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Association of Hymenoptera Parasitoid with Fruit flies *(Bactrocera dorsalis* Complex*)* attacking Guava fruits *(Syzygium aqueum)* in Gianyar, Bali Province, Indonesia

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Abstract Fruit flies are considered a significant insect problem in Indonesia and worldwide as they prey on a wide variety of fruits and vegetables. The study found three types of fruit flies that attack water guava fruit in Gianyar Regency, namely *Bactrocera albistrigata*, *Bactrocera carambolae* and *Bactrocera dorsalis*. The highest percentage of attacks on water guava fruit was found in Gianyar District at 85.00%. The abundance of *B. albistrigata* species had the highest population with an average value of 353.4 individuals. Three species of parasitoid were found to be associated with fruit flies that attack water apple plants in Gianyar Regency, namely *Fopius arisanus*, *Diachasmimorpha longicaudata*, and *Opius* sp. Species *F. arisanus* was the highest parasitization rate of 14.28%, and is an effective natural enemy candidate in controlling fruit fly attacks on water apple. The study suggested that the different host plants of fruit fly species should be further evaluated as an effective control technique and early warning for farmers and policy makers.

Keywords: *Bactrocera dorsalis* complex, *Fopius arisanus,* Hymenoptera parasitoid, *Syzygium aqueum*

Introduction

Water guava *(Syzygium aqueum* Burm f. Alston*)* is a well-known fruit due to its nutrients such as vitamins A and C (Manaharan *et al.,* 2013). In the public health context, water guava is used as a traditional medicine to heal wounds on the mouth and tongue edges, increase immunity, prevent the aging process, relieve fatigue and lethargy, strengthen teeth, prevent and treat mouth sores, control blood pressure, and treat fever (Laily *et al.*, 2015). Furthermore,

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the water guava production in Indonesia fluctuates from year to year. The report stated that the production in 2011 and 2012 totaled 103,156 tons and 104,392 tons, respectively. Water guava production decreased to 91,291 tons in 2013 and increased again in 2014 to 91,983 tons (BPS, 2015).

Fruit fly (Diptera: Tephritidae) is an important pest responsible for the decline in fruit production (Triplehorn *et al*., 2005; Badii *et al.,* 2015; Aryuwandari *et al.,* 2020). Currently, fruit flies from the Tephritidae family are distributed almost throughout the Asia-Pacific region by attacking more than 26 types of host plants. This pest causes losses to the guava quantity and quality, such as low or rotten water guava production. Fruit flies generally attack unripe fruits, causing yield loss of about 90-100% depending on the fruit fly population, environment, variety, and season (Sarwar *et al.,* 2014). This pest is very difficult to control in many tropical countries including Indonesia (Clarke *et al*., 2005; Susila and Supartha, 2020). Farmers also require significantly large funds to cope with fruit fly attacks. Therefore, several countries such as Japan and Australia implemented a strict system of importing fruit products to maintain product quality and prevent invasive pests from entering their countries (Dominiak and Ekman, 2013; Vargas *et al*., 2015).

Fruit flies consist of two well-known genera, namely *Dacus* and *Bactrocera* (Prabhakar *et al*., 2012). The adult fruit fly has important features on its head, chest, wings, and abdomen. They have a habit of laying eggs in water guava fruit tissue. The spawning grounds are marked with a small, fuzzy black dot (Dias *et al.,* 2017). This small spot is an ovipositor puncture which is then followed by a surrounding necrotic appearance. Meanwhile, the larvae that hatch from eggs will eat the flesh of the fruit, causing the appearance of small brown spots. The larvae also destroy the fruit pulp, causing the fruit to rot and fall before ripening. This fallen fruit must be collected and destroyed immediately to prevent larger fruit fly attacks (Wiratama *et al.,* 2017a).

