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THE EFFECT OF PLANT VOLATILES ON PLANT PREFERENCE BY THE PREDATORY INSECT, *Sycanus dichotomus* STAL. (Hemiptera: Reduviidae) IN OIL PALM PLANTATION

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ABSTRACT

A study of choice test and no-choice test was conducted to test plant volatiles response to adult orientation behaviour and performance of predatory insect, Sycanus dichotomus *on three beneficial plants* (Turnera subulata, Antigonon leptopus *and* Cassia cobanensis) *and three ground vegetation covers* (Asystasia gangetica, Euphorbia heterophylla *and* Ageratum conyzoides). *Gas chromatography-mass spectrometry (GC-MS) was used to determine the number of volatile compounds in the beneficial plants extracts, namely Trimethyl-3,4-methylenedioxychromane, Phenol,4,6-di(1,1-dimethylethyl)-2-methyl, 2H-1-Benzopyran,6,7-dimethoxy-2,2-dimethyl-, n-Hexadecanoic acid, Dodecanoic acid, 3-hydroxy-, Octadecanoic acid, and 2-(2-hydroxyethoxy) ethyl ester and to confirm the most preferred plant by the predatory insect. We recommend further investigation on the efficiency of various beneficial plants in relation to the activities of* S. dichotomus.

Keywords: *Sycanus dichotomus*, beneficial plants, host finding, plant volatiles, GC-MS.

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INTRODUCTION

Conservation and distribution of natural enemies of insects (predators and parasitoids) in oil palm plantation suppress pests while pollinators increase crop yields. Many beneficial insects rely on useful plants for nectar and pollen or shelter (Jamian *et al.*, 2017). Plants that are commonly recommended to provide these resources are non-native annuals such as *Turnera subulata, Antigonon leptopus* and *Cassia cobanensis* and these plants are actively being implemented by planters to sustain the population of natural enemies (Norman and Basri, 2007). Besides that, beneficial plants can be crucial in providing alternative food (nectar and pollen) and habitat for beneficial insects (Basri and Kevan, 1995). Many predatory insects, pests and parasites use flower nectar as a source of energy while they search for prey (Yusdayati *et al.*, 2014).

Currently, effective control measures of bagworms comprise of different chemical insecticides. In Malaysia, trunk injection technique using monocrotophos and methamidophos is the most popular control method to control pest of oil palm aged six years old and above in Felda plantations (Noor Hisham and Sukri, 2004).

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Although the usage of insecticides is successful in controlling pests (Wood and Nesbit, 1969; Sing, 1986; Chung, 1989), extensive usage can annihilate natural enemy population and consequently encourages pests resurgence that can lead to pest outbreak (Leena and Hazem, 2011). However, Noor Farehan *et al.* (2013) highlighted that cypermethrin, deltamethrin and trichlorfon are the prominent compounds that caused high mortality against insect predators. Commonly, natural enemies of bagworms such as predators and parasitoids are abundantly available on beneficial plants (Ho *et al.*, 2003; Norman and Basri, 2010; Jamian *et al.*, 2017). Strategic use of flowering plants to increase plant biodiversity in a targeted manner can provide natural enemies with shelter and food source to enhance biological control and reduce dependence to chemical pesticides. Therefore, the propagation and abundance of natural enemies are strongly dependent on the availability of suitable beneficial plants as sources of nectar (Norman and Basri, 2010). Planting of beneficial plants such as *T. subulata, C. cobanensis* and *A. leptopus* were adopted in oil palm plantation to provide food and shelter to many natural enemies particularly predators and parasitoids (Ho, 2002).

Predators and parasitoids need the plants and flowers that provide moisture, shelter, alternative prey and immediate nutrition from nectar and pollen. Floral nectar composition is vital in species pollination system and relationship with potential pollination vectors (Heil, 2011). Different groups of animals will have different physiology and nutritive requirements; therefore, the composition of sugars and amino acids in nectar is usually highly correlated with the specific nutritive requirements of a flower's pollinators (Gonzalez-Teuber and Heil, 2009). Besides that, by manipulating the habitat of floral resources in agroecosystem, it could provide additional food for beneficial insects and potentially enhancing their fitness and efficacy. Nutrients such as carbohydrate-rich nectars are believed to provide energy, and pollens, may provide nutrients for egg production in some species (Jervis *et al.*, 1996) and longevity of parasitoids (Basri *et al.*, 1999).

