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Detection of fruit flies (Diptera: Tephritidae) using cue-lure and methyl eugenol in Depok City and Bogor District, West Java, Indonesia

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Abstract. *Tarno H, Octavia E, Himawan T, Setiawan Y. 2022. Detection of fruit flies (Diptera: Tephritidae) Using Cue-Lure and Methyl Eugenol in Steiner Traps in Depok City and Bogor District, West Java, Indonesia. Biodiversitas 23: 4202-4208.* Fruit flies (Diptera: Tephritidae) are one of the most economically important insect pests worldwide. Two types of attractants are recommended for monitoring and controlling fruit flies: methyl eugenol (ME) and cue-lure (C-L). The objective of this study was to investigate the species richness and abundance of fruit flies (Diptera: Tephritidae) using methyl eugenol (ME) and cue-lure (C-L) in Depok City and Bogor District, West Java, Indonesia. The study was conducted in three sites of fruit orchards in Bogor District i.e., Malay apple (*Syzygium malaccense*), lemon (*Citrus limon*), papaya (*Carica papaya*), and two sites of fruit orchards in Depok City i.e., carambola (*Averrhoa carambola*) and guavas (*Psidium guajava*). Male fruit flies were trapped using Steiner traps baited with methyl eugenol (ME) and cue-lure (C-L). There were 11 fruit fly species collected from this study i.e., *Bactrocera albistrigata, B. carambolae, B. dorsalis, B. neocognata, B. umbrosa, B. verbascifoliae, Dacus conopsoides, D. longicornis, Zeugodacus calumniata, Z. caudatus,* and *Z. cucurbitae*. In this study, *B. albistrigata, D. conopsoides, D. longicornis,* and *Z. caudatus* were only collected in Steiner traps baited with C-L and *B. dorsalis,* and *B. umbrosa* also only responded to ME. During sampling periods, 20,217 male adult fruit flies were collected. The highest relative abundance attracted to ME was *B. carambolae* and C-L was *Z. cucurbitae*. In this study, *B. albistrigata, B. calumniate, D. longicornis, Z. caudatus,* and *Z. cucurbitae* were significantly more attracted to C-L than ME. Whereas, *B. carambolae, B. dorsalis, B. umbrosa,* and *B. verbascifoliae* were significantly more attracted to ME than C-L. There were no invasive species in Depok City and Bogor District, West Java, Indonesia. Our study can be used as a method for monitoring and controlling fruit flies, namely Steiner trap with C-L for *Z. cucurbitae* and Steiner trap with ME for *B. carambolae, B. dorsalis,* and *B. umbrosa* in Depok City and Bogor District, West Java, Indonesia.

Keywords: *Bactrocera carambolae, Bactrocera dorsalis*, cue-lure, methyl eugenol, Steiner trap

INTRODUCTION

Fruit flies (Diptera: Tephritidae) are one of the most economically important insect pests worldwide (Qin et al. 2015). Fruit flies cause economic losses to fruit crops worldwide. Fruit damage caused by fruit fly infestation has been reported to range from 5% to 100% in Asia (Rauf et al. 2013). The family Tephritidae is reported to consist of approximately 4,911 species, and this family is one of the most species-rich in the order of Diptera (Brown et al. 2018; Salazar-Mendoza et al. 2021). Fruit flies are frugivorous species, and several species are important species of plant quarantine in fruit-growing areas (Almeida et al. 2016). The most important Tephritid genera are *Bactrocera, Ceratitis, Anastrepha, Dacus,* and *Rhagoletis* (Jiang et al. 2018). In Indonesia, important Tephritid species were reported from 35 plant species in 18 families: *Bactrocera* spp., *Atherigona orientalis,* and *Dacus longicornis* (Suputa et al. 2010). Therefore, environmental conditions affecting host availability are factors influencing the distribution of several species of Tephritidae. The abundance and diversity of these fruit fly species in tropical regions have been increased by the high diversity of hosts and climate conditions (Salazar-Mendoza et al. 2021).

