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POLLINATION OF THE PASSION FRUIT *PASSIFLORA EDULIS* SIMS.F. *FLAVICARPA* DEGENER IN LUCBAN, QUEZON PHILIPPINES

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ABSTRACT

This study was conducted in Lucban, Quezon from July 2018-August 2019 to study the foraging behavior of the pollinators of passion fruit and their impact on crop yield. Passion fruit is a strictly cross-pollinated plant, but exhibits self-incompatibility. Among the flower visitors of passion fruit, the carpenter bees, *Xylocopa s*pp. were observed to be the primary pollinators, and their foraging activity synchronized with the opening of the flowers. The random flight pattern of carpenter bees and the distance of flight of more than 6 m between flowers facilitate pollination, avoiding flower visitation within the same plant. The number of foraging bees was minimal during early morning hours and gradually increased from 1100h to1400h with a peak at 1300h. The population gradually declined until 1700h. The time spent by the bee on the flower, or the handling time was highest at 1100h which coincided with the peak of anthesis. The hand self-pollinated flowers coming from the same plant and from the control did not bear fruit when the effect of pollination on fruit yield was evaluated. This indicates that passion fruit is self-incompatible and requires outcrossing with other conspecific plant. The fruit sets in hand cross-pollinated and open (carpenter bee) pollinated flowers were 75% and 35%, respectively. The low percentage fruit set in open pollination may be due to the low pollinator-plant ratio in the farm. To sustain and conserve pollinators in an agricultural landscape, planting of bee plants that flower throughout the year is highly recommended.

Key words: carpenter bee, foraging, Lucban, passion fruit, pollination

INTRODUCTION

The yellow passion fruit, *Passiflora edulis* Sims.f. *flavicarpa* is grown in the Philippines for industrial and fresh consumption. In Lucban, Quezon, the average annual yield ranged from 2.25MT/ha to 10.34MT/ha with a mean yield of 4.56MT/ha. (Espino & Espino, 2011) The fruit is known for its anti-oxidant properties, medicinal uses and as a rich source of nutrients, including vitamin A and C (Wong et al., 2014; Thokchom & Mandal, 2017). Passion fruit was originally cultivated by tropical countries such as Brazil, Ecuador and Peru (Silva et al., 2015). The yellow passion fruit was introduced from tropical America to Europe and Australia and was later cultivated in Southeast Asian Region including the Philippines due to its preference for warmer climates and lower elevations (Sharma et al., 2009). The reproduction of the yellow passion fruit is strongly influenced by pollinator activity because it is a protandrous, self-incompatible crop species (Silva et al., 2015). Additionally, because of its large flower, its reproduction relies on the services of large pollinators, mainly *Xylocopa* bees. The reproductive success of the yellow passion fruit relies on cross pollination. Its large flower with plentiful pollen and copious amount of nectar attracts pollinators. Ensuring a pollination of the flowers in cultivation is decisive for fruit formation and juice content. (Fischer et al., 2018). Gutierrez-Chacon et al. (2015) demonstrated the high dependency of passion fruit on wild pollinating insects and highlighted the crucial role of large insects in fruit production. As reported by Rodriguez & Cervancia (1999), carpenter bee is the most effective pollinator because its large, hairy body touches the anther and stigma while obtaining nectar. They further observed that honey bees cannot pollinate passionfruit because they immediately return to the hive after collecting pollen from one flower. Since passion fruit is self-incompatible (Madureira et al., 2014), only flowers from different plants can be pollinated.

This study described the foraging behavior of *Xylocopa* spp and quantified the impact of pollination on fruit yield.

MATERIALS AND METHODS

Study Area

The study site is located at 14°118294’ North 121°5556833 East at Barangay Abang, Lucban, Quezon which is approximately 525 meters above sea level (Figure 1). It has a tropical climate with mean annual rainfall and temperature of 2671mm and 25.1°C, respectively. The experimental plots of passion fruit belong to the board members of Passion Fruit and Vegetable Growers Association of Lucban, Inc. (PAFVEGMAL, Inc.). An experimental plot measuring 40m x 20m was established in the study area. It was divided horizontally into two, and vertically into five, making 10 sub-plots with 4m x 20m dimension.

