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Population dynamics of *Bactrocera* spp., and parasitization efficacy of *Opius* sp. (Hymenoptera: Braconidae) in citrus varieties

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Abstract. *Rahmawati YF, Leksono AS, Rizali A, Gama ZP. 2024. Population dynamics of* Bactrocera *spp., and parasitization efficacy of* Opius *sp. (Hymenoptera: Braconidae) in citrus varieties. Biodiversitas 25: 4350-4359.* Citrus crops play an important role in the agricultural sector of Indonesia. However, farmers face major challenges due to the infestations by the fruit fly, *Bactrocera* spp., which result in significant economic losses. Therefore, this research aimed to identify the periods of fruit fly infestation and evaluate the effectiveness of parasitoids. The population dynamics of *Bactrocera* spp. and parasitoids were analyzed across four citrus varieties in Malang District from January to December 2023. *Bactrocera dorsalis* (Hendel, 1912), *Bactrocera carambolae* Drew & Hancock, 1994, *Bactrocera umbrosa* (Fabricius, 1805), and *Bactrocera cucurbitae* (Coquillett, 1899) were identified using pheromone traps and rearing methods. The results showed that *B. dorsalis* was the dominant species in all citrus varieties, with the highest abundance in siam madu variety. The Duncan Multiple Range Test (DMRT) tested the fluctuation of *Bactrocera* spp. and the Bray-Curtis Index was used to measure the abundance in each variety, which was then visualized using Non-metric Multidimensional Scaling (NMMS). Meanwhile, *Opius* sp. exhibited parasitism rates ranging from 13.52 to 69.10% in the four citrus varieties. Environmental factors, variety characteristics, and monoculture influenced fruit fly abundance and parasitoid effectiveness. July was an important period for pest management interventions, as *Opius* sp. entered an active time to control fruit flies. Understanding the population dynamics of these pests can help predict outbreaks and optimize the timing of control measures using parasitoids.

Keywords: *Bactrocera*, diversity, ferokop traps, *Opius* sp., population dynamics

INTRODUCTION

The fruit flies belong to Tephritidae are key pests in fruit crops and diminishing their quality and yield of harvests. Within the Tephritidae, Genus *Bactrocera* has approximately 750 species distributed across the Asia-Pacific and Afrotropical regions (Drew and Hancock 2022). The *Bactrocera* spp. lays eggs into the fruits. Subsequently, the eggs hatch into larvae and feed on the fruit flesh (Gupta and Regmi 2022). Infested fruits may rot faster and fall off the tree prematurely. Those *Bactrocera* spp. are a polyphagous pest attacking fruit commodities in Asia-Pacific and Afrotropical regions. In Mozambique, *Bactrocera dorsalis* (Hendel, 1912), *Bactrocera cucurbitae* (Coquillett, 1899), *Bactrocera carambolae* Drew & Hancock, 1994, and *Bactrocera zonata* (Saunders, 1842) caused 41, 42, 83, and 18% damage to mango (Cugala et al. 2020). These species also attacks melon in Rajasthan, India (Jakhar et al. 2020) and three citrus varieties in Pakistan (Ashfaq et al. 2020). In Indonesia *Bactrocera* spp. attacks fruits and vegetables such as mango, sweet citrus, papaya, starfruit, guava, apple, tomato and chili (Agustina et al. 2024; Setiawan et al. 2024; Susanto et al. 2022; Tarno et al. 2022; Yuliadhi et al. 2022).

Citrus is a widely cultivated horticultural crop in Indonesia, including sweet varieties. This plant grows in wider elevation range from lowlands to highlands and has high economic value (Sofiyanti et al. 2022). The citrus production contributes significantly to total agricultural output, provides employment, foreign exchange, and alternatives to traditional crops, and supports the downstream sector. However, the citrus commodity has an average potential damage of 44%, resulting in decreased production due to *Bactrocera* spp. attack (Ariningsih et al. 2022). Malang District located in East Java, is one of Indonesia's significant citrus production centres. The region supports optimal growth of citrus at an altitude of 600-1000 asl (above sea level) and an average temperature of 22-25°C (Mufidah et al. 2024). These agroclimatic conditions are suitable for citrus cultivation and its fruit flies pests. Based on research by Hanif and Ashari (2021), the Dau Sub-district experiences fruit fly attacks with fairly high intensity, reaching 34% on citrus plants. Various citrus varieties cultivated in this region provide an opportunity to analyze the preferences of *Bactrocera* spp. and the level of parasitation of *Opius* sp. throughout its host (Rashid et al. 2021). According to local farmers, infestations result in production losses of 45% during the rainy season and 5% during the dry season. This significant seasonal difference is largely due to the rainy season's humidity and temperature, which accelerate the fruit fly life cycle to 18-20 days compared to 30 days in the dry season (Inskeep et al. 2021). Additionally, citrus fruits have softer skin during the rainy season (Nawaz et al. 2021), making them more susceptible to fruit fly attacks. Previous studies reported that *Bactrocera* spp. attract several citrus varieties, such as mandarin, sweet orange, grapefruit, Siam, and tangerines (Ashfaq et al. 2020; Nirwana et al. 2024).

