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Species composition of *Bactrocera* fruit flies (Diptera: Tephritidae) and their parasitoids on horticultural commodities in Batu City and Malang District, East Java, Indonesia

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Abstract. *Setiawan Y, Hamdoen FM, Muhammad FN, Hata K, Tarno H, Wang J. 2024. Species composition of* Bactrocera *fruit flies (Diptera: Tephritidae) and their parasitoids on horticultural commodities in Batu City and Malang District, East Java, Indonesia. Biodiversitas 25: 305-311.* *Bactrocera* Macquart, 1835 fruit flies (Diptera: Tephritidae) are among the most important pests in horticultural commodities worldwide, including horticultural crops in Indonesia. Trapping is suggested to control, eradicate, and suppress fruit flies. The presence of natural enemies is a very important factor in the indicators of agroecosystem balance. This study aimed to monitor the species composition of *Bactrocera* fruit flies and their parasitoids in several horticultural commodities such as apples, large chili, red guava, sweet citrus, and tomatoes in Batu City and Malang District. Trapping and host-rearing methods were used in this research. A total of 7,124 individuals of *Bactrocera* were collected using a Steiner trap baited with methyl eugenol. They consisted of six species: *Bactrocera carambolae* Drew & Hancock, 1994; *Bactrocera cucurbitae* (Coquillett, 1899); *Bactrocera dorsalis* (Hendel, 1912); *Bactrocera verbascifoliae* Drew & Hancock, 1994; *Bactrocera papayae* Drew & Hancock, 1994; and *Bactrocera umbrosa* (Fabricius, 1805). The *B. carambolae*, *B. dorsalis*, and *B. papayae* were the three predominant species collected using Steiner traps baited with methyl eugenol. Based on the host-rearing method, *B. carambolae* and *B. dorsalis* were found in red guava fruit. Only one parasitoid species, *Opius* sp., was found in red guava fruit. These results provide valuable insights into the distribution of *Bactrocera* on horticultural commodities in Batu City and Malang District and the potential challenges regarding parasitoid presence.

Keywords: *Bactrocera*, diversity of fruit fly, fruit fly parasitoid, methyl eugenol, Steiner trap

INTRODUCTION

Fruit flies (Diptera: Tephritidae) are one of the most important pests in fruit crops worldwide (Salazar-Mendoza et al. 2021). One of them is the genus *Bactrocera* Macquart, 1835, which is widely distributed throughout tropical regions in Asia, the Pacific Islands, Northern Australia, Eastern Africa, and several areas in the USA (Capinera 2008), and is reported as the most economically important pests in Indonesia (Suputa et al. 2010). Since the beginning of the 20th century, *Bactrocera* fruit flies have spread rapidly around the world, and they have caused a severe threat to the economy and environment (Zeng et al. 2019).

This insect group is a polyphagous pest on various fruits and vegetables. Many fruit commodities in Indonesia have been attacked by *Bactrocera* fruit flies, such as mango, citrus, papaya, guava, and chili (Abdullah et al. 2021; Octavia et al. 2021; Susanto et al. 2022; Tarno et al. 2022). Each species of *Bactrocera* fruit fly has a wide and different host plant. For example, *Bactrocera dorsalis* (Hendel, 1912) was reported to attack 40 host plant species (Nanga et al. 2022). In comparison, *Bactrocera carambolae* Drew & Hancock, 1994 was reported to have 21 host plant species (Castilho et al. 2019). Losses due to fruit fly infestation are estimated to be 20-78%, and even high attack intensities can reach 100% (Marchioro 2016). Fruit fly attacks resulted in economic losses of up to USD 14.9 million in Fujian Province, China (Kumari et al. 2021; He et al. 2023). Fruit flies have also become a fruit export barrier in Indonesia (Hidayat et al. 2023). For these reasons, *Bactrocera* fruit fly poses serious threats to horticultural farmers.

Fruit fly control requires high costs and usually uses synthetic pesticides (Mumford 2004; Kardinan 2014), and pesticide application negatively impacts natural enemies (Pandey et al. 2022). Natural enemies known as fruit fly parasitoids are Opiine wasps (Hymenoptera: Braconidae: Opiinae) (Camargos et al. 2018). Those braconid parasitoids parasitize the pre-adult phase of fruit fly inside the attacked fruit (Aliniazee and Croft 1999; Mahat and Clarke 2021). Unfortunately, their existence is threatened due to excessive use of insecticides (Pinheiro et al. 2020).

