FLORAL TRAITS AND POLLINATION OF SPANISH CHERRY (*Mimusops elengi Linn.*) IN UNIVERSITI MALAYSIA TERENGGANU CAMPUS

HAIKAL HAZMI SUKRI, FELICIA SYUPIE GUNONG, NAZURAH IDIN AND NOR ZALIPAH MOHAMED*

Faculty of Science and Marine Environment, Universiti Malaysia Terengganu, 21030 Kuala Nerus, Terengganu

*Corresponding author: zalipah@umt.edu.my

Abstract: Some plants are self-compatible thus do not require pollinating agents for fertilisation of the ovule. Pollinating agents however are essential to promote cross-fertilisation, thus beneficial for the long-term survival of the plant species. In this study, we investigate the flower characteristics (flower size, colour, breeding system and nectar reward) and flower visitors of the Spanish cherry, *Mimusops elengi*, one of the most commonly planted ornamental trees in Universiti Malaysia Terengganu campus. Observations of flowers and the flower visitors were conducted between October 2016 and February 2017. The small sized flower, 19.88 ± 1.70 mm (mean $\pm$ SD) in height and 11.38 ± 0.87 mm (mean $\pm$ SD) in width; low volume with 11.90 ± 5.55 µl (mean $\pm$ SD) but with high sugar concentration with 26.57 ± 3.72 % (mean $\pm$ SD) nectar reward indicated insect pollination syndrome in this species. Xenogamy breeding system was determined for this species from the pollen-to-ovule ratio observation, which showed pollinating agents are required for cross-pollination to occur. Notes on the flower visitors revealed that two hymenopteran bee species, *Xylocopa confusa* and *Heterotrigona itama* as the pollinating agents of *M. elengi*. Not only these two species showed high visitation to the flowers, but they also land on the flowers to feed on the flower nectar, which could then potentially transfer the conspecific pollen grains on their bodies to the stigma for fertilisation of the flowers they visited.

Keywords: Xenogamy, *Heterotrigona itama*, *Xylocopa confusa*, visitation rate, conspecific pollen, landing

Introduction

Pollination is vital for the survival of plant species. Successful plant pollination occurs when pollen from the anther reach the stigma of the flower for fertilisation of the ovule. This pollen transfer could happen with the help of flower visitors that come to the flowers to feed on nectar or pollen grains. Not all flower visitors however are pollinating agents as during the feeding activity, they may also act as nectar robbers and flower predators (Inouye, 1980). While feeding, these flower visitors may act as pollinating agents depending on their feeding behaviours, visitation frequency and the number of pollen grains they carry on their bodies (Boyd, 2004; Reynolds & Fenster, 2008). A reproductive success such as the quality and the quantity of fruit and seed set is influenced by the service of these pollinating agents. Apart from reducing the quality and quantity of fruit and seed set, a reduction of pollinators' service also

promotes self-pollination in self-compatible plant species (Rodriguez-Perez, 2005).

Previous studies on *Mimusops elengi* Linn. (Spanish cherry) were conducted for the reason that this species has many medicinal and cultural values. For examples, the alcoholic extracts of *M. elengi* were found to enhances cognitive activity to prevent memory impairment (Hadaginhal *et al.*, 2010), has a diuretic potential (Katedeshmukh *et al.*, 2010), and contain antimicrobial producing agents against multidrug resistant microbes (Prakash *et al.*, 2013). While from the cultural aspect, *M. elengi* is considered as a sacred plant by the Hindus (Mitra, 1981).

Although considered as a commonly found ornamental plant in Malaysia, to our knowledge, no study on the pollination of *M. elengi* was conducted, as compared to other highly valuable ornamental plants such as the orchids (Tan *et al.*, 2002). Although the small sized flower is

a characteristic of insect pollination syndrome (Willmer, 2011), on several occasions we observed small birds visiting the trees and sometimes feed on the flowers of *M. elengi*. Kamaruddin and Nor Zalipah (2020) found that the olive-backed sunbird visiting small size *Ixora coccinae* (jungle geranium) flowers in Universiti Malaysia Terengganu (UMT) campus might act as pollinating agents for the species with their large bodies which enable them to carry high pollen loads. Thus, we conducted this study on *M. elengi*, to further determine the role of flower visitors as pollinating agents for this plant species in UMT campus.