Several effective integrated management measures have been implemented to control fruit fly pests. These include chemical control (Scolari et al. 2021), orchard sanitation (Zida et al. 2023), baiting techniques (Ahmad et al. 2023), the use of sterile insect (Plá et al. 2021), male culling (Shelly 2020), entomopathogenic fungi (Shaurub 2023), and parasitoids (Wang et al. 2021). Parasitoids are natural enemies that effectively control fruit fly populations in horticultural commodities. *Bactrocera* sp. is used as a host to complete the life cycle by laying eggs or larvae, inhibiting fruit flies natural proliferation (Li et al. 2020). Several species of parasitoid wasps from the order Hymenoptera have been reported to have an impact on fruit fly decline, namely *Spalangia endius* Walker, 1839 (Pteromalidae) (Zheng et al. 2021), *Psyttalia incisi* (Silvestri, 1916) (Braconidae) (Lin et al. 2021), *Dirhinus giffardii* Silvestri, 1913 (Chalcididae) (Ullah et al. 2021), and *Opius* sp. Wesmail (Hymenoptera: Braconidae) (Setiawan et al. 2024).

The parasitization rate of parasitoids such as *Opius* sp. shows a low percentage due to the decreased density of pests (de Pedro et al. 2021). Therefore, the lack of hosts affects the cyclical decline in parasitization rates (Wang et al. 2020). In addition, the practice of monoculture in citrus farming also reduces alternative habitats. Since parasitoids often rely on diverse habitats for survival, the fluctuations of these pests and parasitoids are essential to understand. These population dynamics explain the timing of pest populations (Jervis et al. 2023), the influencing factors, and the potential effectiveness of biological control of *Opius* sp. The population dynamics predict pest populations, optimize control, and evaluate the long-term effectiveness of different management methods.

Environmental variables, including temperature, humidity, and availability of food sources, significantly affect the population dynamics of *Bactrocera* sp. and parasitoids (Ibrahim et al. 2022). Seasonal and weather conditions can also affect population development (Salazar-Mendoza et al. 2021). For example, warm and humid conditions tend to favor the population growth of *Bactrocera* (Putri et al. 2024). Parasitoids tend to be active and effective in controlling fruit flies when environmental conditions are suitable. This study analyzed the population dynamics of *Bactrocera* spp. species and parasitation effectiveness on different citrus varieties and explored the relationship between plant variety and parasitoid performance in controlling fruit fly pests. Therefore, this research aimed to provide a comprehensive understanding of fluctuation in *Bactrocera* populations and the effectiveness of *Opius* sp. as a biological control agent in four citrus varieties. Improved strategies and pest management can be developed by reducing pesticide dependence, increasing yields, and promoting sustainable citrus farming.

MATERIALS AND METHODS

Research area

This research was conducted in Sumbersekar Village, Dau Sub-district, Malang District, East Java Province, Indonesia, at approximately 7.908957 South latitude 112.567155 East longitude (Figure 1). The orchards were situated at 635 to 650 meters above sea level. A total of four orchards with four different citrus species and varieties were used, namely siam madu (*Citrus nobilis* Lour.), siam Pontianak (*Citrus nobilis* Lour.), tangerine batu 55 (*Citrus reticulata* Blanco), and Tangerine Rimau Gerba Lebong/RGL (*Citrus reticulata* Blanco), which had been in production for 3-5 years. Monoculture crop management was adopted by farmers, where planting a type of citrus plant continued for a long period with a single species. These four varieties were widely cultivated in Malang District, with a production of 328 thousand tons in 2022 (One Data of Malang Regency 2023). The research was conducted from January to December 2023 to assess the population dynamics of fruit fly pests and the presence of parasitoids. The orchards selected for the study had citrus populations ranging from 90 to 135 trees, covering areas of 1,200-1,300 m^2.

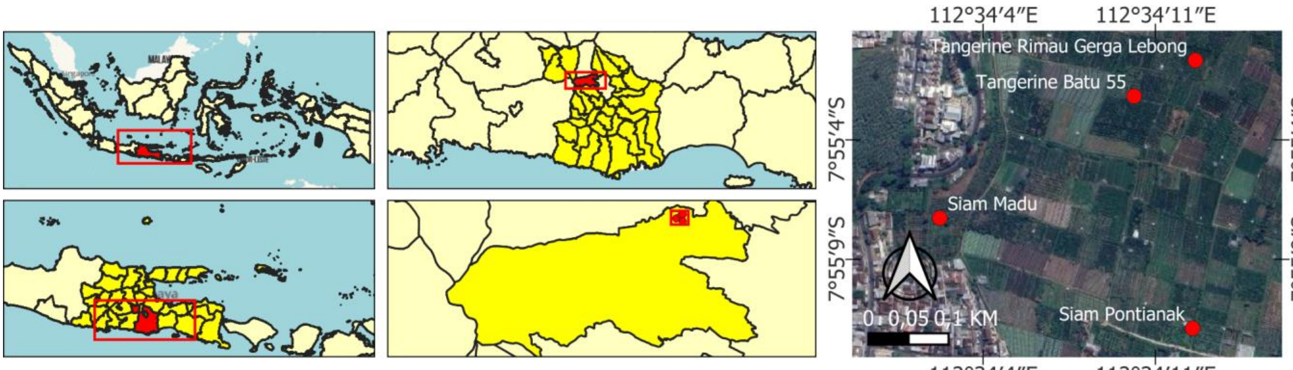

Figure 1. Research sites of fruit fly research at citrus orchards in Sumbersekar Village, Dau Sub-district, Malang District, East Java Province, Indonesia