Foraging Pattern of Xylocopa spp.

A quadrat measuring 6m x 7m along the plant trellis, 1.82 m above the ground was established. Carpenter bees visiting the experimental area were identified as *Xylocopa* spp. since it is hard to discern on the species level.

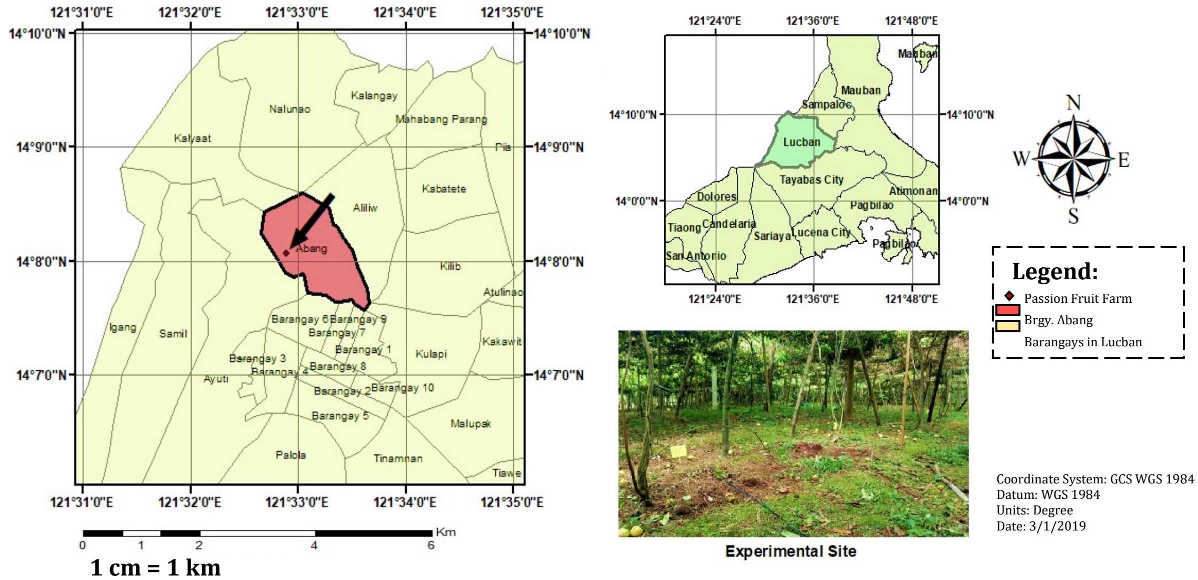

Figure 1. GIS Map (Source: Google Map) of Barangay Abang, Lucban, Quezon showing the study site.

Ocular observation was done to observe the foraging activity of *Xylocopa* spp. in terms of foraging pattern, time of visitation, and handling time. During the ocular observation, one observer (observer 1) sat on the trellis from 7 AM to 5 PM using a podium ladder 1.5 meters away from the experimental area to avoid disturbing the bees, while another observer (observer 2) stayed under the trellis. The foraging distance was assessed through measurement estimation of the distance of the flowers foraged by the carpenter bee. Once the carpenter bee started to forage on the flower, a signal was cued by observer 1 to observer 2. Observer 2 then put labeled sticks parallel to the flowers where the bee foraged to serve as markers in order to precisely measure the foraging distance. The observation ended once the carpenter bee is out of sight (Gautam et al., 2018). Any flight distance of carpenter bees that is out of the experimental area was presented as >6.5m. A video camera was used for documenting the length of stay of carpenter bee in one flower. However, the majority of the observations were based on ocular observation. The length of stay of the carpenter bees was measured using a stop watch, which was switched on as soon as the carpenter bees started to forage on the flower, and switched off just after the bee left the flower (Gautam et al., 2018). Foraging pattern was evaluated based on the distances of flowers foraged by bees.

Pollination of Yellow Passion Fruit

Eighty uniform flower buds approximately of the same age, measuring 40-45mm, were selected randomly prior to the experiment. This was to ensure that the flowers would open at the same day. Flowers were divided into four treatments including control, hand cross-pollination, hand self-pollination, and natural pollination.