Similar research conducted in Bali Province confirmed fruit fly pests on various fruit commodities. Research by Putra *et al.* (2019) showed that there were two fruit fly species attacking starfruit plants in the Gianyar Regency, namely *Batrocea carambolae* and *B. papayae* with an average attack percentage of 86.33%. Furthermore, Badriasih *et al.* (2019) stated that there were three species attacking mangoes in Buleleng Regency namely *B. papayae, B. carambolae*, and *B. occipitalis*. On citrus plants in Gianyar and Bangli Regencies, two fruit fly species were found, namely *B. crambolae* and *B. papayae* (Wiratama, 2017b).

Pest control is necessary to enhance guava plant productivity in the community. Biological control can be used to control fruit fly attacks (Garcia *et al*., 2020; Supartha *et al*., 2022a). According to Schöller *et al.* (1997), biological control is a component of integrated pest control. Although the results of research by Ovruski *et al.* (2000) stated that there was a lack of success in the introduction of exotic fruit fly parasitoids in various Latin American countries. However, the study of natural biological control/conservation is the main area that is most studied; however, augmentative biological controls had a higher study success rate and potential for success, followed by classical biological controls (Dias *et al*., 2022).

Parasitoids are insects in the pre-adult phase that act as parasites on the bodies of other insects (hosts). These parasitoids play a role in killing or weakening the host (Purnomo, 2010). Utilizing parasitoids provides many advantages because, in addition to being safe for the environment, they are also effective in suppressing the development of certain pest populations in the field (Hidrayani *et al.,* 2011). A study showed that there are several parasitoid species from the *Hymenoptera* order of the *Braconidae* family having the potential to control fruit fly (Zamek *et al*., 2012; Ndlela *et al.,* 2020). In Malaysia, the parasitoid potential from the *Braconidae* family reached 57% and in Italy reached 80-95% (Vijaysegaran and Osman, 1991). *Braconidae* is the dominant parasitoid family in mango, guava, and chili fruits in Benin at 97%, followed by *Eulophidae* (1.6%) and *Pteromalidae* (1.2%). Furthermore, Somta *et al.* (2010) observed that out of 1,105 parasitic pupae collected from Malabar yellow almonds in Thailand, four types of parasitoids were found, *Fopius arisanus, Psyttalia* sp., *Diachasmimorpha longicaudatus* Ashmead, and *Spalangia* sp. The parasitization rate of *D. longicaudatus* on guava in Timor Leste was 28% (Oliviera, 2015). Putra *et al.* (2019) stated that there were three parasitoid species associated with fruit flies on star fruit in Gianyar Regency, Bali Province, namely *Diachasmimorpha* sp., *Opius* sp, and *Fopius arisanus*.

The study aimed to identify the types of fruit fly species associated with water guava fruit, calculate the percentage of fruit fly attacks, identifed the types of parasitoid species associated with fruit flies, and the parasitization level of the parasitoid on fruit flies attacking water guava in Gianyar Regency.

Materials and methods

Study area

This study was conducted on a field and laboratory scale. The field-scale research was carried out in Gianyar Regency, Bali, while the laboratory research was carried out at the Laboratory of Integrated Pest and Plant Disease Control, Faculty of Agriculture, Udayana University. The study lasted for 6 months from March to August 2020.

Procedure

Determination of locations and sampling method

The locations were determined using the diagonal method (Figure 1) with the following results namely Sukawati, Gianyar, Ubud, Payangan, and Tampaksiring Districts. Each location had three bearing guava fruit trees (by purposive sampling) with a minimum distance of 3 km. In addition, each tree should have at least 10 fruits attacking by fruit fly. The sampling interval was carried out every 3 days with 6 replications. In all locations, 150 pieces were obtained, therefore the total fruit collected during the research was 900 pieces.

