ASSOCIATION OF FLORAL TRAITS ON POLLINATION EFFECTIVENESS OF INSECTS FORAGING ON *Psidium guajava* (GUAVA)

BY

NUR ATHIRAH ZARIMAN

A dissertation submitted in fulfillment of the requirement for the degree of Master of Science

Kulliyyah of Science
International Islamic University Malaysia

DECEMBER 2023

ABSTRACT

Plant-pollinators are mutualistic relationships where both are dependent and benefit from each other. In order to ensure successful pollination, it is important to have good flower conditions and pollen quality to attract pollinators to forage on flowers. To understand the association of floral traits on guava pollination, general characteristics of guava tree were described. Flowers and pollen grains of *Psidium guajava* cultivars and *Psidium cattleianum* were analyzed and measured for their appearance, shape and sizes. Pollen grains viability were measured with colorimetric method using 2,3,5-triphenyl tetrazolium chloride (TTC) and Iodine Potassium Iodide (IKI) staining solution. Guava flowers are white with multiple of five petals arranged in two whorls for *P. guajava* but only one whorl for *P. cattleianum*. The guava pollen grains were small, triangular amb, tricolprates, brevicolpate aperture type with exine pattern of granulate/scarbate in all *P. guajava* cultivars but rugulate pattern in *P. cattleianum.* There are slight differences in morphology particularly in sizes and numbers of certain aspects among cultivars and species which may be the result of coevoluation with their pollinators. The IKI is the better staining solution as it produces a higher viability percentage (>85%) compared to TTC staining. Kim Choo White has the highest pollen viability among *P. guajava* cultivars studied. Meanwhile, field observations on pollinator visitation frequencies and pollen carrying capacity were conducted at intervals of 2 hours from 0900 h to 1600 h in 5 and 3 random sampling sessions, respectively, from January to October 2022, to identify efficient pollinator species for guava and understand their behavioral that resulted in successful pollination. The most common pollinators that visit guava were *Xylocopa latipes*, *Xylocopa aestuans*, *Apis dorsata*, *Apis cerana*, *Heterotrigona itama* and *Ceratina lieftincki.* It was determined that the insect's body size positively influenced the pollen carrying capacity. Bees from genus *Apis* and *Xylocopa* were found to be efficient pollen carriers and visited more guava flowers compared to the other pollinators.

ملخص البحث

إن العلاقة بين النباتات والحشرات الـمُلقّحة علاقة تعاونية متبادلة حيث يستفيد كل منهما من الآخر، ويتطلب التلقيح الناجح ظروف جيدة للزهرة ولحبوب لقاحها. قام البحث الحالي بدراسة السمات الظاهرية لأنواع من الجوافة؛ لفهم تأثيرها على جودة التلقيح. تم دراسة زهور جوافة الـ*Psidium guajava* والــ*Psidium cattleianum* وحبوب لقاحها من حيث المظهر والشكل والحجم. تم قياس صلاحية حبوب اللقاح باستخدام صبغة 2، 3، 5-تريفينيل تترازول كلورايد (TTC) وصبغة يوديد البوتاسيوم (IKI). تتكون زهرة الجوافة من خمسة بتلات بيضاء مرتبة في اثنين من الدُّوَّارَات لجوافة *P. guajava*، وبتلة واحدة في جوافة *P. cattleianum*. كانت حبوب اللقاح صغيرة، وثلاثية الأضلاع، وثلاثية الثقوب ونوع فتحة الحبوب اللقيطة القصيرة مع نمط جدار خارجي حبيبي/نقشي في جميع أصناف جوافة *P. guajava* ونمط جدار خارجي مموج في *P. cattleianum*. هناك اختلافات طفيفة في الشكل الظاهري بين الأنواع والتي قد تكون نتيجة للتطور المشترك مع الحشرات الملقحة الخاصة بهم. صبغة IKI هي الأفضل كونه أظهر نسبة أعلى من الصلاحية (> 85٪) مقارنة بـ TTC، وكان صنف كيم شو الأبيض (Kim Choo White) أكثر صلاحية للحبوب اللقاحية بين أصناف *P. guajava* المدروسة. أجريت دراسة ميدانية على ترددات الحشرات وقدرة حملها لحبوب اللقاح على فترة كل ساعتين من 09:00 إلى 16:00 في 5 أو 3 جلسات من يناير إلى أكتوبر 2022، لتحديد أنواع الملقحات الفعالة للجوافة وفهم سلوكها الذي أدى إلى تلقيح ناجح. أكثر الحشرات الملقحة هي نحل نجار *Xylocopa latipes* ونحل *Xylocopa aestuans* ونحل العسل العملاق ونحل العسل الشرقي ونحل *Heterotrigona itama* ونحل*Ceratina lieftincki*، كما أن حجم الحشرة يؤثر على القدرة الحمل. وجد أن النحل من جنس *Apis* و *Xylocopa* يكون ناقلاً فعالاً لحبوب اللقاح وزيارة المزيد من زهور الجوافة مقارنة بالملقحات الأخرى.

APPROVAL PAGE

I certify that I have supervised and read this study and that in my opinion, it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Master of Science

……………………………….
Nurul Huda Abdul
Supervisor

……………………………….
Name
Co-Supervisor

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Master of Science

……………………………….
Che Nurul Aini Che Amri
Examiner

……………………………….
Nur Adibah Mohd Ishadi
Examiner

This dissertation was submitted to the Department of Plant Science and is accepted as a fulfilment of the requirement for the degree of Master of Science

……………………………….
Maizatul Akma Ibrahim
Head, Department of Plant Science

This dissertation was submitted to the Kulliyyah of Science and is accepted as a fulfillment of the requirement for the degree of Master of Science

……………………………….
Zarina Zainuddin

Dean, Kulliyyah of Science

DECLARATION

I hereby declare that this dissertation is the result of my own investigations, except where otherwise stated. I also declare that it has not been previously or concurrently submitted as a whole for any other degrees at IIUM or other institutions.

Nur Athirah Zariman

Signature…………………………… Date……………………

INTERNATIONAL ISLAMIC UNIVERSITY MALAYSIA

DECLARATION OF COPYRIGHT AND AFFIRMATION OF FAIR USE OF UNPUBLISHED RESEARCH

ASSOCIATION OF FLORAL TRAITS ON POLLINATION EFFECTIVENESS OF INSECTS FORAGING ON *Psidium guajava* (GUAVA)

I declare that the copyright holder of this thesis/dissertation are jointly owned by the student and IIUM.

No part of this unpublished research may be reproduced, stored in a retrieval system, or transmitted, in any form or by any means, electronic, mechanical, photocopying, recording or otherwise without prior written permission of the copyright holder except as provided below

1. Any material contained in or derived from this unpublished research may only be used by others in their writing with due acknowledgement.

2. IIUM or its library will have the right to make and transmit copies (print or electronic) for institutional and academic purpose.

3. The IIUM library will have the right to make, store in a retrieval system and supply copies of this unpublished research if requested by other universities and research libraries.

By signing this form, I acknowledged that I have read and understand the IIUM Intellectual Property Right and Commercialization policy.

Affirmed by Nur Athirah Zariman

……………………………… …………………..

Signature Date

This dissertation is dedicated to my parents for their endless love, support and encouragement.

ACKNOWLEDGEMENTS

Alhamdullillah, praise be to Allah, the Almighty of God the most Gracious and the most Merciful, and whom ultimately we depend for sustenance and guidance throughout the duration of my study. I am grateful for the countless blessing, knowledge and opportunity granted to me, so that I have been finally able to complete this thesis and also for giving me the tranquility of mind to handle all the obstacles and difficulties in finishing this task properly.

It would be a pleasure for me to express my gratitude and appreciation to each of the many people who inspired, supported, offered guidance, and even made comments that aided me in completing this thesis.

A special thanks to my dedicated supervisor, Asst. Prof. Dr. Nurul Huda Abdul, for the help, guidance and enthusiasm. She has provided positive encouragement and warm spirit throughout the whole process of completing this thesis. It has been a great pleasure and honour to have her as a supervisor. I would also like to thank my parents, Zariman Mohamed and Norazlina Setapa for their prayers, understanding and faith in me and also towards my siblings for giving me continuous support and encouragement throughout the process.

I am grateful to Kulliyyah of Science, IIUM for providing its laboratory facilities in accomplishing this study. Additionally, I am very grateful for my friend, Nurul Alia Omar who helps me develop my ideas and never stops entertaining me in the process of completing my study.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS

℃	Degree Celsius, refers to a specific temperature on the Celsius scale or a unit to indicate a difference or range between two temperatures
μ	Micro, factor of 10^{-6} (one millionth)

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND OF THE STUDY

Pollination process involves plants and animals that can mutually be dependent on each other. The animals regard as pollinators transfer pollen grains to the stigma for fertilization and germination while it search for food, shelter or even pheromones in the flowers (Gupta *et al.*, 2014). Pollinators are responsible for assisting flowering plants to reproduce. Pollinators including small mammals and insects are responsible to reproduce healthy crops to make food, fibers, edible oils, medicines, and any other products (Das *et al.*, 2018). Bees are the most common pollinators in fruit crop production. Besides bees, butterfly, beetles and flies are important pollinator during daytime while bats and moths pollinate during night-time (Halder *et al.*, 2019).

Pollinators are important in crop production of many plant species. According to Norliyana *et al.* (2019), insects are said to pollinate more than 50% of tropical plant which contribute greatly in maintaining and conserving biodiversity. Similarly, Pashte and Kulkarni (2015) also stated that pollinators plays efficient role in pollination of wild plants and several crop species such as apples, blueberries, blackberries, cherries, cranberries, raspberries and strawberry. Like other tropical fruits, guava (*Psidium guajava*) can either be self-pollinated or cross pollinated. However, *P. guajava* bears a hermaphrodite flower with protruded stigma above the anthers and due to this, even self-pollination is unusual without the help of pollinators (Kadam *et al.*, 2012). Main insect species that visited guava flowers belong to Hymenoptera, Diptera, Lepidoptera and Coleoptera species (Kadam *et al.*, 2012; Vinod & Sattagi, 2018).

Pollination is the classic example of co-evolution between angiosperms and their pollinators. Crop yield may be cultivar dependent, however, pollination success is equally important. There is little information regarding which floral traits determine the pollination success. To ensure successful pollination, it is important to have a good pollen quality based on its viability as it is an essential stage in life cycle. Pollen viability is important for the success of sexual reproduction in plants (Bots & Mariani, 2005; da Silva *et al.*, 2017). On the other hand, good pollens gathered by insect act as a source of protein and provide fats or lipids, minerals, and vitamins for their growth. In addition, nectar productivity is also predominant in successful pollination. This is due to the fact that nectar-producing plants have a higher probability of attracting insects that accomplish pollination success (Lange *et al.*, 2017). Flower's nectar are likely able to control the type, schedule, and behavior of pollinator visits and thus it will eventually affect the transfer of pollen and plant's fitness (Luo *et al.*, 2014).

Therefore, association of floral traits on pollination has been conducted on *Psidium* sp. mainly on *Psidium guajava* which is the commercial guava for fruit production. However, some part of the studies included another species, *P. cattleianum,* which has small berry fruits and is commonly grown as ornamental plant for comparison. In order to increase the quality and output of guavas, this study looked at the association of floral traits on pollination effectiveness by insect pollinators. It is crucial to understand the importance of certain traits that attract insect pollinators to maximize their visit.

1.2 PROBLEM STATEMENT

Pollinators are attracted towards flowers as they feed on either pollen or nectar of the flower. Therefore, pollen viability and nectar productivity may be the main influences of pollinators foraging behavior and thus impact pollination effectiveness. The population of pollinator in a particular area measures overall ecosystem's fitness (Das *et al.,* 2018). However, if the areas that have low production also experienced the decline in the pollinator population, it may leads to low possibilities for future agricultural expansion (Giannini *et al.*, 2017). This study aims to examine the pollinator behaviors, pollination efficiency, and their contribution to guava production. Certain limitations such as weather conditions, usage of chemicals and nearby plants may influence both pollinator activity and pollination. Thus, a better understanding of pollination process and cause of decline in pollination are needed to access pollinator limitations.

1.3 SIGNIFICANT OF STUDY

To increase healthy crop production and a better quality of fruits and their abundance, it is important to identify the species that leads to pollination effectiveness as well as food that they feed on such as pollen and nectar. Previous studies found that Hymenoptera, Diptera, Lepidoptera and Coleoptera species as the main pollinators for guava flowers (Kadam *et al.*, 2012; Vinod & Sattagi, 2018). Nonetheless, new pollinator species may be discovered and new insights such as how to control the pollinators towards achieving successful pollination may be found. This study may also provide useful guidance for crop production that might benefit pollinators and pollination improvement.

1.4 OBJECTIVE OF STUDY

Pollinators have a great influence on pollination effectiveness as well as crop production of *Psidium guajava*. Thus, the objectives of this research are:

i. To study the morphological characteristics of flowers and pollen grains of *P. guajava* in comparison with *P. cattleianum* (wild type) and their influence on pollination effectiveness.
ii. To identify and compare the percentage of the pollen viability of guava flowers of different cultivars.
iii. To identify the guava flower visitors and its behavioral activities.
iv. To investigate the relationships between insect body sizes and pollen carrying capacity.

CHAPTER TWO

LITERATURE REVIEW

2.1 GUAVA (*Psidium guajava*)

Guava or scientifically known as *Psidium guajava* belongs to the family of Myrtaceae of Myrtales Order. Guava is sometimes referred to as "the apple of the tropics" (Bose *et al.*, 2019). *Psidium* has around 100 species of tropical shrubs and small trees of the genus and they originate in Mexico, the Caribbean, Central America and the northern part of South America but are now found in all tropical and subtropical regions (Kumari *et al.*, 2013). The tropical and subtropical nations around the world that commercially grow guava including Egypt, Thailand, Colombia, Indonesia, Venezuela, Sudan, Bangladesh, Cuba, Vietnam, the US, Malaysia, Puerto Rico, and Australia and thought to have been introduced to other parts of the world by the Portuguese and Spaniards (Singh, 2011).

The *Psidium guajava* trees is a shallow rooted shrub or a small branching tree around 3 to 10m in height that has a smooth, greenish or reddish brown bark that peels off in thin flakes (Bapat *et al.*, 2020; Kumari *et al.*, 2013). Medina and Herrero (2016) also stated that, as soon as the trunk reaches a diameter of about 20 cm, it becomes hard and woody with a distinctive smooth, light-colored speckled bark that flakes off in tiny flakes. Besides, due to variations in anthocyanic pigmentation, young twigs can be green, yellow, reddish, or red, quadrangular, and downy, with green or brownish-reddish young leaves (Medina & Herrero, 2016). The leaves are slightly aromatic when crushed (Kumari *et al.*, 2013). The light green mature leaves are opposite, smooth, sub-chartaceous, fragrant, and have noticeable lateral veins on the underside with variety of elliptic to oblong-lanceolate shape, seldom round, 4-10 cm long and 2.5-6 cm wide, with an attenuated, apiculate, or widely cuneate apex that is typically obtuse or acute, and short petioles that are 2-7 mm long (Pontikis, 1996; Medina & Herrero, 2016). Apart from that, other species of *Psidium* namely *Psidium cattelainum* or commonly known as Strawberry guava classified as a small fructiferous evergreen shrub or ornamental shrub of a drooping tree habit around 1m to 4m in height, has a slender, smooth and sturdy branches with the ovate leaf shape that appeared dark green in color when matured, glossy and tough like leather (dos Santos Pereira *et al.*, 2018; Patel, 2012; Sharma *et al.*, 2010). The fruit is small with the length of 2.63 cm and diameter of 2.47 cm (Sharma *et al.*, 2010).