Materials and Methods

Study Site and Species Studied

This study was conducted at Universiti Malaysia Terengganu (UMT) campus in Kuala Nerus

District, located 25 km away from the city of Kuala Terengganu (Figure 1). *Mimusop elengi* from the Family Sapotaceea can be found in south and southeast Asia as well as in northern Australia. There are approximately 65 genera in the Family Sapotaceae, consisting of more than 800 species of evergreen trees and shrubs. In Malaysia, *M. elengi* is an ornamental plant, cultivated in gardens for its fragrant flowers and as a shade tree. It is a woody plant that can grow up to 15 m in height. The flower is star-shaped and is white (Figure 2). This small flower however produces sweet fragrance even when the flowers had finished blooming and have dried (Mitra, 1981). The leaves are variable in shape which include elliptic, oblong, short or long acuminate, margin undulate and closely but faintly veined. In UMT, the trees are commonly planted throughout campus, with the tree ranging between 1-8 m in height.

Figure 1: Location of sampling sites in Universiti Malaysia Terengganu (UMT) campus. 1) Makmal Biologi Umum, 2) Bina Syarahan, 3) Rumah Universiti, 4) Kompleks Makmal Berpusat, 5) Pusat Pengajian Informatik dan Matematik Gunaan, 6) Canselori

Figure 2: The flower of *Mimusop elengi* (Spanish cherry) in full bloom observed on a tree located near the Makmal Biologi Umum building

Observations of Flowers

Observations of flower characteristics were conducted between October and November 2016. For each observation, nine full bloom flowers were collected from three different trees (three flowers per tree) in the early morning before 1000 hr and were brought to the laboratory for measurements (height and width) of the flower, stigma and anthers using a plastic vernier caliper. The height of the flower was measured from the base of its peduncle to the stigma. The width of the flower was measured from end-to-end of the opposite petals of the open flower. After measurements of anthers and stigma, three anthers for each flower were collected and kept in the microcentrifuge tube containing 1.0 ml of 70 % ethanol solution for future pollen count. The flowers were then dissected under a dissecting microscope attached with an eyepiece camera (Dino-eye AM 423X, Anmo Electronics Corporation, Taiwan) with 70x magnification for a maximum observation at 210x magnification. For each flower, the number of ovules was then recorded, and the size of the ovules measured from the photos captured using the Dino capture software.

To count the number of pollen grains, the microcentrifuge tubes containing the anthers were manually shaken to separate the pollen grains from the anthers, and 1.0 µl of the ethanol solution was then taken using a micropipette

and put in a glass slide. The solution was then covered immediately with a coverslip to spread the pollen grain, before being observed under a light microscope attached with an eyepiece camera with a maximum observation of 2800x magnification. For each flower, pollen counts were conducted for 10 slides and the total number of pollen grains was extrapolated for 1 ml of ethanol. Then, the number of pollen grains for a single anther was calculated by dividing the number of pollen grains with three anthers. The total number of pollen grains for the individual flower was estimated by multiplying the number of pollen grains per anther with the total number of anthers for the same flower. The pollen-ovule ratio of the flower was then calculated following Cruden (1977).

For the nectar standing crop, the selected flowers were bagged with a veil bag before its opening to avoid the nectar from being robbed by flower visitors (usually 2-3 days before flower opening). Then, the flowers were observed until its full bloom. To record the volume, nectar was extracted from the flower using 0.5 µl glass capillary tube (Minicaps, 9000100, Hirschmann Laborgerate, Germany) at 1000 hr. To record the nectar sugar concentration, the flower nectar was extracted using 10 µl micropipette. Then, the sugar concentration (sucrose in % Brix) of the nectar was measured by using a digital sugar refractometer (HI 96801 Refractometer, Hanna Instruments Inc., USA).

Observations of Flower Visitors

Observations of the flower visitors were conducted in October 2016 until February 2017. For this observation, a branch that encompasses approximately 10-15 flowers was selected for observation. A DSLR camera (NIKON D3300 with NIKON AF-S DX NIKKOR 18-55 mm lens) then was placed approximately 1 m from the selected flowers to record the visitation by the flower visitors. The recordings were conducted in three sessions: morning (0700-1000 hr), afternoon (1100-1400 hr) and evening (1500-1900 hr). For each observation hour, the recording was conducted only for the first 10 minutes. In total, observations were conducted on five different branches of five different trees, thus the total observation conducted was 650 min/tree. After observations, all flower visitors were caught using an insect net. The insects were later identified by referring to the insect collection at the UMT Insectarium.