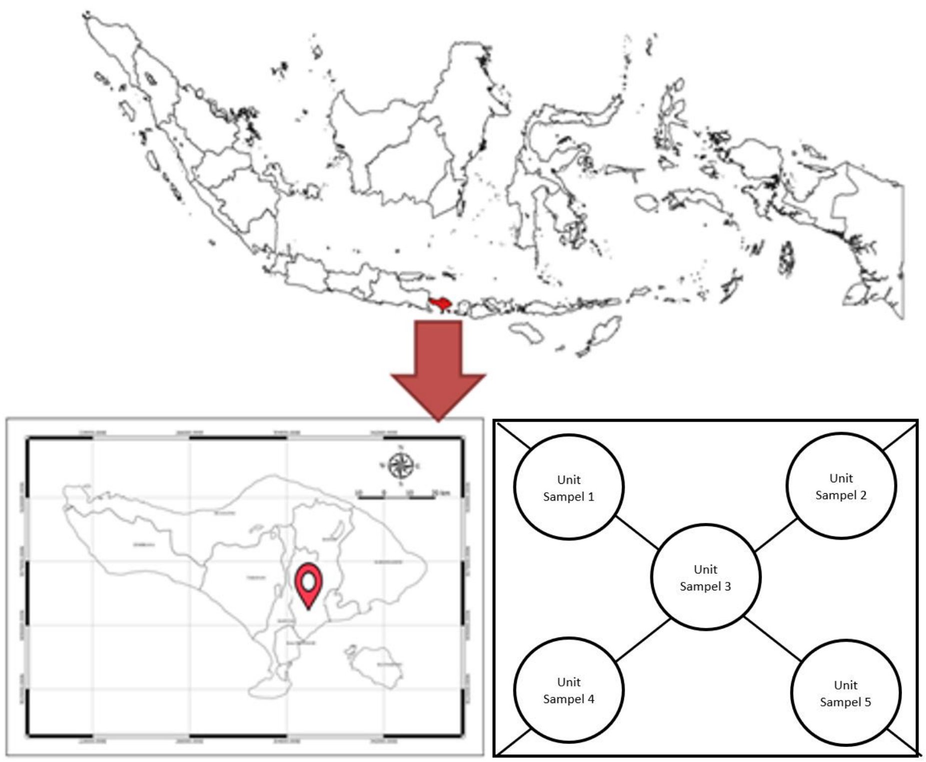


Figure 1. The sampling design in Gianyar Regency using diagonal method
Note: 1. = Payangan District (8°26'17"S, 115°16'0"E), 2. = Tampaksiring District (8°24'30"S, 115°19'18"E),3. = Ubud District (8°32'0"S, 115°15'57"E), 4. = Sukawati District (8°34'33"S, 115°17'12"E) and 5. = Gianyar District (8°33'20"S, 115°20'12"E)

Maintenance of fruit flies

The water guava fruit showing symptoms were collected from the field and placed in a 23.5 cm high and 8.5 cm diameter transparent plastic container (Figure 2).

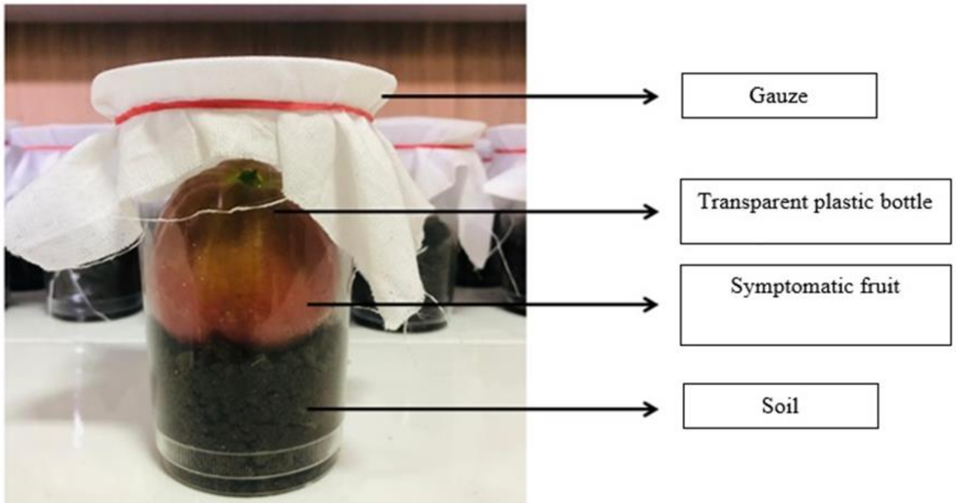


Figure 2. Fruit fly rearing container

As a medium for pupa formation, the container had soil media mixed with sand in a ratio of 1: 1 with 10 cm height, a cover, and air ventilation. The room temperature during the study was 23.00℃ and 64% humidity. Furthermore, maintenance was carried out until the emergence of flies and adult parasitoids.