P. guajava fruit has a variety of shapes such as round, oval, cylindrical, pyriform, oblate, ellipsoidal, or any combination of those shape with rough to smooth surface and free of pubescence (Medina & Herrero, 2016; Singh, 2011). The outer skin may be silky and delicious or tough and can have a bitter taste with different thickness between species, and it is typically green before it is fully matured and turning yellow, maroon, or green when ripe (Kumari *et al.*, 2013). Guava fruit is a berry with the soft and tasty pulp that appears white, cream, pale pink, pink, dark pink, orange-pink, salmon red and has yellowish seed cavity that may be small to large with a lot of hard to semi-hard incorporated into it (Pontikis, 1996; Singh, 2011) . Nonetheless, some uncommon types feature chewable, squishy seeds (Medina & Herrero, 2016). Fruits are medium to large in sizes, weighing an average of 100 to 500 g, measures 5 to 12 cm long by 3 to 10 cm wide (Medina & Herrero, 2016; Singh, 2011). Bapat *et al.* (2020) also described that guava fruit is globose, ovoid, or pyriform, 4–10 cm in diameter, weighs about 100–450 g and it is encircled by calyx lobes. Mature fruit has a rich, sweet aroma that is occasionally accompanied by varying degrees of a musky aroma (Medina & Herrero, 2016).

Guava has an exceptional good flavor and tastiness with high digestive and nutritional value that are easily accessible at comparatively average price (Bose *et al.*, 2019). Guava fruits that are fully grown have exceptional nutritional content and should be consumed regularly (Medina & Herrero, 2016). They are riches in vitamins, fatty acids, fibre, tannins, phenols, triterpenes, flavonoids, essential oils, saponins, carotenoids, and lectins (Chambers, 2011; Medina & Herrero, 2016). Kumari *et al.* (2013) mentioned that, guava has 200 gm of vitamin C per 100 gm. Researches also suggest that guava have multipurpose in medicinal applications and are beneficial to human health since the chemical compound in *P. guajava* have pharmacological effects (Barbalho *et al.*, 2012). Likewise, a number of chemicals isolated from guava plants have shown promising activity in many human trials (Kamath *et al.*, 2008). Guava leaves are used for treating coughs and lung diseases, the bark is used for treating diarrhea among children and the flowers are used for treating bronchitis, eye sores and cooling the body (Kumari *et al.*, 2013). The main components of guava leaves are phenolic compounds, isoflavonoids, gallic acid, catechin, epicathechin, rutin, naringenin, kaempferol, meanwhile the pulp are abundant with ascorbic acid, carotenoids (lycopene, β-carotene and β-cryptoxanthin) and not to forget the seeds, skin and barks possess glycosids, carotenoids and phenolic compounds (Barbalho *et al.*, 2012). Besides, *P. guajava* is primarily known for its antispasmodic and antibacterial effects and used as a hypoglycemic medication apart from possess an antioxidant, hepatoprotective, anti-allergy, antimicrobial, antigenotoxic, antiplasmodial, cytotoxic, antispasmodic, cardioactive, anticough, antidiabetic, antiinflammatory, and antinociceptive activities (Gutiérrez *et al.*, 2008).

2.1.1 Floral Traits

Flower is the reproductive structure in plants that consists of organs required for reproduction. Therefore, flowering is an important process in the reproduction of most plants. Guava bears the hermaphrodite flower that produces gametes which normally associated with both male and female sexes. Partap (2010) mentioned that the typical hermaphroditic flower consists of four parts namely, calyx (sepals) that provides protection to other floral parts in the bud stage, corolla (petals) that helps attract insect pollinators and provides a platform for insects to land, androecium (male sex organ) and gynoecium (female sex organ). Androecium is composed of stamens consisting of filaments and anthers which produces pollen grains, on the other side, gynoecium is made up of pistils which contains ovary, a style, and a stigma where pollen grains attached when receptive. The calyx is whole in the bud but divides into irregular four to six lobes that are reflexed, hairy, and persistent and measure between 1 and 1.5 cm (Bapat *et al.*, 2020). The flower type, shape, color, odor, nectar and structure are very important for the types of pollinators that visits them (Halder *et al.*, 2019).

Guava flowers appeared white in color (Alves & Magalh, 2006; Medina & Herrero, 2016; Pontikis, 1996). Color is predominantly derived in living flowers through the presence and interactions of pigments such as carotenoids and flavonoids, including anthocyanins, supplemented by chlorophyll and metal ions, and frequently amplified by structural features (Rudall, 2020). In general, carotenoids, flavonols, and anthocyanin pigments are responsible for producing the colors yellow, white, blue, purple, and red in flowers, respectively (van der Kooi, 2021). Because pollinators have fundamentally different visual systems from humans, including sensitivity to distinct wavelength ranges, flowers shouldn't just be categorized based on how colorful they appear to an observer (Arnold *et al.*, 2010). White flowers, which appear so plentiful to humans, are almost entirely UV absorbing and thus not white (equally reflected over the optical spectrum) to insects (Kevan *et al.*, 1996). Guava flowers are big and emit sweet odor during the day, besides it flower without depth and consists of anthers with a lot of pollen which indicates a tendency towards melitofilia (Alves & Magalh, 2006; Medina & Herrero, 2016). The white bisexual or perfect flowers with measurement around 2.5 cm in diameter appeared solitary or in clusters of two to three produced in new growth from mature branches with numerous stamen and abundant pollen (Pontikis, 1996; Medina & Herrero, 2016). Moreover, Bapat *et al.* (2020) stated that guava flower are perfect and epigynous which can be found singly or in two- to three-flowered axillary cymes around 2.5 to 3 cm diameter. Both terminal and lateral stems are capable of bearing flowers (Sharma *et al.*, 2017). Flowers having four to five petals, but they can frequently have ten petals arranged in two sets of five, the petals are white, pubescent of 1 to 2 cm long obovate, while the pedicels are 1.5 to 2.5 cm long and pubescent besides having numerous stamens around 1 cm with many pollen grains and pale yellow anthers at the tip (Medina & Herrero, 2016). The numerous stamens are arranged in rows on a disc. The ovary has four to five locules with a capitate stigma and greenish-yellow filiform style (Bapat *et al.*, 2020).

2.1.2 Floral Phenology

Guava undergoes continuous flowering and fruiting throughout the year under mild subtropical and tropical conditions (Singh, 2011). In Bangladesh, guava normally undergoes flowering twice a year, once in March to April and another in October to November (Bose *et al.*, 2019). In India, flowering seasons were observed throughout the year during spring (April-May) and autumn (August-September) under semi-arid conditions (Sharma *et al.*, 2017), and during the rainy season (May-June) and winter (October-November) (Sachin *et al.*, 2015; Vinod & Sattagi, 2018). The time and duration of flowering vary depending on the cultivars and from region to another region. Research done by Singh (2011), recorded that different cultivars start flowering in different time in different season, for instance, the cultivars Hisar Surkha and Lucknow-49 were the earliest to start flowering in spring and autumn season, respectively. In addition, the writer stated that among cultivars studied, Hisar Safeda and Hisar Surkha had the longest blooming periods i.e., 41 and 45 days and 38 and 43 days, whereas, Lucknow- 49 and Allahabad Safeda had the shortest blooming periods i.e., 37 and 40 days and 33 and 38 days during both the season of flowering i.e., spring and autumn, respectively. According to Salazar *et al.* (2006), mid-spring marks the start of the vegetative growth period, which lasts until autumn where the guava tree (*P. guajava* L.) goes through various physiological changes during this time that can be seen as increases in the diameter of the trunk, shoot growth, bud swelling and breaking, and reproductive phases like flower initiation, fruit setting, and ripening. However, regardless of the season, flowers can be produced on newly growing lateral branches, allowing for continual flowering (Medina & Herrero, 2016). Furthermore, the flowering time can predict the harvesting season. For instance, flowers during the spring season started to bloom in February and March, while the fruits were gathered in July and September (rainy season), also winter flowering (October–November) generated fruit in March–April, and then monsoon season blossoming produced fruit in November–January (Singh *et al.*, 2015). Besides, guava tree pruning is a crucial management practice that enables year-round staggered fruit production (Silva *et al.*, 2016). Padilla-Ramirez *et al.* (2012), stated that the time from pruning to flowering ranged from 60 to 115 days in 2011, depending on the pruning dates, whereas the time from flowering to the start of harvest ranged from 100 to 180 days.

The growth phase of guava can be split into the process of flowering from the shoot and bud development and the process of fruit maturity. Studies by Bose *et al.* (2019), stated that different guava genotypes requires an average of 19.70 days for flower bud to develop where the shortest day required is 17.60 days and the longest is 23.20 days. In order to reach flowering, the floral bud underwent eight separate stages of development (Table 2.1), taking 39–41 days in the spring flowering season and 36–40 days in the fall flowering season (Sharma *et al.*, 2017). Bose *et al.* (2019), recorded different genotype required different days for bud development with the longest time recorded is 23.20 days while the shortest time to 17.60 days. Meanwhile, depending on when the pruning took place, there were 11 to 15 days between the pruning and the start of the bud sprout, in comparison the unpruned tree displayed bud emergence in 2–3 days (Singh *et al.*, 2015). Sharma *et al.* (2017), discovered that axillary flower buds are not produced uniformly across the shoot and seem dispersed since on the same shoot buds in the axils of some leaves produce flowers whereas others do not. Even though flowering was dependent on the location and orientation of the branches on the tree, the pattern of growth of specific buds from chosen trees typically resembled that of the entire orchard (Salazar *et al.*, 2006).

The duration of guava flower also differs from genotype to another. Bose *et al.* (2019) said that the duration of the flowering period is a crucial trait in a genotype of high quality. According to Sharma *et al.* (2017), the guava cultivars studied showed a different flowering duration starting from bud development until blooming in spring and autumn where, longer duration of flowering around 38 to 45 days happens in autumn and around 33 to 44 days in spring. Meanwhile Bose *et al.* (2019), recorded that an average days for the flowering duration is 11.64 days where the shortest flowering duration is 10.40 days and the longest duration is 14.20 days. In addition, the flowering period during February and September pruning ranged from 78 to 93 days from the opening of the flower (Singh *et al.*, 2015). The length of the flowering period may vary based on genetic or environmental factors Bose *et al.*, (2019).

Table 2.1 Stages of guava flower development (Sharma *et al.*, 2017).

Stages	Days	Characteristics
1 to 2	7 – 11	Two thin, green bracteoles serve as a shield.
2 to 3	9 – 11	Conical shaped with clearly distinct pedicels
3 to 4	5 – 7	A constriction appears below the middle.
4 to 5	4 – 7	Constriction became more visible, clearly delineating the ovarian portion below, while the upper portion plummeted.
5 to 6	4 – 6	The upper or free part began rounding and developing faster than the lower or adnate part. The bud's color remained green until this point.
6 to 7	4 – 6	Completely formed bud. At this stage, their size and shape were distinct to each cultivar.
7 to 8	3 – 5	Known as balloon stage which is characterized by the calyx splitting, that occurs 13 to 26 hours before the flower opens. The calyx detached as a cap or divided unevenly.
8	1	Flower fully bloomed

The number of days needed for fruit maturity may vary based on the genetic makeup of the plant or the availability of water and other nutrients Bose *et al.*, (2019). Typically, it takes 100 to 150 days for guava fruit to mature from full bloom to harvest (Singh, 2011). Meanwhile, Sachin *et al.* (2015) recorded that fruits mature between 105 and 140 days after fruit set. In other studies, guava reaches maximum maturity 128 days following fruit set (Salazar *et al.*, 2006). Also, maturity of fruit depends on genotypes where the highest number of days noted is 115.8 days while the lowest number of days is 85.8 days for fruit maturation (Bose *et al.*, 2019).

Furthermore, fruit exhibits changes such as a reduction in hardness, chlorophyll, and tannins during the final stage of growth, along with the greatest increases in weight and diameter (Singh, 2011). Bose *et al.* (2019) stated that the greatest fruit length recorded is 9.34 cm while the shortest fruit is 6.16 cm, with the diameter of the highest fruit of 26.28 cm and lowest diameter of 16.30 cm. According to Singh (2011), the mature size of the seedless fruit was less than half that of the seeded fruit and reached their greater diameter on the 120th day following pollination. Guava tree phenology variations between sites or trimming times were attributable to the rate of heat unit accumulation at each location and stages pruning to fruiting and fruiting to beginning of harvest of guava trees required 800–850 and 1,950–2,000 heat units, respectively resulting in a total of 2,850 to 2,900 cumulative heat units needed for guava trees to finish their growth cycle (Padilla-Ramirez *et al.*, 2012).

The environmental factors and cultivar type also have a significant impact on how long each growth phase lasts (Singh, 2011). Similarly, Salazar *et al.* (2006) said that the growth cycle is influenced by both climate factors and plant genotype. In guava cultivars studied by Sharma *et al.* (2017), the number of days needed to complete one stage before next stage gradually decreased as the season progressed. Studying the guava's phenological stages is crucial for excellent agricultural practices and crop management, in addition to aiding in the planning and programming of pruning and harvesting (Silva *et al.*, 2016).

2.2 POLLEN ANALYSIS

Pollen is male reproductive cells produced by anther. Pollen grains or microgametophytes are fine powders that seed plants produced which are necessary for plant sexual reproductions. Pollen found on or in insects can provide information about the plant species visited, the pollinator's capacity for long distance dispersal, migration paths, and whether the pollinators are visiting and dispersing genetically modified plants. Besides, the study of pollen morphology is able to identify the pollination process, foraging resources, and source zones of pollinators. According to Jones and Jones (2001), the use of pollen in these studies is justified for a number of factors in a way that pollen grains are distinct, quickly recognized, and oftentimes species-rank specific which makes it possible to gather very specific knowledge. Next, sporopollenin, the component of pollen, is resilient and resistant to decomposition as well as the geographic location of the plant from which the pollen originated can be identified based on the pollen's identification. Pollinators visits flowers and able to yields crops such as apples, almonds, peaches, melons, and other fruits, however some are harmful to orchards, gardens, and crops so it is important to identify the pollen on insects' bodies to determine what other plants besides crops these pests attack and whether they "migrated" from somewhere else (Jones, 2012).

Additionally, it is crucial to understand the basic characteristics of a pollen grain, such as its symmetry, shape, size, number, and position of apertures, as well as any ornamentation. Pollen comes in a variety of shapes such as spherical, elliptic, or triangular. Areola, clava, echinus, foveola, fossula, granulum, gemma, plicae, reticulum, rugulae, striae, and verruca are only a few examples of the ornamentation elements that can have a wide range in size and shape including exine ornamentation of meshed, granular, grooved, spined, striated, or smooth (Evrenosoğlu & Misirli, 2009; Halbritter *et al.*, 2018). Exine pattern of pollen are important for several reasons such as, to interacts with biotic and abiotic pollination vectors, affects the surface area of the stigma interface, to mediates stigma adhesion, retains pollen coat and affects wall strength and elasticity (Edlund *et al.*, 2004).

Pollen sizes ranges from less than 10 µm to more than 100 µm. It is advised to classify pollen size into the following ranges: very small (10 µm), small (10–25 µm), medium (26–50 µm), large (51–100 µm), and very large (>100 µm) (Halbritter *et al.*, 2018). Pollen sizes are important for pollinators preferences, both biotic and abiotic, and fluid dynamics (Edlund *et al.*, 2004). Hao *et al.* (2020), suggested that pollen grain size and pollinator activity are related where large pollen would be preferred in species with little pollen exploitation, whereas large numbers of smaller pollen grains may improve reproductive success in species visited by pollen-collecting forages by increasing the likelihood that some pollen grains may not be groom. Species whose pollen is collected or consumed have noticeably smaller pollen grains. Besides, pollen size may influence three aspects of pollen transport including pollen removal from anthers or secondary presentation sites, loss during transit, and/or pollen deposition on stigmas. These conditions will inadvertently affect plant pollination.

2.2.1 The Viability of Pollen Grains

Pollen quality is evaluated based on the viability, speed of germination of pollen grains and pollen tube growth (Sulusoglu & Cavusoglu, 2014). Pollen viability is important for the sexual reproduction success in plants (Bots & Mariani, 2005; da Silva *et al.*, 2017). The characteristics of pollen viability can be influences by genetic factor, environmental factor and the age of plant (da Silva *et al.*, 2017). Besides, pollen viability may be affected at various stages of development, however, the most of the factors that influence pollen viability occurs after release from anther as the most direct interaction with the environment occurs at that stage (Bots & Mariani, 2005). Pollen viability can be assessed by different methods such as staining, in vitro and in vivo germination tests or analyses the final seed set (Abdelgadir *et al.*, 2012). Pollen germination rates provides reliable data on pollen viability compare to vital stains (Bots & Mariani, 2005). Staining tests are faster and easier than pollen germination tests, however, it is necessary to perform germination tests to observe the actual viability of pollen as staining method often shows false positive results (Bots & Mariani, 2005; Sulusoglu & Cavusoglu, 2014).