The management of fruit flies is challenging; because their eggs are inserted into the fruit, and the larvae live and eat inside the fruit before leaving the fruit to pupate in the soil (Castilho et al. 2019). Therefore, they can escape the application of insecticides (Dias et al. 2018). Monitoring has been used to control fruit fly pests in Indonesia. Traps with attractants have been used for monitoring this pest (Bali et al. 2021) because they can catch larger numbers of species (than those without attractants) and absolute numbers of fruit fly pests in fruit orchards (Querino et al. 2014).

Monitoring systems are suggested for the control, eradication, and suppression of fruit flies by trapping systems (Manrakhan et al. 2017). Fruit fly trapping systems often use odors such as pheromones, host plant volatiles, and chemical compounds to attract the insects to a trap (Stringer et al. 2019). The use of chemical compounds is one way to monitor and manage fruit flies. Traps with chemical attractants are commonly an effective control used for estimating species richness, studying population dynamics, predicting outbreaks, and mass trapping to control pests (Suckling et al. 2014). Two types of attractants are recommended for monitoring and controlling fruit flies: methyl eugenol (ME) and cue-lure (C-L) (Royer and Mayer 2018). This chemical compound is a product containing phenylpropanoid and related compounds of plant origin (Tan and Nishida 2012). ME is a natural product from plants and an attractant that can entice the fruit fly *Bactrocera dorsalis* (Liu et al. 2018). With its strong attractant properties, ME is a male lure used for mass trapping and monitoring male fruit flies for the management of fruit flies, especially *Bactrocera* spp. (Haq et al. 2018). C-L is known as a natural product, except as hydrolyzed derivatives, and attracts a greater population of male fruit flies *Zeugodacus cucurbitae* and *B. dorsalis* (Arya et al. 2022).

West Java, Indonesia, which has an administrative area of 35,377.76 km^2, is one of the provinces in Indonesia, a horticulture center with various kinds of fruits that can be found in this region (BPS 2020a). BPS (2020b) also reported that fruit production in West Java in 2020 amounted to 2,829,159 tons, out of the total national production for Indonesia from 34 provinces comprising 26 fruit commodities of an amount of 24,872,974 tons. Many types of fruit plants are cultivated in West Java, such as carambola (*Averrhoa carambola*), guavas (*Psidium guajava*), papayas (*Carica papaya*), oranges (*Citrus sinensis*), bananas (*Musa paradisiaca*), mangoes (*Mangifera indica*), and others, which are generally hosts of fruit flies. Bogor District and Depok City are two areas in West Java that have a high diversity of horticultural plants, which affects the presence and diversity of fruit flies in this region.

The intensity of damage caused by fruit flies on horticultural crops has always been a concern. Control efforts, species diversity inventory, and host range renewal at each location need to be carried out. The extent of the composition and diversity of fruit fly species in Depok City and Bogor District, West Java, Indonesia is still limited. A report has stated that 63 species of fruit flies in Indonesia have been found (AQIS 2008). Larasati et al. (2013) reported as many as 18 species of fruit flies found in Bogor and its surrounding areas. Therefore, the objective of this study was to investigate the species richness and abundance of fruit flies (Diptera: Tephritidae) using ME and C-L in Depok City and Bogor District, West Java, Indonesia.

MATERIALS AND METHODS

Study site

The study was conducted in three sites of fruit orchards in Bogor District, West Java, Indonesia, i.e., Malay apple (*Syzygium malaccense*), lemon (*Citrus limon*), papaya (*Carica papaya*), and two sites of fruit orchards in Depok City i.e., carambola (*A. carambola*), and common guava (*P. guajava*) (Table 1 and Figure 1). The GPS coordinates and altitudes of each site were presented in Table 1. Traps were cleared weekly for 12 weeks from June to August 2021. All trees were fruiting when trapping was conducted. This study was conducted during the rainy season and all study sites had mean daily rainfall of 4.40 mm and a mean daily temperature of 21°C (minimum 16.2°C, maximum 28.6°C).