Fruit flies and parasitoids identification

The fruit flies and adult parasitoids that appeared in each rearing container were identified using a stereo-trinocular microscope (Olympus, Japan). Identification was carried out based on the morphological characteristics of adult fruit flies with standard protocol according to Schutze *et al.* (2018); International Center for the Management of Pest Fruit Flies (ICMPFF) & Ministry of Agriculture Republic of Indonesia (KEMENTAN) (2006); Suputa *et al.* (2006). Meanwhile, adult parasitoids were identified using the key of determination from Sharkey and Wahl (1992) and Carmichael *et al*. (2005). The identified adult fruit flies and parasitoids were recorded and documented.

Data analysis

The relative abundance index was calculated using the equation adopted from Krebs (1989). Meanwhile, the parasitization level was calculated using the formula from Buchori *et al.* (2008):

Population abundance

$$Abundance\ (K) = \frac{Number\ of\ species\ found\ at\ location\ x}{Total\ number\ of\ species\ found\ at\ site\ x} \times 100\%$$

Percentage of fruit fly attacks

$$Attack\ percentage\ (I) = \frac{The\ number\ of\ fruit\ attacked}{The\ total\ number\ of\ fruits} \times 100\%$$

Parasitization levels

$$Levels\ of\ parasitization\ (P) = \frac{The\ number\ of\ adult\ parasitoids\ A}{Number\ of\ adult\ fruit\ flies + the\ number\ of\ parasitoids\ A\ adult} \times 100\%$$

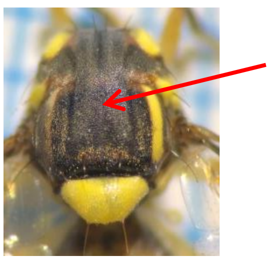
Note: Parasitoid A = The number of adult parasitoids that appeared and
Adult fruit flies = Total number of adult fruit flies from unparasitized pupae

Results

Identification of fruit fly species associated with water guava

Three species of *Bactrocera* spp. were identified, namely *Bactrocera albistrigata*, *Bactrocera carambolae* and *Bactrocera dorsalis*. The results of morphological identification showed that the three fruit fly species observed in this study.

1a There was no medial post sutural vittae in the scutum (Fig.3a)…**Genus *Bactrocera*** 2



(a)

Figure 3. The character of the medial post-sutural vitae of the *Bactrocera* spp. thorax

2a Face with a pair of medium-sized round dark spots (Fig.4a)……………………………………………………………………..**3a**

2b Face with a pair of large oval black spots (Fig.4b)…………………………………..**3b**


(a)


(b)

Figure 4. The spot shape at the top of the mouth (a) Round; (b) oval

3a The dark brown scutum
(Fig.5a)…………………………………………………………………**4a**

3b The shiny black scutum
(Fig.5b)…………………………………………………………………**4b**


(a)


(b)

Figure 5. Scutum color. (a) Dark brown; (b) Black sparkle

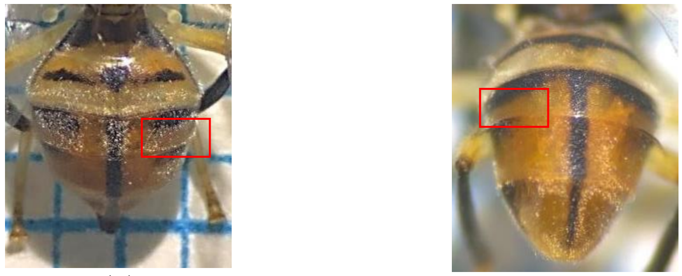

(a)


(b)