The visitation frequencies of each flower visitors were determined from the video footage recorded. A visit was considered when a flower visitor is in close distance to the observed flowers (ca. 1-2 cm from the petals). Visits were recorded irrespective to previous visits by the flower visitors to the focal flower. The visitation behaviours were categorised into either landing or hovering to feed for nectar, or scouting. Landing is when the flower visitors come in contact with the flower by landing onto the flower, while hovering is when the visitors only hover near the flower (without coming into contact with the flower) to obtain nectar. Scouting is considered when the visitor flies close to the flower but did not attempt to feed on the flower. For landing and hovering, the time taken for each visitation was recorded from the time code of the footage. The time spent at the flower was recorded starting from when the visitor first approached the flower (ca. 1-2 cm distance from the flower) until it flies away.

The two most frequent flower visitors were captured using an insect net while visiting the flowers between 0900 hr and 1300 hr.

Each of the captured visitors was then kept in individual plastic containers and euthanized with ethyl acetate to collect the pollen grains on their bodies. A needle was used to collect the individual pollen grains by rubbing the tip of the needle to the pollen basket (corbicula) of the captures, and the needle was then dipped into 1.0 ml of 75 % ethanol in a centrifuge tube to preserve the pollen grains for further observation. Observations on the pollen grains collected were conducted using the same procedure as for the pollen count for each flower. Pollen grains were grouped as either conspecific (*M. elengi* pollen grains) or heterospecific (other pollen grains).

Data Analysis

Chi-Square test was used to compare the total visitation frequency between the flower visitors, and to compare the number of behaviours (landing, hovering and scouting). Mann-Whitney tests were performed to compare the time spent between two flower visitors which showed landing and hovering behaviour respectively. Paired sample t-tests were conducted to determine the significant difference in the number of conspecific and heterospecific pollen grains for the two main flower visitors of *M. elengi*. All analyses were conducted using IBM SPSS ver. 20.0 (Chicago, USA) statistical software.

Results and Discussion

Floral Traits

The flower of *M. elengi* is small, which only about 20 mm in height and 11 mm width (Table 1). Each flower consists of a single stigma located in the middle of eight anthers. The ovary contains only two ovules, and each flower is estimated to have approximately 6786 pollen grains. The pollen-to-ovule (P/O) ratio was estimated to be 93392.89 ± 3711.72 (mean $\pm$ SD). Each flower produces approximately 12 μl of flower nectar with about 26 % sugar concentration.

Table 1: Breeding characteristics of *Mimusops elengi* (n = 9 flowers). Mean $\pm$ SD is used throughout

Variables	Result	
Number of anthers	8	
Number of stigma	1	
Number of ovules	2	
Number of pollen per flower	6785.78 ± 7423.44	
Pollen-to-ovule ratio	93392.89 ± 3711.72	
Nectar volume (µl)	11.90 ± 5.55	
Nectar sugar concentration (%)	26.57 ± 3.72	
	Height/Length (mm)	Width (mm)
Flower size	19.88 ± 1.70	11.38 ± 0.87
Anther size	3.22 ± 0.26	0.86 ± 0.10
Stigma size	0.56 ± 0.09	0.44 ± 0.03
Ovule size	0.68 ± 0.11	0.43 ± 0.10

Flower characteristics such as the flower size and the reward offered (nectar) are essential factors in attracting pollinating agents to come to the flowers (Jacquenmart *et al*., 2015). However, some plants do not require pollinating agents for pollination by undergoing self-fertilisation (autogamy) in hermaphroditic flower (ie. flower that consists of male and female reproductive organs) (Martínez-Palle & Aronne, 1999). For these self-compatible plants however, flower visitors may help to promote cross-pollination, thus, producing visually attractive floral displays and floral rewards is a way for animal-pollinated plants to promote the outcrossing breeding (Barrett, 2003; Goodwillie *et al*., 2010; Fisogni *et al*., 2011).

The white coloured and small sized flower of *M. elengi* indicate its strategy for insect pollination. Insects used ultraviolet light as a nectar guide to find the flowers as they could not detect the colour of the flowers (Cronk & Ojeda, 2008). The bright coloured flowers which usually contain high amount of nectar on the other hand, are more favourable to be visited by birds (Van der Niet *et al*., 2015). The nectar produced by *M. elengi* consists mainly of sucrose component (Freeman *et al*., 1991), and the volume recorded in this study falls into the category of medium/large bee-pollinated flowers (Willmer, 2011). The high quality and quantity of nectar produced influenced the visitation of insect pollinators to the flowers (Jacquemart *et al*., 2015).

