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Research Article

Repellency of Some Essential Oils against *Drosophila melanogaster*, Vector for Bacterium Blood Disease in Banana Plantation

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Abstract

Background and Objective: *Drosophila melanogaster* is one of insect vectors for bacterium blood disease on banana plantation in Indonesia. The most common method to control these insect vectors is by using a synthetic insecticides, although it is now considered not to use as it causes pollutions. Alternatively, using essential oils that are environment friendly in order to stop insect visiting flower, as the fruits are self-fertilized. This research was carried out to identify the optimum concentration and the effective duration time for repellency of three essential oils from citronella, ginger and basil against *D. melanogaster*. **Materials and Methods:** By using a 5-arm test arena, each essential oil with the concentrations of 0 (control), 25, 50, 75 and 100% (with Tween-80 emulsifiers) was tested. Collected data was analyzed using a non-parametric test (Kruskal-Wallis), with a significant value $p<0.05$. GC-MS was used to identify the compound contents in each oil. **Results:** The result showed that the average number of *D. melanogaster* visits to the test arm with essential oils (25, 50, 75 and 100%) was significant different with control. The optimum concentration and the effective duration time for, repellency of each essential oil were as follows; citronella oil 75%-135 min, ginger oil 50%-90 min and basil oil 50%-105 min. Three highest concentrations of the compound in each essential oil were identified as follow: Citronella oil with citronellol, caryophyllene and geranyl isobutyrate, ginger oil with eucalyptol, camphene and geraniol; and basil oil with geraniol, bisabolene and sulcatone. These compound were presumably contributed in insect visiting. **Conclusion:** Essential oils from citronella, ginger and basil can be used as an alternative method to protect the banana plant from insect visiting flower and were effective to repel *D. melanogaster* with various optimum concentrations and various optimum duration times of repellency.

Key words: Repellent, essential oil, citronella, ginger, basil, *Drosophila melanogaster*

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

As an agricultural country, Indonesia produce various fruit including banana. Between 2011-2014, the number of banana production is always at a rate of above six million t per year and reached more than seven million t in 2015. Almost all regions in Indonesia are banana producers and this is due to the appropriate climate for banana plantation[1]. Indonesia is rich in producing different types of banana, to date, more than 200 types of banana has been recorded. Now a day, the production is decreasing and it was reported that 18 out of 33 provinces in Indonesia loss their production as much as 26.38%. The highest is in the provinces of North Sumatra, South Sumatra and DKI Jakarta with more than 50%[1]. Tropical Fruits Review Center at Bogor Agricultural University, Indonesia, stated that bananas facing various problems in the development process. The data shows that existing commercial bananas are generally susceptible to pest and disease; apart that they are various in quality and in short projection life[2].

At the moment, one disease that spreads very fast and causes decrease in banana production is bacterium blood disease. The disease is caused by a bacteria that attack the vessel network systemically[3]. The leaves will have a broken base, midrib weakened and hang drooping while the stems will release a reddish bacterial mucus when transversely cut[4].

Several studies showed that the spread of bacterial blood diseases involve insects that visit banana flowering. Types of insect visitors that supposedly acted as vectors were Diptera[5] and Lepidoptera[6]. Previous study in Padang, West Sumatra showed that there were 7 types of insects visiting banana flower[7], while study in Subang, West Java showed that there were 15 species[8].

The common method used to control the insect visiting flower is by using a synthetic insecticide however insect resistance and insect resurgence, as well as environmental pollution is another problem. To overcome these problems and since banana plantation are self-fertilized to produce fruit, research with repellency mechanisms strategy is become important. Indonesia with mega biodiversity was reported to have many plants with metabolites contents that affect insect activities. Preliminary study using citronella, ginger and basil essential oils as a repellent agent in the field indicated that there was a reduction in number and in species[9].

In this study, the specific ability of citronella, ginger and basil to repel insects were reported. The aim of this study was to identify the optimum concentration and the effective duration time of repellency from three essential oils, citronella, ginger and basil against 1 insect visiting banana flower as a model, *D. melanogaster*.

MATERIALS AND METHODS

This research project was conducted from February-September, 2017.