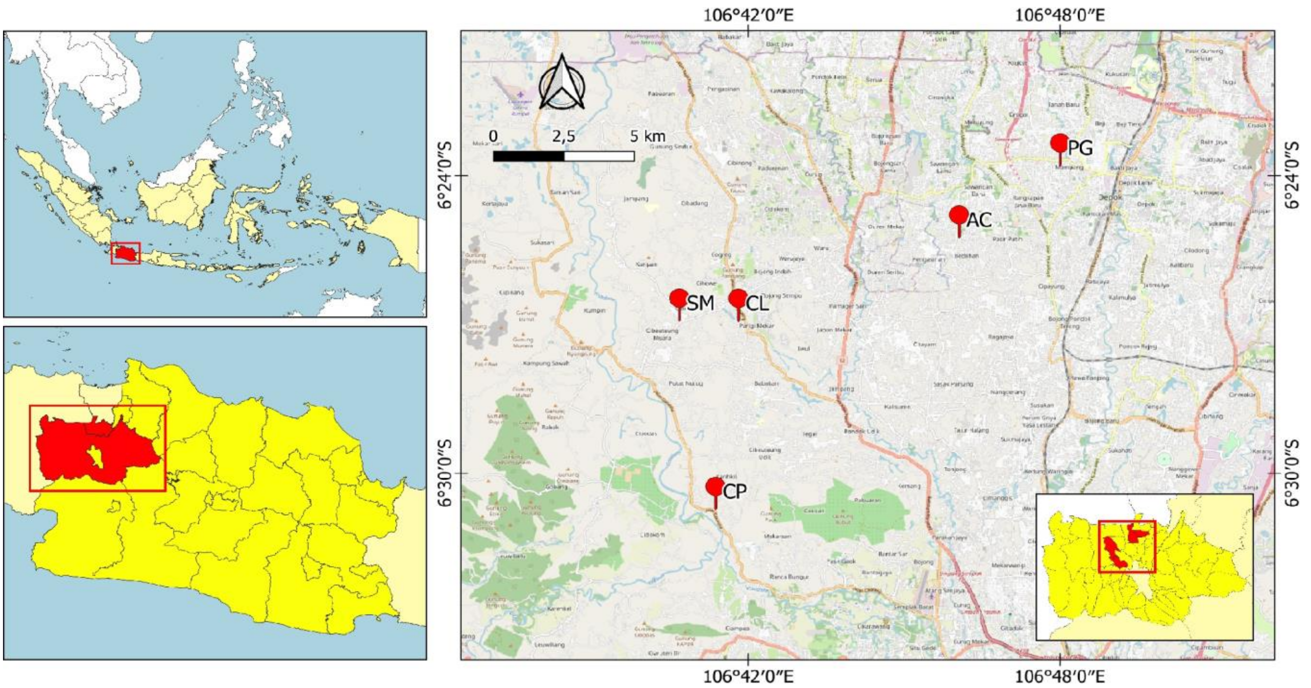

Figure 1. Map of the sampling sites in Depok City and Bogor District, West Java, Indonesia. Note: SM: Babakan, Bogor District; CL: Parigi Mekar, Bogor District, CP: Mekarsari, Bogor District; PG: Pancoran Mas, Depok City; AC: Bedahan, Depok City.

Table 1. Collection sites in Depok City and Bogor District, West Java, Indonesia

Study locations	Site code	Fruit orchards	Coordinates	Altitude (m.a.s.l.)
Babakan, Bogor District	SM	*Syzygium malaccense*	6°26’57.33”S; 106°40’40.57”E	95
Parigi Mekar, Bogor District	CL	*Citrus limon*	6°26’57.59”S; 106°41’48.94”E	106
Mekarsari, Bogor District	CP	*Carica papaya*	6°30’44.90”S; 106°41’22.41”E	131
Pancoran Mas, Depok City	PG	*Psidium guajava*	6°23’49.78”S; 106°48’00.50”E	87
Bedahan, Depok City	AC	*Averrhoa carambola*	6°25’16.73”S; 106°46’03.71”E	97