The study of pollen and ovule within the flowers explained the mating mechanisms and breeding system of the plant (Cruden, 1977). Following the classification of plant breeding systems based on P/O ratios, *M. elengi* is within the range of species showing xenogamy pollination (>5000 P/O ratio). Xenogamy is cross-fertilisation that happened between different individual plants (Crawley, 1997). The group of the plants with xenogamy pollination usually requires the help of pollinating agents such as insect, bird and wind for pollination to occur. According to Barrett (2003) and Charlesworth (2006), the quantity and quality of offspring and consequently the movement of the genes in time and space depend on the reproductive strategies of the organism. Cross-fertilisation mating strategy is one of the strategies that are showed by many plants. However, because of natural selection during interaction with different pollination agents, the floral adaptation and reproductive strategies have evolved in the flowering plants (Barrett, 2010; Goodwillie *et al*., 2010). Therefore, the reproductive strategy depends on the form and function of the sexual units (stamens, pistils, flowers and/or inflorescences) that determine the pattern of pollen dispersal and receipt, together with post-pollination mechanisms such as genetically controlled incompatibility system (Barrett, 2003; 2010; Charlesworth, 2006).

Flower Visitors as Pollinators

The visitors recorded were insects from the order Hymenoptera (four species) and Lepidoptera (one species), and a single species of bird, *Cinnyris jugularis* (olive-backed sunbird) (Table 2). *Xylocopa confusa* (carpenter bee) was recorded as the most frequent visitor with the total number of 149 visitations (4.58×10^{-2} visit/min), followed by *Heterotrigona itama* (stingless bee) with the total number of 127 visitations (3.91×10^{-2} visit/min). The lowest visitation was recorded from an unidentified butterfly (order Lepidoptera) with only six visitations (1.85×10^{-3} visit/min).

Table 2: List of flower visitors and their visitation rate (visit/min) to the flowers. Number in parentheses indicates the number of visits (in %) recorded by each flower visitors observed in the first 10 minutes of every observation hour

Order	Famili	Species	Visitation rate
Hymenoptera	Apidae	*Xylocopa confusa*	4.58×10^{-2} (38.5)
		Heterotrigona itama	3.91×10^{-2} (32.7)
		Unidentified wasp	2.31×10^{-2} (19.3)
Coleoptera	Cerambycidae	Unidentified long-horned beetle	3.69×10^{-3} (3.1)
Lepidoptera	-	Unidentified butterfly	1.85×10^{-3} (1.5)
Passeriformes*	Nectariniidae	*Cinnyris jugularis*	5.85×10^{-3} (4.9)

*Class Aves

Observations on the behaviour of the flower visitors showed that the majority of the visitations was by landing (71 %), and followed by hovering (20 %), while the least was for scouting with only 9 % of the total visitations (Chi-Square test, $\chi^2 = 46.087$, df = 2, p < 0.001). The landing was observed only for *X. confusa* and *H. itama,* while hovering was recorded for unidentified wasp and butterfly (Table 3).

For landing, the time spent (mean ± SD) at the flowers recorded for *X. confusa* (6.19 ± 1.86 sec) was significantly longer than *H. itama* (5.26 ± 1.80 sec) detected from the Mann-Whitney test conducted ($U = 6.85 \times 10^3$, p < 0.001). For hovering, unidentified butterfly (5.17 ± 1.47) was found to hover longer (Mann-Whitney test, $U = 3.72 \times 10^2$, p < 0.05) at the flowers as compared to unidentified wasp (3.51 ± 0.84).

Table 3: Time spent (in sec) at the flowers (mean ± SD) of the flower visitors with landing (*) and hovering (**) behaviour while feeding at the flowers. n indicates the number of observations

Visitor species	n	Time spent	Range
*Xylocopa confusa**	149	6.19 ± 1.86	3-10
*Heterotrigona itama**	127	5.26 ± 1.80	2-10
Unidentified wasp**	75	3.51 ± 0.84	2-5
Unidentified butterfly**	6	5.17 ± 1.47	3-7

*For visitors with landing behaviour, time spent was significant at p = 0.001
** For visitors with hovering behaviour, time spent was significant at p = 0.05