Based on the previous pollen viability research, it is possible to increase the productivity and fruit quality by selecting the pollinating genotypes, however, it is necessary to have broader understanding of the characteristic of pollen viability in species (da Silva *et al.*, 2017). The study of pollen viability rates may estimate the outcrossing risk where the longer periods that pollen remains viable will have more chance on outcrossing but it can be achieved through an experimental study by using bait plants and sensitive techniques to detect outcrossing events (Bots & Mariani, 2005). In addition, pollen viability study able to provide information about the genotypes of guava that has a good potential to generate viable pollen which will be useful for genetic breeding experiments (Coser *et al.*, 2012; da Silva *et al.*, 2017)

2.3 POLLINATION BIOLOGY

Pollination is an ecological process that provides essential services to humans (Gupta *et al.*, 2014). Recently, the Convention of Biological Diversity (CBD) acknowledge pollination as a main factor in the preservation of biodiversity as well as functional ecosystems (Partap, 2010). Pollination can be described as the process of transferring pollen to the stigma of plants that allows fertilization and reproduction to take place, it holds great importance as it gave rise to the formation of fruits and seeds that continues the plant life cycle (Vinod & Sattagi, 2018). The pollen is transferred from anthers, which are the "male" or pollen-producing parts of the flower, to the stigma, or the "female" component of the flower. From the perspective of biological evolution, the goal of flower pollination is the survival of the most fit and the best possible reproduction of plants in terms of both quantity and fitness which also regards as the process of plant species optimization (Yang, 2012). Pollination can happen within the same plant or even the same flower, which is known as self-pollination, or it can happen between two other plants, which is known as cross-pollination. Angiosperms have two mating systems namely, xenogamy where cross-pollination occurs as a result of pollen transfer between different flowers of different plants of the same species, and autogamy where self-pollination occurs within the same flowers or it occurs between different flowers of the same plants (Delaplane *et al.*, 2013).

2.3.1 Mode of Pollination

Most angiosperms require pollination to set seeds and fruits, which rely on agents to vector the pollen. There are two main types of pollination which are abiotic and biotic. Approximately 90% of flowering plants relies on biotic pollination, in which pollen is spread by a pollinator like insects or other animals, whereas abiotic pollination, which doesn't require pollinators, accounts for about 10% of all pollination (Yang, 2012). Abiotic pollination relies on environmental factors like wind and water to transfer pollen between flowers. Water pollination is said to be uncommon, accounting for fewer than 2% of all flowering plants (Les *et al.*, 1997). According to Friedman and Barrett (2009), around 10% of angiosperm species rely on wind pollination when pollinators are in short supply to provide reproductive assurance. Pashte and Kulkarni (2015) mentioned that a well-pollinated flower will contain more seeds, with increased ability to germinate, producing larger and better-shaped fruit thus proper pollination will increase fruit size on yield, accelerate maturity, and produce fruits of more symmetrical shape.

Biotic pollination which is far more frequent, involves the assistance of other organisms called pollinators to spread pollen. Entomophily, also known as insect pollination, is a type of pollination in which insects spread pollen from plants, primarily but not exclusively flowering plants. Partap (2010) reported that the more than 70% of plant species rely on biotic pollination agents to transport the pollen. It is estimated that more than 1,300 species of plants are grown worldwide for food, beverages, medicines, condiments, spices and even fabric and are almost 75% pollinated by animals (Das *et al.*, 2018). According to Vinod and Sattagi (2018), the survival and reproduction of several wild plant species is dependent upon pollinating agents. The common entomophily flowers are large, hermaphrodite, rarely monoecious or dioecious, exhibits abundant of sticky pollen with ornament, pollen kit or devices for mass transport, generally has many ovules, stamen and stigma often inside corolla (Pacini, 2015; Yamasaki & Sakai, 2013). Additionally, insect pollination is also a necessary step in the production of most of the fruits and vegetables that we eat and in regrowth of many feed crops used by livestock (Gupta *et al*., 2014). Malaysia is rich in native pollinator such as bees, stingless bees and bats where some of these species can be used for pollination of some agricultural crops such as starfruits, guava, citrus, mango, watermelon, durian and coconut (Norliyana *et al*., 2019). Guavas are also one of the most important crops that were pollinated by insects. Previous studies by Vinod and Sattagi (2018) found three different insect's order that visited organic and conventional guava ecosystem which includes Hymenoptera, Diptera and Lepidoptera species where Hymenoptera species was the most dominant pollinator group that accounted for maximum relative abundance of 95.85% for organic guava ecosystem and 94.42% for conventional guava ecosystem. Whereas Kadam *et al.* (2012) found in their researches that the guava insect visitors belong to Hymenoptera, Diptera and Coleoptera order. Vinod and Sattagi (2018) mentioned that the important pollinators for guava ecosystems consists of *Apis dorsata*, *Apis cerana*, *Apis florea*, *Tetragonula iridipennis*, *Amegilla cingulifera*, *Xylocopa* sp., *Lasioglossum* sp. etc. of Hymenoptera.

2.3.2 Pollination Effectiveness

Pollinators attributes and characteristics are greatly contributing to the effectiveness of pollination. According to Faheem *et al.* (2004), pollinators vision, olfaction and taste, anatomy, food preferences, behavior and learning ability are partly responsible for pollination effectiveness. Flowers are dependent on vector either wind, water, birds, insects and other animals that visits flowers to move pollen (Halder *et al.*, 2019). Flowers usually attract pollinators and in return, pollinators will contribute to plants' reproductive success. The pollinator's effectiveness of the insects is important to estimate the nature of insects to transfer pollen between flower and umbel by determine the follower visitation rate in a unit of time (Jacobs *et al.*, 2010). Pollinators visit flowers continuously in order to gain rewards apart from nectar and pollen, they also received caloric reward, energetic, protection and oviposition sites (Faheem *et al.*, 2004). Insect's vision are said to be extends from ultraviolet at ca 300 nm (UV) to yellow orang at ca 650 nm (Faheem *et al.*, 2004). In addition, the most common form of color vision in insects is the presence of three color receptors sensitive for UV, blue, green that evolved before floral color evolved which proved that the basal insect lineages are much older than flowering plant and thus, it suggests that floral color evolved due to insect color-vision system under broad perceptual bias (Schiestl & Johnson, 2013). Other than that, olfaction and taste also affect pollination effectiveness. Honeybee's olfactory capability are said to be 40 times better than human thus it can locate food resources and communication inside and out of shelter (Faheem *et al.*, 2004). Schiestl and Johnson (2013) suggests that wasp can rely entirely on olfaction to locate flower. Not to mention, flower scents are important for long distance signal when colored objects cannot be seen from far away, as an example, bees rely on achromatic signals provided by green receptor contrasts and not colored signal and thus, bees should only distinguish flower colors from background environment or differentiate between colors when near to the flower where at a point they already immersed in floral odors (Dötterl & Vereecken, 2010). According to Frankie and Thorp (2009), insect-pollinated flowers produces signals such as odors, colors, shapes, textures, and tastes that combined into patterns that has been acknowledged as syndrome related to the type of pollen vector. For instance, butterfly-pollinated flower that would be red, have an odor and landing platform which nectar usually hidden deep in the tube base that can be reached by coiled butterfly proboscis and contains nectar that high in amino acids and flowers bloom during the day and in contrary, a hawk moth-pollinated flower usually white colored that has a strong sweet odor but lacking a landing platform, have long stamen with freely swinging anthers and bloom at night (Frankie & Thorp, 2009).

2.5 ROLE OF POLLINATORS IN AGRICULTURAL ECOSYSTEM

Pollinators are animals that are commonly known as pollinating agent which includes birds, butterflies, bats, flies, beetles, wasps, and bees. It is very important for pollinators to forage on plants or flowers to ensure fruit production for crop productivity. An abundance of pollinators sets a greater proportion of early flowers, results in an earlier and more uniform crop with higher quantity as well as fruit quality (Halder *et al.*, 2019).

Bees are the most numerous and successful pollinators as they rely entirely on flowering plants and they only consume pollen and nectar throughout their lives (Halder *et al.*, 2019; Willmer & Finlayson, 2014). *Apis cerana* or honey bees are also the most dominant and most efficient pollinator of mango (Deuri *et al.,* 2018), guava (Kadam *et al.,* 2012), and eggplant (Gemmill-Herren & Ochieng', 2008). Equally important to bees, other insects such as beetles, wasps, butterflies and moths also pollinate at certain rates and among mammals, bats are primarily responsible for pollination of a large numbers of plants such as agave and cactus while the main pollination birds are hummingbirds, honeyeaters, sunbirds and perching birds (Das *et al.*, 2018).

Moreover, pollen attached to the body of an insect also determines the reproductive success of guava. Pollinator species may have varying pollen carrying capacity (Huda *et al.*, 2015). This may be due to different sizes, shape and morphology of each individual species. Gupta *et al.*, (2014) also mentioned in his research that, the abundance and diversity of pollinators eventually results in healthy pollination between plants and their pollinators. It is also important to observe the behavioral activities of insects during foraging because different species of insects often display different foraging behavior.

The modification of insects behavior also affects the evolution of floral characteristics where their behavior often affected by weather, distance of food source, food quantity and quality (Faheem *et al.*, 2004). Insects foraging behavior varies with opening period of flower, temperature and light intensity and the peak foraging activity of insects on guava flower are at peak during 11.00 h of the day where the air, temperature and light intensity are sufficient for insect activity (Amin *et al.*, 2019). Application of pesticides may affect foraging activity, therefore, it should be permissible after total cessation of foraging by pollinators occurs as it would help in augmentation and conservation of pollinators while maximizing the pollination effectiveness to increase crop productivity (Kadam *et al.*, 2012).

2.6 FACTOR INFLUENCES POLLINATION AND POLLINATOR ABUNDANCE

Decreasing pollinators may directly impact the food production stability and consumer prices, while declining availability of fruits and vegetables can affect consumer health worldwide (Das *et al.*, 2018). Certain factors need to be considered as it may influence the pollinator activity on foraging as well as affecting the pollination services. According to Bose *et al.* (2019), fruits that are produced in different or even in the same areas may have different types of variations and some may due to the genetic and some may be because of the environment. Weather plays a significant role in determining the pollination success. Cold weather and wind affect the activity of bees in search for food and therefore, the colony should be place in a sunny, sheltered location that prevents wind to encourage maximum flight in springs (Partap, 2010). In addition, pollinators have the potential to face the worst conditions due to climate change and might affect crop production (Giannini *et al.*, 2017)

Besides, the loss of natural pollinators are due to the chemical distributions in agriculture, for example, the use of pesticides and fertilizers (Gupta *et al.*, 2014). Das *et al.* (2018) also suggested that the main problem confronting the bee breeding industry is the loss of bees due to pesticides usage. All environmental toxins that affect the health of the colony also affect the effectiveness of the colony as a pollinating unit by changing the activity of finding food and other effects can also be due to the intentional use of chemical attractants and repellents on flowering plants (Delaplane *et al.*, 2013). Similarly, Kadam *et al.* (2012) also proposed that the application of pesticides at blooming stage will prevent the productivity in comparison to crop protection by dint of pollination deficit.

Research done by Partap (2010), found that bees sometimes neglect crops to pollinate and feed on other more attractive crops such as weeds near crop areas, therefore, it is necessary to eliminate weeds to avoid competition in attracting bees and to prevent reduction of crop productions. The population of wild, native and controlled pollinators is decreased at an alarming rate due to the changes in food and nesting habitats, depletion of natural ecosystems, pesticide poisoning, alien species, diseases and pests, over-collecting, human activity, climate change, smuggling and trading of certain rare and endangered species (Das *et al.*, 2018).

2.7 CROP PRODUCTION AND FRUIT PRODUCTIVITY

Studies on the economic benefits of essential agricultural ecosystem services, such as crop pollination by insects, are fundamental to sustainable food production and farm management (Das *et al.*, 2018). Pollination ensures better crop yields whether in grain, seed or fruit crops and is also required for the preservation of biodiversity (Partap, 2010). Insects also plays a huge role as pollinators in agriculture crop and have direct impact on flora and fauna due to its pollination activities (Vinod & Sattagi, 2018). Halder *et al.* (2019) reported that insects accounts for 15% to 30% of global food production. In accordance with Pashte and Kulkarni (2015), animal pollination is an important ecosystem service because plant crops comprise 35% of global plant-based food production benefiting from animal-mediated pollination hence having adequate pollinators during flowering is essential to producing a sustainable crop. Pollinators such as bees, birds and bats affect 35% of the world crop production which increases production of 87 of the world's leading food crops and the most benefiting items are fruits and vegetables (Das *et al.*, 2018). Although climate change may worsen the conditions of pollinators, pollinators can find new suitable areas that have the potential to increase crop production (Giannini *et al.*, 2017).

Guava is said to be an important fruit crop in the tropical and subtropical region (Boora *et al.*, 2016; Rai *et al.*, 2010; Singh, 2011). Hiwale (2015) stated that guava is a popular fruit crop in India because of its wider adaptation to soil and climatic conditions as well as its ability to produce fruits throughout the year. Moreover, *P. guajava* is a popular fruit crop due to its high nutritional value with abundant sources of citric acid, minerals and vitamins C and contribute significantly in food and nutritional security (Kadam *et al.*, 2012). Insect-pollinated crops can provide a substantial nutritional resource to pollinator communities, which may help maintain their populations over generations besides improving their reproductive potential of pollinators and promoting more efficient pollination of crops in current and subsequent years (Bailes *et al.*, 2015).

In addition, the importance of guava fruit can be further amplified along the evolution of a diversification which possesses a combination of big fruit size, appealing red flesh, good flavour and seedlessness (Sharma *et al.*, 2017). The guava yields fruit is important for fresh consumption and for industrial processed products such as jams, jellies, pastes, fresh-cut salads, juice, nectar, paste, puree, concentrates, candy bars and other similar products (Pontikis, 1996; Medina & Herrero, 2016; Singh, 2011). In general, red-fleshed cultivars are recommended for processing and white-fleshed cultivars for dessert (Singh, 2011). Not to forget, natural selection and breeding procedures produce a variety of genotypes with the primary goal of enhancing fruit market attributes in terms of agronomical characters and the content of antioxidant molecules, vitamins, and minerals, all of which are crucial for food and human health (Medina & Herrero, 2016).

CHAPTER THREE

MATERIALS AND METHODS

3.1 STUDY SITES AND PLANT MATERIALS

The field samplings were conducted at the private owned orchard located in Kg. Lebak Temerloh, Pahang at coordinate 3°23'20.9"N 102°24'44.3"E (Figure 3.1). Guava trees are grown in red soil, which is commonly used for crop cultivation due to their fertile and well-drained nature. Guava trees make up the majority of the orchard's plantings, but there are also a few durians, mangosteen, and banana trees planted scarcely in the area. The study has been conducted from January to July 2021. There is no fixed time for flower sampling as flower availability depends on the season and cultivar. However, pollinator studies were conducted from March to July 2021 at fixed interval as explained in section 3.4.

The plot covered an area of about 1 acre, planted with approximately 125 Lohan guava trees around 3 years old. The guava trees were planted with a distance of 2 m x 2 m but there is some bare area (or planted with different fruit tree) when guava trees were removed due to the damaged or dead trees. Figure 3.2 shows the arrangement of guava trees in the study plot. The orchard was managed conventionally with the application of Legacy insecticide and NovaTec® Premium 15-3-20(+2+TE) fertilizers, which were conducted regularly once every month. Besides, the neighborhood area has a diverse range of crops, including durian, banana, and rubber tree. The daily mean temperature in the area ranges between 25°C and 29.7°C with mean relative humidity of 74.0% to 89.5%, precipitation (08-08 MST) of -33.3 mm to 68.8 mm and mean wind of 0.4m/s to 1.2 m/s.

Figure 3.1 Lohan guava plot in Kg. Lebak, Temerloh, Pahang.