Caging/ Exclusion Experiment

Exclusion experiments (Corbet & Wilmer, 1980) were conducted to determine the effects of pollination in the fruit set of the yellow passion fruit. Selected flower buds were caged and tagged correspondingly. When the flowers started to open, twenty flowers were caged to exclude pollinators.

Hand and Natural Pollination in Passion fruit

For hand pollination, forty flower buds were used. The flowers were divided into two treatments, namely self-pollinated and cross-pollinated flowers. When the flowers started to open, pollination by hand for cross-pollination was done. Using sterile forceps, pollen from a single anther of a different flower source was dusted on the stigma of previously tagged flower from hand cross-pollination. The forceps were washed with alcohol every time dusting was done to prevent cross contamination of pollen. Self-pollination by hand was done by dusting the pollen of the target flower on its own stigma. The flowers for both methods of hand pollination were covered with a fine mesh, 0.44mm in diameter, after dusting to prevent unwanted visitation of insect pollinators. Flowers from the natural pollination were left open and uncaged to facilitate natural pollination.

Data Analysis

Different fruit characteristics such as average circumference, weight, length, number of seeds and juice weight were determined. Student t-test at 0.01 or 0.05 level of significance was used to compare the mean of the traits. The data for foraging distance and foraging pattern were subjected to the Kolmogorov-Smirnov test of uniform distribution (Collevatti et al., 2000) while the distribution of flowers and distances foraged by bees were presented using histogram.

RESULTS AND DISCUSSION

Foraging Pattern of *Xylocopa* spp.

The time in which the pollinator started to visit the experimental area is essential for it dictates the visit frequency and duration (King et al., 2013). Three species of carpenter bees have been observed foraging on passion fruit, namely *Xylocopa (Koptortosoma bombiformis) bombiformis, X. (K) chlorina* (Cock) *and K. (X) bakerina* (Cock). During flight, the species could hardly be distinguished from one another, thus, the species in this study were simply designated as simply *Xylocopa* spp. The carpenter bees were observed hovering over the passion fruit farm as early as 0700h until 0800h when the flowers were just starting to open. This behavior of carpenter bees is called reconnaissance flight (Wagner et al., 2013) which includes flight distance within the farm area (Visscher & Seeley, 1982). The number of bees gradually increased as time progressed and peaked from 1100h to 1400h, and gradually decreased from 1500h until 1700h (Figure.2). The number of bees increased as the number of open flowers increased, reaching a peak for both at 1300h. While the number of open flowers remained constant until 1700h, the number of bees kept on declining. The peak number of foragers synchronized with anthesis, which is between 1200-1300h. This is the pollination window, since anthesis is the time when the stigma is most receptive (Kishore et al., 2010).

The peak of visitation was observed at 1000h with a maximum of 8 bees and 12 open flowers (Figure 2). The highest number of observed open flower was observed at 1200 h (n=32) which remained constant from 1300h to 1700h. The trend in the number of open flowers relative to the time of day is skewed to the left (Figure 3) suggesting a sudden increase in flower opening as time progressed. On the other hand, the trend in the number of bees visiting the experimental area relative to the time of day was approximately symmetrical, wherein the peak in the number bees (n= 19) was observed at 1300h. A similar observation in maximum flower blooming of *P. edulis* was observed by Banu et al. (2009). Nishida (1963) also observed that the number of visitations decreases as time progresses.

Bees can be classified as an efficient pollinator or just as a mere pollen robber in terms of handling time (Nishida, 1963). The mean handling time of carpenter bee increased from 55 ±12.7 seconds at 10:00 AM to 304 ±480.8 seconds at 11:00 AM then abruptly decreased to 16±22.6 seconds at 12:00 noon. A slight decrease was recorded at 1400h (10±8.6), a slight increase to 22 ±20.1 seconds at 1500h and then a decrease down to 11.5 ±8.7 seconds at 1600h (Figure 3). The trend in the mean handling time relative to the time of day was skewed to the right, suggesting a sudden decrease in the handling time as time progressed. The longest handling time was recorded at 1100h which coincided with the observed peak hour of flower opening.

