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Effective Behavior of Insects Pollinators of Flowers in Gadung Mango Clone 21 Variety

(Perilaku Efektif Serangga Penyerbuk Bunga Mangga Gadung Varietas Clone 21)

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ABSTRACT

Pollinating insects are referred as support services, namely services by the processes in the ecosystems that support human well-being by maintaining or enhancing ecosystem services. The availability of flowers, diversity, and behavior of visiting pollinator insects affect the effectiveness of pollination to increase the formation of fruit sets of mango plants. The purpose of this study is to observe the behavior of pollinator insects visiting the mango gadung clone 21 varieties and their effectiveness in increasing the formation of fruit sets. Research has been carried out on two flower seasons: March-May (Off season) and July-September (On season) 2020. Visiting behaviors observed were the number of visits per minute (foraging rate) and the length of visit per flower (flower handling time). Pollination effectiveness was measured from the number of fruit sets formed from the open flower panicles and confined with a tangerine gauze. The results of the study obtained seven species including Apis sp., *Trigona* sp., *Xylocopa* sp., *Polistes* sp. 1, *Polistes* sp. 2, *Chrysomya* sp., and *Eristalis* sp. Visits of pollinating insects on the highest number of mango panicle flowers were (31.69 ± 7.69) flowers / 60 seconds by *Trigona* sp insects, and the lowest numbers were (2.70 ± 0.67) flowers / 60 seconds by *Eristalis* sp. insects. The longest visits of pollinator insects on mango flowers were obtained by *Eristalis* sp for (25.3 ± 8.50) sec/individual/flower and the shortest visits were by *Trigona* sp for (1.8 ± 0.63) seconds/individual/flower. Insect pollination increases fruit formation by 267.5%.

Keywords: insect visits, pollinating insects, fruit formation, gadung 21 varieties mango

ABSTRAK

Serangga penyerbuk dikenal sebagai penyedia jasa ekologis, yaitu jasa yang mempertahankan atau meningkatkan layanan ekosistem dalam prosesnya untuk mendukung kesejahteraan manusia. Ketersediaan bunga, keragaman, dan perilaku serangga penyerbuk yang berkunjung memengaruhi efektivitas penyerbukan untuk meningkatkan pembentukan set buah tanaman mangga. Tujuan penelitian ini adalah mengamati perilaku kunjungan serangga penyerbuk pada mangga gadung varietas klon 21 dan efektivitasnya dalam meningkatkan pembentukan buah. Penelitian telah dilakukan pada dua musim berbunga, yaitu Maret-Mei (*Off season*) dan Juli-September (*On season*) 2020. Perilaku berkunjung yang diamati adalah jumlah kunjungan per menit (*foraging rate*) dan lama kunjungan per bunga (*flower handling time*). Efektivitas penyerbukan diukur dari jumlah set buah yang terbentuk dari malai bunga terbuka dan ditutup dengan kain kasa. Hasil penelitian memperoleh tujuh spesies serangga penyerbuk di antaranya *Apis* sp., *Trigona* sp., *Xylocopa* sp., *Polistes* sp. 1, *Polis* sp. 2, *Chrysomya* sp., dan *Eristalis* sp. Kunjungan serangga penyerbuk tertinggi pada bunga malai mangga mencapai (31,69 ± 7,69) bunga/60 detik oleh serangga *Trigona* sp, dan kunjungan terendah hanya (2,70 ± 0,67) bunga/60 detik oleh serangga *Eristalis* sp. Hasil lama kunjungan serangga penyerbuk pada bunga mangga diperoleh paling lama oleh serangga *Eristalis* sp selama (25,3 ± 8,50) detik/individu bunga dan terpendek oleh *Trigona* sp selama (1,8 ± 0,63) detik/individu bunga. Penyerbukan oleh serangga menunjukkan peningkatan pembentukan buah sebesar 267,5%.

Kata kunci: kunjungan serangga, serangga polinator, pembentukan buah, mangga gadung klon 21

INTRODUCTION

Food and fiber production, plant-derived pharmaceuticals, ornamentals and other aesthetics, genetic diversity, and general ecosystem resilience are just some of the benefits that pollination brings to the ecosystem service (Bauer & Wing 2010; Khalifa *et al.* 2021). Pollination of insects is an important process of the ecosystem to increase agricultural yields and increase production by up to 71% (Bartomeus *et al.* 2014; Efendi & Rezki 2020; Hanley *et al.* 2015). Nearly 75% of pollinating insects play a role in the formation of seeds and fruit seats, especially in horticulture plants (Vasiliev & Greenwood 2020). In Asia, research about pollinating insects on horticultural plants (fruits and flowers) has been reported, including 43 pollinating insects from the Orders *Hymenoptera*, *Diptera*, and *Lepidoptera*. Pollinating insects are dominated by *Apis*

