Australian Journal of Crop Science
AJCS
AJCS 16(09):1078-1083 (2022) ISSN:1835-2707
doi: 10.21475/ajcs.22.16.09.p3562

Effectiveness of Pollinator Insects in Increasing Fruit Formation of Pummelo (*Citrus maxima* (Burm.) Merr.)

Tri Atmowidi[1*], Muchamad Nur Cholis[2], Arif Maulana[2], Windra Priawandiputra[1], Sih Kahono[3]

[1]Department of Biology, Faculty of Mathematics and Natural Sciences, Bogor Agricultural University (IPB University), Bogor, Indonesia
[2]Student of Animal Biosciences Study Program, Graduate School, Bogor Agricultural University (IPB University), Bogor, Indonesia
[3]Research Center for Ecology and Ethnobiology, The National Research and Innovation Agency (BRIN), Bogor, Indonesia

***Corresponding author: atmowidi@apps.ipb.ac.id**

Abstract

Pummelo (*Citrus maxima* (Burm.) Merr.) is a type of plant which have commercial value, and at least 24 cultivars are spread across various regions in Indonesia. Some cultivars have self-incompatibility (SI) mechanisms as genetic barriers to fertilization, but this can be reduced through cross-pollination by insects. Therefore, this study aims to measure the effectiveness of wild pollinator insects, as well as human and bee pollinations in pummelo. A total of six treatments were set up, namely closed pollination or control, open treatment, human pollination using pollen from the same tree (geitonogamy) or different trees (xenogamy), as well as supplementation colony of honey bee (*Apis cerana*), and stingless bee (*Tetragonula laeviceps*). The highest increase of pummelo fruit formation was found in human-pollination (xenogamy), followed by *A. cerana*, *T. laeviceps*, open treatment, and geitonogamy pollination with 63%, 54%, 48%, 41%, and 14%, respectively. Based on the results, bees (*A. cerana*, *T. laeviceps*, *Ceratina* sp., *Xylocopa confusa*, *X. latipes)*, fly (syrphid species), as well as butterflies (*Papilio demoleus*, *Catopsilia pyranthe*, and *C. pomona*) are potential pollinating agents of pummelo.

Keywords: Butterflies, fruit formation, honey bees, stingless bee.
Abbreviations: IPB_Institut Pertanian Bogor; SI_self-incompatibility;

Introduction

The pummelo (*Citrus maxima* (Burm.) Merr.) is a cultivated citrus species with a high economic value and has numerous cultivars scattered throughout Indonesia (Susanto et al., 2013). It also has a compound flower and one inflorescence consists of 8-13 flowers with radial symmetry, consists of 5-6 sepals, five petals, one pistil, 26 stamens, a strong floral scent, as well as a bright color that attracts insects (Cholis et al., 2020). The flowering depends on water irrigation, drought stress, and environmental temperature (Iglesias et al., 2007). In Indonesia, pummelo plantations are traditionally managed and their flowering depends on water irrigation. These conditions prevent flowering throughout the year, except for trees that grow close to water sources. Meanwhile, the peak usually occurs in October or November at the beginning of the rainy season (Cholis et al., 2020). Pummelo has a perfect flower, having both sexes (Susanto et al., 2013) and self-pollination can occur. However, most pummelo varieties have been self-incompatibility (SI).
Plant pollination and fertilization are not often optimal because of the constraint caused by the SI mechanism (de Nettancourt, 1977) which occurs in plants with low genetic variation (Busch and Schoen, 2008) and is controlled by a related gene known as *"S-locus"* (de Nettancourt, 1977). The rate of occurrence varies in each citrus variety (Paudyal and Haq, 2007). Furthermore, the existence of the SI mechanism might disrupt fruit production in the absence of cross-pollination assistance (Wright and Barrett, 2010).
Insects contribute to pollination in agricultural land (Klein et al., 2007), meanwhile, successful pollination can be achieved when the pollen is received by the receptive stigma (Dafni, 1992). The ability of pollinators to escalate pollination success in agricultural systems is influenced by abundance, activity patterns, visitation rate, per-visit efficiency, and interspecific influence (Rogers et al., 2013). In East Java, Indonesia, twelve species in the pummelo plantation belonging to three orders namely Hymenoptera, Diptera, and Lepidoptera were reported. The Hymenoptera had the highest percentage followed by Lepidoptera, and Diptera with 68.65%, 26.73%, and 4.61%, respectively (Cholis et al., 2020). A previous study also showed that honey bees (*A. cerana* and *A. mellifera*) increase fruit formation of pummelo cv. Huangsha Yu (Luo et al., 2019). However, information on the utilization of insects as a pollinator in pummelo is still limited in Indonesia. Therefore, this study