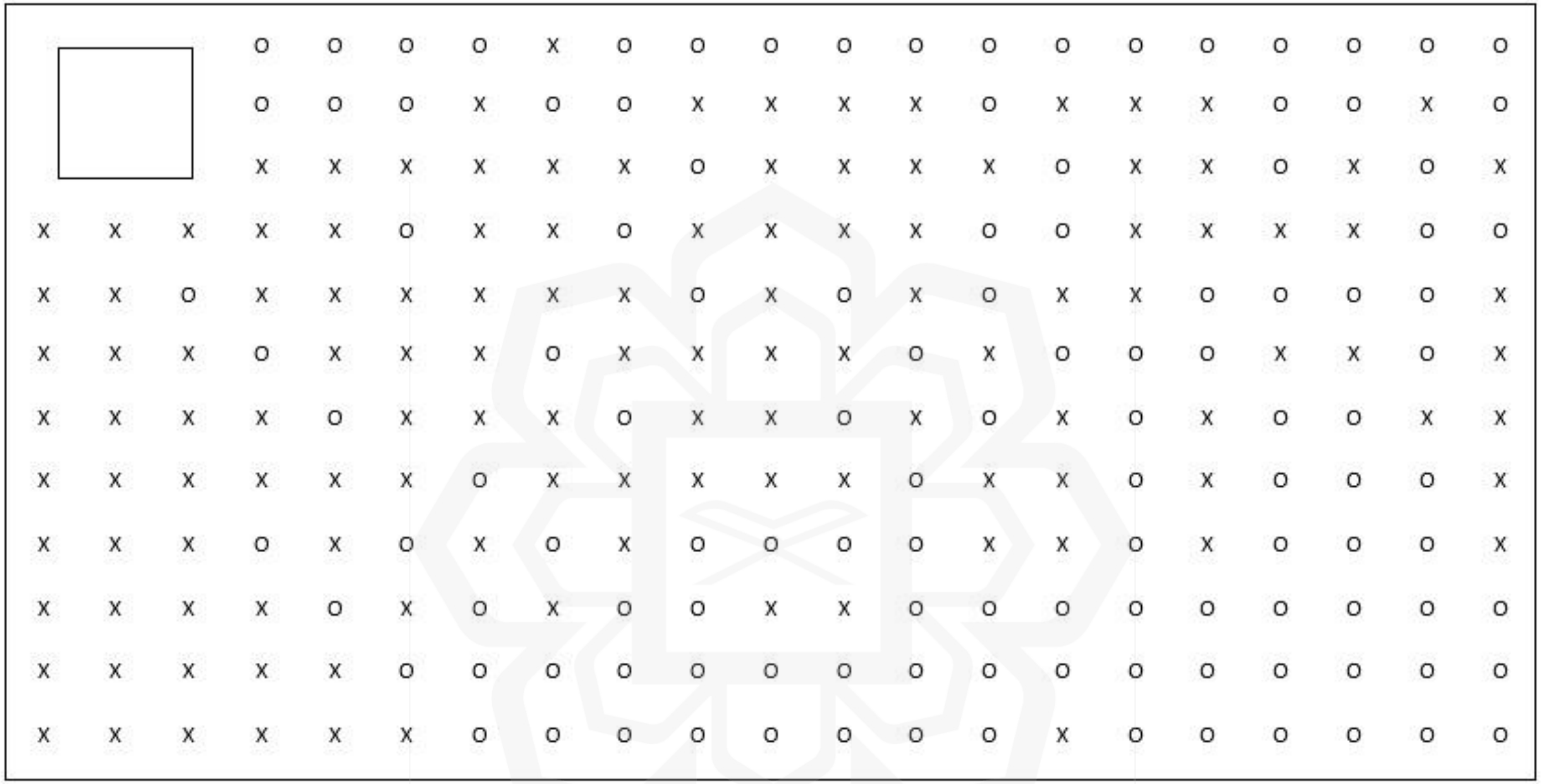


Figure 3.2 The arrangement of guava orchard. X: guava tree, O: absence plot, □: rest hut.

In addition, different cultivars of guava, such as Vietnam Seedless, Bo Chi, Kim Choo Red, Kim Choo White, Kristal and Pink heirloom as well as another species of *Psidium* (*Psidium cattleianum*) were also collected from a random number of trees in Kuantan and Kuala Terengganu to be used for specific experiments in this study. The number of cultivars used is not standardized for all experiments due to limited access to available flowers. Other than Lohan that was collected from the farm with abundant trees, while others were planted as ornamental plants in the housing area. Furthermore, seasonal flowering, which varies between cultivars, makes the sampling more difficult to organize.

3.2 MORPHOLOGY OF *Psidium guajava* and *Psidium cattleianum*

The general characterization of different parts of the guava tree (i.e., trunk/bark, leaf, shoot, flower, and fruit) and fruit development stages of *Psidium guajava* were recorded and photographed for the Lohan cultivar from Temerloh guava orchard. Meanwhile, *Psidium cattleianum* were photographed from house garden in residential area at Kuala Terengganu. However, the explanation on guava morphology only being made briefly on other parts (for identification purpose) except for floral structure as it mainly involved in the pollination process.

3.2.1 Characterization of Floral Structure in Different Guava Species

Fresh flowers of *P. guajava* cv. Lohan and *P. cattleianum* were collected from the field and placed individually in a specimen container (urine bottle, 60 ml), then, brought back to the laboratory for the study of floral structure under a dissecting microscope. The different floral characteristics such as the colors of sepals and petals, the quantity and arrangement of floral parts were recorded. The measurements were recorded for the flower sizes (size of sepal and petal), number of petals and sepals, size of anther, stigma and filament and estimated anther per flower.

3.2.2 Preservation and Preparation of Pollen Grain Specimens

Pollen grains from several guava cultivars (*P. guajava* cv. Lohan, Kristal, Bo Chii, Kim Choo Red), Pink (heirloom) and species *P. cattleianum* were processed by going through a certain step before being ready for observations under light microscope and scanning electron microscope. The purpose of pollen preparation is to ensure that the sample is not damaged or shrunk as well as no changes happened on the cell structure in order to examine the pollen morphological characteristics.

3.2.2.1 Preparation of Pollen Samples

Flower samples obtained from the field underwent a fixation process immediately after being collected because the flower samples are very easily damaged and shrink. Flower samples were preserved in a bottle containing 80% ethanol to avoid damage (modification from Huda *et al.*, 2015). Before pollen is observed under a light microscope and a scanning electron microscope, pollen samples are cleaned to remove any pollen kit, wax coating or impurities that are on the surface of the pollen so that the image of the pollen is cleaner, and the shape and surface of the pollen can be better observed.

3.2.2.2 Washing Pollen Kits

Pollen samples were washed and cleaned using a method modified from Erdtman (1960) acetolysis technique to avoid the presence of pollen kits. The anthers of the flower are inserted inside a 2 ml Eppendorf tube. Pollen was washed using 0.5 ml of 1% Teepol and spun in the microcentrifuge (Mikro 200, Hettich) for five minutes at a speed of 3000 rpm. 0.5 ml of glacial acetic acid was then added, and the tube is spun again for five minutes. The liquid and sediment are then removed through decantation, and only the anthers are left in the Eppendorf tube. Next, 1 ml of glacial acetic acid was added to the Eppendorf tube containing anthers and soaked in hot water with a temperature of 90 °C to 100 °C for two to three minutes. This acid was added to remove any impurities on the surface of the pollen (descaling agent).

The solution was allowed to cool before being spun at 3000 rpm for five minutes. After discarding the residue, distilled water is added, and the tube was spun for another five minutes to wash away the residue and ensure pollen was deposited at the base of the tube. After discarding the residual distilled water, glycerin was added into the Eppendorf tube as a fixative solution. Pollen samples were then transferred straight from Eppendorf tube to glass slides for light microscopy inspection and subsequently to stubs for scanning electron microscope observation.

3.2.3 Observations Under Light Microscope

Observation of pollen under a light microscope requires appropriate techniques so that the pollen image obtained is in an equatorial view state and accurate polarity views to minimize errors in measurements pollen size. Measurement pollen at the polar view (P) and the equatorial view (E) are applied onto at least three grains of pollen.

In this procedure, a fine hair or eyelashes was used by attaching it to the tip of the skewer using glue (Figure 3.3). The eyelashes pick was used because the pollen sizes are very small as well as not easily broken if used while moving the pollen in the solution. Pollen that has been washed using microcentrifuge in an Eppendorf tube were transferred onto a glass slide carefully so that the structure and shape of the pollen are not damaged. One small drop of clear glycerin was placed on another part on top of the glass slide as an area for individual observation of pollen. Observations were performed under a light microscope Leica DM500 with camera connections. Pollen image taken using Leica Application Suite (LAS EZ) software version 3.0.

3.2.4 Observations Under Scanning Electron Microscope

Cleaned pollen grains were obtained and transferred to the stub before being observed under a scanning electron microscope. Pollen was transferred individually onto the stub with carbon tape (Figure 3.4). Pollen from Eppendorf tube with glycerine as fixative solution was taken and air dried overnight in a glass desiccator before undergoing spray coating process.

The process was continued the next day and the finished stub is then undergoing a spray coating process with gold in the sputter coater (Leica EM SCD005). The observation was done under a scanning electron microscope (ZEISS EVO® 50) because the pollen grains individually are too small and difficult to be find on the stub. Both polar view and equator view were observed, and the sizes of pollen were measured for its length and width.

Figure 3.3 The eyelash pick Stub with carbon tape.

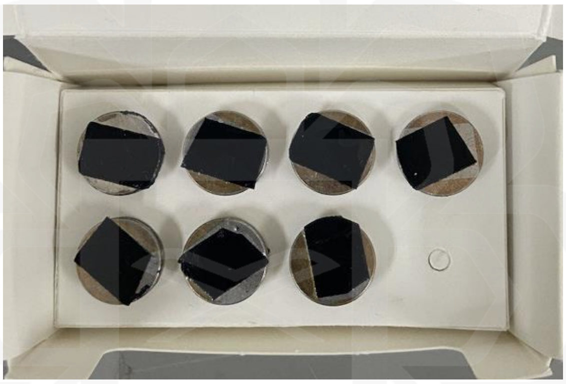


Figure 3.4 Stub with carbon tape.

3.3 POLLEN VIABILITY TEST

Pollen viability is commonly associated with pollination process. Duration of pollen viability varies greatly between species and is related to the type of pollination. In general, plants with entomophilous pollination have pollen with longer and greater viability than those with anemophilous pollination. To examine pollen viability in different cultivars and species of guava, six cultivars of *Psidium guajava* (Lohan, Bo Chii, Kim Choo Red, Kim Choo White, Pink heirloom and Vietnam Seedless) as well as species *P. cattleianum* were used in this test.

3.3.1 Identifying Best Stages of Guava Flower for Pollen Viability Test

A simple study was done to look for the best flower stages and storage conditions that are suitable for the observations of pollen viability under the colorimetric method. Guava buds of different stages (i.e., fully closed buds and buds that nearly at anthesis) on Kristal variety were collected from different trees in private farm located in Lanchang, Pahang. Additionally, fully open flowers were also obtained and stored under different conditions before being brought back to the laboratory for observations under two different staining techniques. Fully opened flowers were brought back fresh as they are, immersed in water and immersed in 80% ethanol to identify the best storage method to travel from the orchard to laboratory. Samplings of the flower were conducted approximately at 8.30 am and flower in each stages were collected in 3 replicates.

3.3.2 Assessment of Pollen Viability by Colorimetric Method

This method was performed to estimates pollen viability with two different staining methods followed from Sulusoglu and Cavusoglu, (2014). Flowers from random trees of each cultivar were used in this study. Unopened flowers were collected at balloon stage and placed on black paper under incandescent lamp on the table overnight to dry. The next day, pollen grains were obtained from the bud by randomly picked 5 anthers and placed into the micro-centrifuge tube. Guava has many anthers (more than 400), therefore only 5 anthers were picked to estimate the percentage of pollen viability. Thus, the data reported in the result is percentage of pollen viability of 5 anthers.

The first staining method is using 1% aqueous solution of 2,3,5-triphenyl tetrazolium chloride (TTC). A drop of staining mixture around 0.1 ml was dropped in a micro-centrifuge tube. The pollen viability count was made after two hours the pollen was placed in a TTC solution. Pollen grains that stained orange or red are considered viable. Secondly, staining was also done using Iodine Potassium Iodide (IKI) and pollen viability counts were made five minutes after pollen were placed in an IKI solution. The observation for the viability of pollen was made using Neubauer-improved haemocytometer at x40 magnification using a compound microscope Leica DM500. Staining percentage was determined by dividing the number of stained pollen grains by the total number of pollen grains per field of view and expressed as a percentage;

$$\text{Staining percentage per 5 anthers (\%)} = \frac{\text{Number of estimated pollen grains}}{\text{Total number of pollen grains per field of view}} \times 100\%$$

3.4 ASSESSING POLLINATOR'S CONTRIBUTION TO PLANT REPRODUCTIVE SUCCESS

There are many methods to assess pollinator contribution to plant reproductive success. In this study, pollinator contribution was evaluated through identification of pollinator visitation frequency and their behavioral during the visits as well as their pollen carrying capacity.

3.4.1 Visitation Frequency and Behavioral Observations

In this study, random field observations were conducted every 6 weeks. The number of single flowers visited by each insect species from the 5 most common genera per 2 minutes observations period were marked as visitation frequency (each individual pollinator's visit activity per unit time). Fifteen individuals from each species were observed for their handling time (periods of insect spend on flowers to collect nectar or pollen) on flower panicles in a 2-minutes observation period using a stopwatch. This is considered as pollination effectiveness of individual species (Jacobs *et al.*, 2010). Meanwhile, the number of visitors per flower per unit time or per patch/panicles of flower in standard 2 minutes observation periods were also be recorded (Gemmill-Herren & Ochieng', 2008). About 20-25 flowers were observed at intervals of 2 hours over 8 hours (09:00 to 16:00). For each visit, the behavior of insects was observed and recorded.

3.4.2 Pollen Carrying Capacity by Pollinators

Individual insects may vary in pollen deposition performance based on their body size as well as their behavior and foraging experience. In this study, pollen load on insects were estimated with the hypothesis that insect with higher pollen carried in its body is more effective pollen depositor. Ten to fifteen individual of each common insect species (*Xylocopa aestuans, Xylocopa latipes, Apis dorsata, Heterotrigona itama, Apis cerana, Ceratina lieftincki*) visited flowers were captured carefully using small zip locked bag for small insects and using net for big insects (> 10mm) and to prevent pollen loss and cross-contamination that might occur if pollinators were collected in nets or plastic bags as well as to prevent the insect groom pollen from their bodies. Each captured pollinator was soaked and washed thoroughly with 2 ml 80% ethanol in a small universal bottle and the dislodged grains were counted. Following the method of Huda *et al.* (2015), the number of pollen grains adhering to insect's body were counted under a stereo microscope Leica DM500 using Neubauer-improved haemocytometer. The haemocytometer is a microscope slide with two counting chambers that have a surface area of 9 mm^2 each with a depth of 0.1 mm when covered with a cover slip. The chamber is divided into nine 0.1 ml small chambers, and 18 small chambers can be studied at once. (Two counting chambers x nine small chambers). The pollen sample in the solution was mixed thoroughly and a drop of suspension was extracted using a dropper and added to the haemocytometer chambers. The entire 2 ml pollen suspensions were withdrawn in five extractions. Each of the haemocytometer's small chambers was able to hold 1/20,000 of the pollen suspension that had originally been placed in each vial, which resulted in a total of 90/20,000 ml (18 small chambers x five drops) of suspension observed for each sample. All pollen grains that were contained in the tiny chambers were counted. Estimated pollen count can be described as;

$$\text{Estimated pollen count} = \frac{\text{A}}{\text{B}} \text{ x C}$$

where A is the number of pollens observed, B is the total chambers x number of extraction and C is the volume of pollen suspensions.