Environmental friendly control is a pheromone trap; for example, using pheromones was highly recommended for controlling fruit flies in Nepal (Adhikari et al. 2020). Trapping is suggested to control, eradicate, and suppress fruit flies (Manrakhan et al. 2017). The trapping system often uses odors such as sex pheromones to attract fruit flies (Stringer et al. 2019). Traps with chemical attractants are effective for estimating species richness, studying population dynamics, predicting outbreaks, and mass trapping (Suckling et al. 2016). Methyl eugenol and cue lure are commonly used for trapping fruit flies (Vargas et al. 2015). Methyl eugenol is an organic compound found naturally in the essential oils of several plant species (Liu et al. 2018). In previous research, several *Bactrocera* species were more attracted to methyl eugenol (Tarno et al. 2022).

Bactrocera fruit flies are widely distributed in Indonesia, such as East Java (Suputa et al. 2010). The central horticultural fruit production in East Java was in Batu City and Malang District, and both regions have several horticultural crop commodities that can only be produced in the highlands. These conditions suit cultivating horticultural crops such as citrus, guava, and apple. Due to their polyphagous behavior, a study on the *Bactrocera* was needed to gain information about their abundance and species composition on different Batu City and Malang District commodities. The evaluation of trapping with pheromones to control fruit flies in Batu City and Malang District is also important to be studied. The existence of natural enemies of fruit flies also needs to be observed to determine the species and their abundance in different commodities. This study aimed to monitor the species composition of *Bactrocera* fruit flies and their parasitoids on several horticultural commodities such as apples, large chili, red guava, sweet citrus, and tomatoes in Batu and Malang, East Java, Indonesia.

MATERIALS AND METHODS

Study site

This study was conducted at five sites in three locations i.e., Junrejo and Batu Sub-districts, Batu City and Pujon Sub-district, Malang District, East Java, Indonesia (Table 1, Figure 1). Horticultural commodities used in this study were large chili (*Capsicum annuum* L.), red guava (*Psidium guajava* L.), sweet citrus (*Citrus* sp.), tomato (*Solanum lycopersicum* L.), and apple (*Malus* sp.). The study locations ranged from 606 to 1,198 meters above sea level (m asl.). During observation, the temperatures at both locations ranged from 22.3°C to 29.5°C.

Table 1. Coordinate and altitude at the five study sites in Batu City and Malang District, East Java, Indonesia

Study Site Code	Study Locations	Study Sites (Commodities)	Coordinate	Altitude (m asl.)
AP	Pujon Sub-district, Malang District	Apple	7°49'4.19"S, 112°28'35.62"E	1,198
CH	Junrejo Sub-district, Batu City	Large chili	7°54'39.80"S 112°34'43.55"E	606
RG	Batu Sub-district, Batu City	Red guava	7°53'53.27"S 112°31'34.84"E	996
SC	Batu Sub-district, Batu City	Sweet citrus	7°53'51.81"S 112°31'51.08"E	953
TO	Pujon Sub-district, Malang District	Tomato	7°49'48.96"S 112°28'24.24"E	1,130