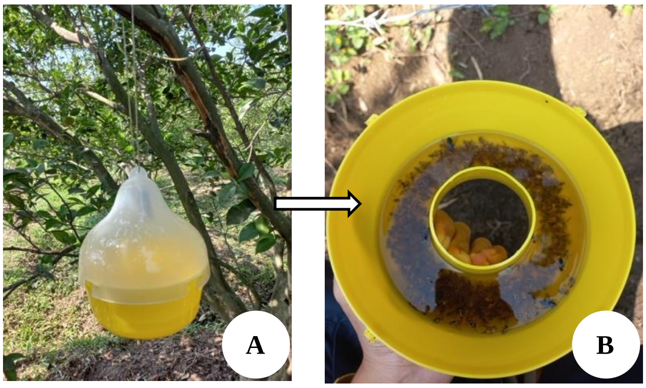

Figure 2. A. Ferokop trap with wooden blocks to capture *Bactrocera* spp. hanging from citrus tree branches; B. The arrow indicates the *Bactrocera* spp. trapped in the ferokop

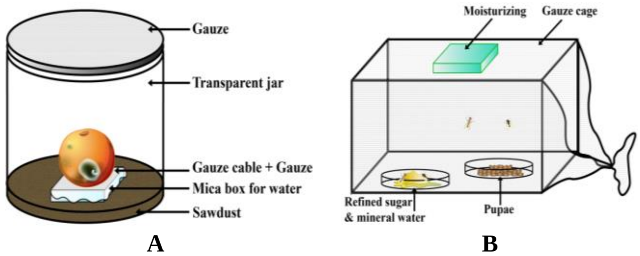

Figure 3. A. Containers of decayed citrus fruits for rearing *Bactrocera* spp./*Opius* sp. larvae; B. *Bactrocera* spp./*Opius* sp. rearing containers at the pupae to adult stage

Sample

The research location of the traps was identified using a purposive sampling method by selecting fruiting plants and farming with a single cropping system. The trap was a 90 BB ferokop using wooden blocks made of polypropylene material with a size of 140×170×70 mm. This trap was set in the field using the Flight-T design with methyl eugenol bait. Methyl eugenol is made by extracting plant essential oils from cloves processed using the steam distillation method. It can be directly used as a fruit fly trap in ready-to-use form. A yellow container was Water mixed with a few drop of detergent was poured into with approximately five cm depth and it hung on the branches of citrus trees (Figure 2). Meanwhile, fruit fly specimens were collected every two weeks 12 times. Wooden blocks were replaced every two months to maintain the effectiveness of capturing fruit fly pests. Traps were placed at each corner and one piece was in the center of each research site. Therefore, five traps were set for each citrus variety and the research was conducted between January and December 2023.

Fruit rearing

Fruit flies and parasitoids were obtained by cultivating host species in a controlled laboratory environment. An estimated one kilogram of fallen fruits with signs of being infected were collected from each location every week. The fruits were stored in containers and rearing cages for the host species. The enclosures were constructed from plastic and draped with gauze. Subsequently, the container was filled with cotton saturated with refined sugar and mineral water (Figure 3). This served as food for adult fruit flies or parasitoids for 7 to 14 days. The fruit fly was transferred to microtubes and stored in the refrigerator at -10°C until the identification process was completed. This procedure persisted until the whole collection of fruit flies died. The number of fruit flies and parasitoids was observed and counted.

Preservation and identification

The fruit flies caught in pheromone traps and the specimens established from host rearing (including fruit flies and parasitoids) were identified at the Laboratory of Animal Diversity and Environmental Technology of Biology Department at Universitas Brawijaya, Malang, Indonesia. The fruit flies and parasitoids have been preserved in vials filled with cotton and 95% ethanol for preserving. Every vial was marked with the specific location, research site, and sampling date. The species captured in traps were identified using a stereo microscope with lucid keys specifically designed for fruit flies, particularly those belonging to the Dacininae subfamily (Doorenweerd et al. 2018). The Nikon D5300 DSLR and Raynox DCR-250 macro lens were used to pin and document every species.