Note: *m.a.s.l.: meters above sea level

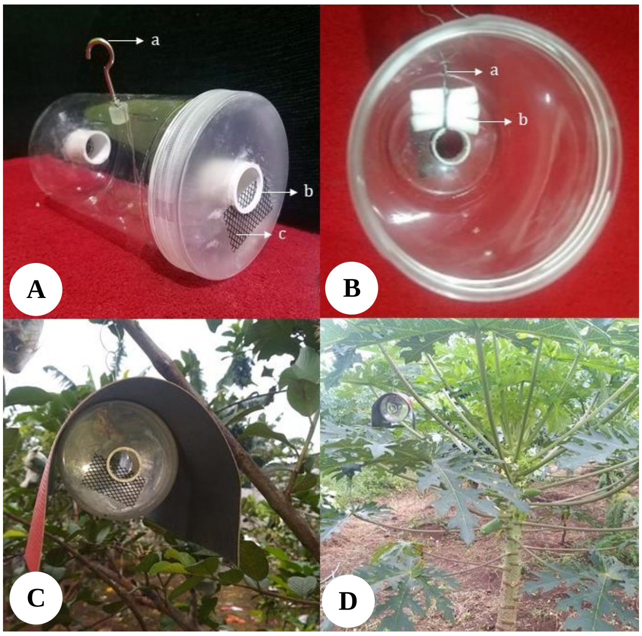

Figure 2. Steiner trap used in this study. A. Outside part (a. iron hook; b. trap entrance; and c. wire gauze), B. Inside part (a. wire to hang cotton and b. cotton wick with a lure), C. Traps covered with tarpaulin to protect from rain, and D. Steiner trap installed on *C. papaya*

Trapping

Male fruit flies were trapped using Steiner traps baited with methyl eugenol (ME) (4-allyl-1, 2-dimethoxybenzene-carboxylate) and cue-lure (4-(p-acetoxyphenyl)-2-butanone) (C-L). The distance between ME and C-L traps in one site was at least 25 meters. In total, 10 traps (five traps each for ME and C-L) were used per site or 50 traps overall across all sites. Traps were set on tree branches 1.5 m above the ground. Steiner traps were made of transparent cylindrical plastic jars, 15 cm high and 10 cm in diameter, with the bottom of the funnel protected with wire mesh. At the top of the trap, an iron hook was used to hang the trap on a tree branch, while the inside was connected to hang cotton. They contained cotton wicks impregnated with 0.25 mL of a mixture with a ratio of 4 mL of attractant and 1 mL of insecticide (Deltamethrin 2.8 EC 0.0028%) (Figure 2). Traps were observed every seven days and the bait was replaced. The fruit flies trapped in the Steiner traps were then collected and transferred to collection bottles lined with tissue paper, given silica gel and camphor, and labeled for further identification.

Identification of fruit flies

Fruit fly specimens were identified to the species level based on their morphological characteristics using an Olympus SZ61 stereo microscope (Olympus Optical Co. LTD, China). Identification of the fruit flies to the species level according to morphological characteristics used as identification keys for the tropical fruit flies of Southeast Asia. The used references were Drew and Romig (2013) and Plant Health Australia (2018). Specimens were then sent to the Plant Quarantine Laboratory Center for Diagnostic Standard of Agricultural Quarantine, Agricultural Quarantine Agency, Indonesia, to confirm the fruit fly’s species.

Data analysis

A pivot table was used to process the trapping data (survey dates, study sites, and male adult fruit fly captured). The significant differences between the mean of fruit flies that were attracted by both attractants (C-L and ME) were obtained using Student’s t-test. The Shapiro-Wilk test was used to verify the normality of the data ($\alpha = 0.05$). To achieve a normal distribution, data on the abundance of fruit flies were transformed into log (x + 1). All data were analyzed using the statistical software R version 3.3.3 (R Core Development Team 2019).