Nevertheless, flower may attract beneficial insects through the released chemicals. Liu *et al.* (2013) reported that flowers of *Mucuna sempervirens* produce nectar whose composition include sugars, proteins, phenolics, hydrogen peroxide and aromatic compounds. While, the flowering plants namely *C. cobanensis, Crotalaria usaramoensis, Asystasia gangetica* and *Euphorbia heterophylla* contain nectar composed of sucrose, fructose and glucose (Basri *et al.*, 1999). Many predators and parasitoids are attracted to flowering plants, where they obtain pollens and nectars that help to increase their longevity and ability to lay eggs. Jamian and Nur Azura (2018) reported the combination of flowering plants (*T. subulata*) and its prey (*M. plana*) would sustain their longevity and fecundity of predatory insect. We are not aware of any research being carried out on the efficacy of beneficial plants to improve performance of insect predators as potential biocontrol agents in oil palm plantation. Therefore, the aims of this study were: (i) to investigate the efficiency of various beneficial plants towards the activities of *S. dichotomus*, and (ii) to evaluate the nectar content of different type of plants.

MATERIALS AND METHODS

The study was carried out under a controlled environment in the glasshouse of the Faculty of Agriculture, Universiti Putra Malaysia (UPM), Selangor, Malaysia. The glasshouse had 12:12 hr day:night photoperiod, 21°C-36°C temperature range and relative humidity of 55%-65%. Six types of plants were used in this study, namely, *T. subulata, A. leptopus, C. cobanensis, Asystasia gangetica, Euphorbia heterophylla* and *Ageratum conyzoides*. Experiment was separated to no-choice and choice test categories.

No-choice and Choice Glasshouse Test

The tested plants were placed in a cage, (40 cm x 40 cm x 60 cm) (*Figure 1*). The sides and the top were made of transparent perspex, and the base was made of wood. The front of the cage was converted into a door with a dimension of 40 cm x 60 cm to facilitate access into the cage for introducing the various plants prior to the start of the experiment and for cleaning purposes. A hole of 20 cm diameter was made at the left and right sides, and on the door, to introduce insect predators. To prevent insect escaping the rearing cage, the holes were equipped with a sleeve of muslin cloth that can be folded. To diffuse the light coming from the ceiling, the top of the cage was covered with a layer of clean plain white paper. For no choice test, five pairs (males and females) of *S. dichotomus* and six types of plants, 2-3 pots per plant were used for *T. subulata, C. cobanensis, E. heterophylla, A. gangetica,* and *A. conyzoides,* except for *A. leptopus* with only one pot. Each plant type was located in different cages; they were introduced into the cage when they have flowers which were completely opened. Whereas, for the choice test, five pairs (males and females) of *S. dichotomus* and six types of plants were introduced in the same cage (100 cm x 100 cm x 170 cm). The parameters observed were: (i) the number of predatory insects feeding on nectar or resting on any parts of the plants between 9.00 am-11.00 am for one to four days after introduction into the cage, and (ii) the number of eggs laid. All experiments were replicated 10 times.