Data analysis

An Analysis of Variance (ANOVA) was conducted to examine the variations in the abundance of the four research sites and the changes in *Bactrocera* spp. population during the observation period. The ANOVA was followed by Duncan's Multiple Range Test (DMRT) to evaluate the data. In addition, the research sites were compared for variations in the composition of *Bactrocera* using the Analysis of Dissimilarity (ANOSIM) method based on the Bray-Curtis Index. The results were visualized using Non-Metric Multidimensional Scaling (NMMS). The analyses were conducted through RStudio, using the vegan and agricultural packages (R Core Team 2023). The parasitization percentage was calculated using the following formula.

$$P = (\sum \text{parasitoid imago} / \sum \text{fruit fly imago} + \sum \text{parasitoid imago}) \times 100\%$$

RESULTS AND DISCUSSION

Bactrocera spp. in four citrus varieties

A cumulative quantity of 11,371 fruit fly specimens were captured using ferokop traps at four Dau Village, Malang District. These specimens belong to four distinct species, including *B. dorsalis*, *B. carambolae*, *Bactrocera umbrosa* Fabricius, 1805, and *B. cucurbitae* (Figure 4). The predominant fruit fly identified were 6,622 *B. dorsalis* (58.24%), 4,074 *B. carambolae* (35.83%), 642 *B. umbrosa* (5.64%) and 33 *B. cucurbitae* (0.29%).

Figure 5 showed that *B. dorsalis* and *B. umbrosa* species increased in the seventh month before declining. In *B. carambolae*, the number increased in the sixth and seventh months before decreasing. Based on further tests, the group formed in the two species only has the letter 'a'. Therefore, there is no significant disparity in the number of fruit flies of *B. dorsalis*, *B. carambolae*, and *B. umbrosa* between month 1 (January 2023) to 12 (December 2023). For *B. cucurbitae*, the number obtained at the research site was relatively stable compared to other species, with a slight increase in months 10 to 12. In November, the number of *B. cucurbitae* fruit flies was significantly higher than in other months. Groups 'a', 'b,' and 'ab' show post hoc test results reporting significant differences in certain months. These four species suggested peak population periods occurring in the sixth to eighth month related to the harvesting period of citrus fruits, environmental factors, and insect life cycle.

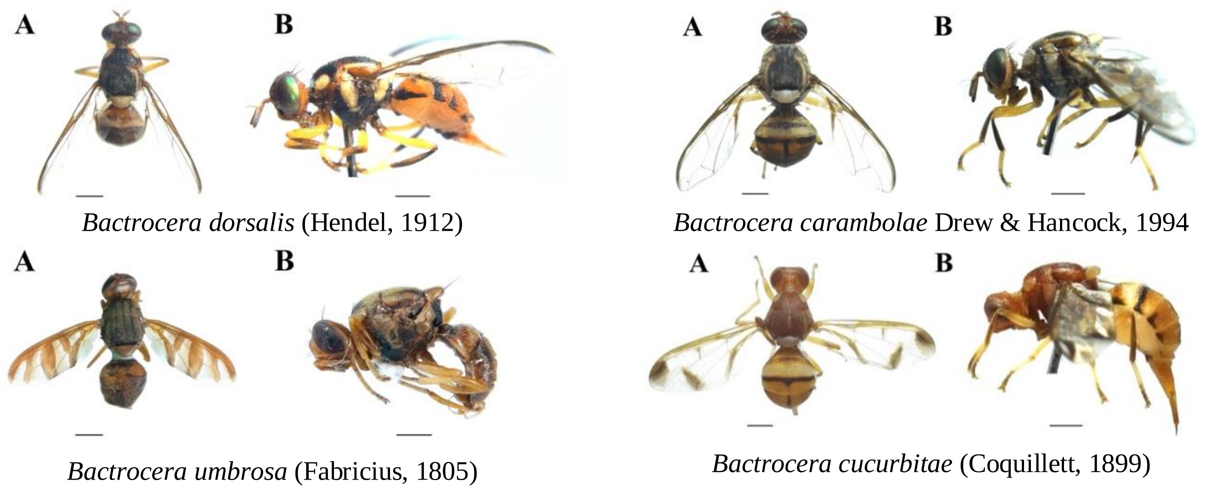

Figure 4. Fruit fly species of *Bactrocera* spp. on four citrus varieties (scale bars: 1 mm)