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mellifera (Hymenoptera: Apidae) followed by *Eristalis cerealis* (Diptera: Syrphidae), *Tetralonia nipponensis* (Hymenoptera: Apidae), *Xylocopa appendiculata* (Hymenoptera: Apidae), *Eristalis tenax* (Diptera: Syrphidae), *Helophilus virgatus* (Diptera: Syrphidae), and *Artogeia rapae* (Lepidoptera: Pieridae) (Funamoto 2019; Holloway 1976; Kato *et al.* 2008). It has been shown by a number of researchers that the mango is primarily an anemophilous plant. However, in terms of its morphology or physiology, the mango does not display any modifications that are tailored for wind pollination. Because there is only one anther, it only generates a very low number of pollen grains (about 200 or 300), and the stigma is quite tiny so it may better aid in capturing the pollen grains. Mango trees are subject to entomophilous pollination, which may be deduced from the fact that they produce nectar for the purpose of attracting insects (Kumar *et al.* 2016). Insects such as flies, wasps, bees, butterflies, moths, beetles, ants, and different bugs visit the inflorescence of mango (*Mangifera indica* L.) owing to the vast quantities of nectar and pollen, which play a key role in boosting fruit set (Vishwakarma & Singh 2017). The Diptera include the syrphids *Episyrphus balteatus*, *Melanostoma orientale*, *Syrphus corollae*, and *Eristalis tenax*. *Apis dorsata*, *A. mellifera*, and *A. cerana indica* of the Hymenoptera are also seen, with *A. mellifera* representing for 28.03 percent of the overall 40.95% insect pollinators visits on mango flowers (Usha *et al.* 2014). Research information on the behavior of feed seeker pollinators on radish (*Raphanus sativus L.*) plants has also been reported, including the maximum rate of the visit of *Episyrphus balteatus* as much as 0.73 / individual / flower / 60 seconds followed by *Andrena* sp. as much as 0.53 / individual / flower / 60 seconds. The maximum time recorded by *Eristalinus laetus* is 41.76 seconds / flower and by *E. aeneus* is 39.64 seconds / flower (Sung *et al.* 2006). The study of the intensity of the main pollinators of mango flowers shows that the intensity of the stingless bee, *Tetragonula* sp. is high (11.50 / panicle / hour) followed by honeybees, *Apis indica Fab.* (6.40 / panicle / hour) and blow fly, *Chrysomya* sp. (5.85 / panicle / hour) during the inflorescence period and the pollinator peak intensity is 4 to 6 weeks (Klein *et al.* 2007). Information on the study of insect pollinator behavior on Gadung 21 mango has never been done. In this research, a study of the behavior of pollinator insects was carried out in the formation of fruit sets.

MATERIAL AND METHODS

This research was conducted at the farmer's Mango Garden in Watu Lunyu Village, Oro oro Ombo Kulon, Rembang District, Pasuruan Regency, East Java with a topography of 60 meters above sea level at 7° 38' 44.92" LS 112° 45' 49.51" BT. The Mango Garden has Gromosol soil type, and the average temperature is 26–32°C with a minimum humidity of 80–88%. The area of the Mango Garden of Gadung 21 variety is seven hectares.

Village of Oro oro Ombo Kulon and Oro oro Ombo Wetan, Rembang Subdistrict, Pasuruan Regency is a special area of the mango plant with the type of mango Gadung Clone 21 variety. This research was conducted twice, namely, outside the mango inflorescence season in March to May 2020 by giving Paklobutrazol Growth Regulating Substance in December 2019 and mango inflorescence season in July–September 2020.

Insect Behavior

The method used in this study was a sampling scan (Altmann 1984). Visiting behaviors observed were the number of visits per minute (foraging rate) and the length of visits per flower (flower handling time). Behavioral observations were carried out on ten open mango plants that were used in the insect diversity study of mango flower visitors. Observations were carried out for 29 days (mango inflorescence period) in each inflorescence season, starting in March–April 2020 and July–August 2020. Observation of insect behavior on mango flowers was also done by recording using ~~a~~ camera audio-visual for 10-20 minutes (effective time of insects). Observation of the pollination insect behavior in mango plantations is terminated after the inflorescence phase begins to form a fruit set.

Formation of Fruit Set

Research on the formation of fruit sets was carried out by covering ten mango panicles. This treatment was done to prevent insects from visiting flowers. The other ten flower panicles were left open (as a control), so the pollinating insects could still visit. The number of male flowers, female flowers, and fruit formed from one panicle per plant was calculated. This pollination's success is measured by comparing the percentage of fruit formed (%).