Figure 6. The lateral width of the post sutural vittae. (a) Narrow; (b) Width

5a Black and narrowed T pattern of the III-V abdominal terga, rectangular pattern of terga III
(Fig.7a)…………………………………………………………………6**b**

5b Black and widened T pattern of the III-V abdominal terga, triangular pattern of terga III
(Fig.7b)…………………………………………………………………6**a**

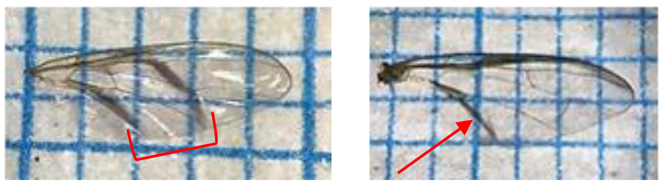


(a) **(b)**

Figure 7. Abdominal pattern: (a) Quadrilateral (b) Triangle

6a 2 transverse bands from the costal boundary to the bottom
(Fig. 8a)……………………………………………….***Bactrocera albistrigata***

6b One transverse band on the anal part (under the wing)
(Fig. 8b)……………………………………………......7

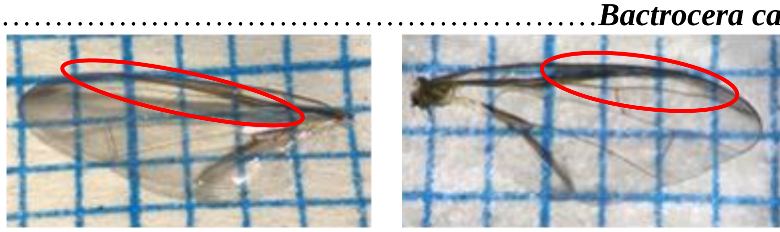


(a) **(b)**

Figure 8. The number of bands across the flange (a) 2 bands (b) one band

7a The coastal band confluents to R_{2+3} and do not extend along the tips of the wings
(Fig. 9a)……………………………………………….***Bactrocera dorsalis***

7b The coastal band overlaps with R 2 + 3 with the same width until the ends of R_{2+3}
(Fig.9b)……………………………………………….***Bactrocera carambolae***



(a) **(b)**

Figure 9. The coastal band (a) Confluent to R_{2+3};(b) Overlapping against R_{2+3}

Percentage of fruit fly attacks

The results showed that fruit fly attacks on water guava in Gianyar Regency had various percentages. These include Sukawati District (83.33%), Gianyar District (85.00%), Ubud District (84.44%), Payangan District

(83.89%), and Tampaksiring District (82.78%) with an average attack percentage in Gianyar Regency (83.89%) (Figure 10).