This observation is similar to the assumption of Esenberg (2013) who stated that dense floral patches will more likely have short handling time, while sparse floral resources are predicted to a have long handling time. The observation was further supported by Gautam et al. (2018), who claimed that foraging activity of carpenter bees decreases as time progresses.

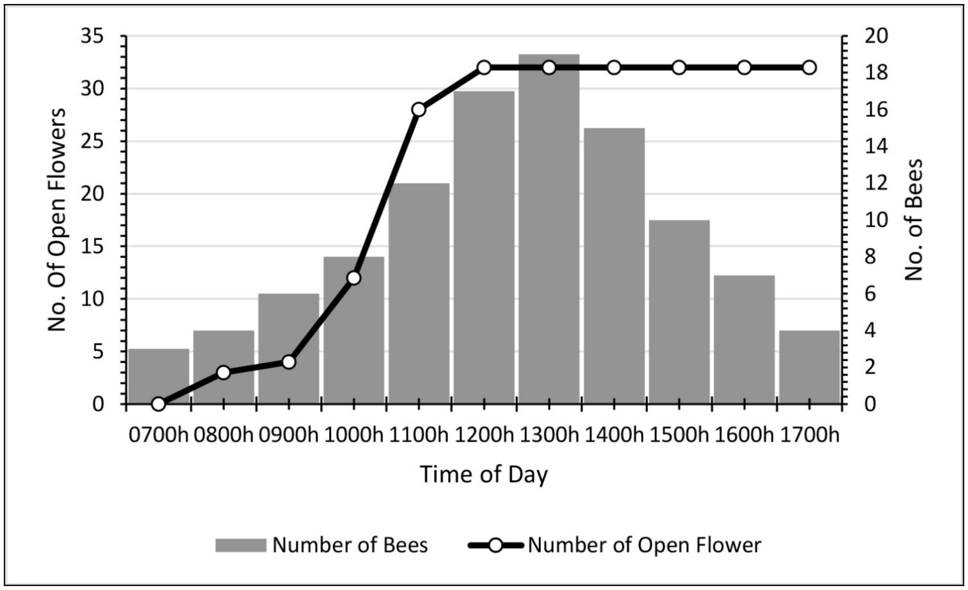

Figure 2. Number of bees visiting the experimental plot as time progresses from 0700h to 1700h with reference to the number of open flowers.

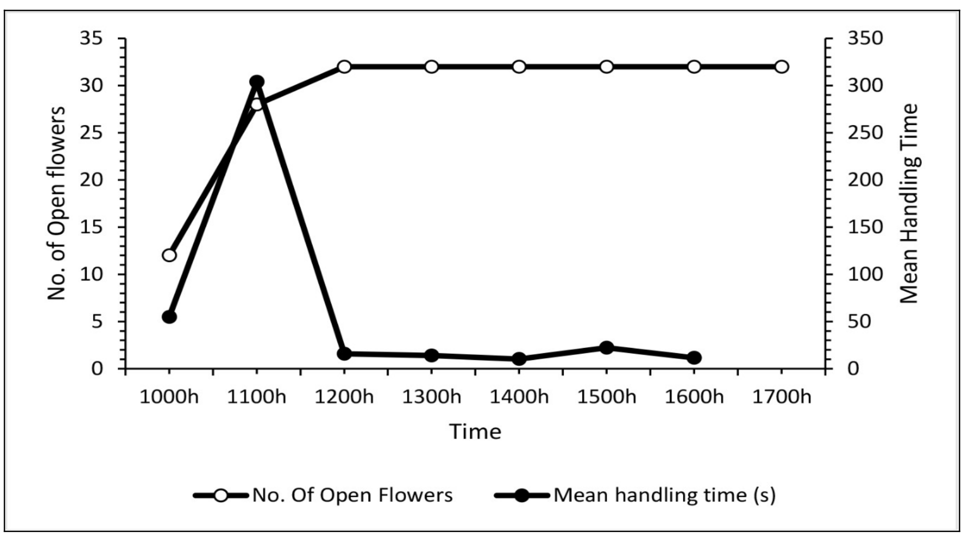

Figure 3. Mean handling time of carpenter bee in flowers as time progresses from 0700h to 1700h with reference to the number of open flowers

During foraging, the carpenter bee uses its hairy legs to grasp the corona filament enabling it to stand in place with its head facing the androgynophore and mouthparts towards the flower cavity (Figure 4). The carpenter bee was observed to efficiently gather the nectar of the passion fruit by moving around and digging its mouthparts in the flower cavity.