Data Analysis

Analysis of data of visit behavior of seven pollinator insect species on mango flowers was displayed in table and box plots and analyzed using the analysis of variance (ANOVA) followed by LSD test level $\alpha = 0.05$.

RESULTS AND DISCUSSION

Mango Flower Phenology

The results of the observation showed that the whitish mango flower had a strong scent, produced pollen and nectar. Mango flowers were found in one panicle and were compound flowers where there were male flowers predominantly from hermaphrodite flowers (perfect flowers). Flowers start budding until the initial opening takes 7–10 days. In general, the mango flowers are open not simultaneously in either one tree or one panicle. The total time of open flowers as a whole requires 10–30 days, and at one panicle flower

requires 6–29 days. Perfect flowers (hermaphrodites) have pistils and stamens that are sterile and fertile in one flower, while male flowers only have five stems of stamens (Figure 1). The characteristics of the flower's morphology are in accordance with the description of the superior variety of gadung mango 21 as a result of research conducted by Prasetyono *et al.* (2016).

Figure 1 Mango Flower Morphology, Male (A); Hermaphrodite (B).

Length of Insect Visitation per flower

The results of the study found seven species of insect visitors to the mango flower, namely *Trigona* sp., *Apis* sp., *Xylocopa* sp., *Polistes* sp. (2 species), *Chrysomya* sp., and *Eristalis* sp (Figure 2). Some of these insect species are active pollinators of horticultural plants (fruits and vegetables) (Choi & Jung, 2015; Fajardo *et al.* 2008; Klein *et al.* 2007; Sung *et al.* 2006; Tej, 2017; Usama Zameer *et al.* 2017). Some social and solitary bee species, namely *Trigona*, *Apis,* and *Xylocopa*, are commonly found visiting mango flowers and being the main pollinators (Dag & Gazit 2000; Fajardo *et al.* 2008; Gogoi *et al.* 2018; Sung *et al.* 2006). The length of visitation of seven species of pollinating insects on mango plant flowers varies (Figure 3). The longest visit of pollinator insects was carried out by *Eristalis* sp. (25.93 ± 7.57 seconds/flower) followed by *Polistes* sp. (15.75 ± 1.36 seconds/flower), *Chrysomya* sp (9.91 ± 1.89 seconds/flower), *Polistes* sp. (7.75 ± 1, 01 seconds/flower), *Apis* sp. (3.21 ± 0.30 seconds/flower), *Xylocopa* sp. (2.39 ± 0.15 seconds/flower), and abbreviated as *Trigona* sp. (1.76 ± 0.23 seconds/flower) (Table 1). Based on the results of the analysis of the length of insect visit to mango flowers per second in mango plants, it was shown that there were a very different lengths of visits between insects

Figure 2 Observations of pollinating insect visits on the mango flowers during on season and off season. (A) *Apis* sp. (B) *Trigona* sp. (C) *Xylocopa* sp. (D) *Polistes* sp.1. E) *Polistes* sp.2. (F) *Eristalis* sp. (G) *Chrysomya* sp.

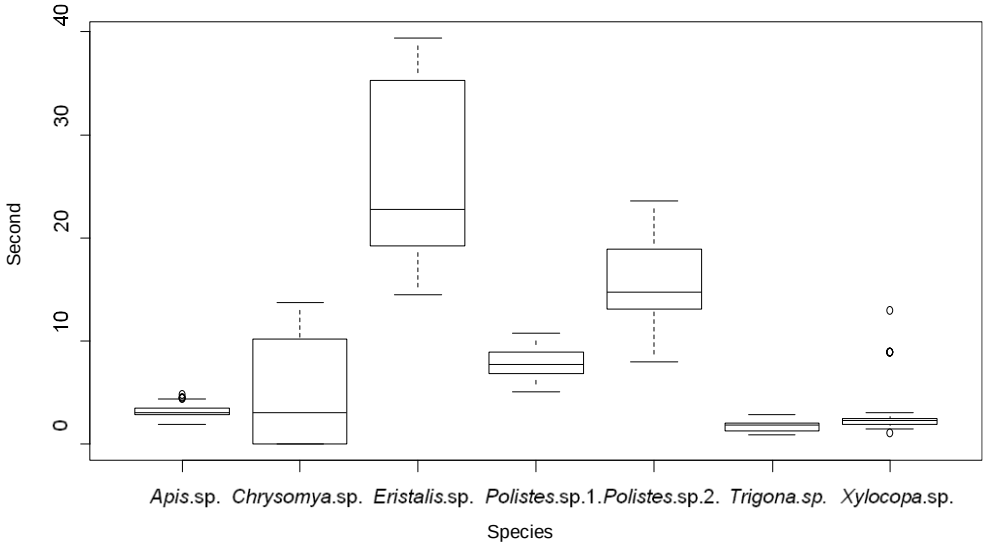

Figure 3 Length of insect species visit per mango flower Box Plot.