RESULTS AND DISCUSSION

Species richness and abundance of fruit flies

During the sampling period, there were 11 fruit fly species from Depok City and Bogor District, West Java, Indonesia. The collected fruit flies were from the three genera of *Bactrocera*, *Dacus*, and *Zeugodacus*. The genus of *Bactrocera* consists of six species: *Bactrocera albistrigata*, *B. carambolae*, *B. dorsalis*, *B. neocognata*, *B. umbrosa*, and *B. verbascifoliae* (Figure 3 and Table 2). The genus of *Dacus* consists of two species: *D. longicornis* and *D. conopsoides*. The genus of *Zeugodacus* consists of three species: *Z. calumniata*, *Z. caudatus*, and *Z. cucurbitae*. Nine of these species were attracted to the cue-lure (C-L) i.e., *B. albistrigata, B. calumniate, B. carambolae, B. neocognata, B. verbascifoliae, D. conopsoides, D. longicornis, Z. caudatus* and *Z. cucurbitae*. Seven species were attracted to methyl eugenol (ME) i.e., *B. calumniate, B. carambolae, B. dorsalis, B. neocognata, B. umbrosa, B. verbascifoliae* and *Z. cucurbitae* (Table 2).

Table 2. Abundance of fruit flies collected in Steiner trap with C-L and ME in Depok City and Bogor District, West Java, Indonesia

Fruit fly species	SM		CL		CP		PG		AC		Total (C-L)	Total (ME)
	C-L	ME	C-L	ME	C-L	ME	C-L	ME	C-L	ME		
Bactrocera albistrigata (Meijere)	7	-	3	-	1	-	45	-	10	-	66	0
Bactrocera calumniate Hering	8	1	2	-	10	-	1	-	26	1	47	2
Bactrocera carambolae Drew & Hancock	1	2,372	-	707	-	166	3	2,511	-	4,005	4	9,761
Bactrocera dorsalis (Hendel)	-	1,109	-	1,252	-	381	-	2,732	-	3,043	0	8,517
Bactrocera neocognata (Hardy)	-	-	-	-	1	1	-	-	27	2	28	3
Bactrocera umbrosa Fabricius	-	109	-	336	-	17	-	38	-	381	0	881
Bactrocera verbascifoliae Drew & Hancock	-	1	-	5	-	1	-	10	2	4	2	21
Dacus conopsoides (Meijere)	1	-	-	-	-	-	-	-	1	-	2	0
Dacus longicornis Wiedemann	3	-	1	-	-	-	-	-	2	-	6	0
Zeugodacus caudatus (Fabricius)	10	-	11	-	6	-	2	-	49	-	78	0
Zeugodacus cucurbitae (Coquillett)	150	2	349	3	56	-	3	-	234	2	792	7
Total	180	3,594	366	2,303	74	566	54	5,291	351	7,438	1,025	19,192
Species richness	7	6	5	5	5	5	5	4	8	7	9	7

Note: C-L: cue-lure; ME: methyl eugenol; SM: Babakan, Bogor District; CL: Parigi Mekar, Bogor District, CP: Mekarsari, Bogor District; PG: Pancoran Mas, Depok City; AC: Bedahan, Depok City.

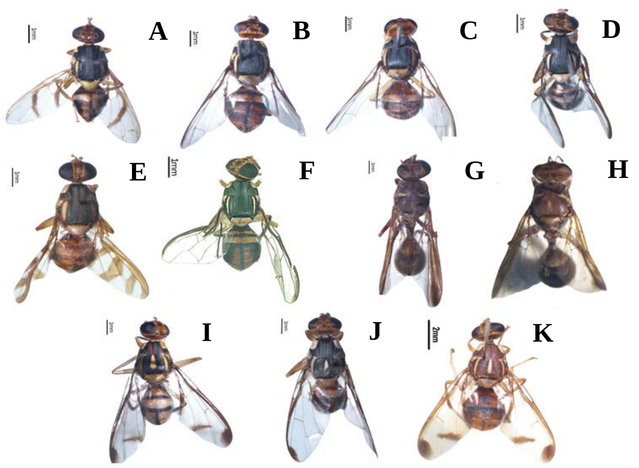

Figure 3. Morphological characteristics of fruit fly species in male lure traps in Depok City and Bogor District, West Java, Indonesia. A. *B. albistrigata;* B. *B. carambolae;* C. *B. dorsalis;* D. *B. neocognata;* E. *B. umbrosa*; F. *B. verbascifoliae;* G. *D. longicornis;* H. *D. conopsoides;* I. *Z. calumniata;* J. *Z. caudatus;* K. *Z. cucurbitae*