Insects: Wild type of *D. melanogaster* was obtained from Genetics Laboratory, School of Life Sciences and Technology, Institut Teknologi Bandung. The insects were reared in a jar and fed with mixture of banana, plain agar and sugar. To obtain eggs, a piece of tissue paper was dipped into food as a place for insect's nesting. This research was conducted at Laboratory of Entomology, School of Life Sciences and Technology, Institut Teknologi Bandung from February to September 2017, with average room temperature between 25-29°C and relative humidity between 60-71%.

Essential oils: To obtain the oil, each plant was washed and was cut before entering the distillation process with steaming procedure[10]. The Na_2SO_4 was then added to the oil to remove the remaining water. All of this distillation procedure was conducted at Experimental Field of Manoko, Indonesian Spice and Medicinal Crops Research Institute. Each essential oil was then diluted in Tween-80 emulsifiers to make 5 different concentrations consisting of 0 (control), 25, 50, 75 and 100%[11]. GC-MS used to determine the essential oil's compounds was conducted at Indonesian Center for Rice Research, Subang-West Java Indonesia.

Bioassay: Each essential oil was tested on a 5-arm test arena made from acrylic glass (Fig. 1). The test arms used were modified from a 6-arm olfactometer[12]. The test arena consists of 3 parts, namely; the vertical tubular center section (d = 20 cm, h = 30 cm), which is the start point of *D. melanogaster* having 5 holes towards the arm; the

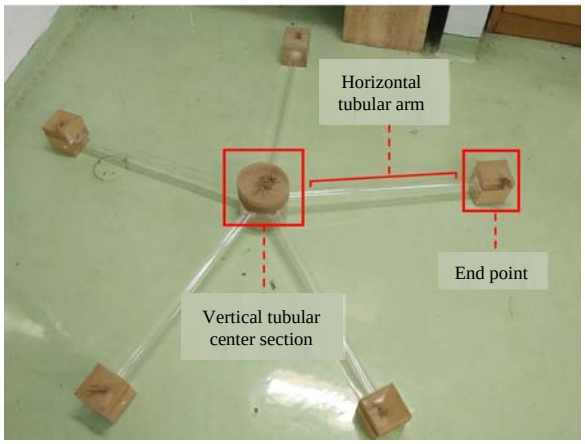

Fig. 1: 5-arm test arena

horizontal tubular arm (d = 5 cm, l = 50 cm) and the end point of the arm. As much as 50 *D. melanogaster* were placed in the center section. Essential oils with five different concentrations were placed at the end point of the 5-arm, each arm for each type of concentration. The experiment was conducted by counting the number of *D. melanogaster* approaches the essential oil at the end point and was count every 15 min during 150 min observed (10 periods of observations). All treatments were replicated for 4 times.

Statistical analysis: The data collection were analyzed by using non-parametric test of Kruskal-Wallis method at 95% confidence level using SPSS version 23 software.

RESULTS AND DISCUSSION

Results indicated that application of different concentrations of essential oils triggered different number of visiting *D. melanogaster*. Each essential oil applied gave different in optimum concentration and in effective duration time of repellency.

Data analyzed showed that there was a significant difference between control and treatment oil (25, 50, 75 and 100%) as it showed in Table 1.

Different number of *D. melanogaster* visits apparently caused by the concentration of active compounds in the essential oils and showed that the higher concentration the small number of insect visits. These results were similar to the study of *Spodoptera litura* against *Azadiracta indica*, *Eupatorium inulifolium* and *Tagetes* spp. essential oils that the higher concentration of oil gave the greater amount of toxins to insect. It caused insects to respond quickly to stay away, to eat less and even to cause death[13].