Table 1 Length of insect species visit per mango flower

Family	Sub Family	Species	Number of visits/minute ± standard deviation		
			Off season	On season	Average
Apidae	Apininae	*Apis* sp.	3.12 ± 0.43	3.30 ± 0.16	3.21a ± 0.30
		Trigona sp.	1.64 ± 0.27	1.88 ± 0.19	1.76a ± 0.23
	Xylocopinae	*Xylocopa* sp.	2.31 ± 0.12	2.48 ± 0.18	2.39a ± 0.15
Vespidae		*Polistes* sp1.	8.04 ± 1.08	7.47 ± 0.95	7.75a ±1.01
		Polistes sp2.	13.30 ± 0.38	18.20 ± 2.34	15.75b ±1.36
Calliphoridae		*Chrysomya* sp.	9.91 ± 1.89	-	9.91a ± 1.89
Syrphidae		*Eristalis* sp.	25.24 ± 8.49	26.63 ± 6.65	25.93c ± 7.57

Description: The same letter in the same column shows no different from the 95% Anova level test followed by the LSD test.

(F6.1673 = 5.789; P ≤ 2 x 10–16)***. Many *Polistes* species are found visiting plant flowers in search of feed for their needs. Tabuhan (Tawon) insects are mostly predatory species in fruit pest insects (Khan *et al.* 2018), but some also play a role in the process of pollinating flowers when sucking on nectar. *Polystes* species are found to help pollinate fruit group plants (Choi & Jung 2015). Insect groups from the family *Callphoridae* and *Shyrphidae* are generalist pollinators and have an important role in the process of pollinating the second flowering plant after *Hymenoptera* (Ssymank *et al.* 2008).

Number of Insect Visits per Minute

The visits of seven species of pollinating insects on mango panicles were varies (Figure 4). The highest visit of pollinator insects was carried out by Trigona sp. [(30.29 ± 4.11) flowers/60 seconds] followed by *Xylocopa* sp. [(21.47 ± 1.31) flowers/60 seconds], *Apis* sp. [(19.45 ± 2.32) flowers/60 seconds], *Chrysomya* sp [(6.25 ± 1.50) flowers/60 seconds], *Polistes* sp.1 [(5.86 ± 0.52) flowers/60 seconds], *Polistes* sp. 2 [(2.84 ± 0.45) flower/60 seconds], and the lowest visit was carried out by *Eristalis* sp. [(2.61 ± 0.44) flower / 60 seconds] (Table 2). Based on the results of the analysis of the number of insect visits on mango flowers per minute in mango plants, it shows that insects have a number of visits that are very different from each other (F6.1673 = 2.491; P≤ 2x10–16)***. In general, the visitation behavior of seven species of insects on mango flowers can increase the effectiveness of insects as pollinators. Insect behavior can be measured by the number of insect visits per minute to get flowers and the length of insect visits per flower (Sjödin 2007; Zhang *et al.* 2019). Another indicator found a collection of stamens that attach to the insect tibia. Bee-type insects collect pollen, by combing it with the limbs, and collecting it into the corbicula / pollen-basket located on the outside of the limb tibia (Grüter & Ratnieks 2011; Matsuki *et al.* 2008; Saunders 2018). Unlike the insect type of fly that does not have a corbicula structure so that the pollen sticks to the hairs on all or part of the body. *Eristalis tenax* flies collect pollen in most of their its hairy body, thorax, and abdomen including at the front of the eye, tarsi, and tibia part of the legs (Holloway 1976; Howlett & Gee 2019; Wacht *et al.* 2000).

