, B. & Azhar, I. 2007. Handling of cocoa black ants as a biological control agent against cocoa pod borer in monococoa ecosystem. In *Proceedings 2007 Conference on Plantation Commodities*, pp 48-54.

Sirirungsa, P. 1998. Thai zingiberaceae: Species diversity and their uses. *Pure Application Chemical* 70: 98-110.

Sirirungsa, P., Larsen, K. & Maknoi, C. 2007. The Genus Curcuma L. (Zingiberaceae): Distribution and classification with reference to species diversity in Thailand. *Gardens Bulletin Singapore* 59 (1&2): 203-220.

Stoate, C., Boatman, N.D., Borralho, R.J., Carvalho, C.R., de Snoo, G.R. & Eden, P. 2001. Ecological impacts of arable intensification in Europe. *Journal of Environmental Management* 63: 337-365.

Sueur, J., Pavoine, S., Hamerlynck, O. & Duvail, S. 2008. Rapid acoustic survey for biodiversity appraisal. *PLoS One* 3:e4065.

Sundaram, K.M.S., Campbell, R., Sloane, L. & Studens, J. 1995. Uptake, translocation, persistence and fate of azadirachtin in aspen plants (*Populus tremuloides* Michx.) and its effect on pestiferous two-spotted spider mite (*Tetranychus urticae* Koch). *Journal of Crop Protection* 14 (5): 415-421.

Tripathi, A., Prajapati, V., Verma, N., Bahl, J., Bansal, R. & Khanuja, S. 2002. Bioactivities of the leaf essential oil of *Curcuma longa* (var. ch-66) on three species of stored-product beetles (Coleoptera). *Journal of Economical Entomology* 95: 183-189.