Citronella essential oil: The average number of *D. melanogaster* visits to citronella essential oil during 150 min with 10 periods of data collection was showed in Fig. 2. Analysis with non-parametric Kruskal-Wallis test showed that the highest number of visiting insects was in

Table 1: Average number of *D. melanogaster* visits to the essential oils

Concentration of essential oils (%)	Average number of *D. melanogaster* visits to each oil		
	Citronella	Ginger	Basil
0	3.75	6.725	4.475
25	3.275	3.85	3.20
50	2.325	0.60	2.025
75	0.875	0.60	1.75
100	0.575	0.85	0.475

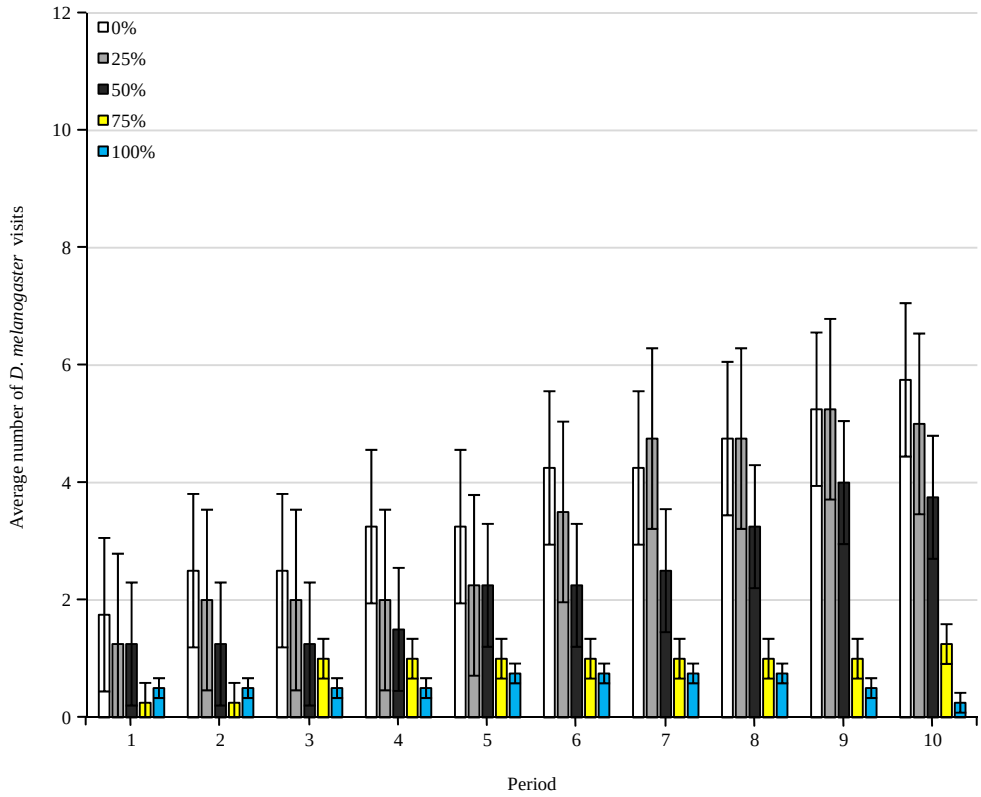

Fig. 2: Average number of *D. melanogaster* visits to citronella essential oil

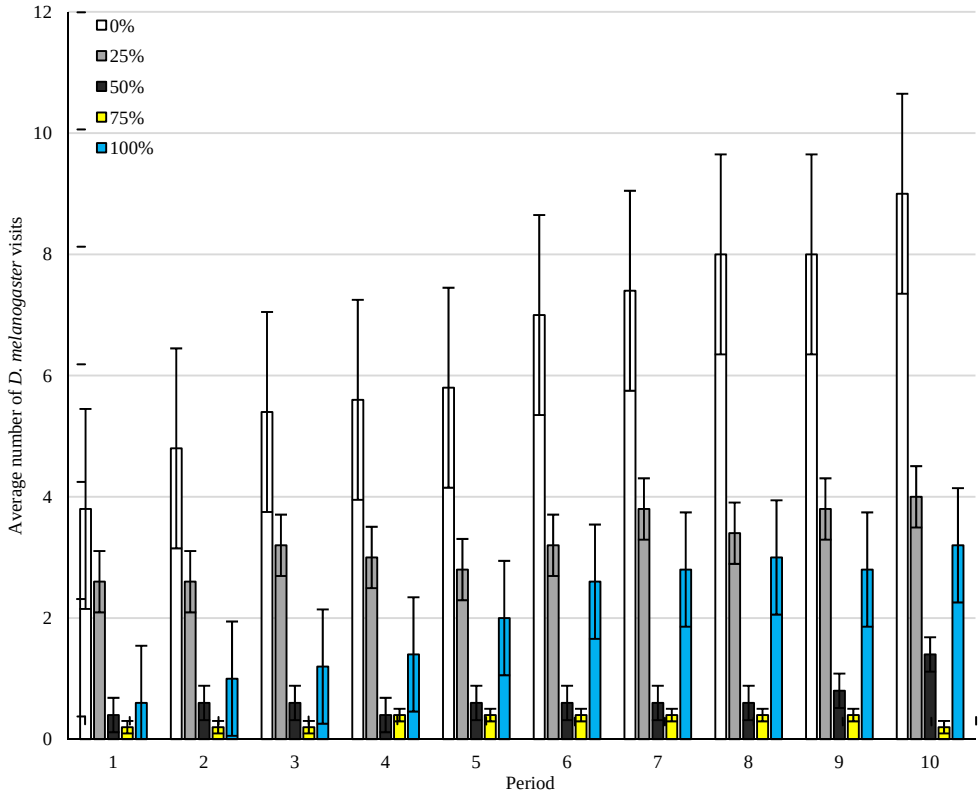

Fig. 3: Average number of *D. melanogaster* visits to ginger essential oil

Table 2: Rank of average number of *D. melanogaster* visits to citronella essential oil

Concentration of essential oil (%)	Rank of average number	Difference with the previous rank
0	39.65	-
25	36.15	3.50
50	30.50	5.65
75	13.80	16.70
100	7.40	6.40

Table 3: Rank of average number of *D. melanogaster* visits to ginger essential oils

Concentration of essential oil (%)	Rank of average number	Difference with the previous rank
0	43.15	-
25	34.30	8.85
50	24.15	10.15
75	15.20	8.95
100	10.70	4.50

Table 4: Rank of average number of *D. melanogaster* visits to basil essential oils

Concentration of essential oil (%)	Rank of average number	Difference with the previous rank
0	37.6	-
25	32.6	5.0
50	26.4	6.2
75	23.9	2.5
100	7.0	16.9

control compare to the lowest was in the experiment with concentration of 100%, while the effective duration time for repellency were at 135 min. The mean value difference between concentration of 75% and concentration of 50% has the highest value, therefore, the optimum concentration was at the concentration of 75% (Table 2).

Ginger essential oil: The average number of *D. melanogaster* visits to ginger essential oil during experiments was showed in Fig. 3.

Analysis with non-parametric Kruskal-wallis test showed that the highest average rating was 0% and the lowest was 100% while the effective duration time for repellency were at 90 min. The mean value difference between the concentration of 50% and the concentration of 25% has the highest value, therefore the optimum concentration was at the concentration of 50% (Table 3).

Study with *Blatella germanica* indicated that ginger essential oil is potential as a repellent although it was concentration dependent. The concentration with at least 50 were repels 100% of insect in 48 h[14].

Basil essential oil: The average number of *D. melanogaster* visits on basil essential oil showed that oil with 25% of concentration was able to decrease the number of visits (Fig. 4).