Visiting behavior of seven pollinator insect species has different characteristics. Insect visiting behavior in flowers is reversed, if the duration of the visit is short, the number of flowers is more. And vice versa if the flower visit takes a long time, then the flowers are infested less. This can be seen in the box plot (Figures 5, 6, and 7). The results of the research were the fastest visit behavior and the highest number of flowers obtained from *Trigona* sp. i,e., (1.76 ± 0.23)

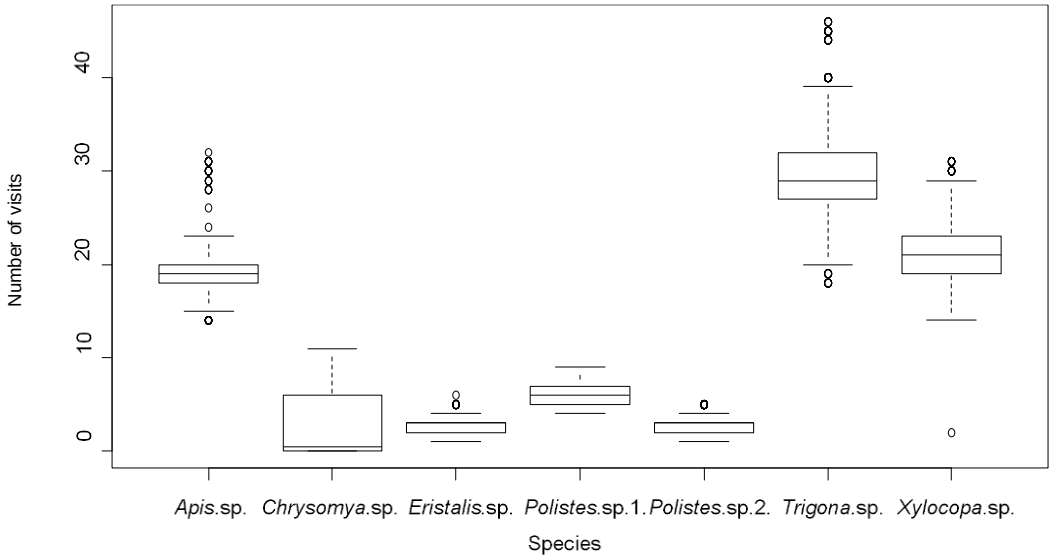

Figure 4 The number of visits per minute of seven insect species on the mango panicle flower Box plot

Table 2 The number of visits per minute of seven insect species on the mango panicle flower

Family	Sub Family	Species	Number of visits/minute ± standard deviation		
			Off Season	On Season	Average
Apidae	Apininae	*Apis* sp.	20.55 ± 3.69	18.36 ± 0.94	19.45^{c} ± 2.32
		Trigona sp.	31.41 ± 7.37	29.18 ± 0.85	30.29^{e} ± 4.11
	Xylocopinae	*Xylocopa* sp.	21.07 ± 1.73	21.86 ± 0.89	21.47^{d} ± 1.31
Vespidae		*Polistes* sp1.	6.25 ± 0.78	5.47± 0.26	5.86^{b} ± 0.52
		Polistes sp2.	3.38 ± 0.73	2.29 ± 0.16	2.84^{a} ± 0.45
Calliphoridae		*Chrysomya* sp.	6.25 ± 1.50	-	6.25^{b} ± 1.50
Syrphidae		*Eristalis* sp.	2.87 ± 0.63	2,34 ± 0,25	2,61^{a} ± 0,44

Description:The same letter in the same column shows no different from the 95% Anova level test followed by the LSD test.

seconds/flower to obtain (30.29 ± 4.11) flower/60 seconds. Conversely, the longest activity of insect behavior and the minimal number of flowers visited is *Eristalis* sp. i.e., (25.93 ± 7.57) seconds/flower and earn (2.61 ± 0.44) flower/60 seconds. Optimal foraging theory is that insects collect food as much as possible by utilizing minimal energy and time (Hall *et al.* 2022; Wäckers *et al.* 2007).

Insects search activities on vegetable flowers and fruit flowers have also been reported. Stingless bee insect species *Tetragonula iridipennis* extracts nectar for (7.8 ± 1.6)/flower/second and pollen for (3.9 ± 0.5)/flower/second in cucumber flowers (Tej 2017). *A. florea* 0.47/individual/flower/60 seconds and *Apis dorsata* 0.33/individual/flower/60 seconds in *Raphanus sativus* L. flowers. *Eristalinus aeneus* (2.1 ± 1.4)/flower/60 seconds, *Chrysomya albiceps* (7 ± 50)/flower/60 seconds, and *Apis mellifera* (8.9 ± 3.3)/flower/60 seconds in mango flowers (Dag & Gazit 2000). In the panicle, the insecticidal mango flower from the Calliphoridae fly group can pollinate as many as 37 flowers (Saeed *et al.* 2016).

Formation of Fruit set of Mango Fruit

Pollinating insect diversity has a positive effect on the fruit set. Twelve pollinating insect species that visit mango flowers (order *Hymenoptera*, *Diptera,* and *Lepidoptera*), only seven species are effective as pollinating insects included in the two orders, *Hymenoptera* and *Diptera*. These flower pollinators are included in four families, from 72620 individuals. The seven species of insects are *Apis* sp., *Trigona* sp., *Xylocopa* sp., *Polistes* sp. 1, *Polistes* sp. 2, *Eristalis* sp., and *Chrysomya* sp.