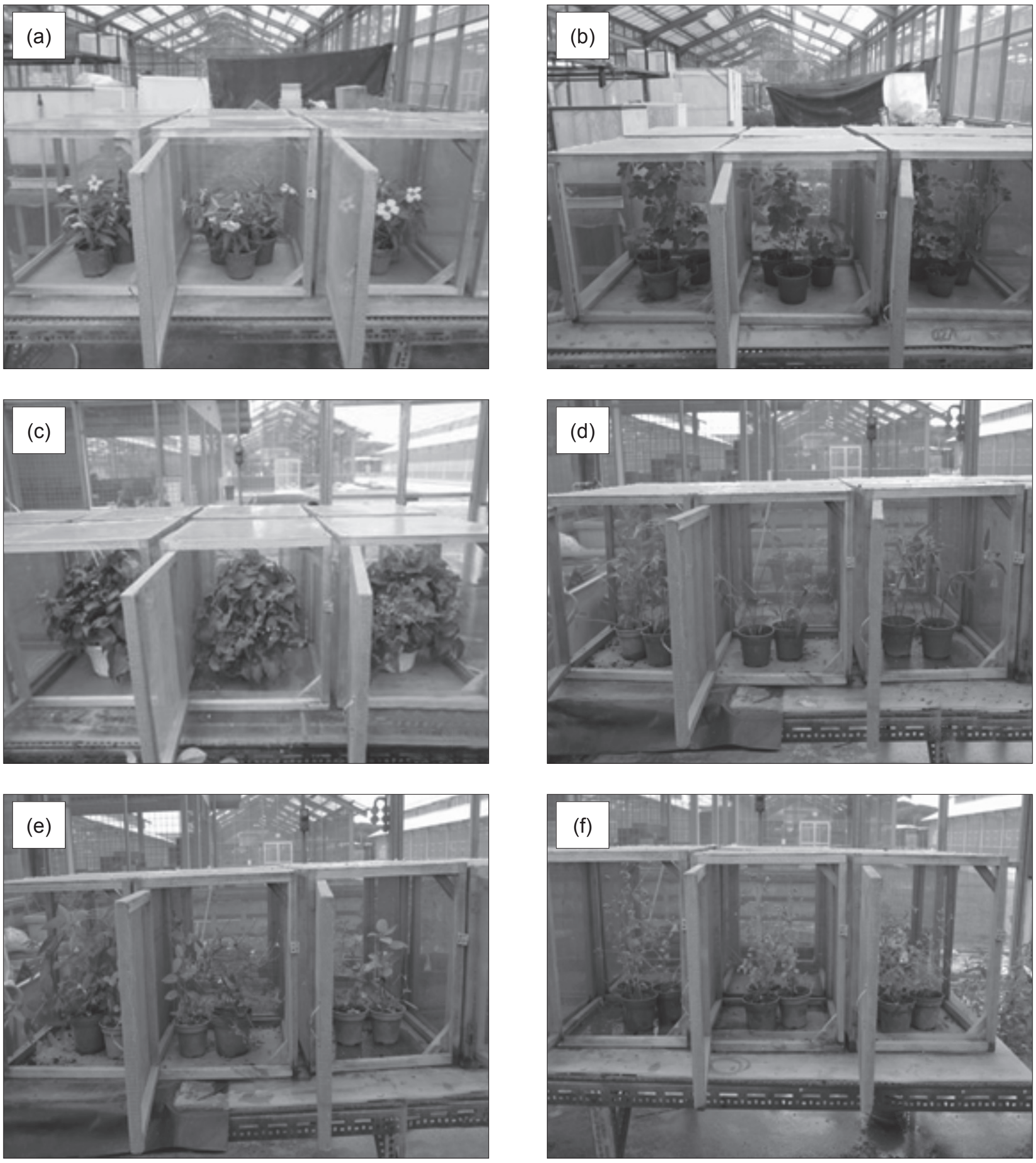

Figure 1. Demographic of plants, (a) T. subulata, *(b)* C. cobanensis, *(c)* A. leptopus, *(d)* E. heterophylla, *(e)* A. gangetica, *and (f)* A. conyzoides.

Chemical Composition of Nectars

Fresh nectars were collected from the six plant species in Field 2 and the Glasshouse Unit at UPM. A wick was used to collect nectars from 10 flowers of *T. subulata, A. leptopus, C. cobanensis, A. gangetica, E. heterophylla* and *A. conyzoides* as described by Liu *et al.* (2013). Collected nectars were extracted using n-hexane solvent by shaking at room temperature for 1 hr. The extract was then centrifuged at 12 000 rpm for 30 min at 4°C and concentrated under a stream of N^2. The extract was analysed using gas chromatography (GC) [Trace EC Ultra, column: 0.25 mm x 30 m (TG-5MS)] with temperature programming 80°C to 350°C at 3°C min^{-1}, or to gas chromatography-mass spectrometry (GC-MS) [TSQ Quantum XLS (Trace EC Ultra, column: 0.25 mm x 30 m) (TG-5MS)] under the following conditions: electron-impact (EI) mode (70 eV), constant current 1.0 ml min^{-1}, and temperature programming from 80°C to 240°C at 3°C min^{-1}. Identification of volatile compounds was performed using X Calibur 2.1 software for Windows. Peak identification was made by comparing retention times and the mass spectra of eluting compounds to those in the NIST/Wiley database.

Data Analysis

Obtained data sets of choice and no-choice experiments were subjected to analysis of variance using computer software (MINITAB 14.0 for Windows) statistical package. Treatments with significant differences were compared at P = 0.05 level of probability using Tukey's test.