aims to measure the pollination effectiveness of insect pollinators on pummelo.

Results

Visiting activities of insects on flowers

Insects' frequency and visit duration on pummelo flowers varied among the different species. Wasp (*Vespa affinis*) showed the highest visit namely 68.71 flowers per 5 minutes with the shortest duration of 4.90 seconds per flower. Meanwhile, the lowest visit was observed in the syrphid fly namely 5.89 flowers per 5 minutes with the longest duration of 57.03 seconds per flower (Figure 1 and 2). Furthermore, ants *(Dolichoderus thoracicus* and *Oecophylla smaragdina)* only visited one pummelo tree for 5 minutes of observation (Figure 2), while carpenter bee (*Xylocopa latipes)* visited 5.06 trees with duration of 104.97 seconds per tree. Based on the results, the honey bee (*Apis cerana)* showed the greatest potential as a pollinator of pummelo with a high average visitation activity of more than three trees per 5 minutes with visit duration of 84.80 seconds per tree (Figure 2).

Pollination effectiveness

On average, xenogamy pollination produced 8.12 fruits, which was the highest percentage of normal fruits namely 78.44%, while the lowest of 1.80 fruits was from closed treatment with 13.33% normal fruits. Fields of pummelo with a supplemental hive of *A. cerana* produced large fruits up to 19.10 cm of longitudinal and 21.52 cm of transversal diameters. Similarly, fields with supplemental hives of *T. laeviceps* produced large fruits reaching 18.62 cm of longitudinal diameter. Furthermore, the fruit weight produced from open pollination did not differ significantly with xenogamy, the supplemental hive of *A. cerana*, and *T. laeviceps* (p=1.00, p=0.16, and p=1.00, respectively). The closed pollination produced the lowest fruit weight of 0.80 kg, while the highest increase of fruit formation occurred in xenogamy pollination with 63% (Table 1). The number of fruits produced in open and xenogamy pollinations, as well as the supplementation of *A. cerana* and *T. laeviceps* were higher than the closed and *geitonogamy* pollinations. The normal fruit of pummelo was characterized by a spheroid shape with a larger diameter in the middle, while abnormal fruits have an irregular shape. Normal fruit also exhibited larger weight and diameter, while ripe fruits were characterized by yellowish-green color (Figure 3a, b). Moreover, normal fruits had large segments, while the abnormal had small and overlapping segments (Figure 3c-f).

Discussion

Insect visiting activities on pummelo flowers

Various factors influence insect activities, such as environmental conditions and food sources (Faheem et al., 2004). Meanwhile, these activities affect the success of pollination and determine the level of contribution of a particular species (Vázquez et al., 2005). Wasp (*Vespa affinis)* visited flowers and trees the most frequently per unit time, but their body structure was less supportive of an effective pollinator. Ants (*D. thoracicus* and *O. smaragdina*) also have less potential as pollinators because they only visit a single tree for a short duration, hence, cross-pollination is unlikely to occur. However, ants have been found to help pollinate of *Turnera ulmifolia* (Cuautle and Rico-Gray, 2003). Two species of *Anoplolepis* and *Prenolepis* were also reported as a visitor to *Jatropha curcas* flowers (Rianti et al., 2010), while *D. thoracicus* and *O. smaragdina* visited pummelo flowers (Cholis et al., 2020).