The presence of insect visitor of flowers on mango plants in this study provides indirect benefits to the increase in mango production. The mango tree flower is a plant whose cross pollination is aided by insects (Kumar *et al.* 2016). Different flowering times between male and female flowers, in each panicle, and the position of male and female flowers causes insects to play a role in cross pollination (Usman *et al.* 2001). Cross pollination has contributed to a large increase in the mango set (Nurul Huda *et al.* 2015). The process of cross pollination is usually aided by honey-sucking insects and bees, which try to suck honey from the flower (Usman *et al.* 2001).

The results showed that the number of fruit sets per panicle of mango flowers that were open was higher than the yield of the panicle flower treated with hood. In open flower panicles, there is an increase in the number of fruit formation by 265% (excluding the flower season) and 270% (flower season), the average percentage is 267.5% (Table 3). In general, plants need pollination by insects, and 75% of plants pollinations depend on insects. About 20% of plant pollination by insects is needed to produce quality and

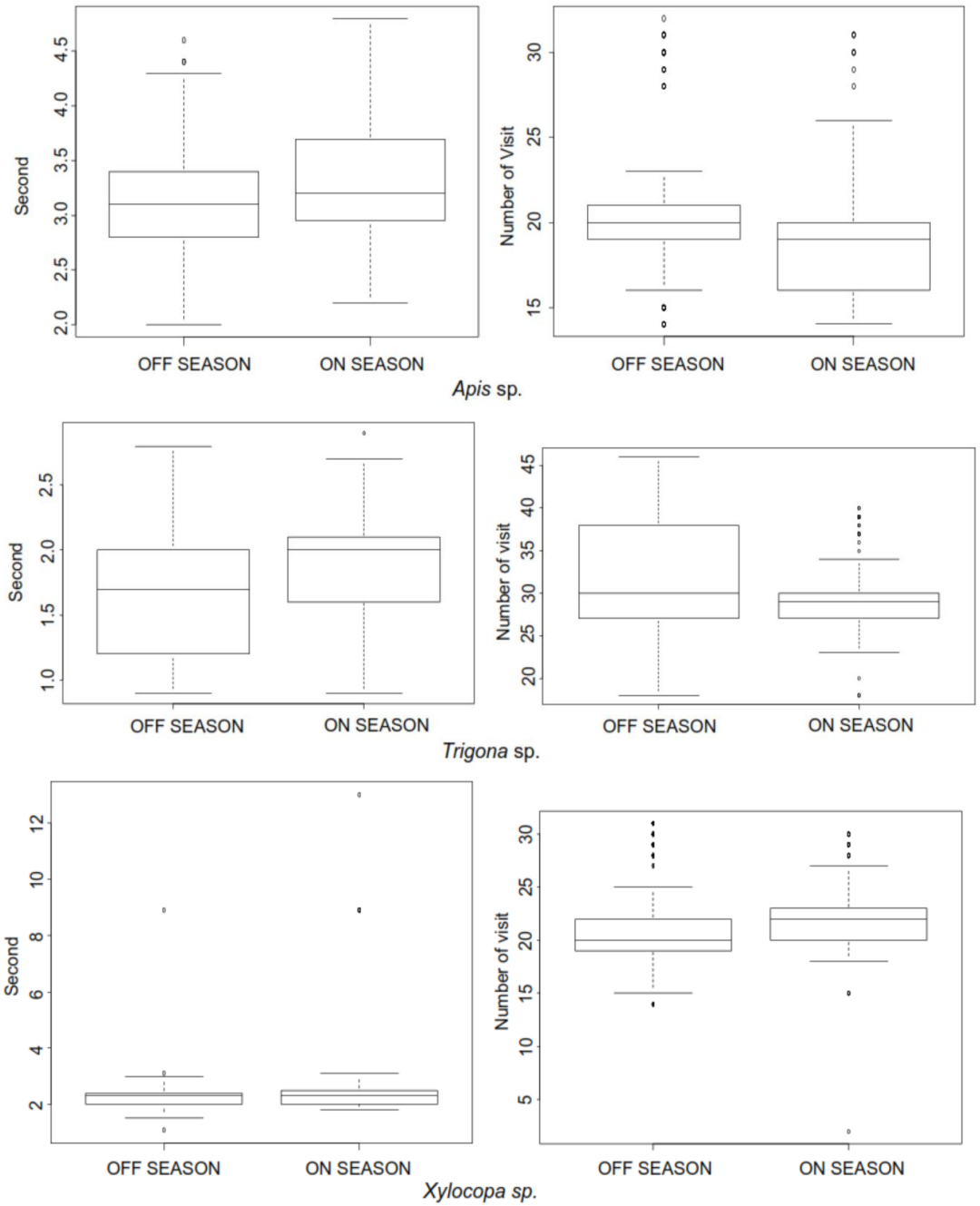

Figure 5 Duration (Left) and number (Right) of visits of *Apis* sp., *Trigona* sp. and *Xylocopa* sp. on mango flowers in the Off season and On season.