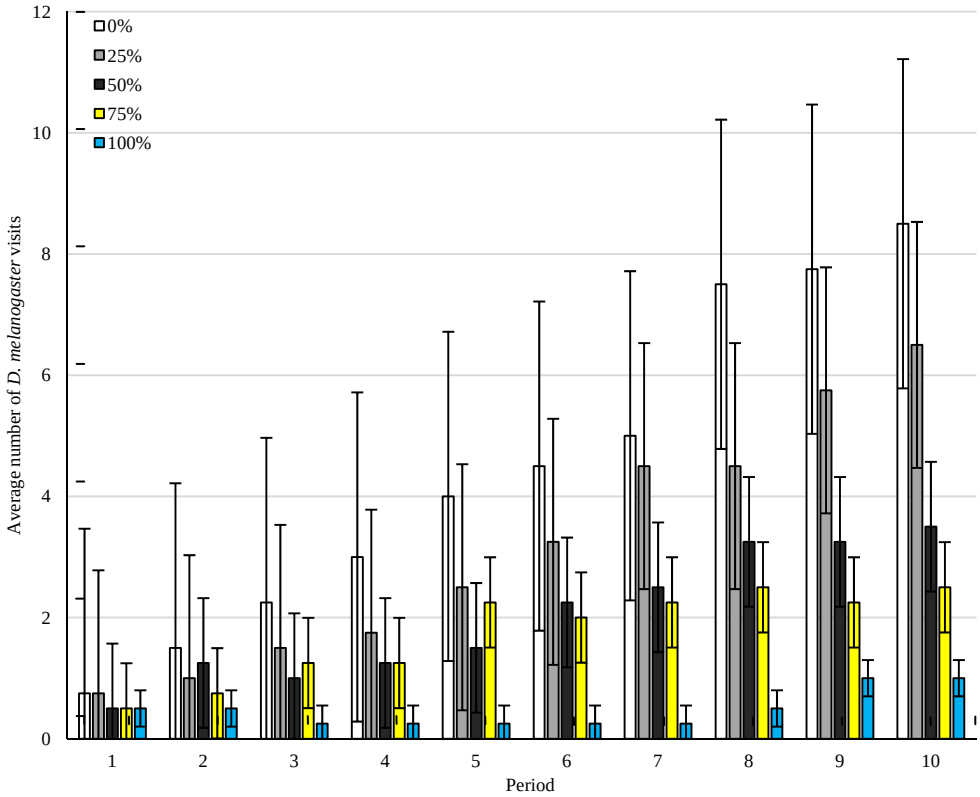

Fig. 4: Average number of *D. melanogaster* visits to basil essential oil

Table 5: Three compounds with the greatest abundance in each essential oil

Type of essential oils	Type of compound	Retention time (min)	Abundance (%)
Citronella	Citronellol	28.917	9.538
	Caryophyllene	37.172	2.601
	Geranyl isobutyrate	35.296	1.724
Ginger	Eucalyptol	19.883	30.906
	Camphene	16.017	17.958
	Geraniol	30.765	7.292
Basil	Geraniol	30.865	38.098
	Bisabolene	41.620	2.571
	Sulcatone	17.638	2.480

Analysis with non-parametric Kruskal-wallis test showed that the highest average rating was 0% and the lowest was 100%. The mean difference between the concentration of 50% and the concentration of 25% was higher than between the concentration of 0% and the concentration of 25%, as well as the concentration of 50% and the concentration of 75%, therefore the optimum concentration was at the concentration of 50% (Table 4).

Basil essential oils was also reported to be effective in killing two majors stored product adult pest *Ephestia kueniella* (Zeller) and *Plodia interpunctella* (Hubner) (Lepidoptera: Pyralidae)[15].

Compounds in essential oils: Based on the GC-MS results, it showed that essential oils from citronella, ginger and basil were composed of different compounds, both in terms of the type and abundance (Fig. 5-7).

Three compounds with the greatest abundance in each essential oil were varied as it showed in Table 5.

Although 3 highest compounds in each essential oil were identified, only 1 majority compound was showed significantly higher in each essential oil, namely citronellol (9.538%) from citronella essential oil, eucalyptol (30.906%) from ginger essential oil and geraniol (38.098%) from basil essential oil. Presumably these compounds from each essential oil play important role and caused *D. mellanogaster* to avoid as the number of visiting these insects in the test arena were declined.