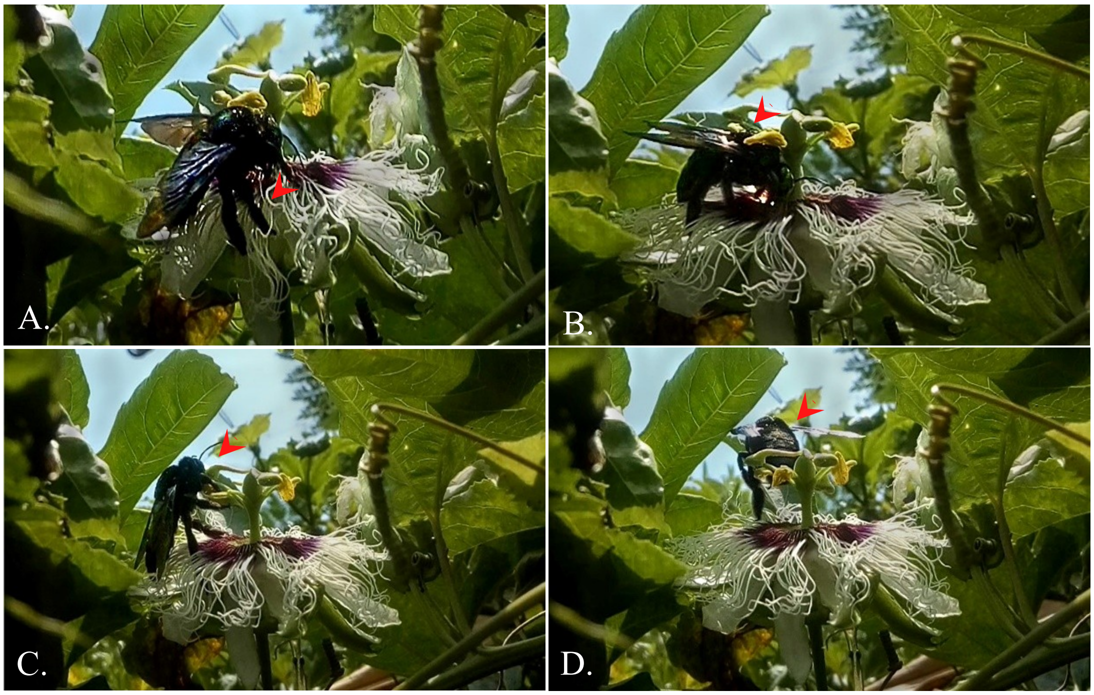

Figure 4. Foraging behavior of carpenter bee in yellow passion fruit during nectar gathering. (A) Use of legs to grasp the corona filament, (B) Contact of thorax on the anther, (C) Contact on the stigma and (D) Departure.

As the bee moves around the flower cavity, the pollens on the anther are being transferred around its thorax. This classifies the carpenter bee as an efficient pollinator compared to another insect such as honey bee. Aside from effectively collecting the pollen, the carpenter bee can also effectively deposit it to the stigma. This behavior was similar to the observation of Baran et al. (2017). Throughout the day, the average handling time spent by carpenter bees was 23 ±85 seconds. In contrast, Aguiar-Menezes et al. (2002) recorded an average handling time of 8.54 seconds in carpenter bees. The differences in observation on handling time may be due to variations in the environment of the two sites. Light and temperature affect the anthesis and consequently, the nectar secretion and pollen viability and stigma receptibility. Silva et al. (2019) reported that seasonality also influences the fruit set of crop species that are pollinated by bees. Climate determines plant flowering patterns and consequently affects pollinator activity.