amount of fruit and 15% to produce seeds (Klein *et al.* 2007).

A number of insect species are reported to contribute to the increasing fruiting to obtain maximum production. *Apis mellifera* and *Trigona spinipes* species that visit cashew flowers can increase seed production by 692.4 kg/ha (Freitas *et al.* 2014). The main pollinators are stingless bee mango species (*Trigona biroi*), blow flies (*Chrysomya* spp.), hoverflies (*Eristalis* spp.), and honeybees (*A. cerana* and *A. mellifera*) increase fruit set by 41% in open flower panicles and 0.7% in flowers without insect pollinators (closed) (Fajardo *et al.* 2008). *Chrysomya* spp. and *Eristalis* spp. are the effective pollinators on the varieties of mango Sala and Chok Anan flowers that can contribute fruit set by 53% (Nurul Huda *et al.* 2015).

CONCLUSION

This study observed the behavior of pollinating insects on the Gadung 21 mango by analyzing the activity of pollinating insects during fruit set formation. Seven different pollination insect species have been seen to spend varying amounts of time on the flowers

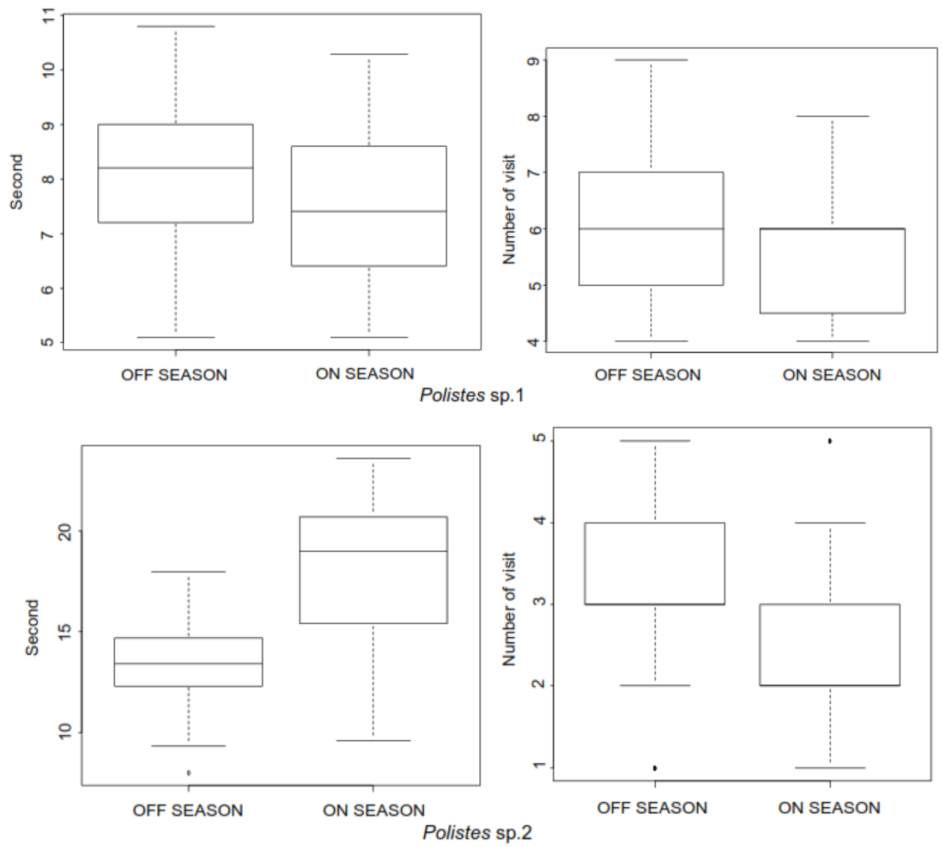

Figure 6 Duration (Left) and number (Right) of visits of *Polistes* sp.1 and *Polistes* sp.2 on mango flowers in the Off season and On season.