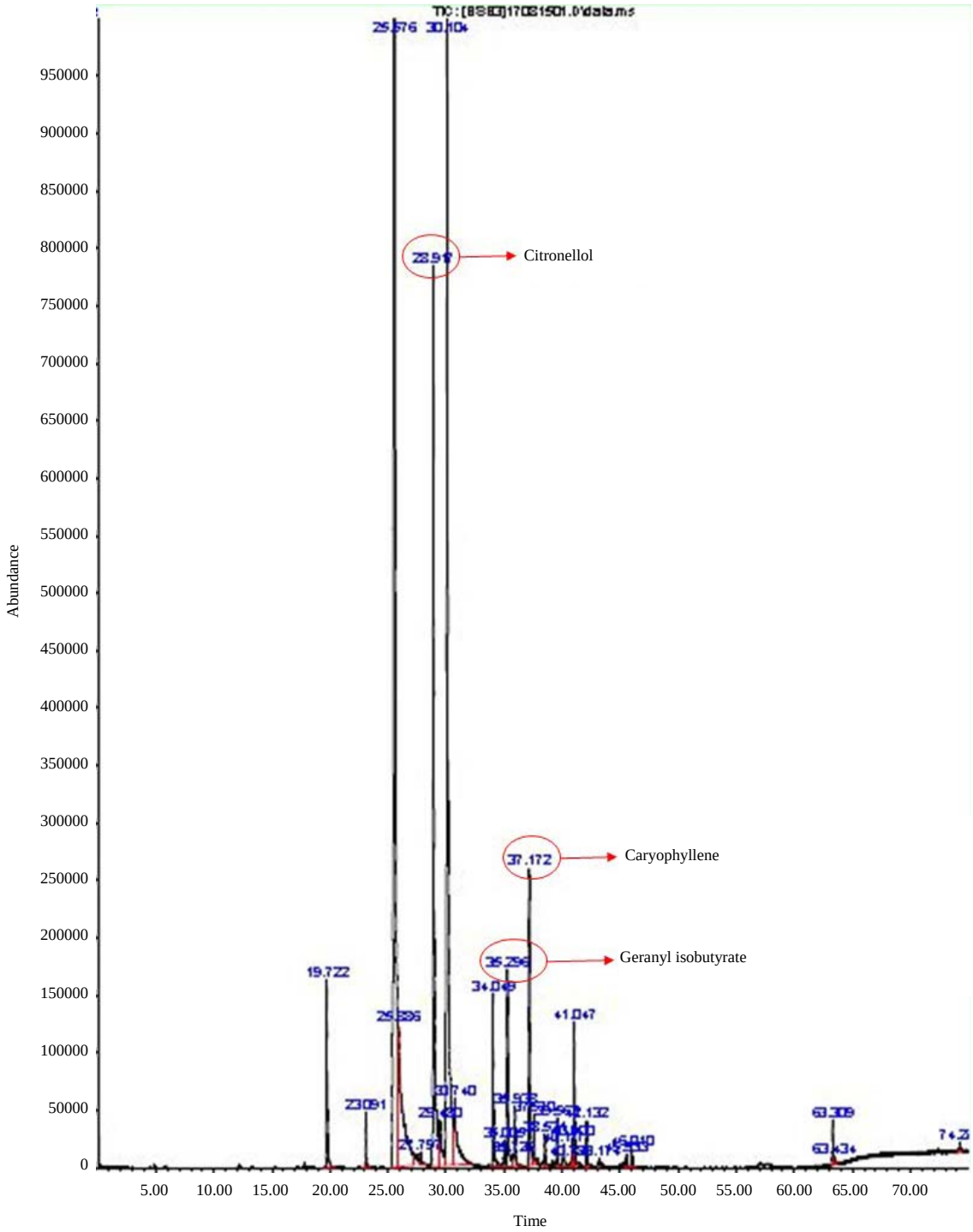

Fig. 5: Compounds of citronella essential oil

Citronellol as a major component of citronella essential oil was reported that can be separated by steam distillation[16]. Several studies showed that citronellol compound interfered the olfactory receptors in insects and elicit a response of reluctance. More than that, citronellol was reported will generate a response to the olfactory co-receptor used to respond the synthetic DEET compound[17]. Citronellol compound also reported to facilitates the avoidance behavior by working on *D. melanogaster* gustatory neurons and not on olfactory neurons[18].