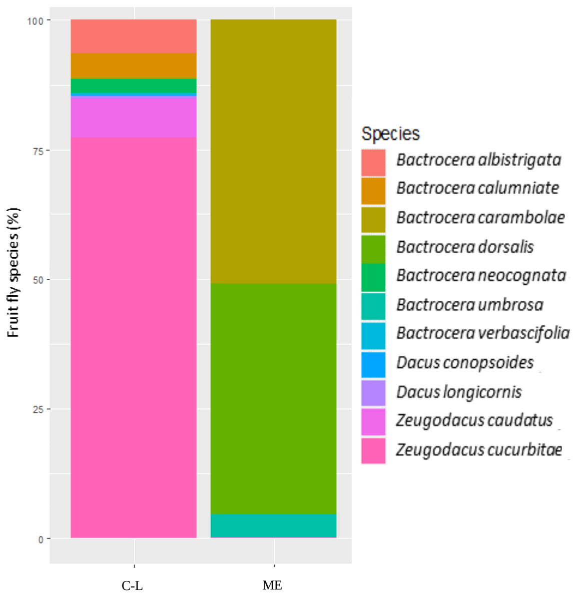

Figure 4. Fruit fly species composition in Steiner trap. Percent of fruit fly species captured in Steiner trap baited with cue-lure (C-L) and methyl eugenol (ME) placed in all study sites in Depok City and Bogor District, West Java, Indonesia

In this study, 20,217 individuals of male adult Tephritidae fruit flies were collected during the sampling period. In total, 1,025 individuals of fruit flies were attracted to the cue-lure (C-L) and 19,192 individuals of fruit fly attracted to the methyl eugenol (ME). The most common species attracted to the cue-lure (C-L) were Z. *cucurbitae*, Z. *caudatus, B. albistrigata*, *B. calumniate,* and *B. neocognata* represented 77.27%, 7.61%, and 6.44% from total individuals trapped, respectively. The most common species attracted to the methyl eugenol (ME) were *B. carambolae*, *B. dorsalis*, and *B. umbrosa* representing 50.86%, 44.38%, and 4.59%, respectively (Figure 4).

In this study, five of the nine species analyzed were significantly more attracted to C-L than ME i.e., *B. albistrigata* (t-test, df=48, p<0.001), *B. calumniate* (t-test, df=48, p<0.001), *D. longicornis* (t-test, df=48, p=0.021), *Z. caudatus* (t-test, df=48, p<0.001) and *Z. cucurbitae* (t-test, df=48, p<0.001). Whereas four of the seven species were significantly more attracted to ME than C-L i.e., *B. carambolae* (t-test, df=48, p<0.001), *B. dorsalis* (t-test, df=48, p<0.001), *B. umbrosa* (t-test, df=48, p<0.001), and *B. verbascifoliae* (t-test, df=48, p=0.013) (Figure 5).