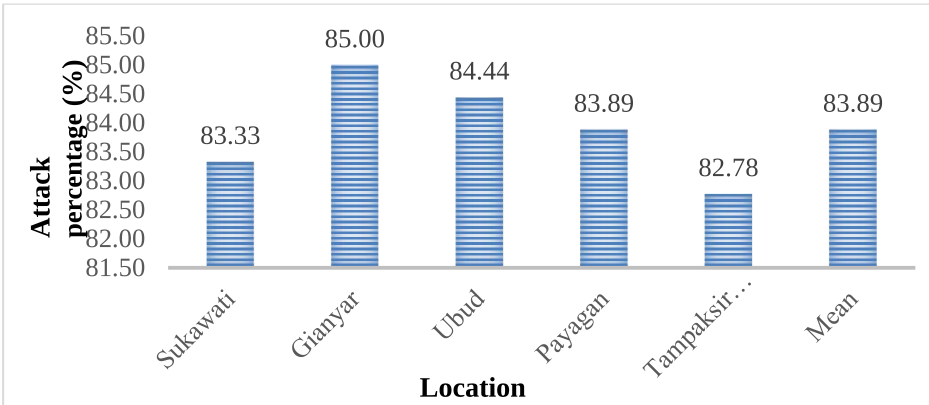


Figure 10. Percentage of Fruit Fly Attacks on Water Guava in Gianyar Regency

Abundance of fruit flies

The abundance of fruit flies attacking the guava plants showed different values in each district. The *B. albistrigata* had the highest population with an average value of 353.4 individuals. This was followed by *B. carambolae* with 349.8 individuals and *B. dorsalis* with 174.8 individuals. In Sukawati District, *B. albistrigata* constituted the dominant species with a population of 314 individuals, followed by *B. carambole* and *B. dorsalis* species with a population of 269 and 140 individuals. In contrast to Sukawati District, Gianyar District was more dominated by *B. carambole* species with a population of 394 individuals, followed by *B. albistrigata* (329 individuals) and *B. dorsalis* (171 individuals). Furthermore, the Ubud and Payangan districts were more dominated by *B. albistrigata* species with 381 and 405 individuals respectively, while the *B. carambolae* species attacking water guava totaled 343 and 388 individuals respectively. In Ubud and Payangan districts, *B. dorsalis* species had a total of 178 and 172 individuals respectively. Tampaksiring District was dominated by *B. carambolae* species of 355 individuals, followed by *B. albistrigata* (338 individuals) and *B. dorsalis* (213 individuals) (Figure 11).

Parasitoid species associated with fruit flies

There were 3 species of parasitoids associated with fruit flies attacking water guava plants in Gianyar Regency, namely *Diachasmimorpha longicaudata*, *Fopius arisanus*, and *Opius* sp. (Table 1).

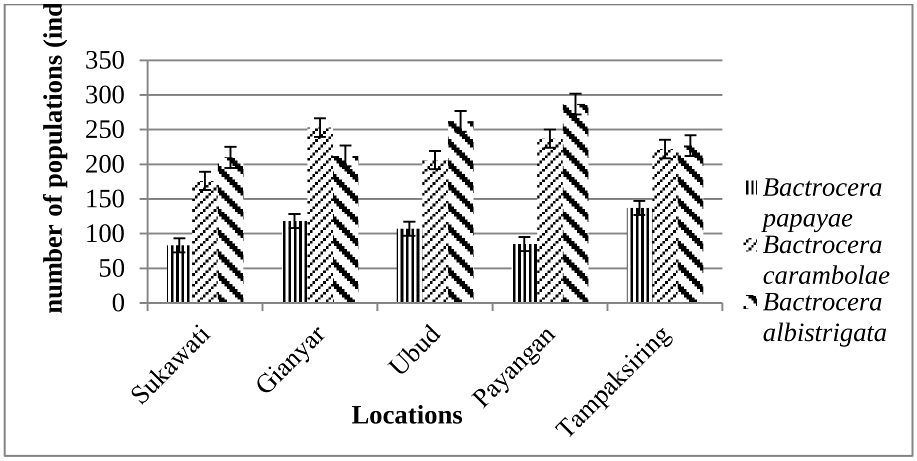


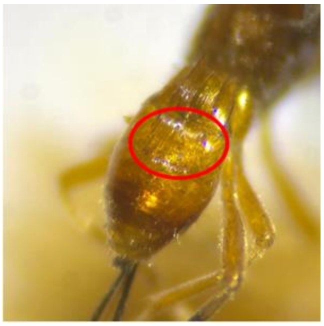
Figure 11. Abundance of Fruit Flies Attacking Water Guava in Gianyar Regency

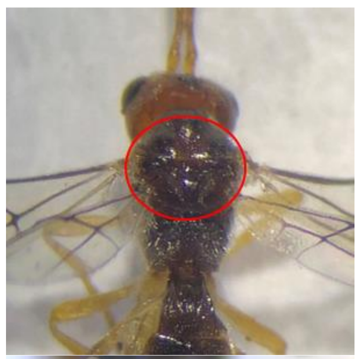
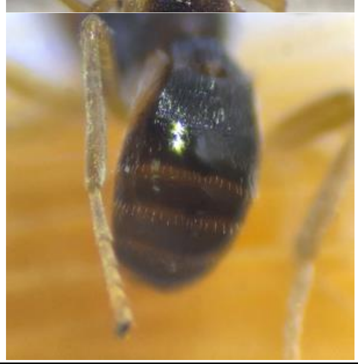
Table 1. Types of fruit fly parasitoid species in water guava in Gianyar Regency