RESULTS

In the no-choice test, the duration of exposure to different plants had a pronounced effect on the cumulative mean number of adult predators. The highest number of adult predators were observed on *T. subulata, A. leptopus, C. cobanensis* and *A. conyzoides* throughout the four days exposure as shown in *Table 1*. The number of adult predators at two days exposure time were significantly highest (P<0.001) on *T. subulata* (3.7 individuals), *C. cobanensis* (3.3 individuals) and *A. leptopus* (2.6 individuals) compared to other plants in the treatments. However, it was observed a decline in the number of predators in those plants at Day 3 of exposure. The number of predators on the weed plants ranging from 2.1 to 2.2 (*A. gangetica* and *E. heterophylla*) was lower compared to the other plants with the exception of Day 3, throughout the study. At Day 4, *T. subulata* had similar number of predators as *A. leptopus, C. cobanensis* and *A. conyzoides* but significantly different from *A. gangetica* and *E. heterophylla*.

The same result was obtained from the choice study where the exposure to different plants had a pronounced effect on the mean number of adult predators. The highest number of adult predators was observed on *T. subulata* and *C. cobanensis* throughout the four days exposure times (*Table 2*). In this study, the presence of predators after two days on *T. subulata* (3.0 individuals), *A. leptopus* (3.0 individuals) and *C. cobanensis* (2.1 individuals) were observed and they were significantly higher than the other plants (*A. gangetica, E. heterophylla* and *A. conyzoides*). Although the number of predators declined at Days 3 and 4 of observation, *T. subulata* and *C. cobanensis* remained the highest with 2.2 and 2.3 number of predators, respectively. Meanwhile, *A. leptopus* showed significant declined in the number of predators and the number was lower compared to the weed plants from Days 3 and 4 of observation. In both no-choice and choice tests, there were no eggs produced even though *S. dichotomus* was provided food from nectar. Essential nutrients such as protein and amino acids, lipids, vitamins and mineral are also vital to enhance or optimise growth, development and reproduction.

Based on the results of earlier study, extract of six plants namely *T. subulata, C. cobanensis, A. leptopus, A. gangetica, E. heterophylla* and *A. conyzoides* were included in the next study because they contained high concentration of volatile compounds. From the result, it is shown that about 88 to 92 volatile compounds were identified in the nectar and pollen using GC-MS based on NIST/ Wiley database, respectively. The quantification of identified compounds accounted for 88%-92% of the total volatiles (*Table 3*). Additional compounds were detected at retention times as shown in *Figure 2*. They were grouped into four major classes (alkane, alcohol, carboxylic acid and carboxylate ester) based on their biosynthetic origins. None of these four classes was dominant in the six plants.

DISCUSSION

Number of Predatory Insects

These findings supported Yusdayati *et al.* (2014) who reported that diversity and distribution of population of natural enemies can be observed on beneficial plants (*C. cobanensis* and *Turnera* spp.) while Norman and Basri (2010) reported that parasitoids and predators are the most abundant on *C. cobanensis*. However, Ho *et al.* (2003) reported that the number of parasitoid activities in the field increased with the presence of *E. heterophylla* and *C. cobanensis* in the field.

Few predatory insects and parasitoids feed on non-prey foods such as pollens and nectar (Hoffman and Frodsham, 1993; Hickman and Wratten, 1996; Kroon, 2009). Predators may be attracted to allelochemicals when released into the environment. Host colour and shape can be important in plant characteristic visual features and for predators that are day flying, although visually mediated responses are usually relatively unspecific (Bernays and Chapman, 1994). Many experiments have shown that the presence of flowers in an agroecosystem can increase the abundance of natural enemies. Results revealed that the tested predator showed strong attraction to the beneficial plant, *T. subulata* and these findings are similar to Yusdayati (2008), who reported that *Turnera* sp., *A. leptopus* and *C. cobanensis* are more favoured by natural enemies. Chaney (1998) highlighted that the number of beneficial insects increased in lettuce in the presence of a range of flowers species. Leaf roller parasitoids, *Dolichogenidea tasmanica* (Cameron) were found in yellow sticky trap and the number increased when buckwheat flowers were present in an apple orchard (Irvin *et al.*, 2000) and a vineyard (Berndt *et al.*, 2002).