Notes: m asl.: meters above sea levels

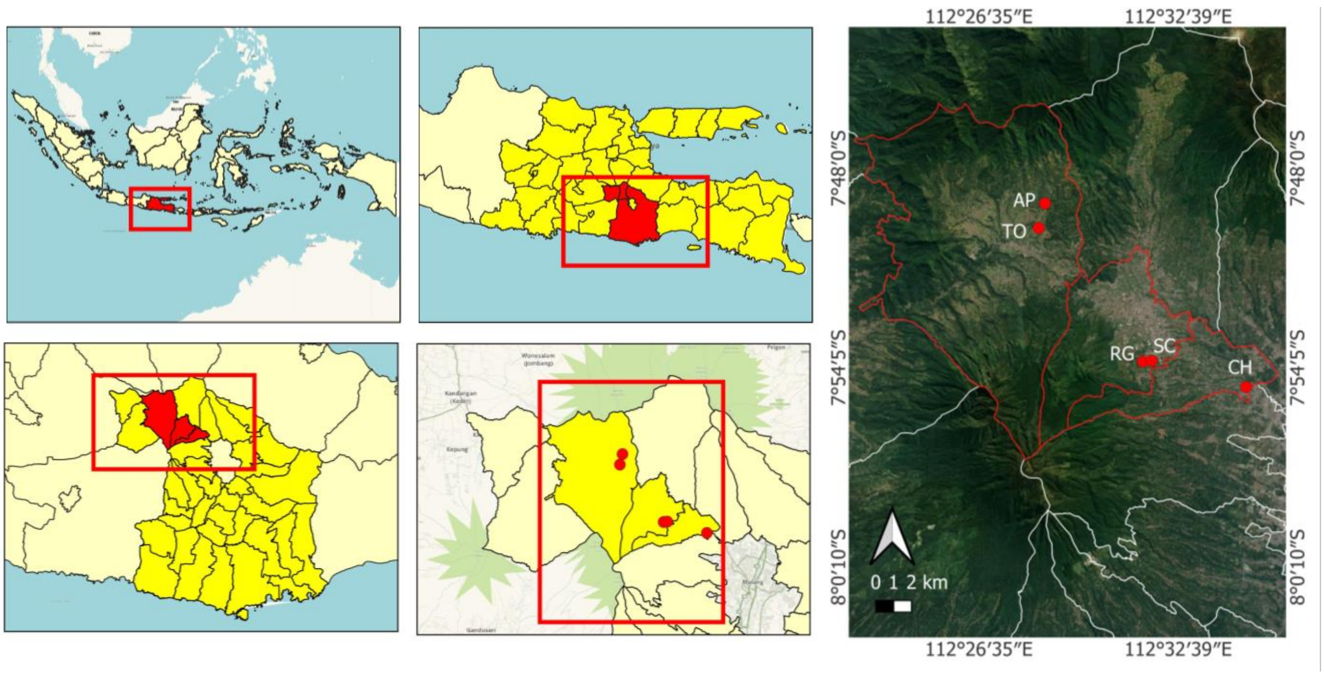

Figure 1. Study sites for *Bactrocera* specimen collection in Batu City (Junrejo Sub-district and Batu Sub-district) and Malang District (Pujon Sub-district) of East Java, Indonesia. Note: AP: Apple; CH: Large chili; RG: Red guava; SC: Sweet citrus; and TO: Tomato

Figure 2. Installation of a Steiner trap on an apple plant branch (A) and bamboo stakes in the tomato study site (B)

Sampling method

Trapping

Study sites for trapping methods were determined using the purposive sampling method with the following criteria: the plant is in the fruiting phase and cultivated with a monoculture cropping system. The trap used in the study was a cylindrical tube Steiner model with methyl eugenol and deltamethrin insecticide (Tarno et al. 2022). Small holes on the right and left sides of the trap are a place for fruit flies to enter. The rope was used to hang the trap on branches of guava, citrus, and apple plants (Figure 2. A). However, in tomato and chili fields, traps are hung on bamboo stakes 1.5 meters above ground level (Figure 2. B). The methyl eugenol was placed in a Steiner tube on cotton hanging in the middle. The cotton was also dripped with insecticide to kill the trapped fruit flies. The ratio of methyl eugenol and insecticide dripped on cotton was 4:1. Fruit fly specimens were collected weekly and carried out eight times. The cotton was replaced with a new one containing methyl eugenol and deltamethrin insecticide in the same ratio after specimen collection. In each study site, the traps were placed at each corner and one in the middle. In total, five traps were placed at each study site. This research was conducted from December 2022 to February 2023.

Host fruit rearing

In addition, the collection of fruit flies and parasitoids was also carried out through the host fruit-rearing method. The activity was carried out five times weekly by picking up fallen fruit and fruit with symptoms of fruit fly in each study site. A total of one kg of fruit was collected every week. The collected fruits were maintained in a host-rearing cage. The cages were made of plastic topless covered with gauze. Cotton with honey was placed in the cage to feed the adult fruit fly. This process lasted until all fruit flies died, and no adult fruit flies emerged. The fruit flies, and parasitoids that emerged were counted.