The distances to which the carpenter bee foraged are essential in determining its efficiency in overcoming the self-incompatibility of the yellow passion fruit. The frequency distribution of the distances of flowers foraged by the bees showed that they can forage for a maximum distance of >6 m. The carpenter bee was able to facilitate deposition of pollen by flying greater distances. The trend of the mean foraging distances and the time of day was skewed to the left (Figure 5), suggesting that there was a gradual decrease in the foraging distances of carpenter bee as time progresses. The farthest distance (>6.5m) recorded was at 1000 h and 1100 h which coincided with increasing number of open flowers from 12 to 28 (Figure 5).

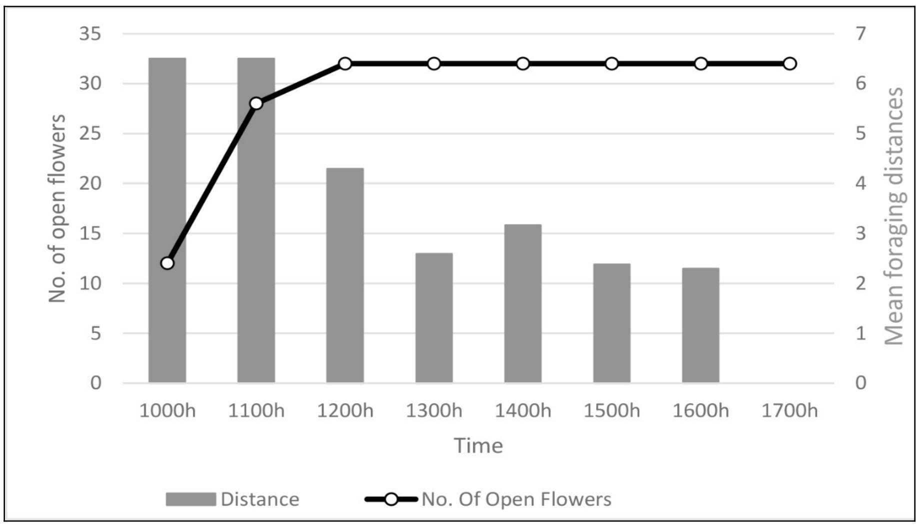

Figure 5. Foraging behavior of carpenter bee in terms of foraging distance and pattern relative to number of open flowers over time of day.

Interestingly, as the number of open flowers increased, the foraging distance of carpenter bee decreased. This suggests that flower patches affect the foraging activity of carpenter bee in terms of distance. When there are more floral resources available, the bees tend to travel in short distances. In this way, the carpenter bee was able to consume the nectar and deposit pollen without spending too much energy in foraging. Furthermore, the mean foraging distance observed is enough for the carpenter bee not to revisit flower it previously visited. Flower revisitation subjects the carpenter bee in spending energy cost (Ohashi & Yahara, 2002; Heinrich, 1975).

The foraging pattern of the carpenter bee does not follow specified distribution at α=0.05. This only shows that the carpenter bee foraged on the passionfruit flower at random distances. Gautam et al. (2018) stated that this could have been resulted from the distance structure of flowers within the area. However, the results show that the symmetrical trend of flower distances does not coincide with the trend of foraging distance of carpenter bee. The trend observed between foraging distances and flower distances were not identical. A graphical comparison of flower distances (Figure 6A) and foraging distance of carpenter bees (Figure 6B) shows that the foraging distance of carpenter bees was independent of distances of the flowers. The foraging distance of carpenter bee exhibits normal distribution (μ= 3.34<σ=7.91) while the flower distances exhibit binomial distribution (μ= 2.40>σ=1.20).