Flies appear to have less potential as a pollinator because of the low visit frequency, although they were reported as a pollinator of *J. curcas* (Raju and Ezradanam, 2002). In Indonesia, *Syrphus balteatus* and *S. argyrocephala* were found to be a pollinator of mustard (Atmowidi et al., 2007), *Parasyrphus* sp. and *Syrphus* sp. in cucumber (Hasan et al., 2017), and syrphid species in pummelo (Cholis et al., 2020). Additionally, Siregar et al., (2016) observed six species of syrphids on different agricultural lands in Jambi, Sumatra.

The butterfly is a good pollinator due to the body scales which trap the pollens. The results showed that three butterfly species observed on pummelo, namely *Papilio demoleus, Catopsilia pyranthe,* and *C. pomona* visited more than three trees as well as 17 to 26 flowers per 5 minutes (Figure 1). A previous study reported that butterflies are general flowers visitor. Atmowidi et al., (2007) reported six species in mustard and *Nyctemera* sp. as dominant visitors among lepidopterans. Another study showed that butterflies visited several flower species, including three species in nutmeg (Rianti et al., 2010), two species in cucumber (Hasan et al., 2017), and three species in pummelo. In the agricultural lands of Jambi, Sumatra, Siregar et al., (2016) reported six species of butterfly.

The results indicated that bees are the most promising agents of pollination, in which the honey bee (*A. cerana*) was the most suitable pollinator based on body morphology and visiting activity. Observation showed that they visited more than two trees of the plant species per 5 minutes with varying durations. Atmowidi et al., (2008) also reported that pollination by *A. cerana* increased the number of nutmeg fruits, while Tylianakis et al., (2007) reported that bees are the most effective and important pollinator than other groups of insects. Workers of *A. cerana* start their foraging early in the morning with peak activity between 09.00 h and 13.00 h (Verma, 1995).

Another species, the stingless bee (*Tetragonula laeviceps*), also has high potency as a pollinator of pummelo. Dense hairs in the body of this species help to trap pollens. Honey and stingless bees have *corbicula* in the hind tibia as pollen collectors during foraging. Subsequently, two carpenter bees, namely *Xylocopa latipes* and *X. confuse*, also visited the pummelo flowers. Based on the frequency and visit duration, these species have a high potential as pollinators, but their large body size might damage the flowers. The flowering period of pummelo is relatively short and the flowers fall off easily, this implies that pollination must occur at the right time. Additionally, carpenter bees were also found to be pollinators of several plant species, such as mustard (Atmowidi et al., 2007), nutmeg (Rianti et al., 2010), tomato (Indraswari et al., 2016), cucumber (Hasan et al., 2017), and pummelo (Cholis et al., 2020).

Insect pollination effectiveness and fruit set

The xenogamy pollination produced the highest increase in fruit formation (63%), followed by the supplementation of *A. cerana* (54%), *T. laeviceps* (48%), as well as open (41%), and geitonogamy pollinations (14%). Although xenogamy pollination showed the highest fruit formation, this method requires more time, effort, and high cost. The pollination of *A. cerana* and *T. laeviceps* increased fruit set (Figure 3) and

Table 1. Pollination effectiveness of pollinator insects on pummelo: close-, open-, geitonogamy, xenogamy, *Apis cerana*, and *T. laeviceps* pollinations. Different superscript letters on the same row indicate significant differences among treatments (ANOVA-Tukey at 5% significance level).