Preservation and identification

All fruit fly specimens were trapped in a Steiner trap, and specimens that emerged from host rearing (fruit fly and parasitoid) were identified in the Plant Pest Laboratory, Department of Plant Pest and Disease, Faculty of Agriculture, Universitas Brawijaya, Indonesia. Fruit flies and parasitoids were preserved in a vial bottle filled with dental cotton containing 95% ethanol. Each vial bottle was labeled based on location, study sites, and sampling date. Fruit flies collected in traps were identified as species using the Olympus SZX7 stereomicroscope and the lucid key for fruit flies, especially the Dacini tribe (Doorenweerd et al. 2022). Each species was pinned using a mounting method for better documentation.

Data analysis

The difference in abundance among the five study sites and population fluctuation during the observation period of the *Bactrocera* fruit fly were analyzed using ANOVA, followed by Duncan's Multiple Range Test (DMRT). In addition, the difference in *Bactrocera* species composition on different study sites was analyzed using analyses of dissimilarity (ANOSIM) based on the Bray-Curtis index and visualized by a non-metric multidimensional scaling (NMDS). All the analyses were conducted using RStudio with vegan and agricolae packages (R Core Team 2023).

RESULTS AND DISCUSSION

***Bactrocera* fruit flies in Batu City and Malang District**

In total, 7,124 individuals of fruit fly were collected in Steiner traps in five study sites in Batu City and Malang District, consisting of six species: *B. carambolae; Bactrocera cucurbitae* (Coquillett, 1899); *B. dorsalis*; *Bactrocera verbascifoliae* Drew & Hancock, 1994; *Bactrocera papayae* Drew & Hancock, 1994; and *Bactrocera umbrosa* (Fabricius, 1805). The most abundance fruit fly collected in this study was *B. carambolae* (2,494 individuals, 35%), followed by *B. dorsalis* (2,386 individuals, 33.49%) and *B. papayae* (1,942 individuals, 27.25%) (Table 2).

In this study, the total number of individuals of *Bactrocera* fruit flies trapped in the red guava and chili study sites was the highest compared to other sites. Besides, *B. carambolae* and *B. dorsalis* were the most abundant species collected in the red guava study site. *B. papayae* was the most abundant collected in the chili study site. Based on ANOVA, the abundance of the six species of *Bactrocera* fruit flies collected using the Steiner trap in this study was significantly different ($F_{5.24}$ = 30.820; p <0.001). In this study, *B. carambolae, B. dorsalis,* and *B. papayae* were reported as more dominant than other collected species (Figure 3).

Population fluctuation of the *Bactrocera* fruit flies

Based on ANOVA, there were differences in the *B. dorsalis* population ($F_{7.32}$ = 3.417; p = 0.007) during observation, with the lowest population found at the seventh and last observation. The population of *B. carambolae* also showed differences during observation ($F_{7.32}$ = 7.202; p < 0.001). However, *B. cucurbitae* showed no differences in population for each observation ($F_{7.32}$ = 0.857; p = 0.550). For *B. verbascifoliae*, there were differences in population during observations ($F_{7.32}$=10.210; p < 0.001), and the abundance increased in the sixth, seventh, and last observations. The *B. papayae* showed differences in population during observations ($F_{7.32}$ = 9.058; p < 0.001). The population of *B. papayae* increased until the fifth observation but decreased in the sixth, seventh, and eighth observations. Meanwhile, *B. umbrosa* also showed differences during observation ($F_{7.32}$ = 4.113; p = 0.002) (Figure 4).

Species composition of *Bactrocera* fruit flies in Batu City and Malang District

The ANOSIM results showed that the composition of fruit flies differed in the five study sites (R = 0.621; p = 0.001). In this study, six species of fruit flies were found in the red guava study site. However, only four species were collected in large chili and sweet citrus study sites. In addition, three species of fruit flies were collected in apple and tomato study sites. The *B. cucurbitae* only appears in red guava study sites, while *B. verbascifoliae* does not appear in apple and tomato study sites. Based on the NMDS plot, the species composition of fruit flies in red guava and sweet citrus study sites were intersected. Intersects were also found between apple and tomato study sites (Figure 5).

***Bactrocera* fruit flies and their parasitoid on five horticultural commodities**

Based on the rearing method, two fruit fly species emerged from red guava and one from sweet citrus. Besides, one parasitoid species was obtained from red guava (Table 3).