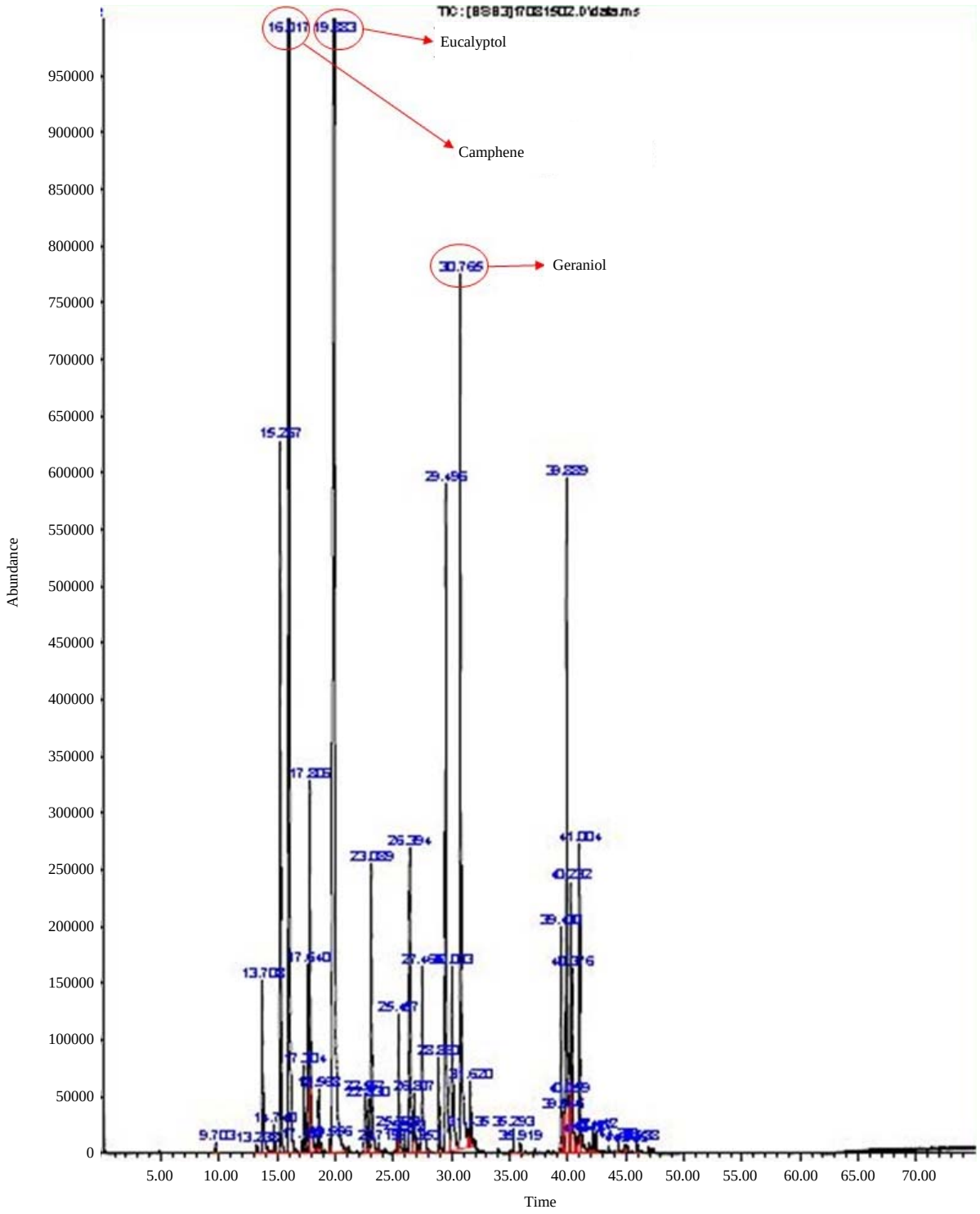

Fig. 6: Compounds of ginger essential oils

Some studies showed that eucalyptol, the majority compound of ginger essential oil can be act as a repellent for insects. The odorant receptor that responds to DEET, a synthetic insecticide caused the same response with the eucalyptol compound in *Culex quinquefasciatus* (Diptera: Culicidae)[19].