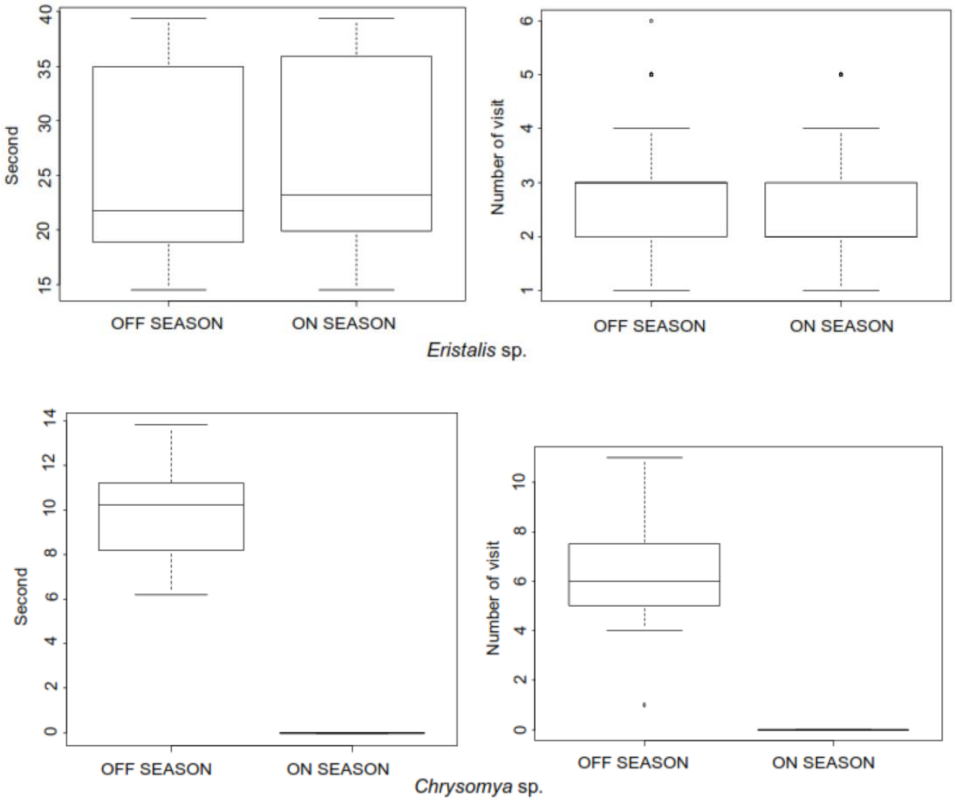

Figure 7 Duration (Left) and number (Right) of visits of *Eristalis* sp. and *Chrysomya* sp. on mango flowers in the Off season and On season.

Table 3 Fruit set formation by treatment of mango panicles

Reproduction component of mangoes	Open flower panicle			Covered flower panicle		
	Off Season	*On Season*	*Average*	*Off Season*	*On Season*	*Average*
Male Flowers	3469,95±245,2	1689,7±238,3	2579,83±123,13	3341,55±340,03	1117,7±229,57	2229,63±284,8
Perfect flower (Hermaphrodite)	1156,5±81,8	328,2±42,31	742,1±62,055	1113,85±29,19	225±44,44	669,43±36,82
Fruit set formation	715,8±81,7	224,4±30,22	470,1±55,96	269,85±113,34	83±26,75	176,43±70,05

mango plants. *Eristalis* sp. made the longest of mango plants. *Eristalis* sp. made the longest pollinator visit, spending around 26 seconds per flower whereas *Trigona* sp. only stayed for around 1.7 seconds. Seven different kinds of pollinator insects have different ways that they visit plants. The way insects act when they visit flowers is backwards. If they stay for a short time, they visit more flowers. Also, if the visit to the flower takes a long time, the flowers are less likely to be infested. The results of the study showed that *Trigona* sp. had the fastest visit behavior and the most flowers. It took 1.76 seconds per flower to get 30 flowers per minute. *Eristalis* sp. takes 26 seconds per flower and earns 2.6 flowers per minute. This is the longest insect behavior and the fewest flowers it visits. The presence of a wide variety of pollinating insects has a beneficial impact on fruit set. Although there are twelve species of pollinating insects that visit mango flowers (orders Hymenoptera, Diptera, and Lepidoptera), only seven of those species are successful as pollinating insects. These seven species are split between the two orders Hymenoptera and Diptera. *Apis* sp., *Trigona* sp., *Xylocopa* sp., *Polistes* sp. 1, *Polistes* sp. 2, *Eristalis* sp., and *Chrysomya* sp. are the seven species of insects that have been studied in this research.

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