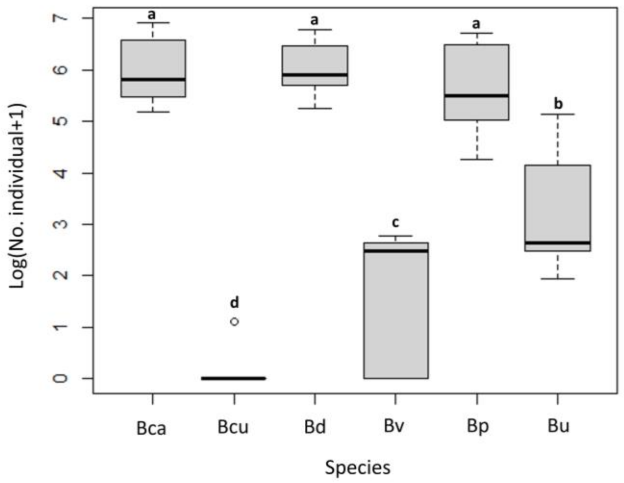

Figure 3. Abundance of *Bactrocera* fruit flies were collected in Steiner traps in Batu City and Malang District of East Java, Indonesia. Bca: *B. carambolae*; Bcu: *B. cucurbitae*; Bd: *B. dorsalis*; Bv: *B. verbascifoliae*; Bp: *B. papayae*; Bu: *B. umbrosa*. Data of fruit fly individuals were transformed to log(x + 1) to achieve normal distribution. Boxplots with different letters were significantly different at p < 0.05 according to Duncan's Multiple Range Test (DMRT)

Table 3. Association of *Bactrocera* fruit fly species and parasitoids found on various host plants based on host rearing

Host plant	*Bactrocera* fruit fly species						Parasitoid species
	Bc	Bd	Bp	Bu	Bv	Bcu	Op
Apple	-	-	-	-	-	-	-
Large chili	-	-	-	-	-	-	-
Red guava	11	7	-	-	-	-	1
Sweet citrus	8	-	-	-	-	-	-
Tomato	-	-	-	-	-	-	-

Note: Bc: *B. carambolae*; Bd: *B. dorsalis*; Bp: *B. papayae*; Bu: *B. umbrosa;* Bv: *B. verbascifoliae*; Bcu: *B. cucurbitae*; Op: *Opius* sp.

Table 2. Abundance and composition of *Bactrocera* fruit flies collected in Batu City and Malang District of East Java, Indonesia, using Steiner trap

Species	Study sites						
	AP	CH	RG	SC	TO	∑	%
Bactrocera carambolae	177	724	1,019	335	239	2,494	35.00
Bactrocera cucurbitae	0	0	2	0	0	2	0.02
Bactrocera dorsalis	188	646	884	368	300	2,386	33.49
Bactrocera verbascifoliae	0	11	13	15	0	39	0.54
Bactrocera papayae	70	826	651	244	151	1,942	27.25
Bactrocera umbrosa	6	13	169	62	11	261	3.66
Total	441	2,220	2,738	1,024	701	7,124	100.00

Notes: AP: Apple; CH: Large chili; RG: Red guava; SC: Sweet citrus; and TO: Tomato