The conducted experiments are the first study in investigating the attraction of predatory insects to beneficial plants namely *T. subulata, A. leptopus* and *C. cobanensis* and three ground vegetation covers, *A. gangetica, E. heterophylla* and *A. conyzoides*. Ho *et al.* (2003) carried out comprehensive trials to evaluate the efficacy of a range of beneficial plants and the result showed three plant species namely *C. cobanensis, A. leptopus* and *T. subulata* to be the most effective to attract natural enemies. In addition, *E. heterophylla* is not favoured for planting because of having difficulty to establish and possesses a short life cycle (Ho *et al.*, 2003); and *C. cobanensis* produces flowers only once a year (Basri *et al.*, 1999). The most preferred plant then is *Turnera*. Schlindwein and Medeiros (2006) has reported that the flowers of *T. subulata* could attract 28 species of insects. Similarly, Barret (1978) has listed as many as 13 species of insects as flower visitors on *T. urmifolia*. In this study, *S. dichotomus* was indeed very attracted to *T. subulata*. This shows the importance of *Turnera* as a source of shelter and nectar for *S. dichotomus* and it is suggested to plant it because the flowers are set throughout the year.

TABLE 1. MEAN NUMBER OF ADULT PREDATORS IN NO-CHOICE STUDY AT DIFFERENT EXPOSURE TIMES

Treatment/exposure time	Day			
	1	2	3	4
Cassia cobanensis	0.0 ± 0.00[a]	3.3 ± 0.90[ab]	2.7 ± 0.68[a]	2.8 ± 1.55[ab]
Antigonon leptopus	0.0 ± 0.00[a]	2.6 ± 1.08[abc]	2.5 ± 1.08[a]	3.2 ± 1.48[ab]
Turnera subulata	0.0 ± 0.00[a]	3.7 ± 1.40[a]	3.3 ± 0.68[a]	4.1 ± 1.20[a]
Asystasia gangetica	0.0 ± 0.00[a]	2.2 ± 0.79[bc]	2.8 ± 1.48[a]	2.1 ± 0.88[b]
Euphorbia heterophylla	0.0 ± 0.00[a]	1.8 ± 1.03[c]	2.2 ± 1.40[a]	2.1 ± 1.10[b]
Ageratum conyzoides	0.0 ± 0.00[a]	2.2 ± 0.79[bc]	2.1 ± 0.88[a]	2.6 ± 1.08[ab]

Note: Means in the column with same letters are not significantly different at P = 0.05 level of probability according to Tukey's test.

TABLE 2. MEAN NUMBER OF ADULT PREDATORS IN CHOICE STUDY AT DIFFERENT EXPOSURE TIMES

Treatment/exposure time	Day			
	1	2	3	4
Cassia cobanensis	0.0 ± 0.00[a]	2.1 ± 0.32[b]	2.3 ± 0.95[b]	2.0 ± 1.16[b]
Antigonon leptopus	0.0 ± 0.00[a]	3.0 ± 0.48[b]	0.5 ± 0.71[a]	0.0 ± 0.00[a]
Turnera subulata	0.0 ± 0.00[a]	3.0 ± 1.50[b]	2.2 ± 1.03[b]	2.0 ± 0.67[b]
Asystasia gangetica	0.0 ± 0.00[a]	0.0 ± 0.00[a]	0.2 ± 0.42[a]	0.4 ± 0.52[a]
Euphorbia heterophylla	0.0 ± 0.00[a]	0.4 ± 0.52[a]	0.8 ± 0.79[a]	0.8 ± 0.42[a]
Ageratum conyzoides	0.0 ± 0.00[a]	0.2 ± 0.42[a]	0.0 ± 0.00[a]	0.4 ± 0.52[a]

Note: Means in the column with same letters are not significantly different at P = 0.05 level of probability according to Tukey's test.

TABLE 3. COMPOSITION OF HOST PLANTS FLORAL VOLATILES FROM SIX CULTIVAR BY GAS CHROMATOGRAPHY-MASS SPECTROMETRY (GC-MS)

Compounds	Number of compounds					
	AC	AG	TS	CC	EH	AL
Alcohol	10	12	15	15	18	11
Carboxylate ester	12	19	9	19	14	22
Alkane	14	21	15	25	30	17
Alkene	13	3	5	5	4	7
Carboxylic acid	19	25	28	12	19	19
Alkyne	1	1	3	1	1	3
Aldehye	2	1	1	1	0	1
Amine	2	1	0	1	1	1
Carboxylate salt	0	2	0	0	0	0
Ester	2	0	0	0	0	0
Ether	0	0	0	1	0	1
Ketone	4	1	2	3	2	3
Organosilicone	2	1	1	0	0	1
Salt	5	2	10	8	1	2
Unknown	2	0	1	1	0	2
Total	88	89	90	92	90	90