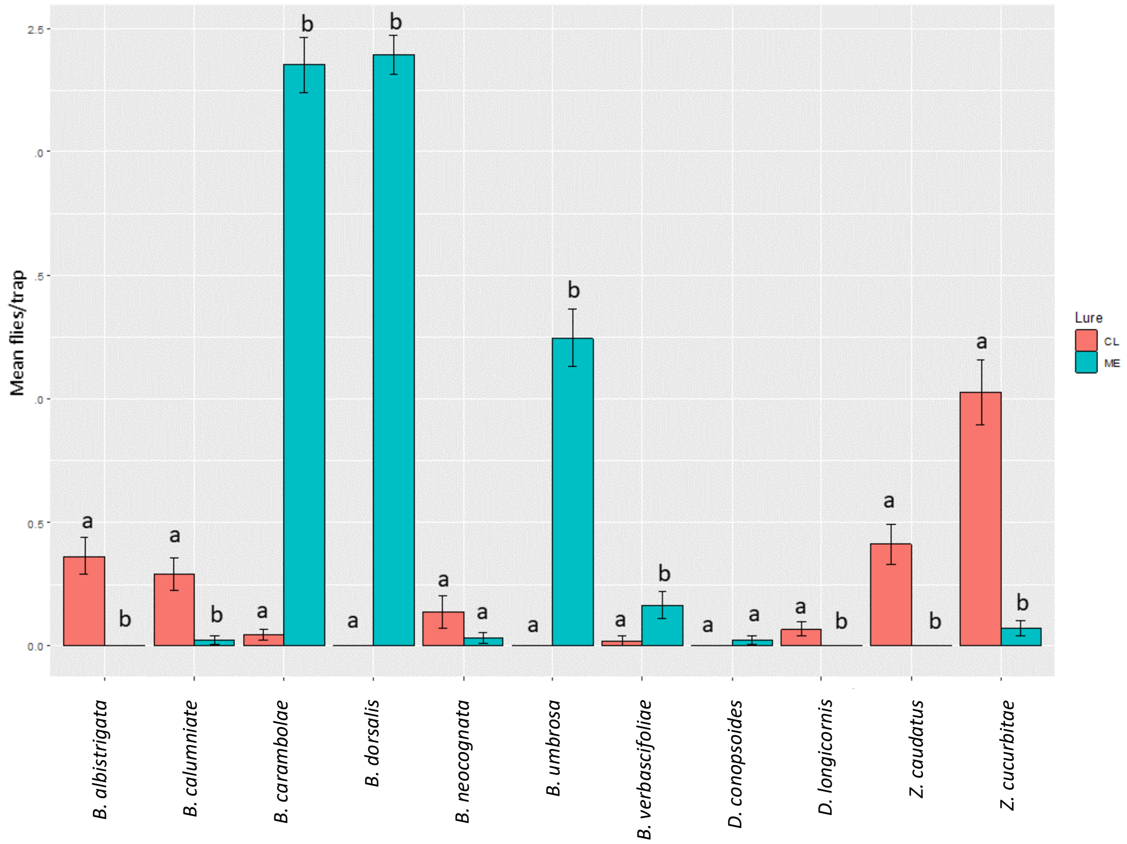

Figure 5. Mean trap catch (±SE) of 11 species into traps baited with C-L and ME in Depok City and Bogor District from June to August 2021. Data were transformed into log (x + 1) to achieve normal distribution. Means for the same species with different letters are significantly different (P < 0.05)

Discussion

In this study, *B. albistrigata*, *D. conopsoides*, *D. longicornis*, and *Z. caudatus* were only collected in Steiner traps baited with C-L. Our findings agree with previous studies that *D. conopsoides* was attracted to C-L in forest habitats and orchard habitats i.e., cashews (*Anacardium occidentale*), mangosteen (*Garcinia mangostana*), and mango (*M. indica*) in Lombok Island, Indonesia (Hudiwaku et al. 2022). Suputa et al. (2010) also reported that *D. longicornis* as an economic importance pest on certain crops attracted to C-L in several regions in Indonesia i.e., Kutai Timur (East Kalimantan), Bantul and Sleman (Special Region of Yogyakarta), Blitar and Kediri (East Java), Buleleng and Denpasar (Bali), Lombok (West Nusa Tenggara), Maros and Soppeng (South Sulawesi), Banawa (Central Sulawesi), and Pohuwato (Gorontalo). *Bactrocera albistrigata* also reported being attracted to C-L in several regions in Java, Indonesia (Suputa et al. 2010). Nair et al. (2021) also reported that *Z. caudatus* was only attracted to C-L in Tripura, N.E. India.

In this study, *Z. cucurbitae* had the highest relative abundance species and was more strongly attracted to C-L than ME. Inskeep et al. (2018) reported that cue-lure was the most attractive attractant for male melon flies (*Z. cucurbitae*) in the field, and the number of captured flies was positively and linearly correlated to the quantity of cue-lure in traps in Kahuku, Oahu, Hawaii. Sulaeha et al. (2020) also reported that *Z. cucurbitae* was attracted to cue-lure on the watermelon plants in South of Sulawesi, Indonesia. Our study also found that *B. carambolae*, *B. dorsalis* and *B. umbrosa* were the most abundant species and more strongly attracted to ME than C-L. Leblanc et al. (2013) reported that adult males of *B. dorsalis* and *B. umbrosa* were only attracted by ME in the Cook Islands, Tonga, the Fiji Islands, the Federated States of Micronesia, New Caledonia, Papua New Guinea, Samoa, Nauru, French Polynesia, the Solomon Islands, and Vanuatu. Wee et al. (2002) also reported that the order of species attracted to ME was *B. dorsalis*, *B. papayae*, and *B. carambolae*. Zida et al. (2020) reported that ME was used to attract *B. dorsalis* and representatives of the subgenus *Pardalaspis* Bezzi in eastern Burkina Faso, West Africa.