Insect collection for pollen load study were conducted at least for 3 random sessions (days) to achieved required sample size. However due to sampling difficulty and pollinator availability, the number of replicates in this study varied among species collected. Moreover, the insect's body sizes were measured to investigate the influences of various body sizes parameter of flower visitors. Body sizes including head width, head length, body width and body length of all pollinators were measured under dissecting microscope (Olympus SZ61) attached with a digital camera equipped with program Toup View (Hangzhou ToupTek Photonics Co. Ltd.). The sex of each insect was also recorded based on its secondary sexual characteristics and body size as well as behavior observed in field before it being collected.

3.5 STATISTICAL ANALYSES

The data were compiled and tabulated properly for statistical analyses on the different parameters under this study. Data was not normally distributed and homogenous thus subjected to non-parametric test. Mann-Whitney test at $p < 0.05$ was conducted to compare the differences of flower measurement between species, pollen viability under different staining methods (i.e., IKI and TTC), pollen carrying capacity of insects between sexes, and measurement of insect's body sizes between sexes. Meanwhile, Kruskal-Wallis test at $p < 0.05$ were conducted to accommodate more than two groups which were to identify the differences of pollen sizes (length and width) under polar view and equator view between different guava cultivars and species, the pollen viability distributions among guava cultivars and species, visitation frequencies of common guava flower visitors observed in two-minutes observation period among different pollinators species, pollen carrying capacity on insect's body between species observed and the measurement of body sizes among insects species. Later, the test was proceeded with multiple mean comparison using Mann-Whitney test with Bonferroni correction at $p < 0.0033$ to compare the visitation frequency (number of flowers visited) by common flower visitors. Lastly, Spearman's rank correlation coefficient was analyzed to determine the influence of insect's body sizes on pollen carrying capacity. All statistical analyses were performed using IBM® SPSS® Statistics Version 23 software.

CHAPTER FOUR

RESULTS AND DISCUSSIONS

4.1 MORPHOLOGY OF *Psidium guajava* AND *Psidium cattleianum*

Psidium guajava L. cv. Lohan (3 years old) from private orchard located in Kg. Lebak, Temerloh, Pahang were used in this study as sample models to describe morphology of *Psidium guajava*. Due to systematic orchard management by the farmer, Lohan guava in this orchard bloom continuously and produce fruit throughout the year. However, field samplings were conducted during the main flowering seasons which is from March until April and June until July. The main fruit harvest seasons are in September, December, and January. Parts of guava trees as shown in Figure 4.1. The guava bark is thin brown colored and has speckled bark that flakes off in tiny flakes. The leaves are green with lateral veins underneath, elliptical, and typically have obtuse or acute apex.

The depiction of stages for guava fruit development can be seen on Figure 4.2. The shoot grew and produced a bud that took around 2 to 3 weeks which then blossomed into flower. Based on the observations, the approximate duration for one flower to reach full blooming stage (Figure 4.2g-h) is about 2 hours which usually starts at 5 a.m. until 7 a.m. Later, the flower drops the petals and flower took only 1 to 2 days to dry up and anthers fell from the blossom which marked the last stage of flowering. The flowering period usually lasts about 3 to 4 weeks. Flower develops into a fruit set around 40 to 45 days after flowering. With optimal condition, the guava fruit matured after 20 to 28 weeks and later ready for consumption.

In orchard management, understanding guava development is important to determine the crucial time for pruning and harvesting. Guava tree pruning is an essential management technique that permits year-round staggered fruit production (Silva *et al.*, 2016). Besides, the schedule for application of insecticides and fertilizers can be planned carefully without interrupting the plant growth process. As mentioned by Salazar *et al.* (2006), it will be easier to determine the ideal time to apply insecticides and growth regulators once the phenological stages of guava (*P. guajava* L.) are properly established.

In addition, *Psidium cattleianum* trees morphology (Figure 4.3) were also described briefly for comparison with *Psidium guajava*. The *Psidium cattleianum* or commonly known as Strawberry guava is a small shrub tree, also has brown bark with tiny flakes (Figure 4.3a) and ovate green leaves (Figure 4.3b). The fruits are small, round and red colored (Figure 4.3e). Patel (2012) described that the fruit strawberry-like flavor and a sweet-tart flavor.

Figure 4.1 Various parts of common guava tree; (a) bark, (b) leaves, (c) shoot, (d) flower and (e) fruits.

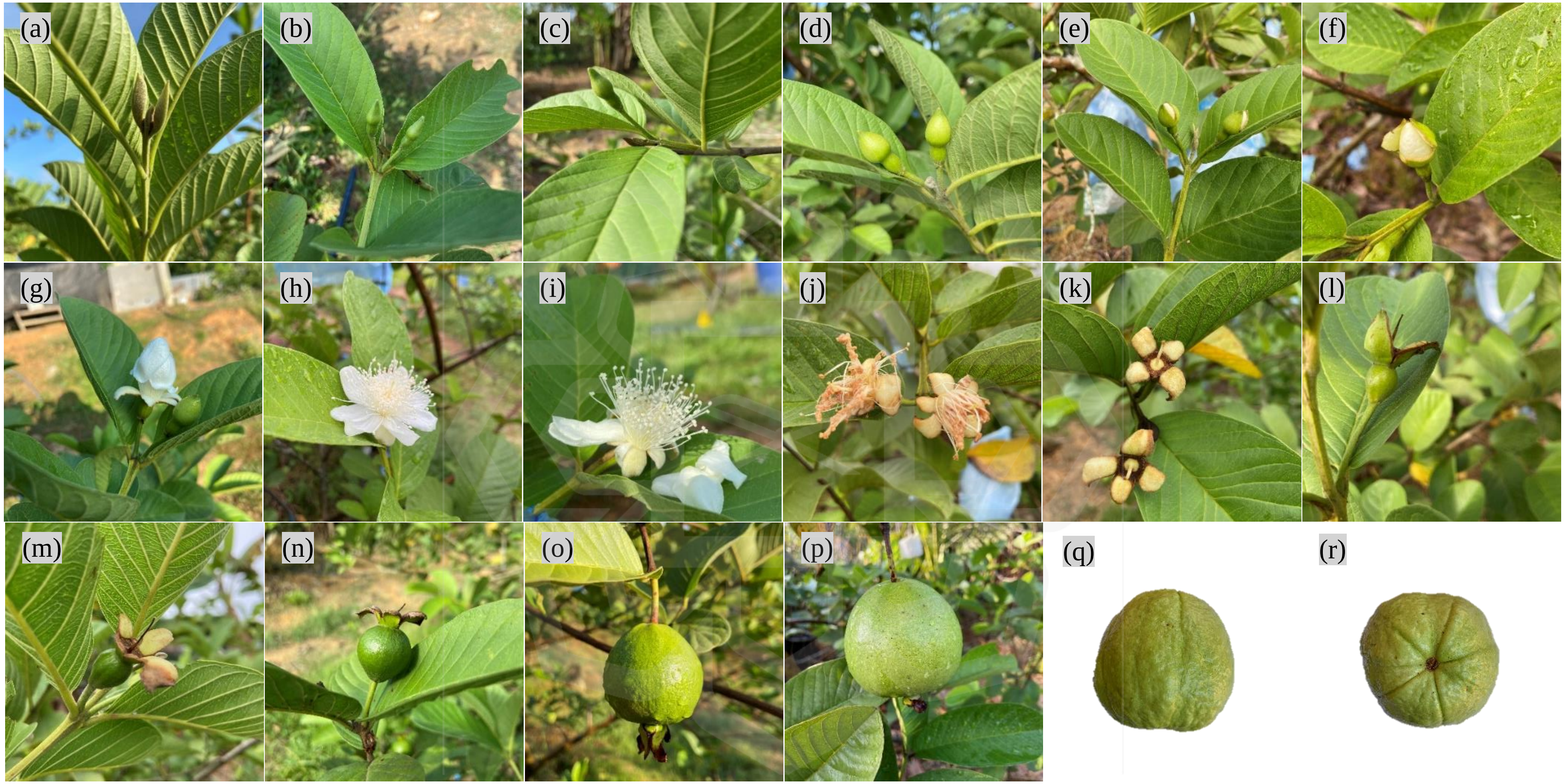


Figure 4.2 Stages of guava fruit development; (a) shoot, (b-f) bud development, (g-i) flower development, (j-k) flower dried, (l-m) fruit set development, (n-r) fruit development.

Figure 4.3 Various parts of Strawberry guava tree; (a) bark, (b) leaves, (c) shoot, (d) flower and (e) fruits (Britannica, 2021).

4.1.1 Floral Structure of Different Guava Species

In this part, the flowers morphology of two different guava species was compared in terms of appearance and measurement. The said species are *Psidium guajava* L. cv. Lohan (Figure 4.4a) and *Psidium cattleianum* or Strawberry guava (Figure 4.4b). Strawberry guava is considered ornamental guava even though the fruit is edible. Basic morphology was similar in both species except for the measurement. Both species appeared to have white colored flowers. Identification of color was made using global flower color based on human visual perception described by Dyer *et al.* (2021). For humans, flowers must reflect blue, green, and red in order to be white, meanwhile flower would need to reflect UV, blue, and green in roughly equal amounts in order for bees and moths to interpret it as white (Erickson & Pessoa, 2022). Many insects, especially hymenoptera and lepidoptera, whose vision extends into the ultraviolet (UV) spectrum, see most flowers that appear white to the human eye as colored because they are reflecting UV light (Rudall, 2020). Humans have a trichromatic visual system with photoreceptors that are capable of efficiently absorbing light with wavelengths of around 421, 530, and 559 nm (blue, green, and red) that allows us to see color while bee pollinators have trichromatic vision, which is based on UV-Blue and Green-sensitive photoreceptors with natural preferences for mostly short-wavelength reflected colors, including blue (Dyer *et al.*, 2021). Regardless, the color determination of *Psidum* species was based on visual perception by human which has been set as white color. *Psidium guajava* have white petals that were arranged in two whorls with five petals in each whorl, meanwhile *Psidium cattleianum* only has one whorl of five white petals. Both have either four or five sepals and have tons of stamen. *Psidium guajava* and *Psidium cattleianum* flowers appeared to be solitary, lateral, or in cymes of three to four.

Flower parts for Lohan and Strawberry guava were shown in Figure 4.5 and Figure 4.6 respectively. Comparison in measurement of these parts between species was reported in Table 4.1.

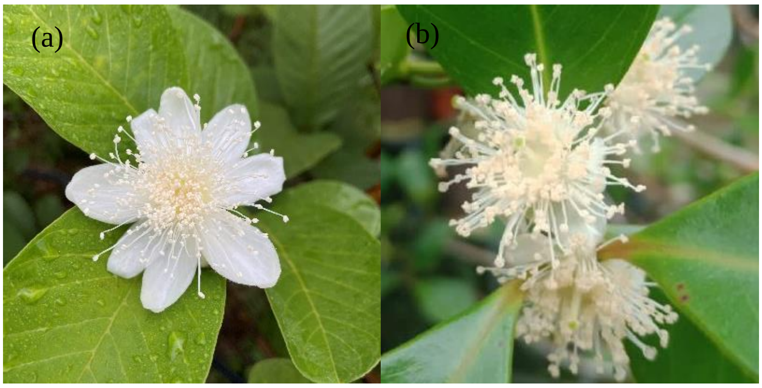


Figure 4.4 Flower of two guava species; (a) *Psidium guajava*, (b) *Psidium cattleianum*.

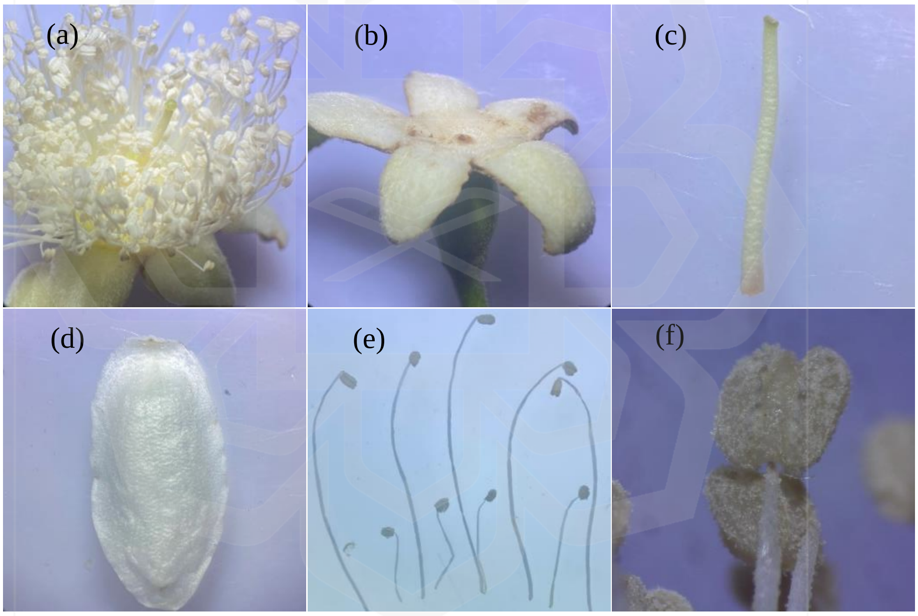


Figure 4.5 The floral parts of *Psidium guajava* viewed under dissecting microscope;(a) flower without petals, (b) sepals, (c) stigma, (d) petal, (e) stamen and (f) anther with pollens.

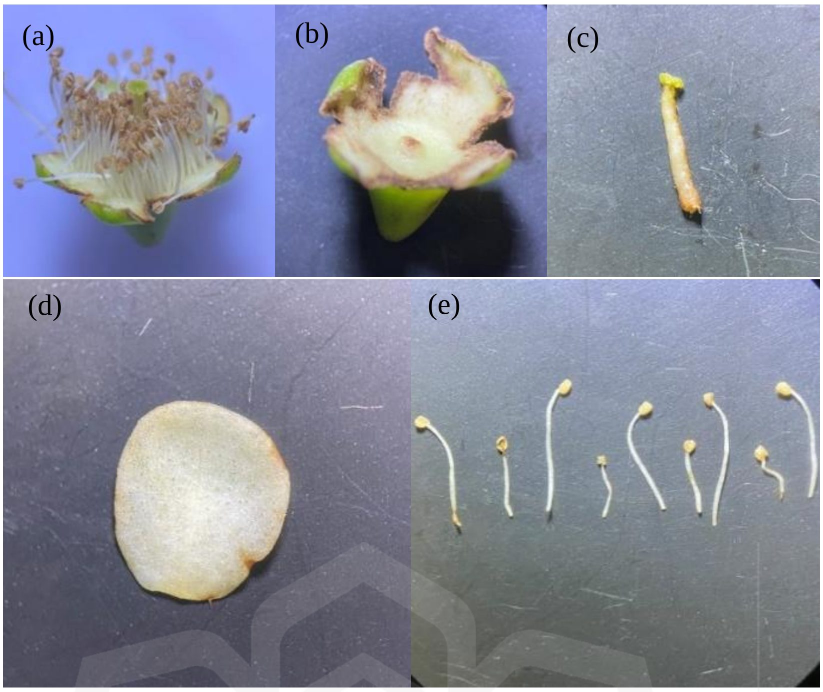


Figure 4.6 The floral parts of *Psidium cattleianum* viewed under dissecting microscope; (a) flower without petals, (b) sepals, (c) stigma, (d) petal, and (e) stamen.

Table 4.1 The comparisons of flower measurement (mean ± SE) for different guava species. Significant difference at $p < 0.05$ was indicated with a * symbol.