Note: AC - *Ageratum conyzoides.*
CC - *Cassia cobanensis.*
AG - *Asystasia gangetica.*
EH - *Euphorbia heterophylla.*
TS - *Turnera subulata.*
AL - *Antigonon leptopus.*

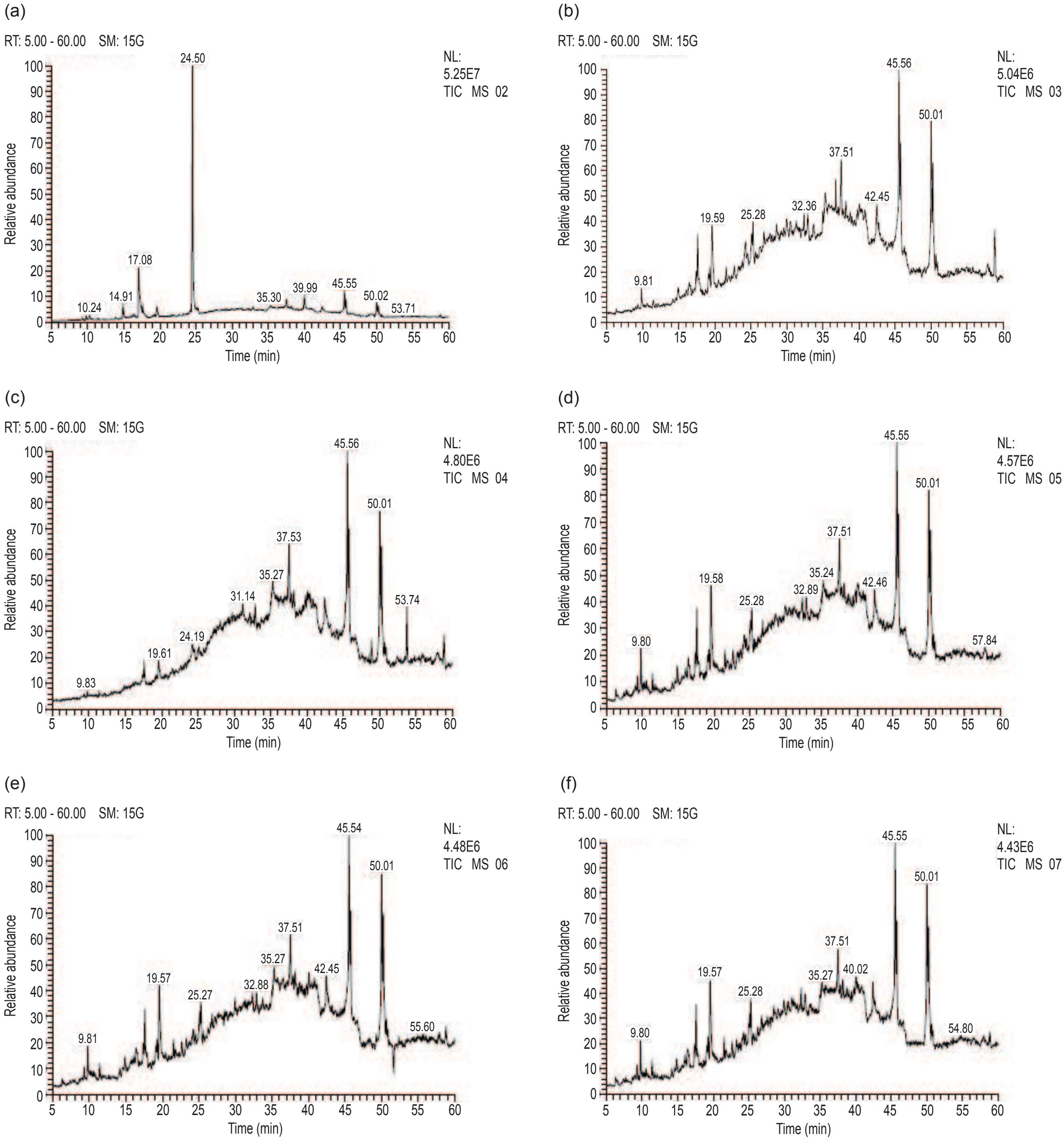

Figure 2. Chromatogram peak of plants, namely, A. conyzoides *(a),* A. gangetica *(b),* T. subulata *(c),* C. cobanensis *(d),* E. heterophylla *(e), and* A. leptopus *(f).*