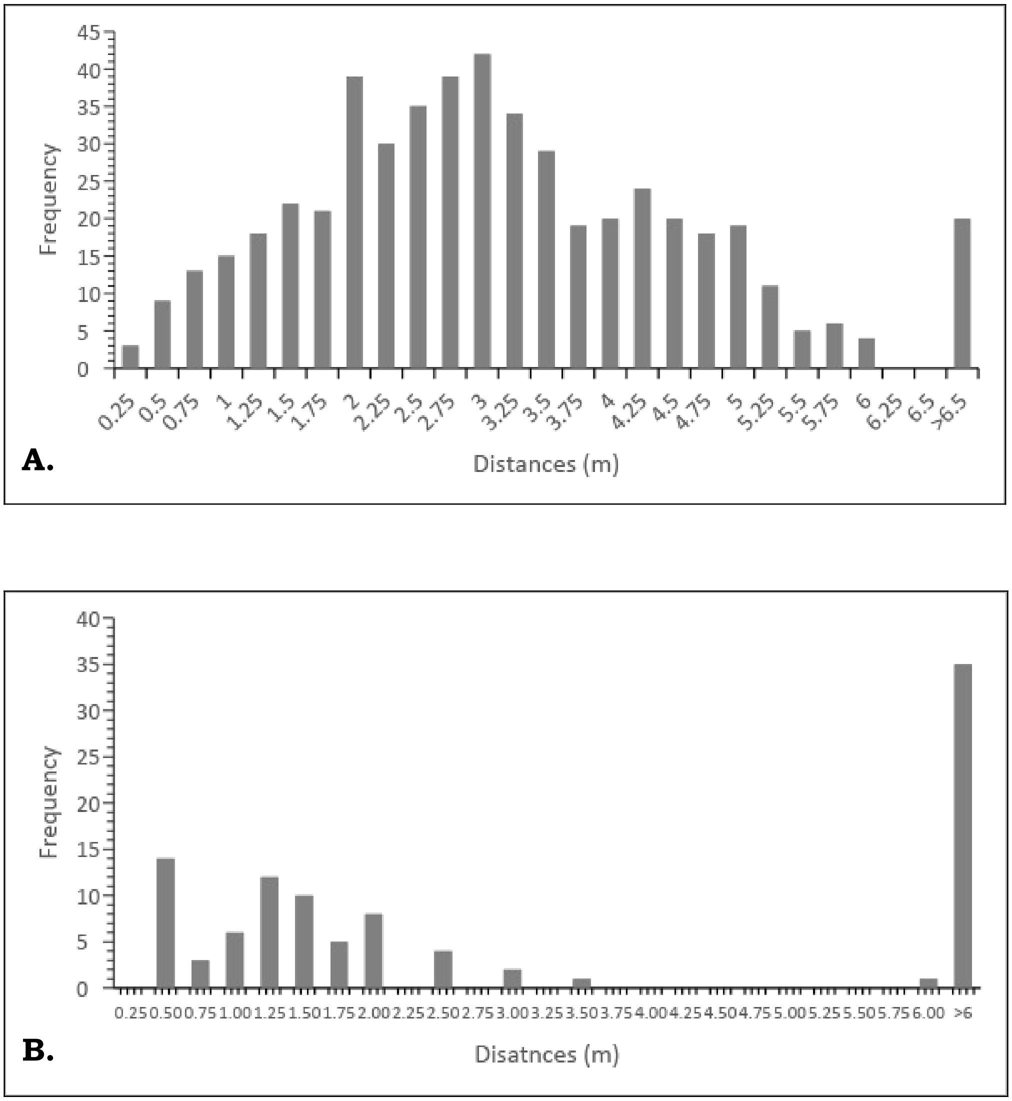

Figure 6. Histogram showing the comparison between the distribution of (A) flower distances around the experimental plot and (B) flower distance foraged by bees around the experimental plot. Dissimilarity in the distances indicates independence of flight pattern of carpenter bees in flower distances

Caging/ Exclusion Experiment

The flowers in the control and hand self-pollinated flowers did not set fruit. This shows that passion fruit strictly relies on pollination services in order for its ovary to mature into fruit due to its self-incompatibility (Junqueira & Augosto, 2016). Although reproductive parts of yellow passion fruit flower are self-incompatible, still it plays an important role in ovary maturation.

Hand and Natural Pollination

In this study, the hands self -pollinated flowers died off 5-6 days after they were dusted with their own pollen. This supports the position that passion fruit is self-incompatible (SI) Hossain (2013). SI It is one of the most important reproduction mechanisms used by angiosperms to promote outcrossing. The self-incompatibility (SI) system in passion fruit was investigated by Madureira et al (2014) using hand pollination. The results revealed that the pollen tubes grew slowly and were often completely arrested in the stigma in an incompatible combination.