Species	*Psidium guajava* L. Lohan	*Psidium cattleianum*
Number of stamens *	464.07 ± 20.819	240.20 ± 14.490
Stamen length (mm) *	12.72 ± 0.276	4.19 ± 0.104
Stigma length (mm) *	13.67 ± 0.361	4.93 ± 0.145
Number of sepals	4.60 ± 0.214	4.67 ± 0.159
Sepal length (mm) *	11.45 ± 0.802	3.68 ± 0.123
Sepal width (mm) *	9.33 ± 0.534	3.88 ± 0.136
Number of petals *	6.73 ± 0.680	3.60 ± 0.290
Petals length (mm) *	21.06 ± 0.919	4.99 ± 0.131
Petals width (mm) *	11.05 ± 0.582	4.06 ± 0.123

Based on Table 4.1, the average counts for stamen recorded from *Psidium guajava* L. Lohan and *Psidium cattleianum* are 464.07 and 240.20 respectively. Lohan guava has a lot of stamens on flowers collected compared to Strawberry guava. Previous study by Sharma *et al.* (2017) calculated the mean number of flower parts with the stamens of 389.5. The number of stamens on each flower may differ greatly among each other. This is because, the stamen will easily fall off the flower due to a few factors such as wind or interference of pollinators. The flowers stamen consists of long and slender filament that supports anthers on top. Generally, guava stamen existed in two different sizes as depicted in Figure 4.5(e) and 4.6(e). A group of short stamen surrounds the stigma in the inner area of flower, while the long stamen found in the outer part of the flower away from stigma. The stamen length also varies greatly between each species. Lohan guava recorded an average stamen length of 12.72 mm while Strawberry guava is 4.19 mm. Apart from that, other measurements recorded also clearly showed that Lohan flowers measurement are bigger than the Strawberry guava flowers. For instance, the stigma length for Lohan guava is around 13.67 mm and 4.93 mm for Strawberry guava. Both flowers usually consist of four to five sepals depending on the pattern of the flower bloomed. However, their sepal sizes differ among each other where Lohan are larger with a length of 11.45 mm and width of 9.33 mm compared to Strawberry with only around 3.68 mm in length and 3.88 mm in width. Similarly, the sizes of flower petals can be easily distinguished between the said species. The mean length and width of Lohan guava flower petals are 21.06 mm and 11.05 mm respectively.

As studied by Bose *et al.* (2019), from 15 genotypes of *Psidium guajava*, the longest petals exhibited is 21.60 mm and shortest petal recorded is 17.00 mm, meanwhile the width of the petal were recorded to be 13.80 mm and 9.00 mm for the highest and the lowest width, respectively. In the meantime, Strawberry guava flower petals are about 4.99 mm in length and 4.06 mm in width. Sharma *et al.* (2017), mentioned that the mean number of sepals and petals of *Psidium guajava* are 4 and 8.75, respectively. Lohan guava flower has a multiple of five flower petals which can have about 10 petals at the time flower bloomed meanwhile, Strawberry guava usually has up to only five petals during flower blooming. Typically, flowers that are pollinated by animals have bigger, more flamboyant petals that are a variety of sizes and shapes to draw in pollinators (Zariman *et al.*, 2022). According to Teixido *et al.* (2016), large flowers have high benefits in pollination, pollinator diversity, visitation rates and reproductive success. Increasing flower size and floral display size was usually associated with increased visitation, independent of gender (Glaettli & Barrett, 2008). Another essential feature of the flower is a platform (petals or stigma) for insects and other pollinators to land on (Jones & Jones, 2001). Besides, both guava species also greatly differ in terms of the fruit sizes produced.

Variation in flower size can contribute to a species' reproductive isolation, which has strong evolutionary impacts (Krizek & Anderson, 2013). Larger flowers provide a considerable advantage in terms of pollination and reproductive success for male function. However, female function is constrained by high floral production and maintenance costs as well as prevalence of florivores (Teixido *et al.*, 2016). Different flower sizes in a population can either benefit or detriment the flower itself or pollinators. Guava flowers are hermaphrodite that enables them to self-pollinate without relying on the pollinators. However, there are different types of arrangement of sex organ that may influence the plant reproduction and pollination. The arrangement of sex organ in guava flowers can be seen in Figure 4.7.

The presence of heterostyly in hermaphrodite flowers may hinder the fertilization of receptive stigmas by viable pollen from the same flower (Jesson, 2016). Heterostyly is a polymorphism of herkogamy within a plant from the same species that promote cross-pollination in flowering plant (Barrett, 2019). Herkogamy is a characteristic of having styles of different lengths in relation to stamens in the flowers from different plants but due to polymorphism, cross pollination may be beneficial and more effective than self-pollination. Heterostyly was divided into two forms which are distyly and tristyly. Structurally, distyly has two coexistence of morphs within the flowers, the pin and thrum form which ensure the pollen collected from the stamens of one type is deposited on the stigma of the other. Li *et al.* (2007), defined pin flowers as a long-styled flowers that have a stigmatic surface near the mouth of the flower and anthers halfway down the corolla tube, contrarily, thrum is short-styled flowers with the stigmatic surface concealed by the corolla tube and anthers are visible at the flower's mouth. On the other hand, tristyly has three morphs in which individuals have either long, moderate, or short styled pistils, with anthers located in the other two positions depending on the filament's length (Figure 4.7).

Guava flowers commonly exist in tristyly. However, in this study guava flowers appear in a moderate style, short and long anthers (Figure 4.7b). Guava flowers are considered to have short morph where the stigma is in the lowest position and the two pairs of anthers above them. This condition may influence guava flowers reproduction. Tristyly functions is to encourage cross-pollination, but changes in stamen position frequently result in self-pollination (Arunkumar *et al.*, 2017). For cross pollination that depends on insect to be effective, it required appropriate dimensional relationship between flower and the insects. As the guava flowers are visited by various species of insects with different sizes and morphologies, tristyly seems to be the best result of a coevolutionary process between the plant and its pollinators.

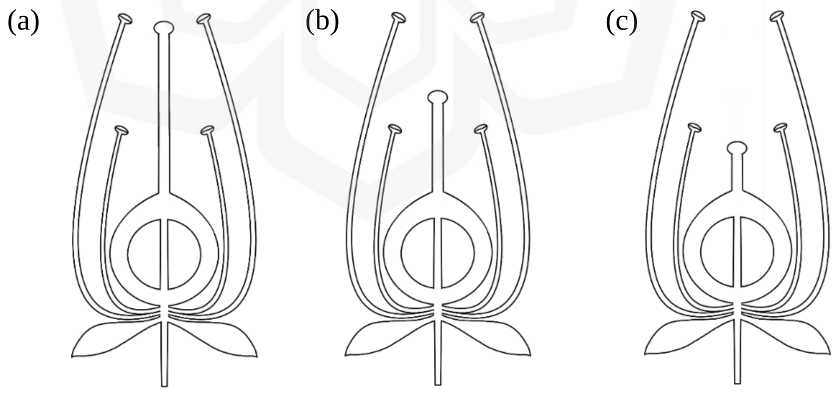


Figure 4.7 Tristyly floral polymorphism of guava; (a) long-styled, (b) mid-styled, and (c) short-styled.

4.1.2 Guava Pollen Morphology

Pollen morphology is beneficial in understanding plant origin. It helps address complex problems of taxonomic interrelationships and determining classification status, especially with regard to families, subfamilies, tribes, genera, species, and subspecies. Pollen morphology may be linked to pollination vectors, and certain pollen characteristics, such as aperture configuration and exine ornamentation, are susceptible to co-adaptation with specific pollinators (Wang *et al.*, 2009). It is crucial to visualize the basic characteristics of guava pollen grains in terms of its symmetry, shape, size and other important features. In this study, the pollen grains of different guava cultivars of *Psidium guava* namely Kristal guava, Lohan, Kim Choo Red, Bo Chii and Pink (heirloom) guava with comparison to *Psidium cattleianum* were studied under both light microscope and scanning electron microscope. Measurement was also taken to understand further about their different sizes especially between species of *P. guajava* and *P. cattlleianum* which differ in terms of their flower sizes. The images of guava pollen were recorded for both polar and equatorial view.

Firstly, the observations of guava pollen grains under light microscope (LM) can be seen in Figure 4.8 for its polar view and Figure 4.9 for its equator view. Observation of the pollen under light microscope can roughly provide the shapes and size but not the ornaments.

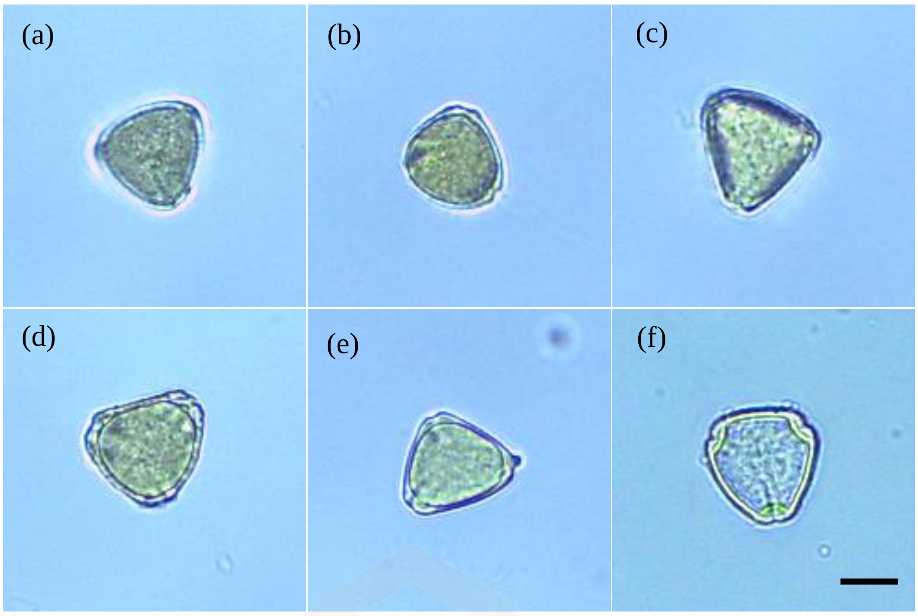


Figure 4.8 Polar view of guava pollen grains under light microscope;(a) Kristal guava, (b) Lohan guava, (c) Kim Choo Red guava, (d) Bo Chii guava, (e) Pink guava and (f) *Psidium cattleianum*. Scale bar = 10 µm.

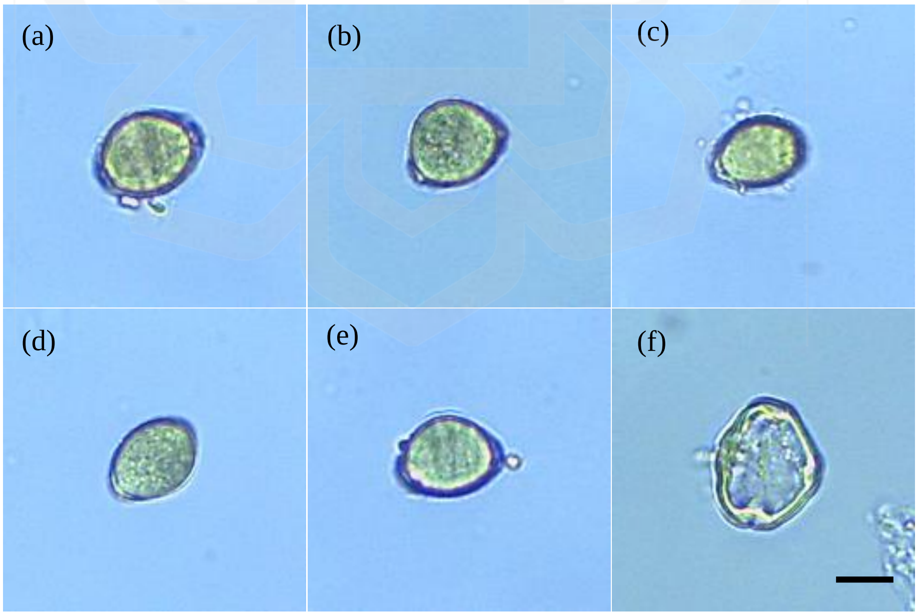


Figure 4.9 Equator view of guava pollen grains under light microscope; (a) Kristal guava, (b) Lohan guava, (c) Kim Choo Red guava, (d) Bo Chii guava, (e) Pink guava and (f) *Psidium cattleianum*. Scale bar = 10 µm.

Based on observations in LM, all *Psidium* pollen grains seemed to be triangular under polar views and oval in equatorial view. This is perfectly consistent with the earlier findings that reported guava pollen grains in polar perspective are triangular with angles having apertures, and in equatorial view, they are elliptic (Nascimento *et al.*, 2019). This shape can be clearly seen as well in scanning electron microscope (SEM) observations in Figure 4.10 (polar view) and Figure 4.11 (equator view). Observations were made under both LM and SEM in order to accurately describe any taxonomic value as suggested by Halbritter *et al*., (2018). LM depicted the 2-Dimesional view of the pollen while SEM visualize a 3-Dimensional image. Besides, in contrast with light microscope, SEM required elaborate procedure to photograph the image. It gives a clearer image because it operates in a vacuum with higher magnification and resolutions. SEM is also able to analyze structures that cannot be detected with a light microscope.

Based on the observations, the general morphological characteristics of pollen grains among guava cultivars and species do not differ from each other. The guava pollen grains in polar view for both LM and SEM observations depicted the convex-triangular outlines. This can simply define the guava pollen grains shape as triangular amb which is in line with previous studies (Souza-Pérez *et al*., 2021; Nascimento *et al.*, 2019; Tuler *et al.*, 2016). Meanwhile, in equator view, all *Psidium guajava* cultivars are elliptic, except for *Psidium cattleianum* that appeared irregular (Figure 4.11f). *Psidium cattleianum* may also be elliptic shape but seems irregular may be because the samples were destroyed or crushed during handling making its hard to identify the shape properly.

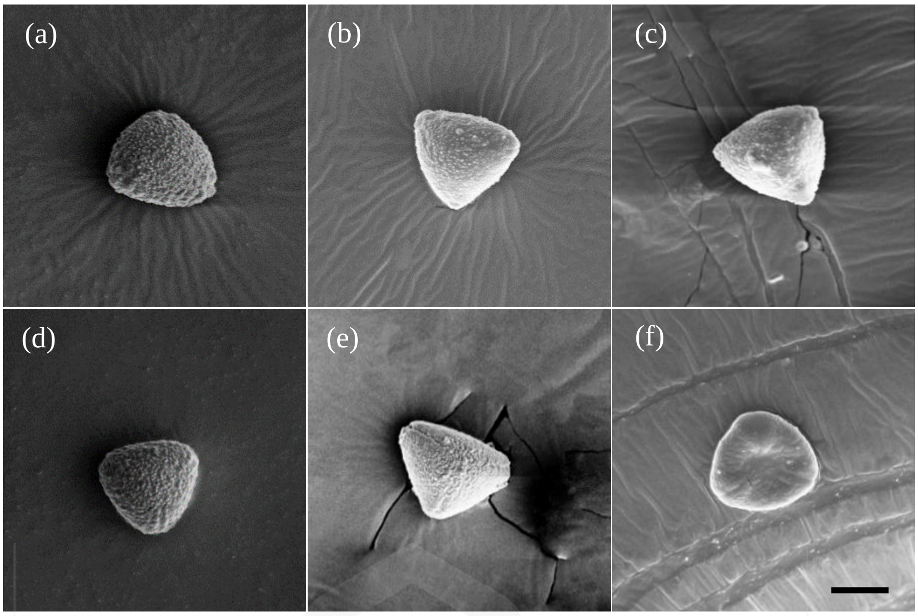


Figure 4.10 Polar view of guava pollen grains under scanning electron microscope;(a) Kristal guava, (b) Lohan guava, (c) Kim Choo Red guava, (d) Bo Chii guava, (e) Pink guava and (f) *Psidium cattleianum*. Scale bar = 10 µm.