Fruit parameters	Pollination treatments					
	Closed (control)	Opened	*Geitonogamy*	*Xenogamy*	*A. cerana*	*T. laeviceps*
Number of composite flowers (inflorescences)	25	25	25	25	25	25
Number of fruits produced	1.80^{e}	5.88^{c}	3.16^{d}	8.12^{a}	7.28^{ab}	6.60^{bc}
Percentage of normal fruits (%)	13.33^{d}	59.02^{c}	42.47^{d}	78.44^{a}	72.47^{b}	64.74^{bc}
Percentage of abnormal fruits (%)	66.67^{a}	$40.98b^{c}$	57.53^{ab}	21.78^{c}	27.53^{c}	35.26^{c}
Transversal diameter of fruits (cm)	18.05^{c}	21.11^{ab}	19.70^{b}	21.04^{ab}	21.52^{a}	21.20^{a}
Longitudinal diameter of fruits (cm)	15.16^{b}	18.32^{a}	15.77^{b}	17.87^{a}	19.10^{a}	18.62^{a}
Fruit weight (kg)	0.80^{c}	1.24^{a}	0.99^{b}	1.26^{a}	1.39^{a}	1.26^{a}
Percentage of increased fruit formation (%)	-	41.00	14.00	63.00	54.00	48.00

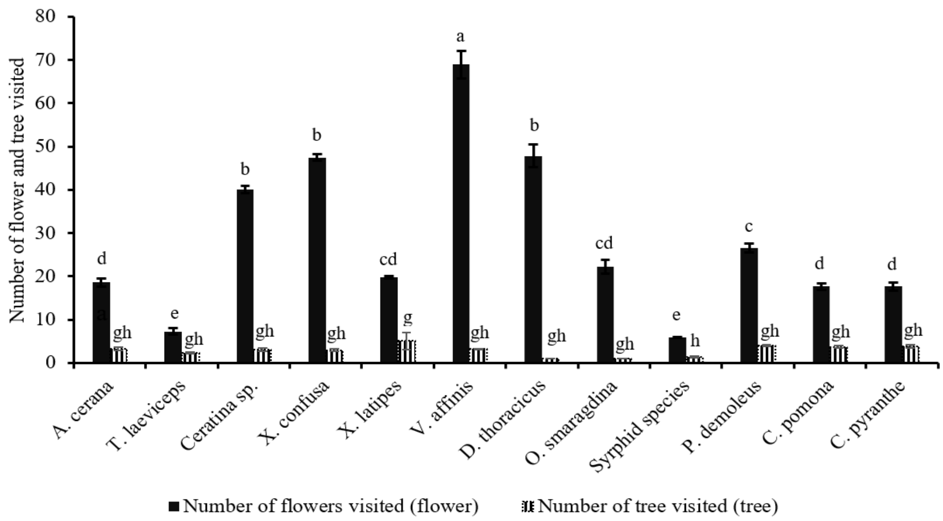

Figure 1. The number of flowers (black areas) and trees (pattern areas) of pummelo visited by insects. Different letters in each bar indicate significant differences among species (ANOVA-Tukey at 5% significance level). Standard errors are shown in the graphic.