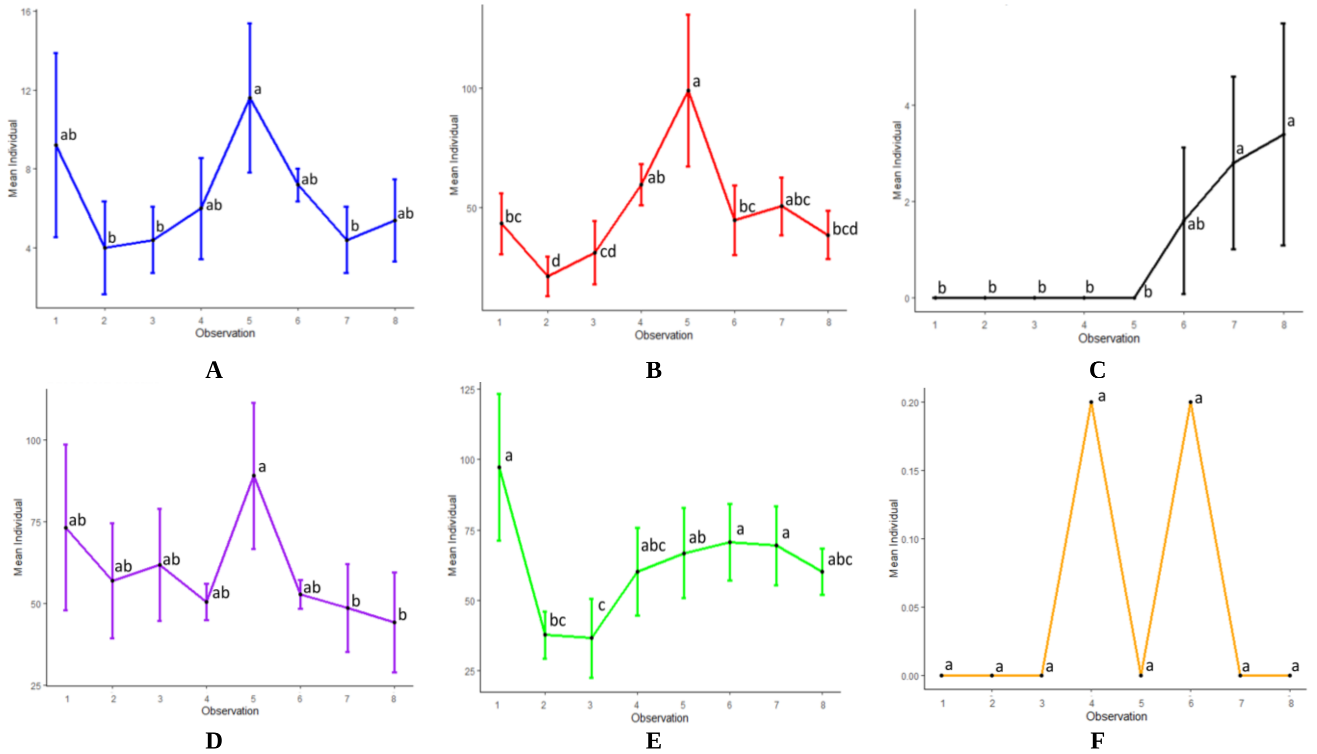

Figure 4. *Bactrocera* fruit fly population fluctuations in eight observations of East Java, Indonesia. A. *B. umbrosa;* B. *B. papayae;* C. *B. verbascifoliae;* D. *B. dorsalis;* E. *B. carambolae;* and F. *B. cucurbitae*

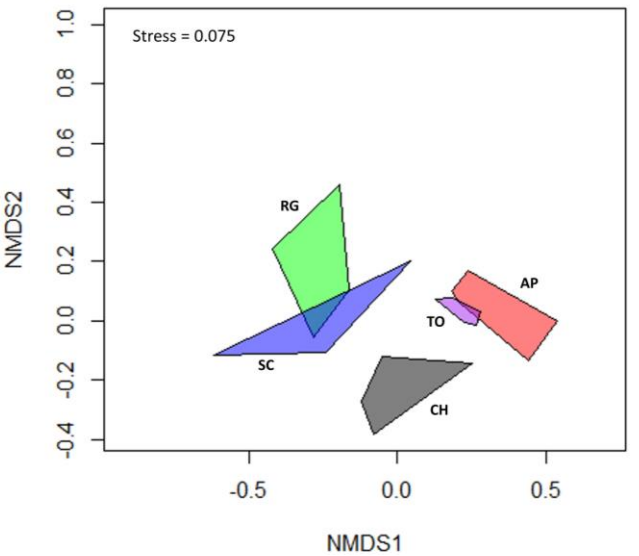

Figure 5. Non-Metric Multidimensional Scaling ordination (NMDS) of fruit fly species composition by study sites based on a Bray-Curtis dissimilarity index. AP: Apple; CH: Large chili; RG: Red guava; SC: Sweet citrus; and TO: Tomato

Discussion

This study collected the three predominant fruit fly species using Steiner traps baited with methyl eugenol, i.e., *B. carambolae*, *B. dorsalis*, and *B. papayae*. According to Vargas et al. (2015), *B. carambolae* and *B. dorsalis* are Category A species characterized by polyphagous generalists and highly destructive specialists that have become established outside of their native range. The current distribution of these three species is the Pacific Asia and exotic pests in Hawaii and South America (Drew and Hancock 2022). From an economic viewpoint, these three species are important pests on several agricultural commodities. Aryuwandari et al. (2020) reported that *B. carambolae* and *B. dorsalis* attacked eleven fruit species of 23 species surveyed in Sleman, Indonesia. Marchioro (2016) reported that *B. carambolae* attacked eight important fruit commodities in Brazil. For *B. papayae*, Suputa et al. (2010) reported that 28 species of fruit commodities in Indonesia are known as hosts of *B. papayae*.