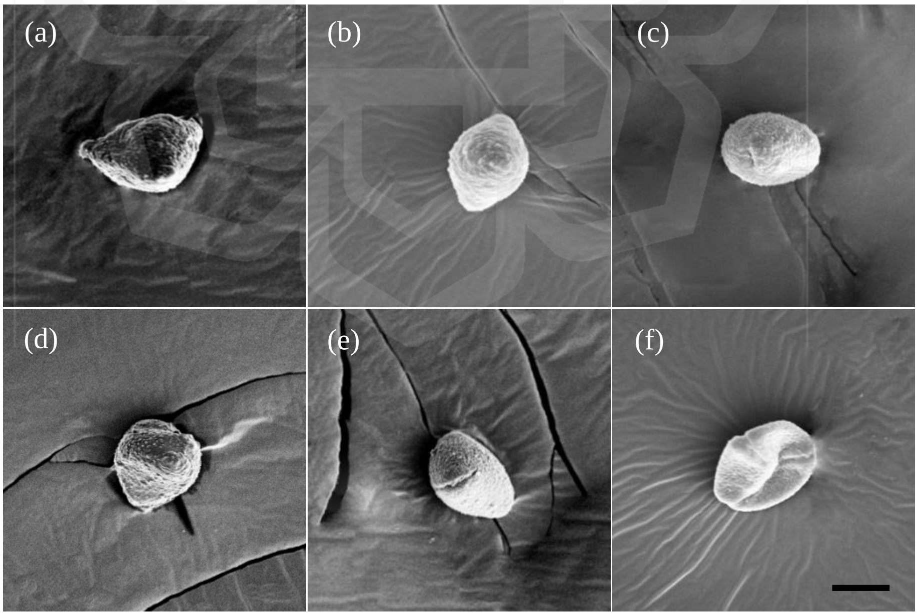


Figure 4.11 Equator view of guava pollen grains under scanning electron microscope;(a) Kristal guava, (b) Lohan guava, (c) Kim Choo Red guava, (d) Bo Chii guava, (c) Pink guava and (f) *Psidium cattleianum*. Scale bar = 10 µm.

The guava pollen grains studied have 3 apertures or tricolprates with the aperture position situated at the corner. This was also consistent with the earlier findings. Apart from that, the colpal morphology or aperture type appeared to be brevicolpate for all pollen. Brevicolpate were described as colpi that fail to meet at the end of the pole because of the exine layer (Thornhill & Crisp, 2012). Despite this fact, Souza-Pérez *et al.* (2021) stated that *Psidium guajava* L. has a syncolpate aperture type. Instead, other studies found that *Psidium guajava* and *Psidium cattleianum* are parasyncolpate (Nascimento *et al.*, 2019; Tuler *et al.*, 2016). In contrast, other *Psidium guajava* species were also found to be brevicolpate or less commonly syncolpate which described in *Psidium molle* by Vishwakarma *et al.* (2021).

The ornaments on guava pollen can be seen clearly in SEM instead of LM. For instance, the exine surface of pollen grains cannot be identified in LM but able to be seen clearly in SEM. Firstly, for all *Psidium guajava* cultivars, the exine pattern showed a granulate/scarbate pattern (Figure 4.12a). Souza-Pérez *et al.* (2021) and Vishwakarma *et al.* (2021) also described *Psidium guajava* and other species such as *P. cattleianumm, P. molle*, *P. chinensis*, *P. guineense*, as granulate/scabrate or verrucate/scabrate. Next, for *Psidium cattleianum*, the exine pattern displayed regulate with irregular pattern consists of flattened exine segments (Figure 4.12b).

In addition, the measurement of guava pollen grains can provide useful information regarding the pollen. The measurements were taken by measuring the length and width of both polar view and equator view. Figure 4.13 shows how the measurements are taken under LM and SEM. Besides, Table 4.2 displayed the measurement of the pollen grains for all guava cultivars and species.

Table 4.2 Measurement of guava pollen (mean ± SE) under light microscope

Guava cultivars and species	Polar view (µm)		Equator view (µm)	
	Length	Width	Length	Width
Psidium guajava c.v. Kristal	17.0 ± 0.19	19.1 ± 0.19	14.4 ± 0.13	19.6 ± 0.13
c.v. Lohan	18.3 ± 0.98	19.2 ± 0.83	13.7 ± 0.34	19.7 ± 0.69
c.v. Kim Choo Red	18.5 ± 0.17	20.8 ± 0.50	14.4 ± 0.68	19.6 ± 0.87
c.v. Bo Chii	19.7 ± 0.31	20.3 ± 0.20	14.4 ± 0.11	18.9 ± 0.30
c.v. Pink (heirloom)	16.7 ± 0.12	20.7 ± 0.08	14.0 ± 0.11	18.7 ± 0.34
Psidium cattleianum	19.6 ± 0.90	20.0 ± 0.23	15.7 ± 0.54	20.3 ± 0.55

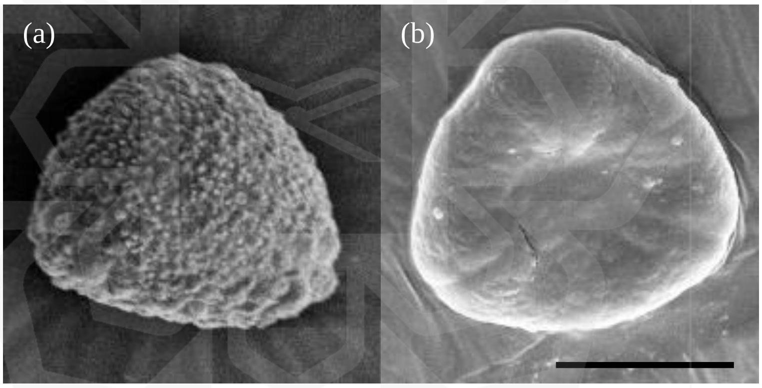


Figure 4.12 Exine pattern of guava pollen grains; (a) Granulate/scarbate and (b) Rugulate. Scale bar = 10µm

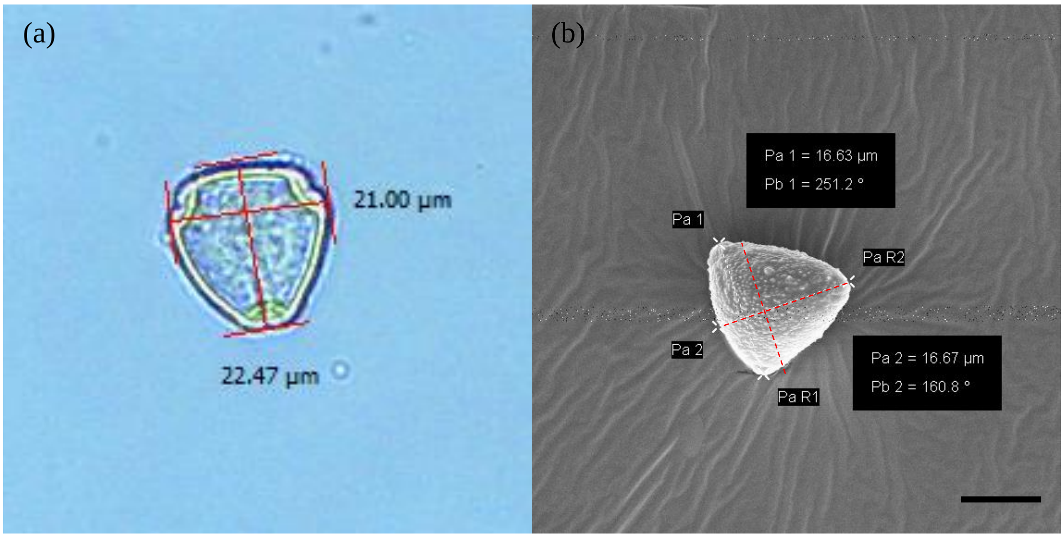


Figure 4.13 The measurement of guava pollen grains taken under different microscopes (a) LM and (b) SEM. Scale bar = 10 µm.

According to the results obtained, there is no significant difference of pollen size in terms of length and width under polar view and width under equator view between guava cultivars and species studied at p = 0.05. However, under the equator view the pollen length was significant for all guava cultivars and species at $p < 0.05$ (χ^2 =16.07, df =5). Based on Halbritter *et al.* (2018), all of guava pollen grains with pollen sizes in ranges of 10-25 µm are considered as small. Similar measurements also reported by Souza-Pérez *et al.* (2021) and Nascimento *et al.* (2019). Although the sizes do not differ much between each other, the smallest pollen grains were recorded in Pink guava, meanwhile, the largest were Strawberry guava (*Psidium cattleianum*) pollen grains. In pollination, the size of pollen grains influences pollinator feeding strategies. According to Hao *et al.* (2020), large pollen grains are linked with species where pollen grains were rarely taken by pollinators, but small pollen grains were associated with species of pollen-collecting foragers. Mango blossoms, for example, with medium-sized pollen grains (~ 30 µm) were favoured by flies over bees (Huda *et al.*, 2015). Meanwhile, many insect species, particularly pollen-collecting foragers like bees, prefer small guava pollen grains (10-25 µm) (Vinod & Sattagi, 2018).

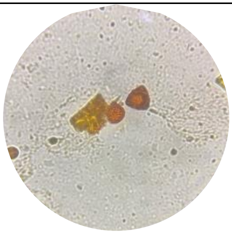
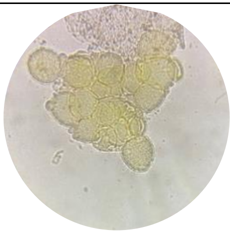
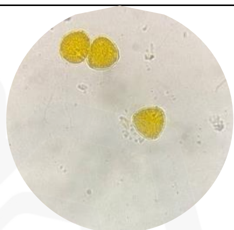
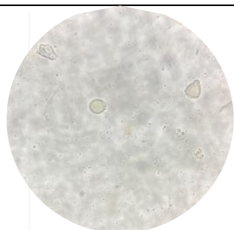
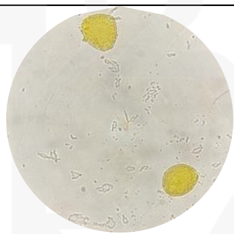
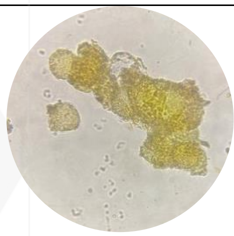
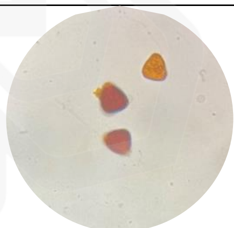
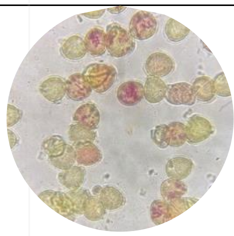
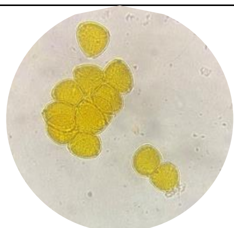
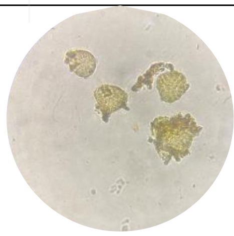
4.2 POLLEN VIABILITY

4.2.1 Identifying Best Stages of Guava Flower for Pollen Viability Test

It is critical to retain the flower sample in the proper storage conditions so that it may be utilized for the experiment. Kristal guava flowers of various phases (closed bud, bud nearing anthesis, and fully bloomed flower) were taken from several trees at a private farm in Lanchang, Pahang. These samples were maintained in a variety of circumstances, including completely opened flowers that were brought back fresh as they were, immersed in water, and immersed in 80% ethanol as described in the methodology (section 3.2.1) before being taken back to the laboratory for analysis. Table 4.3 displays the results of IKI and TTC staining on the pollen of different stages of flowers kept in different storage conditions.

Based on the observation, all fully opened flowers that were collected either freshly stored, immersed in water or immersed in ethanol (ETOH) have less amount of pollen left on the anthers when examined compared to bud nearly at anthesis and fully closed bud. This situation probably happened due to anthers being shed during picking, pollen already fall due to wind or shed when submerged in the solutions (i.e., water or ETOH). Although the viability still can be detected among the available pollens of fully opened flowers (freshly stored) but lack of pollen for counting make it disadvantages to be used in the assessment. In contrast, fresh flower bud nearly at anthesis and fully closed bud have more pollen attached to it which make it more superior in term of numbers. However, no viable pollen was detected from fully closed bud in both staining methods. In this pollen viability assessment, the best flower stage to be used for staining is flower bud nearing anthesis as many viable pollens were detected under both staining methods.

Table 4.3 The sample of Kristal guava flowers and buds tested with IKI and TTC staining under different storage conditions.

No.	Conditions and stages	Descriptions	IKI Staining	TTC Staining
1		Fully opened flower (fresh)		
2		Fully opened flower immersed in the water		
3		Fully opened flower immersed in the 80% ethanol		
4		Bud nearly at anthesis stage (fresh)		
5		Fully closed bud (fresh)		

4.2.2 Assessment of Pollen Viability by Colorimetric Method

The assessment of pollen viability was done using two different staining methods which are 1% aqueous solution of 2,3,5-triphenyl tetrazolium chloride (TTC) and Iodine Potassium Iodide (IKI) on different cultivars of *Psidium guajava* which include Lohan, Bo Chi, Kim Choo Red and Kim Choo White, Pink guava (heirloom) and Vietnam Seedless guava as well as on another species of guava which is *Psidium cattleianum* also known as Strawberry guava. In TTC staining, orange or red stained pollen grains that are stained were counted as viable (Figure 4.14a), meanwhile dark colored stained pollen either dark red or brown color are considered viable in IKI staining (Figure 4.14b). In contrast, pollen grains with irregular shape and have weak or no coloration were considered dead or non-viable.

Table 4.4 shows the mean percentage of viable pollen (of 5 anthers) for each species and cultivars under different staining methods. From the results, it is concluded that the pollen viability of all guava cultivars and species are considered high in IKI staining compared to TTC staining. The staining of all *P. guajava* cultivars pollen under IKI has a high viability percentage (more than 85%) except for Strawberry guava (*Psidium cattleianum*) with only 28.99%.

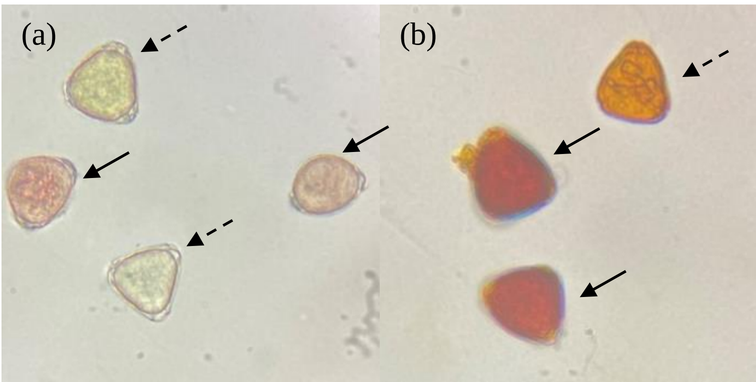


Figure 4.14 Pollen grains that are viable (full arrow) and non-viable (dashed arrow) under different staining methods; (a) TTC staining, (b) IKI staining.

Table 4.4 The mean percentage of viable pollen (of 5 anthers) for each species and cultivars under different staining methods.

Guava species & cultivars	Staining	N	Mean ± SE (%)
Psidium guajava c.v. Lohan	IKI	20	98.47 ± 0.336
	TTC	20	46.95 ± 6.956
c.v. Bochii	IKI	9	98.53 ± 0.719
	TTC	9	15.49 ± 7.326
c.v. Kim Choo Red	IKI	10	97.93 ± 0.557
	TTC	10	2.30 ± 1.110
c.v. Kim Choo White	IKI	4	99.82 ± 0.180
	TTC	4	15.82 ± 9.504
c.v. Pink (heirloom)	IKI	10	99.59 ± 0.259
	TTC	10	8.32 ± 3.898
c.v. Vietnam Seedless	IKI	4	85.08 ± 11.914
	TTC	4	0.00 ± 0.000
Psidium guajava	IKI	8	28.99 ± 7.495
	TTC	8	0.00 ± 0.000

There is a difference in viability percentage under different staining methods even though it is from the same cultivars. The highest pollen viability in IKI were recorded on Kim Choo White guava cultivar followed by Pink heirloom guava, Bochii, Lohan and Kim Choo Red, Vietnam Seedless and Strawberry guava. Meanwhile in TTC staining, the highest pollen viability was observed on Lohan followed by Kim Choo White, Bochii, Pink heirloom and the least pollen viable for TTC staining are Strawberry and Vietnam Seedless with 0%. In general, the TTC staining has a low viability percentage with all species stained for less than 50% of overall pollen. The pollen viability under different staining methods were proven significant at $p < 0.05$ (U = 193, z = -8.999,). The viability of each species and cultivars were found to vary in terms of numbers probably because of staining mechanism that involved detection of amount of certain chemical content in the pollen (i.e., starch and enzymes). It is proved that the pollen viability among guava cultivars and species were found significantly different by Kruskal-Wallis at $p < 0.05$ (χ^2 =18.977, df = 6).