Fopius arisanus			
No	**Morphology**	**Figure**	**Identification**
1	Whole body		• Body dark brown to black. • The caput is dark orange. • Legs light brown.
2	Thorax		Thorax blackish brown. In the mesonotum, the notauli are clearly carved up to the midpit forming a "V"

3	Abdomen		The belly is brownish in adult females. In tergum II there are tightly patterned lines.

Diachasmimorpha longicaudata

No	Morphology	Figure	Identification
1	Whole body		The whole body is brownish orange.
2	Thorax		On the mesonotum, there are three lumps that are clearly visible. Notauli looks smooth and the midpit is clearly visible.
3	Abdomen		In targum II, there is an extensive pattern of longitudinal stripes

No	Morphology	*Opius* sp. Figure	Identification
1	Whole body		Body dark brown to black.
2	Thorax		The meoscutum consists of three lumps.
3	Abdomen		The abdomen is black.

The results showed that the parasitoids associated with fruit flies on guava plants in the Gianyar Regency had different values for each species. *Fopius arisanus* had a very high parasitization level in all locations with an average value of 14.28%, while *Diachasmimorpha longicaudata* had a lower parasitization level and was found in Sukawati, Gianyar, Payangan, and Tampaksiring Districts with an average value of 0.23%. Furthermore, *Opius* sp. had the lowest parasitization level and was only found in Gianyar, Ubud, and Tampaksiring Districts with an average of 0.16% (Figure 12).

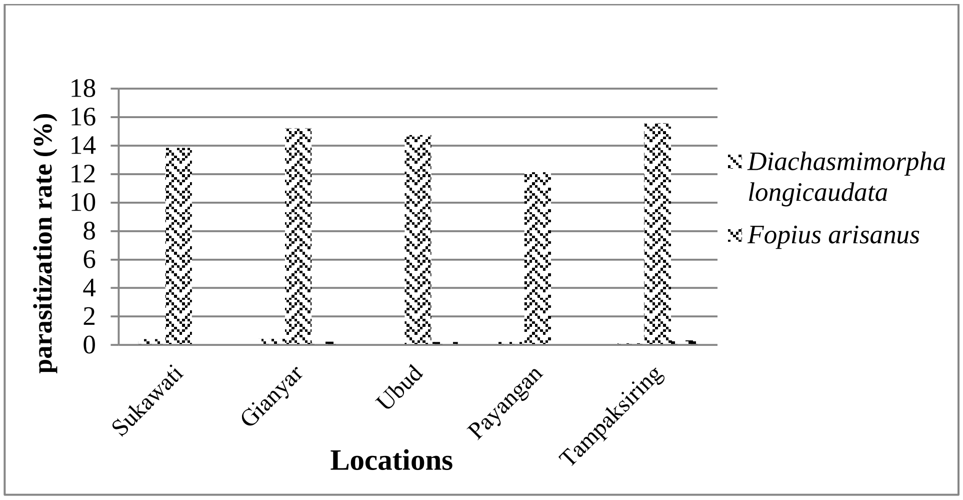


Figure 12. Parasitization Level of Fruit Flies Attacking Water Guava in Gianyar Regency

Discussion

A high percentage of fruit fly infestation has a positive correlation with a high abundance of fruit flies. The abundance of fruit flies attacking water guava plants occurred due to rarely intensive pest control treatment. In addition, the fruiting season also causes an increase in the fruit fly population due to the high number of hosts. This agrees with Radonjić *et al*. (2013) which stated that the abundance of fruit flies population is closely related to the abundance of host plants in the field. Studies also showed that fruit fly infestations also increase as the fruits ripen due to changes in skin texture and chlorophyll fluorescence when ripe (Grechi *et al*., 2021). The percentage of attacks from fruit flies is also influenced by control techniques, namely traps, sanitation, and pesticides (Vargas *et al*., 2015). The guava as a home plant is rarely cultivated widely and is only planted around the house yard. This causes low intensive pest control treatment when compared to other cultivated plants such as citrus, chili, and watermelon. In addition, the prevention of fruit fly infestations also involves adjusting the harvest time, especially by harvesting the fruit at the half-ripe stage (Grechi *et al*., 2021).