In this study, population fluctuations of *Bactrocera* spp. varies on each observation. This may be due to the relative effect of the permanent Steiner traps set up for two months and weekly collected. In line with Vayssières et al. (2009), there was a decrease in the fruit fly population and the length of time the traps were installed. Other factors influencing population fluctuations of *Bactrocera* spp. in this study may be due to variations in geographical conditions, such as the altitude of the experimental location, causing differences in temperature and humidity. Kim et al. (2020) reported that environmental temperature was considered a crucial factor affecting the occurrence and distribution of *Bactrocera* fruit flies. Cai et al. (2023) also state that the combined effects of temperature and other environmental variables can influence adult survival and larval development. The control technique using pheromones from methyl eugenol is called the *Male*

Annihilation Technique (MAT), which can also reduce the population of male fruit flies (Scolari et al. 2021).

The species composition of *Bactrocera* fruit flies in red guava and sweet citrus study sites was similar; the same result was also found between apple and tomato study sites. This shows that one species of fruit fly can be found at more than one study site, which may be the reason for the similarity in fruit fly species composition. This study used methyl eugenol as a pheromone, which may also attract fruit flies surrounding the study sites. Steiner (1952) reported that methyl eugenol can attract various species of fruit fly at a distance of approximately 0.6 km. A previous study also reported that methyl eugenol can attract 123 species of *Bactrocera* (Vargas et al. 2015).

Based on the results of the host-rearing method, *B. carambolae* were obtained on red guava and sweet citrus. Besides, *B. dorsalis* was obtained only on red guava. This indicated that red guava is a host plant of *B. dorsalis* and *B. carambolae,* and sweet citrus is also a host for *B. carambolae*. Koswanudin et al. (2018) reported that *B. carambolae* and *B. dorsalis* prefer the mature host plants as a means of laying eggs with a strong and distinctive aroma, thin skin, and attractive color, one of which is found in the Myrtaceae and Rutaceae plant families. However, we did not obtain fruit flies from apples, large chili, or tomatoes. This study also showed that the fruit fly's parasitoid was only obtained by one individual in the red guava fruit, namely *Opius* sp. Parasitoids of the Opiinae subfamily are parasitoids with a specific host of Dacinae fruit flies (Carmichael et al. 2005). *Opius* sp. has a very short life cycle of 14 days, starting when the female looks for a host to lay her eggs, and a high reproduction rate so that it can potentially reduce the number of fruit fly frequency populations (Putra et al. 2019). In this study, only a few fruit flies and parasitoids were obtained from the host-rearing method compared to the trapping method. This indicated that methyl eugenol could attract various fruit flies in each study site. Another reason may be the intensive application of insecticides more than twice a week for apple, tomato and large chili, while on guava and citrus, it is applied once every two weeks. Pinheiro et al. (2020) explained that pesticides could reduce the population of the fruit fly and cause more negative effects on the survival and reproduction of fruit fly parasitoid. Low parasitization is thought to be due to the adverse influence of insecticide treatment and uncooperative cultivation methods that adversely affect the presence of parasitoids on plants (Overton et al. 2021). One factor that influences the development of parasitoids is the availability of suitable feed and unwise pesticide use (Bueno et al. 2017).

In conclusion, six species of *Bactrocera* were found in five horticultural study sites in Batu City and Malang District namely *B. carambolae, B. cucurbitae, B. dorsalis, B. verbascifoliae, B. papayae,* and *B. umbrosa*. In this study, *B. carambolae, B. dorsalis,* and *B. papayae* are the three predominant species collected using Steiner traps baited with methyl eugenol. Based on the host-rearing method, *B. carambolae* and *B. dorsalis* were found in red guava fruit. Only one parasitoid species, *Opius* sp., was found in red guava fruit. These results provide valuable insights into the distribution of *Bactrocera* spp. on horticultural commodities in Batu City and Malang District and the potential challenges regarding parasitoid presence.

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