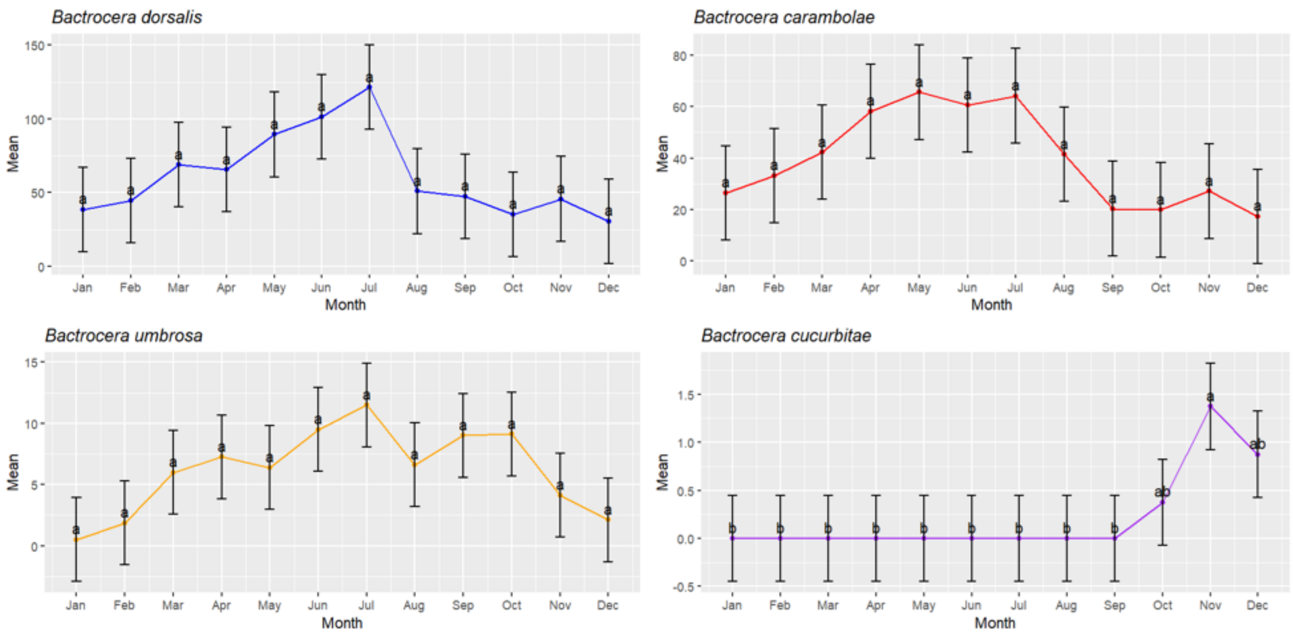

Figure 5. Fluctuations in *Bactrocera* spp. fruit fly populations in citrus fields during January-December 2023

Fluctuation of fruit fly population *Bactrocera* spp.

Based on the ANOVA test, the p-value is <0.05 (0.00896) since there is a difference in the average number of *B. dorsalis* in siam madu (*C. nobilis*), siam Pontianak (*C. nobilis*), tangerine batu 55 (*C. reticulata*), and Tangerine Rimau Gerba Lebong/RGL (*C. reticulata*) (Figure 6). Meanwhile, *B. carambolae* and *B. umbrosa* obtained p-values smaller than 0.05 (<0.001), showing a difference in the average number of species. The results report that there is a significant difference between siam madu citrus varieties with *B. carambolae* and *B. umbrosa* species because the number is the highest compared to others. Conversely, there was a significant difference between the Tangerine RGL variety and *B. dorsalis*, *B. carambolae,* and *B. umbrosa* species because the number is the least. For *B. cucurbitae*, the p-value is greater than 0.05 (0.127) because none of the citrus varieties are significantly different in terms of the number compared to other varieties.

Composition of fruit fly species *Bactrocera* spp. in different varieties

The ANOSIM results showed significant differences in the composition of fruit fly (R = 0.3405, p = 0.001). *B. dorsalis*, *B. carambolae*, and *B. umbrosa* were found in all varieties, while *B. cucurbitae* was only reported in the varieties of siam madu and siam Pontianak. The data groups on the MDS graph show that adjacent and distant points have similar and different data, respectively. In the graph, groups 1 (siam madu) and 2 (tangerine batu 55) are close to each other. Therefore, the composition of fruit fly in siam madu and tangerine batu 55 varieties are more similar to each other than in Tangerine Rimau Gerga Lebong/RGL and siam Pontianak. Groups 3 (RGL) and 4 (siam Pontianak) are also close to each other but are further away from 1 and 2. Therefore, the data in groups 3 and 4 are more similar to each other and different from 1 and 2 (Figure 7).

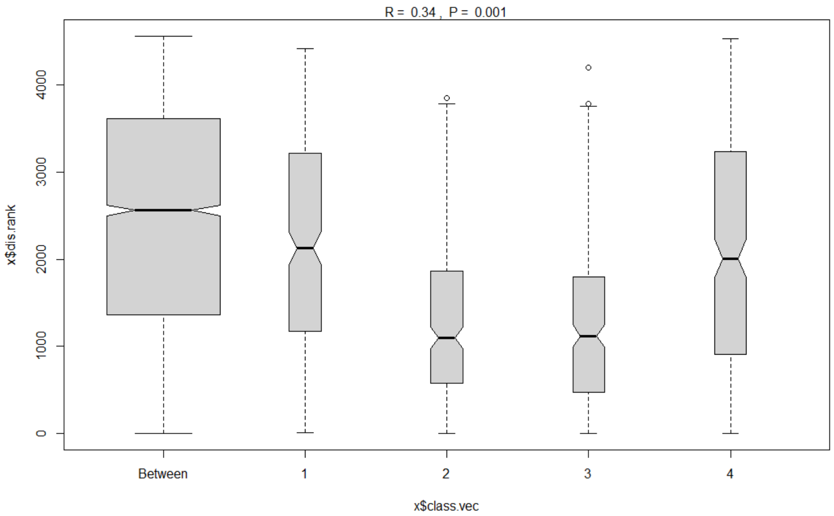

Figure 6. Abundance of *Bactrocera* fruit flies collected by ferokop traps on several citrus varieties