Structural Elucidation Unknown Compound Using GC-MS

Terpenoids are the compounds that are generally thought to be insect attractants (Tholl *et al.*, 2004). In this study using six species of plants, the terpenoids containing volatile compounds were detected as mentioned by Basri *et al.* (1999) who stated that several compounds have been identified in intact host plants nectars (sucrose, fructose and glucose). For this study, the major constituents (48.45%) included Trimethyl-3,4-methylenedioxychromane, Phenol,4,6-di(1,1-dimethylethyl)-2-methyl, and 2H-1-Benzopyran,6,7-dimethoxy-2,2-dimethyl-, whereas the minor constituents (0.45%) were n-Hexadecanoic acid, Dodecanoic acid, 3-hydroxy-, and Octadecanoic acid, 2-(2-hydroxyethoxy) ethyl ester. According to Buchmann and Buchmann (1981) and Baker and Baker (1975), the host plant volatile compounds could attract predatory insects, where carboxylic acids, lipids and other organic compounds were also present in floral nectars. Besides that, the water element in nectar is also important to nectarivores (Willmer, 1986). Smilanick *et al.* (1978) reported field trials where acetaldehyde, ethyl acetate and ethyl alcohol were indicated as the principal attractants of *Carpopilus hemipterus* (Coleoptera: Nitidulidae) in California.

Volatile compounds are said to mediate many interactions between organisms, including plant response to pathogen infection (Shulaev *et al.,* 1997), plant-parasitoid signalling in response to herbivory (Turlings *et al.,* 1990) and plant-pollinator communication during flowering. As pollinator attractants, volatile compounds are the important cues that help insects to locate flowers and contribute to feeding and mating behaviours as well (Knudsen *et al.*, 1993). Plants synthesise and emit a large variety of volatile organic compounds with terpenoids, fatty acids and derivatives as the dominant classes. Floral scent composition of a plant is thought to have evolved partly from adaptations towards the olfactory requirements of efficient pollinators. Some volatile compounds are probably very common to almost all plants while others are specific to only one or a few related taxa (Visser, 1986; Pichersky and Gershenzon, 2002).

Plant odour specificity is achieved by a characteristic ratio of the constituent chemical compounds, which are generally distributed among the plant species (Visser, 1986). Anthers and pollens release distinctive odours (Blight *et al.,* 1995; Barkman, 2003) and floral volatile compounds are formed via plant biosynthetic pathways. The rapid progress in elucidating the biosynthetic pathways, enzymes and genes involved in the formation of plant volatiles allows their physiological activities and functions to be rigorously investigated at the molecular and biochemical levels. Floral volatiles act as attractants for species-specific pollinators. However, the volatiles emitted from the vegetative parts, especially those released after herbivory, protect plants by deterring herbivores and or attracting the enemies of herbivores (Pichersky and Gershenzon, 2002). Most of the floral fragrance compounds are terpenoids, simple aromatics, amines and hydrocarbons. The most common floral fragrance compounds are monoterpenes (Vickery and Vickery, 1981; Williams and Whitten, 1983).

There are suggestions on the use of beneficial plants for the improvement in the quality of natural enemies or beneficial insects (Van Emden, 1963; Altieri and Whitcomb, 1980; Tiong, 1982). Many researchers studied the parasitoid relationship with plants, while little research on predatory insects with plants. Leius (1967) reported on Umbelliferea species having carbohydrates content that is essential in the normal fecundity and longevity of three Icheumonid species. Meanwhile, Wolcott (1941) reported that the successful establishment of the introduced parasitoid, *Larra americana* to control cricket depended on the presence of two weeds, *Borreria verticillata* and *Hyptis atrorubens* as these plants provided the nectar for the adult wasps in Puerto Rico.

CONCLUSION

Predatory insects have been known to respond to plant volatile. Our result indicated that the volatile from *T. subulata, C. cobanensis*, and *A. leptopus* attract *S. dichotomus* adults in cage experiments. To our knowledge, there are few reports on the response of *S. dichotomus* to different plant volatiles. Both sexes of the predatory insect highly preferred the odours emanating from *T. subulata* followed by *C. cobanensis*, and *A. leptopus*. Our finding provides new information on how to increase the predatory insects as a biological control agent in oil palm plantation. Stakeholders need to manage oil palm plantations by planting beneficial plants for predatory insect habitat.

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