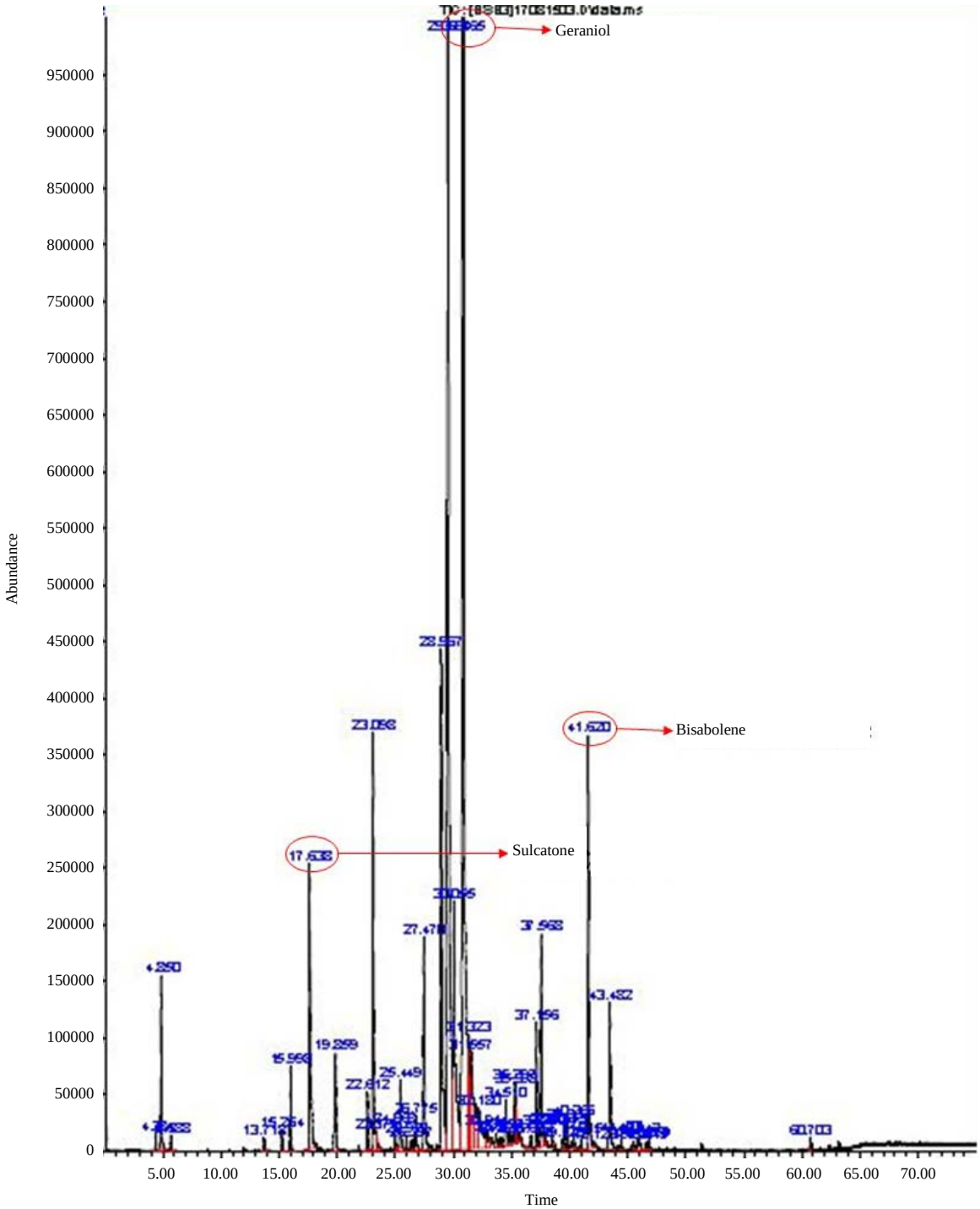

Fig. 7: Compounds of basil essential oils

Geraniol is a compound commonly found in basil[20]. One study reported that Farnesol is detected by odorant receptors resulting in a dodge response and geraniol compound having similar structures produce the same response[21].

CONCLUSION

Essential oils from citronella, ginger and basil were found to be effective as repellent agent against *D. melanogaster*. The optimum concentration was 75% for citronella oil, 50% for

ginger oil and 50% for basil oil. Effective duration time of essential oils to repel *D. melanogaster* was 135 min for citronella oil, 90 min for ginger oil and 105 min for basil oil.

SIGNIFICANCE STATEMENTS

Application of insect repellency mechanism strategy using essentials oil from citronella, ginger and basil into self-fertilized banana plantation, can be an alternative strategy to protect the plant from bacterium blood disease. Each essentials oil has a specific type and amount of compound, therefore, the effectivity to deter *D. melanogaster* was varied in concentrations and in optimum duration times of repellency.

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