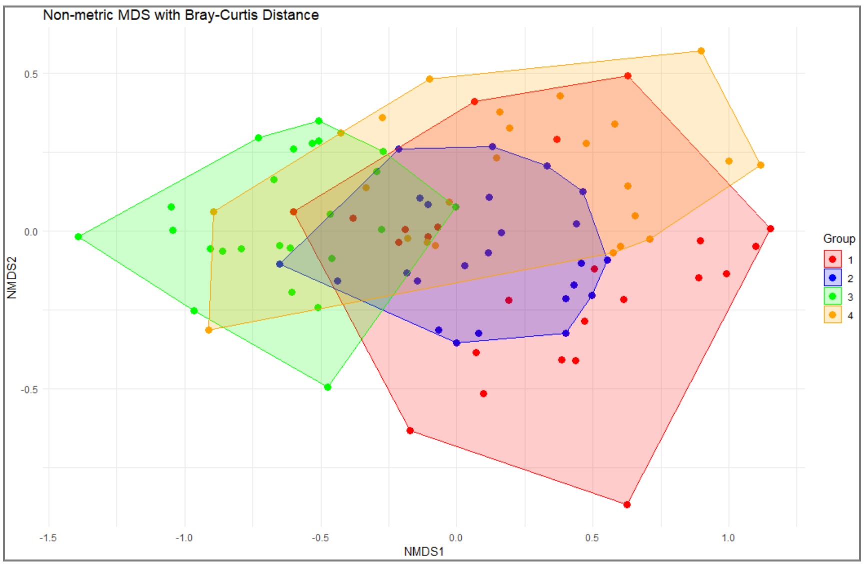

Figure 7. Non-metric test with Bray-Curtis Distance of fruit fly species composition specifically focusing citrus fruit varieties. Group 1: siam madu, 2: tangerine batu 55, 3: Tangerine Rimau Gerga Lebong/RGL, 4: siam Pontianak

The proximity of citrus species in the ordination plots of the siam madu and tangerine batu 55 varieties indicate similarities in the composition of the fruit fly communities that infest the two varieties. This may be due to similarities in fruit morphological characteristics, biochemical composition, or other ecological factors that influence fruit fly species preferences. Conversely, RGL and siam pontianak oranges appear to create clusters separated from the previous two citrus varieties. This condition reflects the different preferences of fruit fly species towards specific fruit characteristics of each citrus variety. This finding also underscores the potential role of host-plant characteristics in shaping fruit fly community structure, where similar citrus varieties tend to support similar fruit fly assemblages, reflected by clustering in ordination space.

Parasitoid presence on some citrus fruit varieties

The population of *Opius* sp. fluctuated in response to changes in *Bactrocera* spp. The parasitoid population increased with the fruit fly, from March to July 2024. There was a delay in the response of *Opius* sp. to the presence of fruit fly populations, with the numbers peaking after *Bactrocera* spp. increased. This is common in predator-prey (or parasitoid-host) dynamics, where natural enemy populations increase in response to host growth. The lack of abundance in certain months, such as July and November, suggests a potential challenge for biological control (Figure 8).

Based on the rearing method, the fruit flies found were *B. dorsalis*, *B. carambolae* and parasitoids *Opius* sp. was also present. This parasitoid was higher in the siam madu variety and lower in the tangerine batu 55. The presence of these parasitoids can suppress the survival of fruit flies, especially in the siam madu variety. The highest parasitization rate was obtained sequentially in the siam madu variety (69.10%), siam Pontianak (42.01%), tangerine batu 55 (22.64%), and Tangerine Rimau Gerga Lebong/RGL (13.52%), as shown in Figure 9.