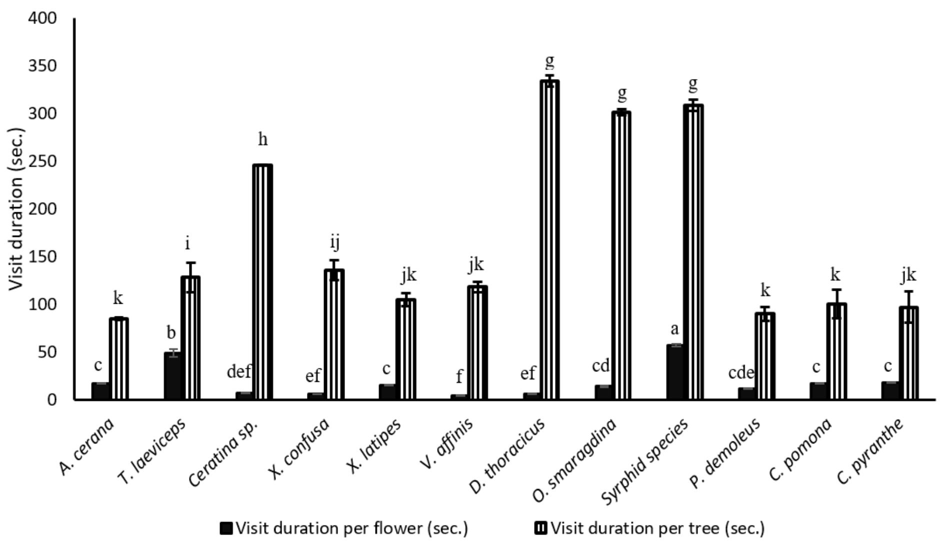

Figure 2. Visit duration of insects on flower (black areas) and tree (pattern areas) of pummelo. Different letters in each bar indicate significant differences among species (ANOVA-Tukey at 5% significance level). Standard errors are shown in the graphic.

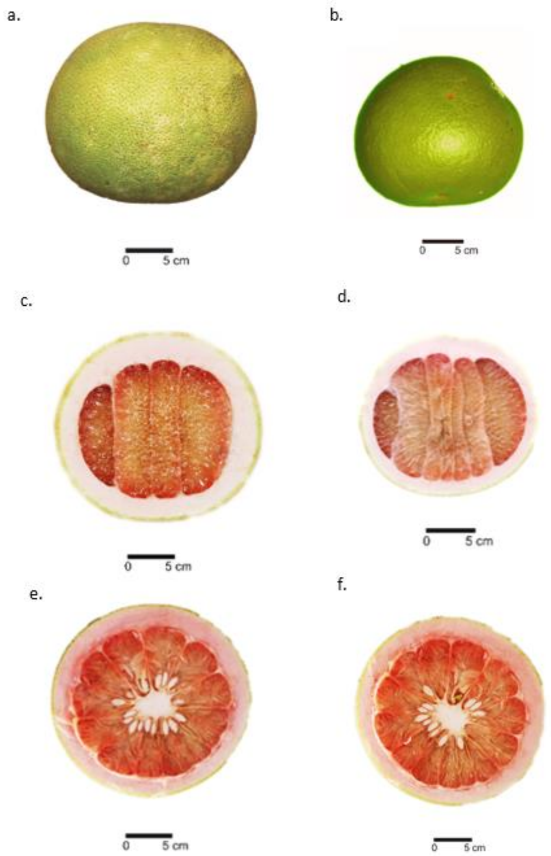

Figure 3. Pummelo fruits after six months of pollination: (a) normal fruit, (b) abnormal fruit, (c) normal fruit (longitudinal section), (d) abnormal fruit (longitudinal section), (e) normal fruit (transversal section), and (f) abnormal fruit (transversal section).

the two species were effective as pollinators of pummelo. *Apis cerana* was a superior pollinator due to its visiting activity and ability to reach all flowers in the inflorescence. In general, honey bee pollination is more reliable when the number of wild insects is insufficient and is important in agriculture because it is versatile, inexpensive, and effective (Southwick and Southwick, 1992; Roubik, 2002; Klein et al., 2007). The effectiveness of honey bees to pummelo pollination has previously been reported, for example, *A. mellifera* increased fruit formation of pummelo cv (Sujitratanunth 1992), while *A. cerana* and *A. mellifera* improved fruit formation compared to self-pollination (Luo et al. 2019).

In the open pollination, pummelo flowers were visited by wild insect species, such as carpenter bees (*X. confusa*, *X. latipes*), wasp (*V. affinis*), syrphid species, and butterflies (*P. demoleus*, *C. pyranthe*, and *C. pomona*). These species promote cross-pollination that allows the pollen transfer among trees. Azevedo and Pio (2002) also reported that cross-pollination produced citrus fruit set. The results showed that geitonogamy pollination increased the low percentage of fruit set (14%) but the self-incompatibility mechanism might occur. Therefore, cross-pollination did not occur in the caged plants and geitonogamy pollination.