For the two most common flower visitors (Figure 3), the number (mean ± SD) of conspecific pollen grains on their bodies were doubled than that of the heterospecific grains observed (Table 4). The Paired sample t-tests conducted showed that the number of conspecific pollen grains was significantly higher than the heterospecific grains detected for both *X. confusa* (t = 7.483, p < 0.001) and *H. itama* (t = 8.419, p < 0.001).
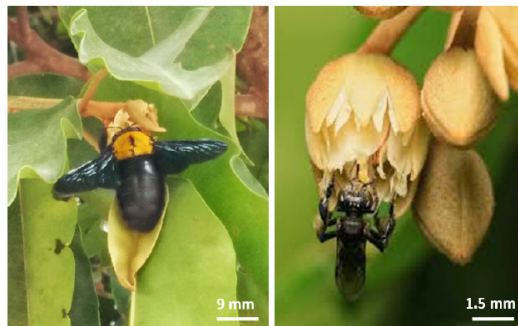
Figure 3: The two main flower visitors of *Mimusop elengi* in Universiti Malaysia Terengganu campus. A) *Xylocopa confusa* (carpenter bee), and B) *Heterotrigona itama* (stingless bee)

Table 4: Number of pollen grains (mean ± SD) recorded for the two main visitors of *Mimusop elengi* in Universiti Malaysia Terengganu campus. n indicates the number of individuals observed

Visitor species	n	Pollen type	Number of grains
*Xylocopa confusa**	8	Conspecific	12.00 ± 3.29
		Heterospecific	4.00 ± 1.77
*Heterotrigona itama**	8	Conspecific	10.00 ± 2.51
		Heterospecific	5.50 ± 2.20

*Significant at $p < 0.001$

A favourable outcome in plant breeding from pollination is influenced by the number of pollinating agents (Martén-Rodríguez *et al*., 2015). The more individual visitors, the higher probability of successful fertilisation of the plants. Among insects; bees, wasps and occasionally ants (Order Hymenoptera), beetles (Order Coleoptera), moths and butterflies (Order Lepidoptera) and flies (Order Diptera) are among the most common pollinating agents for thousands of plant species (Dar *et al*., 2017). In our study, although only six species of flower visitors were recorded, high visitation frequencies particularly by two hymenopterans, *X. confusa* and *H. itama* showed their effectiveness as pollinating agents to the flowers they visited (Latif *et al*., 2019).

Landing on the flower is a common feeding strategy for many insects (Sarkar *et al*., 2014), as hovering feeding requires higher energy expenditure (Dreisig, 1997). *Heterotrigona itama*, with their small body size, enables them to land on small sized flowers to forage for nectar (Kamaruddin & Nor Zalipah, 2020). *Xylocopa confusa* on the other hand, is relatively large as compared to *M. elengi* flower, thus landing

feeding is more preferable to save their energy because they gain only a small amount of nectar from the flowers. The longer time is taken by this bee at the flowers however, probably to collect as much nectar as possible from the flowers to fulfil their energy demand. Hovering usually occurs in short duration at the flowers, not only to save energy but also could reduce exposure to predators (Romero *et al*., 2011).

Not all insect flower visitors are reliable pollinating agents because not all carry pollen grains on their bodies while foraging at the flowers (Gafni *et al*., 2018). As compared to the former two hymenopterans, an unidentified wasp and an unidentified butterfly only hovers at the flowers thus precluding them from acting as pollinating agents for the flowers. Although these two unidentified insects spent a considerable time foraging at the flowers, hovering from a distance limited them from collecting pollen grains from the flowers or transferring any pollen grains on their bodies to the stigma of the flower for pollination. *Xylocopa confusa* and *H. itama* on the other hand, carried a significantly higher number of conspecific pollen grains on their bodies, thus showing higher potentials for

conspecific pollen transfer to the stigma of the flowers to initiate pollination.

Conclusion

Mimusops elengi found to show xenogamy breeding system which requires pollinating agents to undergo cross-pollination. The flower size and colour, together with the nectar reward further indicate its suitability for insects as pollinating agents. Although flowers were visited by six different insect species and a single nectarivorous bird species, only *X. confusa* and *H. itama* showed the highest potential as pollinating agents for *M. elengi* in UMT campus. Not only these two hymenopterans showed high visitation frequencies to the flowers, both species also carried higher amount of conspecific than heterospecific pollen grains on their bodies when visiting the flowers. Thus, while landing on the flowers to consume the nectar, these two hymenopterans could potentially transfer the conspecific pollen from their bodies to the stigma, further indicating their important role as pollinating agents to the plant species they forage at.

Acknowledgements

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