In this study, *B. carambolae* had the highest relative abundance and was attracted to ME in Depok City and Bogor District, West Java, Indonesia. *Bactrocera carambolae* is a native tephritid in Asia and has the potential to damage fruit and cause economic losses for commodities such as cashew (*A. occidentale*), papaya (*C. papaya*), tangerine (*Citrus reticulata*), guava (*P. guajava*), lemon (*C. limon*), orange (*C. sinensis*), mango (*M. indica*) and avocado (*Persea americana*) (Marchioro 2016). *Bactrocera carambolae*, a polyphagous fruit fly, commonly attacks 21 host plants in Brazil (Almeida et al. 2016). In

Southeast Asia, *B. carambolae* was found on 75 plant species of 26 families (Allwood et al. 1999). Lemos et al. (2014) also reported that the highest number of *B. carambolae* adults were obtained from Myrtaceae such as *A. carambola* and *P. guajava*. It has also been reported in *C. limon* and *C. papaya* in Brazil (Almeida et al. 2016; Marchioro 2016).

Several studies have also reported that synthetic ME has been successfully used in fruit fly surveys for purposes such as performing quarantine detection, estimating survival rates in natural ecosystems, analyzing the native fruit fly population dynamics, determining the relationship between fruit phenology and fruit fly oviposition time, monitoring the movement of native fruit flies between different ecosystems, and mass trapping for the control of Tephritid fruit flies (Sikandar et al. 2017; Iamba et al. 2021). In addition, *B. carambolae*, *B. dorsalis*, and *B. dorsalis* are strongly attracted to ME, this may be due to ME affecting the mating behavior of males. This indicates that ME was effective in trapping several species in the genus *Bactrocera*, i.e., *B. carambolae*, *B. dorsalis*, and *B. umbrosa*. Chemicals ingested are either sequestered unchanged or converted into derivative products, which are then stored in male pheromone glands and released as pheromonal components that improve male mating competitiveness (Kumaran et al. 2013). Furthermore, the compounds have been linked to *Bactrocera* mating behavior not only through male pheromone modification but also by acting as mating rendezvous sites or increasing male-male competitive advantage (Kumaran et al. 2014).

In conclusions, there are eleven fruit fly species collected from this study by both C-L and ME i.e., *B. albistrigata*, *B. carambolae*, *B. dorsalis*, *B. neocognata*, *B. umbrosa*, *B. verbascifoliae*, *D. conopsoides*, *D. longicornis*, *Z. calumniata*, *Z. caudatus*, and *Z. cucurbitae*. In this study, *B. albistrigata*, *D. conopsoides*, *D. longicornis*, and *Z. caudatus* were only collected in Steiner traps baited with C-L, and *B. dorsalis* and *B. umbrosa* were also only attracted to ME. During sampling periods, 20,217 male adults of fruit flies were collected. *Zeugodacus cucurbitae* is the highest relative abundance of fruit flies collected in Steiner traps baited with C-L and strongly attracted to C-L than ME. *Bactrocera carambolae* had the highest relative abundance of fruit flies collected in Steiner traps baited with ME and were more strongly attracted to ME than C-L. Our study also reported that the invasive fruit fly species aren't found in Depok City and Bogor District, West Java, Indonesia. In addition, based on our study, the Steiner trap baited with C-L can be used to attract *Z. cucurbitae* and the Steiner trap baited with ME is suitable to attract *B. carambolae*, *B. dorsalis*, and *B. umbrosa* related to the monitoring and controlling fruit flies in Depok City and Bogor Regency, West Java Indonesia.

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