Insects play an essential role in pollination and there is a positive correlation between the number of pollinators and fruit formation. Successful pollination induced some hormones in flower, such as gibberellin, cytokinin, auxin, and abscisic acid. The level of these hormones affects the development of fruits (Iglesias et al., 2007). Gibberellin is crucial in ovule development (Ben-Cheikh et al., 1997) and the concentration increases when flowers bloom. Meanwhile, when the flowers are not pollinated, abscisic acid concentration rises, causing a fall out of the ovary. Decreased gibberellins also caused the abscission of fruits (Talon et al., 1992).

Further study of the diversity and species richness of insect pollinators in the pummelo plantation are needed. When a deficit in pollinators occurs, cross-pollination is proportionally hampered. The depletion of pollinators in an area might be caused by pesticide application, environmental changes, land conversion, and the practice of monoculture systems (Dicks et al., 2016). Support for pollinating insects can be provided through polyculture systems, organic farming, and flower gardens (IPBES, 2016).

Materials and methods

Observation of insect activities on flowers

This study was conducted in pummelo plantation at Tambakmas Village, Sukomoro District, Magetan Regency, East Java, Indonesia, from October 2019 to June 2020. The Nambangan cultivar, which is native to Magetan (Balitbangtan, 2007) was used. The visiting activities of insects were observed using the focal sampling method (Martin and Bateson, 1986). Furthermore, the activities of 12 insect visitor species, previously reported by Cholis et al., (2020), namely *Apis cerana, Tetragonula laeviceps*, *Ceratina* sp., *Xylocopa confusa*, *X. latipes*, *Vespa affinis, Dolichoderus thoracicus*, *Oecophylla smaragdina*, syrphid species, *Catopsilia pyranthe*, *C. pomona*, and *Papilio demoleus* were observed. These activities include the number of flowers and trees visited, as well as the duration of visits (Dafni, 1992). Observations were conducted for 5 minutes at intervals of

55 minutes starting at 07:30 am to 05:30 pm (Wulandari et al., 2017) for 14 sunny days.

Measurement of pollination effectiveness

A total of 25 inflorescences in five pummelo trees were selected for each treatment, and in each inflorescence, ten individual flowers were selected. Subsequently, the selected flowers were caged by gauze to prevent access by pollinators. The effectiveness of pollination was accessed using six different treatments, namely closed (control), in which the inflorescence was caged using gauze (Vaissiere, 2011), open, which allows the natural pollinators to access the flowers, the use of pollen from the same tree (geitonogamy), the use of pollens from a different tree (xenogamy), as well as supplementation with one hive of honey bee (*Apis cerana*) and four hives of stingless bee (*Tetragonula laeviceps*). The pollination effectiveness was measured by the number of fruits produced after a month, while fruit diameter and weight were measured after six months of pollination. Moreover, the effectiveness of fruit formation among treatments was compared and the significance of differences was tested using ANOVA followed by Tukey test at 5% significance level using Paleontological Statistics (PAST) 3.20 (Hammer et al., 2001).

Conclusion

Pollination by the honey bee (*A. cerana*) and stingless bee (*T. laeviceps*) were as good as xenogamy. Their activities and body morphology support the effectiveness of these two species of bees. Based on the results, the natural insect pollinator of pummelo were carpenter bees, the syrphid fly, and butterflies. The highest increase in fruit formation occurred in xenogamy pollination (63%), followed by *A. cerana* (54%), *T. laeviceps* (48%), open (41%), and geitonogamy pollination (14%).

Acknowledgments

This study was funded by the Decentralization Scheme of Basic Research of Higher University (PDUPT- IPB University), Ministry of Research, Technology, and Higher Education, the Republic of Indonesia in 2021 for the corresponding author (1/E1/KP.PTNBH/2021, March 8, 2021).

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