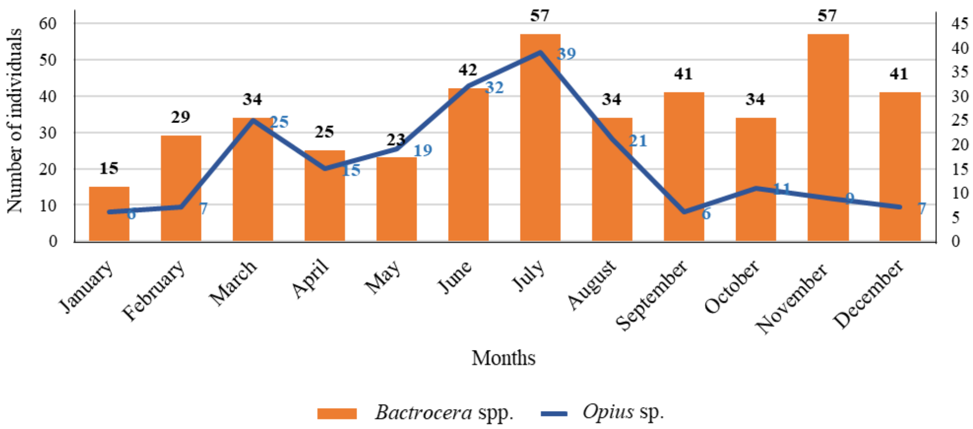

Figure 8. Association of *Bactrocera* spp. and *Opius* sp. on host rearing

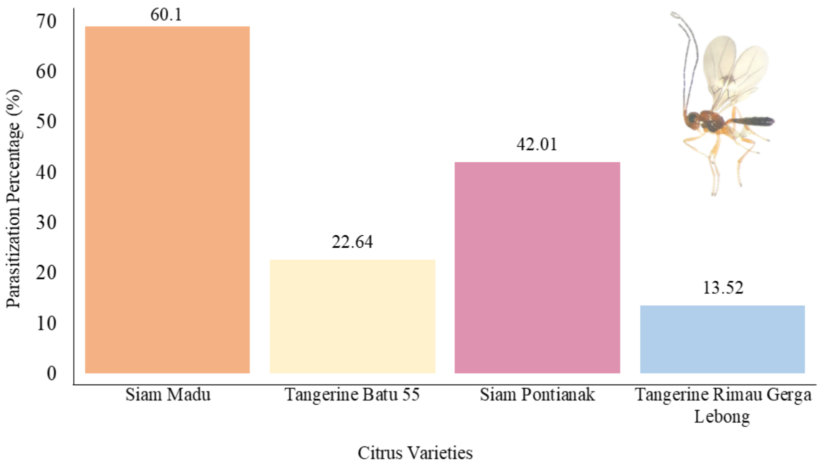

Figure 9. Percentage of parasitization presence in all citrus varieties

Discussion

In this research, four species were collected using ferokop traps with methyl eugenol bait, namely *B. dorsalis*, *B. carambolae*, *B. umbrosa*, and *B. cucurbitae*. According to (Mutamiswa et al. 2021), all fruit flies of this type are species with high reproductive ability and a short life cycle, regenerating optimally every year. The family Tephritidae is reported to attack 18 alternative fruit and vegetable plants (Rasolofoarivao et al. 2022), allowing for a wider invasion of others. This species is an important pest of several agricultural commodities in tropical and subtropical regions. In western Indonesia, 8 species are serving as important pests that attack more than 20 types of fruits (Suwarno et al. 2018). Previous study reported that there were five *Bactrocera* species attacked sweet citrus consisted of *B. carambolae*, *B. dorsalis*, *Bactrocera verbascifoliae* Drew & Hancock, 1994, *Bactrocera papaya* Drew & Hancock, 1994 and *B. umbrosa* (Setiawan et al. 2024). Different location and time may cause the variation in the species collection.

In this study, the population of *B. dorsalis* dominates all varieties and is the primary pest on citrus, especially the siam madu variety. Fruit flies of the genus *Bactrocera* are part of the family Tephritidae, which has a high species diversity and is widely distributed in tropical and subtropical regions. Similar research shows that the species is a significant pest for citrus in Asia and Africa (Qin et al. 2018; Nebie et al. 2021; Li et al. 2024). The population of *B. cucurbitae* was found to be the least since citrus was not the primary host of the species. Even though this species lays eggs quickly, but the development to pupa or adult stage is difficult (Follett et al. 2022). Due to the difficulty of fulfilling the life cycle, the mortality is higher than in other species.

The population of *Bactrocera* spp. showed fluctuations over every sampling interval in the present research. The percentage level of infestation is strongly influenced by the abundance of fruit flies and the availability of citrus. There is a relationship between the number of fruit flies caught with the citrus's maturity level and the harvest frequency (Grechi et al. 2021). The highest and lowest abundances are obtained during the harvest period when the size of the fruit is yellowish-green and greenish, respectively. The greenish-yellow produces an ester aroma when the fruit changes color. The volatile profile dominated by terpenes and terpenoids in citrus causes a strong oviposition response in female fruit flies (Antonatos et al. 2023).

The highest percentage of attacks occurred in the siam madu variety, which had the highest fruit fly population compared to other varieties. Observations indicate that siam citrus varieties are more susceptible to fruit fly infestations than tangerines, as there are more rotten and damaged fruit in the garden. *Bactrocera* spp. are attracted to damaged citrus due to the presence of aromatic compounds such as octane and β-pinene found in very ripe citrus fruits, which can entice *Bactrocera* spp. to lay their eggs (He et al. 2023). Fallen or damaged fruit should be removed from the orchard to avoid the fruit fly's pupa development in the soil (Theron et al. 2023). The availability of paraffin traps, sanitation, the use of pesticides, and technical knowledge in implementing fruit fly pest management are also factors affecting the percentage of attacks (Adhikari et al. 2020; Rahmawati et al. 2024). In addition, environmental factors such as temperature, humidity, wind speed, and the duration of sunlight exposure significantly affect the presence and spread of *Bactrocera* spp. (Choudhary et al. 2021, Susanto et al. 2022). The host-rearing experiment indicated that *B. dorsalis* presented in all four citrus varieties, with the highest percentage in the siam madu. This suggests that the siam madu is the most favorable environment for the species, and fruit flies prefer citrus with a soft texture and complete nutritional content (Saeed et al. 2022). Additionally, the higher harvesting frequency in siam madu allows for the abundance of citrus. Conversely, the Tangerine Rimau Gerga Lebong/RGL variety had the lowest percentage due to its thicker skin (3.10-4.67 mm) than siam (2-3 mm). This thickness complicates and prolongs the egg-laying process for females.