The fruit set in hand-cross pollination was 75% and this was significantly higher than in natural pollination (45%) (Table 1). This is expected in areas where natural pollinators mismatch the number of flowers. In the study site, the density of pollinators may not be enough to cover all the flowers Hand pollination ensures that an adequate amount of pollen is transferred to the receptive stigma. The size of the fruit and the number of seeds will depend on the amount of pollen grains deposited into the stigma as Zhang et al. (2010) observed that in *Pyrus pyrifolia* higher levels of pollen density improved seed viability, fruit set, and fruit quality. Treatments with the highest pollen density showed significantly increased fruit growth rate and larger fruit at harvest. High pollen density increased germination rate and brought faster pollen tube growth, both *in vivo* and *in vitro*. However, seed production is not always directly related to pollen deposition because not all pollen grains that are deposited on a stigma are compatible or conspecific (Wang et al., 2017). Bodlah et al. (2015) discussed that scarcity in the abundance of pollinators is a key factor in unsuccessful pollination of crops. To assess the pollination deficits, it is necessary to compute the pollinator-plant ratio (Rabajante et al., 2019). One approach to increase the pollinator population in managed farm is to develop bee pasture

Table 1. Mean of different parameters assessed in different pollination treatments

PM	NFS	PFS	MFW (g)**	MTD (cm)**	MLD (cm)**	MPT (cm)^{ns}	MPL (g)**	MNS*	MJY (g)*	SS	TA	SS/TA
NP	7	35	89.77±17.69	6.11±0.18	6.55±0.22	5.28±0.94	49.38±8.11	165±69.33	36.99±14.06	15.6±0.84	0.76±0.11	20.88±2.48
HCP	15	75	123.75±23.73	6.75±0.38	7.29±0.44	5.45±1.28	69.65±15.86	238±73.83	51.23±9.46	15.3±0.91	0.746±0.28	22.33±6.08
HSP	---	---	---	---	---	---	---	---	---	---	---	---
C	---	---	---	---	---	---	---	---	---	---	---	---

Note: ** significant at t-test with 0.01 and 0.05significance level
* significant at t-test with 0.05 significance level
ns- not significant
—- Zero/ no value

PM= Pollination Method
NP= Natural Pollination
HCP= Hand Cross-pollination
C= Control
NPF= No. of Pollinated flowers
NFS= No. of Fruit Set
PFS= Percent Fruit Set

MFW= Mean Fruit Weight
MTD= Mean Transverse Diameter
MLD= Mean Longitudinal Diameter
MPT= Mean Peel thickness
MPL= Mean Peel weight
MNS= Mean Number of Seeds
MJY= Mean Juice yield
SS- Soluble Solids

TA= Titratable Acids
SS/TA= Soluble solids and Titratable Acids

consisting of bee plants that bloom throughout the year that will support pollinator population when the target crops are not yet flowering.

There were significant differences in the fruit characteristics per pollination type using student t-test. Only hand cross-pollination and natural pollination were compared since no fruits were obtained from the hand self-pollination and control set-ups (Table 1). The fruit weight (p=0.00088), transverse diameter (p=0.00001), longitudinal diameter (p=0.00002) and peel weight (p=0.00038) showed high significant differences at α=0.01 while pulp weight, number of seeds and seed weight (p=0.02) were found significant at α=0.05. However, peel thickness is not statistically different (α=0.05). In terms of soluble solids (p=0.19) no significant difference was obtained although both natural (15.6 °Brix) and hand cross-pollination (15.3 °Brix) produced values within the technical regulation for standards of identity and quality for passion fruit pulp issued by Brazilian Ministry of Agriculture (11 °Brix). Titratable acid (p=0.43) and SS/TA ratio (p=0.22) showed no significant difference at α=0.05. Additionally, hand cross-pollinated flowers produced more juice yield (51.29g±9.46g) than naturally pollinated flowers (36.99g±14.06g) which is significant at α=0.0. Better fruit set was also obtained in the study of Das et al. (2013) wherein juice weight, fruit weight and number of seeds per fruit were greater in hand cross-pollination than natural pollination. (Dogterom et al., 2000).

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