The differences in the abundance of fruit flies that attack in each area in Gianyar Regency occurred due to several factors, namely biotic and abiotic (Choudhary *et al.,* 2021; Supartha *et al*., 2022c). The biotic factors include life cycle, growth, development, survival, distribution, behavior and reproduction (Supartha *et al.,* 2020). However, the current study of insect ecology is focused on abiotic factors such as environmental temperature which impacts the rate of development, geographic range, risk of invasive alien species and changes in insect survival (Fand *et al*., 2014; Tonnang *et al.,* 2017). Changes in air temperature will have an impact on the abundance, range distribution and potential damage caused by fruit flies (Ahmed *et al*., 2019). Changes in air temperature not only affect the life cycle of insects, but may also change the speed of feeding, growth, the chemical composition of the host plant, the activity of insect pest biocontrol agents, etc. (Dillon *et al*., 2010; Kumar *et al.,* 2020).

The detection of parasitoid species is a strategic step for fruit fly control, especially for controlling the quality of its maintenance and evaluating the level of suppression of fruit flies after the mass release of parasitoids (Liang *et al*., 2015). The results of the investigation by Liang *et al*. (2015) also support the hypothesis that *F. arisanus* and *D. longicaudata* emerged from pupae obtained from tropical almonds (*Terminalia catappa*) infested with *B. dorsalis.* Recent investigations have also shown that *F. arisanus* is found in African mangoes and has a more aggressive ability to repel other parasitoids such as *F. caudatus* (Karlsson *et al.,* 2018). *D. longicaudata* was shown to efficiently kill medfly (*Ceratitis capitata*) in peaches and oranges (Suárez *et al.*, 2019). Three wasps in the genus *Opius* spp. were shown to act as potential control agents against oriental fruit flies, namely *O. longicaudatus*, *O. vandenboschi*, and *O. ophilus* (Holt, 2017).

The parasitoid population is influenced by the host plant and host population (Cuny *et al.,* 2019; Supartha *et al*., 2022b). In this study, the parasitization level of water guava plants were high, this may be due to the high abundance of fruit flies as parasitoid hosts. The parasitoids *F. arisanus* and *D. longicaudata* were able to coexist and attack different developmental stages of *B. dorsalis* in the same landscape (Yang *et al*., 2018). Therefore, it is possible that interspecific competition related to "multiparasitism" occurred where these two parasitoids were able to coexist (Vargas *et al*., 2013; Miranda *et al*., 2015; Gao and Reitz, 2017). This provides an advantage for the parasitoids to carry out high parasitization. In addition, the absence of synthetic chemical pesticide intervention on host plants is also a factor that enables the parasitoids to survive better.

The fruit fly species associated with water guava in the Gianyar Regency were *Bactrocera albistrigata*, *Bactrocera carambolae*, and *Bactrocera dorsalis*. The highest percentage of fruit fly attacks on guava plantations was 85.00% in Gianyar District, while the lowest was in Tampaksiring District of 82.78%. The highest fruit flies population was dominated by *B. albistrigata* with an average value of 353.4, followed by *B. carambolae* with 349.8 individuals and *B. dorsalis* with 174.8 individuals. Three species of parasitoids associated with fruit flies were observed, namely *Fopius arisanus, Diacasmimorpha longicaudata* and *Opius* sp. The parasitization level of *Fopius arisanus* species was very high in all locations with an average value of 14.28%. Further research is recommended for a broader fruit fly study, which is to be carried out throughout Bali Province. The expansion of sampling throughout Bali will reveal other species that have the potential to attack water guava fruit. Furthermore, unknown parasitoid species will then be identified by molecular biology using the DNA Barcoding approach.

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