Compared to the laboratory-rearing method, using methyl eugenol bait resulted in a higher abundance of fruit flies. This research identified several factors contributing to pupal mortality in *Bactrocera* spp. rearing, including parasitoids, fungal attacks, and failure to hatch. Meanwhile, unhatched pupae exhibited a darker blackish-brown color. The parasitoid population affecting fruit flies across various citrus varieties in Malang District included *Opius* sp. The parasitoid population of fruit flies from all citrus varieties in Malang District obtained *Opius* sp. Among the four citrus varieties studied, siam madu had the highest parasitism rate at 69.1%. This demonstrates that the effectiveness of *Bactrocera* spp. varies during the egg, larval, and pupal stages. Therefore, the capability of these natural enemies to control the host population can be evaluated.

Monthly fluctuations in *Opius* sp. parasitism were observed across the four citrus varieties. The periods of parasitism for siam madu, tangerine batu 55, and Tangerine RGL and siam Pontianak were recorded as March to June, June to August, and July to August, respectively. These variations are attributed to differences in the availability of citrus fruits in the garden and the time taken from flowering to harvesting. In siam madu and siam Pontianak, new buds (flowers) appear approximately every three months, while tangerine batu 55 and RGL take four months. However, the peak population occurs in the sixth to eighth month. The frequency of the flowering to the harvest period in the siam variety increases the abundance of fruit and the presence of parasitoids.

This result can serve as a recommendation since *Bactrocera* spp. Increases in July and November. Farmers should focus on pest management practices to increase biological control methods, such as releasing *Opius* sp. The stable presence of *Opius* sp. shows natural biological control. Farmers can adjust pesticide use to avoid damaging *Opius* sp. populations, especially in March and July. *Opius* sp. is an effective biological control agent for *Bactrocera* spp., due to its ability to parasitize host larvae at all developmental stages (Clarke et al. 2022). As an endoparasitoid koinobiont, *Opius* sp. lays its eggs inside the body of fruit fly larvae and allows the host to continue developing while utilizing nutrients from the host, eventually leading to host death when the parasitoid reaches the adult stage (Fidelis et al. 2023). The presence of parasitoids is able to control fruit fly populations through parasitism and may indicate a natural balance in the ecosystem that supports sustainable pest control (Wang et al. 2021). The effectiveness of releasing *Opius* sp. as a biological control agent was reported by Sivinski 2020 that the optimal parasitation rate reached 45% with an average release density of 800 parasitoids per hectare. However, success is highly dependent on environmental conditions, host availability and the quality of the parasitoid (Vosteen et al. 2020). The integration of biological control involving *Opius* sp. is an essential strategy for optimizing sustainable control of *Bactrocera* spp. populations (Vargas et al. 2012).

The fruit fly population was very high in each variety observed, while the parasitoid *Opius* sp. population was relatively low. The imbalance between fruit fly control and *Opius* sp. utilization programs causes the low population and the level of parasitization. Some citrus plants were sprayed with insecticides and environmental sanitation was not carried out. Parasitoids contaminated with insecticides through sprays, contact, or orally from food sources cause toxic effects (Pinheiro et al. 2020). The low parasitization rate is also due to the proximity of other fruit fly host plants, the structure of natural habitats, as well as the ability of ovipositors to reach fruit fly eggs, larvae, or pupae (Karlsson et al. 2018, Mama Sambo et al. 2020).

The results showed that in all citrus varieties, more than one individual was found to have parasitoids due to intraspecific competition. According to (Ode et al. 2022), the population of *Opius* sp. will be high when there is no exploitative competition between females and males to obtain hosts. The parasitoid has excellent potential, as proven in Malaysia, Brazil, Australia, and the South Pacific Region which have successfully used the organism as a fruit fly biocontrol agent (Clarke et al. 2022). Another role of *Opius* sp. was reported in South Florida as the most dominant species found in most vegetable and bean crops with a six-year observation duration, where the presence followed the abundance pattern of the main pest (Seal et al. 2023).

A total of four distinct species of *Bactrocera* sp. were identified in four varieties of citrus in the Dau Sub-district, Malang District namely, *B. dorsalis*, *B. carambolae*, *B. umbrosa*, and *B. cucurbitae*. Among these, *B. dorsalis* was the dominant species obtained using ferokop traps with methyl eugenol and through laboratory-rearing methods. The siam madu variety was the most heavily impacted by fruit flies, as it has the longest harvest period of three months. Further research is necessary to explore the relationship between the nutrition content of siam citrus varieties and the attraction of fruit fly pests and parasitoids. The presence of parasitoid *Opius* sp. as a natural enemy of *B. dorsalis* and *B. carambolae* shows the excellent ability to regulate the balance of the host population. The months of July and November are important for pest management interventions. Farmers are recommended to adjust pesticide use in July to avoid damaging *Opius* sp. when parasitoids actively control fruit fly pests. Meanwhile, the highest parasitization rate was obtained in siam madu citrus at 69.1%. These results provided a valuable contribution to developing effective and timely pest management strategies in citrus production. Understanding the population dynamics of *Bactrocera* sp. can help predict pest outbreaks, optimize the timing of control measures, and assess the long-term effectiveness of future citrus crops.

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