IKI might be reliable because its viability rates are higher than those of TTC, but it might also be unreliable if it can't adequately stain or differentiate between viable and non-viable pollen. TTC staining solution indicates the presence of dehydrogenase enzymes, while IKI solution indicates the presence of starch (da Silva *et al.*, 2017; Soares *et al.*, 2013). This proved that the staining solution may only color the pollen grains that favors their reaction based on specific chemical content of the pollen. Previous studies on sugarcane stated that IKI are preferred and more stable to count pollen viability (Melloni *et al.*, 2013). Study on passion fruits also suggest that IKI staining has higher viability percentage compared to TTC staining (Soares *et al.*, 2013). This is because in TTC staining, the intact pollen grains and viable chromosome could have reduced the level of viability due to low pollen grain enzyme activity since TTC affects the active dehydrogenase and peroxidase enzymes. On pistachio's cultivar, Aldahadha *et al.* (2020), also stated that IKI are more reliable than safranin and TTC staining. In this case, TTC also recorded the lowest viability percentage. Similarly, in cherry laurel and sweet cherry both generally gave the highest values in IKI and stained better compared to TTC staining (Koyuncu & Tosun, 2008; Sulusoglu & Cavusoglu, 2014).

However, Huang *et al.* (2004) who studied the pollen viability on wheatgrass found that TTC are more reliable even though it recorded a low viability percentage. This is because, TTC produced a reasonable indication of viability and it can easily distinguish fresh and heat killed pollen, while IKI produced unrealistically high viability and failed to compare them. In his study, pollen was heat killed for 2 hours at 80°C and no color reaction were observed in TTC. However, in IKI the heat killed pollen were seen similar with fresh pollen. Pollen in TTC stained survived longer than IKI hence why they are reliable.

Nevertheless, in this study, it is concluded that IKI are reliable staining compared to TTC to stain guava pollen due to the fact that it produced a higher viability rate than TTC. A further understanding of the effect of staining on the pollen content is very much needed for a better insight in pollen viability. It is important to have a higher viability because, the higher the viable pollens recorded, the higher the ability of pollen to mature, germinate and reproduce and resulted in a good quality of guava fruits productions particularly for those seeded type of guava.

4.3 ASSESSING POLLINATOR'S CONTRIBUTION TO PLANT REPRODUCTIVE SUCCESS

Insects that visit flowers are called pollinators. Insects and other organisms play a crucial part in raising crop yields, vegetables, fruits, and seeds by visiting flowers and aiding in pollination (Thapa, 2006). They are responsible for pollination as they move pollen grains from anther to stigma which resulted in fertilization. Pollinators visit and forage on flowers primarily for food rewards like nectar and pollen, but they also visit for non-nutritive reasons like breeding grounds, shelter, gathering places, and oviposition sites (Zariman *et al.*, 2022). In this study, pollinators were found actively visit Lohan guava flowers during blooming seasons which is in March to April and June to July. They are seen actively forage on flowers around 7am until 10am and start to slow down at 11am.

4.3.1 Visitation Frequency and Behavioral Observations

In this study, a total of 20 species from 8 families of 3 orders of insects were collected on guava flowers. The pollinators species visited *Psidium guajava* L. Lohan flowers in the orchard includes Family Apidae, Halictidae and Collectidae of hymenopterans and a few Rhiniidae, Tachinidae and Calliphoridae of dipterans as well as Family Mordellidae and Cetoniidae of coleopterans (Table 4.5).

The highest pollinators species that visit guava flowers comes from the order of Hymenopterans. They have the most insect species with 13 species observed. Bees contribute roughly 80% of overall insect pollination, making them among the most prominent pollinators (Thapa, 2006). Bees from the family Apidae recorded the higher number of species. Among them, honeybees are regards as successful pollinators due to the fact that they have body hairs for pollen attachment, shows consistency in visiting flowers as long as there is a food sources, offer the possibility for lengthy working hours as well as live in colonies to supple needs and feed offspring (Partap, 2010). In the meantime, honeybees not only pollinate successfully, but they also sustain local livelihoods by producing high-quality honey (Panda *et al.*, 2019). Apart from that, approximately 5 species of Dipterans were reported to visit guava flowers in this orchard. Dipteran insects are important yet underappreciated pollinators (Kumar *et al.*, 2016). *Stomorhina* and *Chrysomya* are the 2 genera that also was reported to visit mango flowers (Huda *et al.*, 2015). Meanwhile, only 2 species of coleopterans observed on guava flowers. Coleoptera are also acknowledged as pollinators but considered less significant since their visit usually caused damage to the flowers but may accidentally serve as pollinator. The presence of several insect species in large quantities in a guava orchard may have an impact on the productivity and sustainability of guava fruit production (Amin *et al.*, 2019).

Table 4.5 Insect species observed foraging on Lohan guava flower.

Order	Family	Genus	Species
Hymenoptera	Halictidae	*Lasioglossum*	*Lasioglossum deliense*
			Lasioglossum sp.
		Nomia	*Nomia strigata*
		Halictus	*Halictus* sp.
	Apidae	*Heterotrigona*	*Heterotrigona itama*
		Ceratina	*Ceratina lieftincki*
		Xylocopa	*Xylocopa aestuans*
			Xylocopa latipes
		Apis	*Apis dorsata*
			Apis cerana
		Braunsapis	*Braunsapis* sp.
		Tetragonula	*Tetragonula atripes*
	Collectidae	*Hyaleus*	*Hyaleus* sp.
Diptera	Rhiniidae	*Stomorhina*	*Stomorhina discolor*
			Stomorhina sp.
	Calliphoridae	*Chrysomya*	*Chrysomya* sp.
		Lucilia	*Lucilia* sp.
	Tachinidae	*Prosena*	*Prosena* sp.
Coleoptera	Mordellidae	*Hoshihananomia*	*Hoshihananomia* sp.
	Cetoniidae		*Ixorida pseudoregia*

Visitation frequencies of these 20 insect species were observed in a 2-minutes observations period. However, from the total species observed (Table 4.5), 14 species were omitted from the Table 4.6 that shows the visitation frequency because they only exhibit single-flower visit during 2 minutes of observations period (observed for all replicates) leaving only 6 common species from wild bee group. These insects visited guava flowers as early as 7.00 am and remained active until 11.00 am. *Xylocopa latipes* was recorded with the highest visitation rate among other insects followed by *Xylocopa aestuans*, *Apis dorsata*, *Apis cerana* and the least guava flower visited by *Heterotrigona itama* and *Ceratina lieftincki*.

Table 4.6 Visitation frequencies of common flower visitors (mean ± SE) observed in two-minutes observation period on guava flower cv. Lohan.

Insects	Number of flowers visited	Significant differences of mean at $p < 0.0033$
Xylocopa aestuans	3.47 ± 0.274	b
Xylocopa latipes	4.17 ± 0.405	b
Apis dorsata	2.89 ± 0.261	b
Heterotrigona itama	1.07 ± 0.067	a
Apis cerana	1.20 ± 0.200	a
Ceratina lieftincki	1.07 ± 0.067	a

In general, number of flowers visited (visitation frequency) were reported as significant different among species of pollinators based on Kruskal-Wallis test at $p < 0.05$ (χ^2 =58.51, df =5). However, multiple means comparison shows that there is no significant difference in number of flowers visited among *H. itama*, *A. cerana* and *C. lieftincki*. Meanwhile, *Xylocopa* was significantly the most active visitor compared to other genera but shows no significant different between species (i.e., *X. latipes* and *X. aestuans*).

Xylocapa is a fast-flying carpenter bee. In 2 minutes, they are able to visit three to six flowers of different trees and fly further from one flower to another. Next, *Apis dorsata* is able to visit two to three flowers in 2 minutes as they do not spend longer time on one flower and typically visit the nearest flowers. Meanwhile, *A. cerana* spends more time on flower and only able to visits 1 to 2 flowers in the span of two 2 minutes. According to Das *et al.* (2019), bees visited fewer flowers when they spent more time in a single blossom. Other than that, *Heterotrigona itama* and *Ceratina lieftincki* also visit one to two flowers in the period of observation. They tend to spend longer time foraging on guava flowers. These species also prefer to revisit the same flower after some time which means they committed to only one flower and often visit the other flower within the same tree. According to Singh (2009), the length of time bees spends foraging on flowers depends on how much nectar and pollen are available, depending on the kind of flowers and when in its development it is as well as the climate.

Furthermore, insect visitation is often associated with behavioral activities such as feeding, grooming, locomotion, reproduction, flight, migration and host and prey selections. Insects often forage on flowers to gain rewards such as food and nutrients from pollen and nectar. Foraging behaviors are essential for bees to successfully manage and utilize the rewarding blooms (Suneetha & Raju, 2019). *Xylocopa* or carpenter bees is a solitary bee and often female does all the forage to store pollen that serves as main ingredient food which contains sole nutrition sources to feed on bee larvae. However, male usually spend less time on flower compared to female and thus they are capable of visiting more flowers in less time than female. Based on the observation, *Xylocopa* usually hovering around the flower before hanging on the filaments (Figure 4.15) and was seen to forage on anthers where pollens are located. They collect pollen as their food resources and to feed the young. They were rarely seen to forage on the middle of the flower where nectar is located maybe due to the fact that guava flower has little to no nectar. They spent around 2 to 5 seconds on a flower before moving to another flower making it enables to visit many flowers in short amount of time. Additionally, carpenter bees shows a variety of flower-foraging behaviors, including opportunistic, territorial, traplining, buzzing, and others, to effectively use the available food source while rewards plants from these behavioral characteristics during outcrossing (Raju & Reddi, 2000). Both species observed exhibit traplining behavior as they are frequently spotted returning to the same flower after visiting another flower at quite a distance. This behavior shows that they are loyal to a single flowering species, which significantly promotes outcrossing. This observation also in accordance with the finding by Takano *et al.* (2005).

Moreover, two wild honeybee species. Meanwhile, honeybees from genus *Apis* (Figure 4.16), were found foraged on the pollen and often buries themselves among the anthers. Besides, they also spend less time on a single flower which is around 5 to 10 seconds and often visit the nearest flowers of the same tree. As reported by Joshi and Joshi (2010), *A. cerana* took 5.11 ± 0.9 seconds per apple flower. Meanwhile, *Apis dorsata* has maximum foraging speed of being 15.74 seconds time spent on fennel flower (*Foeniculum vulgare* L.) on different dates and visits 4.13 flower/min (Kumar & Rai, 2020). *A. dorsata* spent more time in a single litchi flower than others (A. *cerana indica*, *A. florea*, *A. mellifera*) and visited less number of flowers because of body weight which make it requires more food (Das *et al.*, 2019). In the meantime, compared to other species, *A. cerana* exhibited a substantially greater metabolic rate and made much more visits within the same habitat (Koetz, 2013). *A. cerana* are said to be busier and spend less time on each bloom when gathering pollen from dispersed flowers of various plant species (Koetz, 2013).

Figure 4.15 Carpenter bees foraging on guava flower; (a) *Xylocopa aestuans* and (b) *Xylocopa latipes* foraging on guava flower.

Figure 4.16 Honeybees foraging on guava flower; (a) *Apis dorsata* and (b) *Apis cerana*.

Heterotrigona itama (Figure 4.17a) was observed moving around the flower before landing on the petals and continuously moving from petals to another petals and forage on bottom part of the filaments instead of at the anther where abundant of nectar located. According to Basari *et al.* (2018), *H. itama* preferred sugar concentrations more than 35% especially when temperature increase and often seen to forage on food near their beehives. Benedick *et al.* (2021) also stated that *H. itama* prefers to forage in locations with a variety of food sources that are closer to their nesting place. *H. itama* seen on guava flowers in the orchard seems to be looking for nectar rather than pollen. However, the nest location was unidentified and not reported in this study, thus the relation of nesting habitat to foraging behavior cannot be justified.

Other than that, *Tetragonula atripes* (Figure 4.17b) shows a behavior such that, they start by hovering around the flower and landed on sepals or nearby leaf then move to the anther and spent around 15 seconds to forage on the pollen before they start hovering again to find another target anther. They also may directly land on the anther but most of the time *T. atripes* linger around the flower before forage. Next, *Ceratina* (Figure 4.17c-d) was seen to forage on guava flower by burying themselves among the anthers and filaments and often forage on the middle and inner part of the flowers. They also spend longer time on the flowers to feed on pollen and nectar.

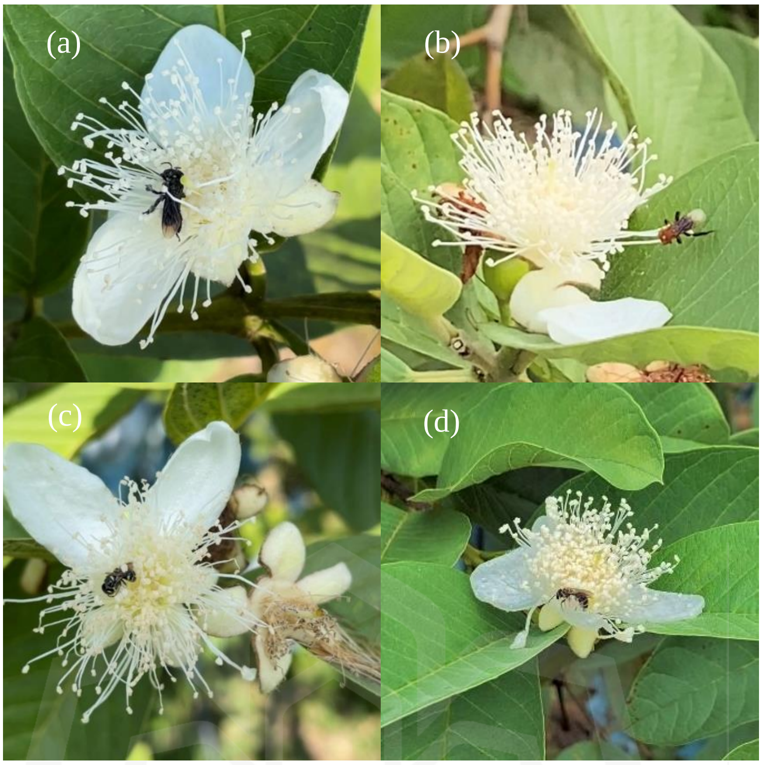


Figure 4.17 Other pollinators from Apidae family; (a) *Heterotrigona itama* (b) *Tetragonula atripes,* (c) *Ceratina lieftincki* and (d) *Ceratina* sp..

Other bees such as *Lassioglossum delicense, Lassioglossum* sp., *Nomia strigata* and *Halictus* sp. from Halictidae family and *Hylaeus* sp. of Collectidae do not show any distinct behavior and spent most of their time only to forage on the middle part of the flower. Both said families showed a similar behavior towards guava flowers. They are moving persistently from anther to the bottom part of the filament or dive directly to the middle part of the flower and bury themselves in the filaments and spent a long time to forage, sometimes more than 2 minutes. Figure 4.18 shows *Nomia striata* foraging among filaments on guava flower.

Apart from Hymenopterans, a few Dipterans as seen in Figure 4.19 were also observed on guava flowers. This includes *Stomorhina discolor, Stomorhina* sp., *Chrysomya* sp., *Lucialia* sp. and *Prosena* sp. Among them, *Prosena* sp. landed directly on the flower and lingering around the anther while the foot grabbed on the filament of the flower and forage on the pollen. Other species such as *Stomorhina discolor, Stomorhina* sp. and *Chrysomya* sp. often landed on the petal of the flower or the leaf and foraged directly on the anther where pollens are located. These species also move around a lot while forage and do not stay in the same place for a long time. They may eat pollen quickly as flies often swallow pollen as a whole. According to Woodcock *et al.* (2014), while other insects puncture the pollen grains and suck off the protoplasm, dipterans swallow the grains whole making their abdomen bloated and yellow from ingesting so much pollen, and it is easy to see digested pollen in their feces. Meanwhile, coleopterans often spent most of 2 minutes on the guava flowers either foraging on pollen or the flower itself and were also possible guava pollinators.

Figure 4.18 *Nomia strigata* foraging on Lohan guava flowers.

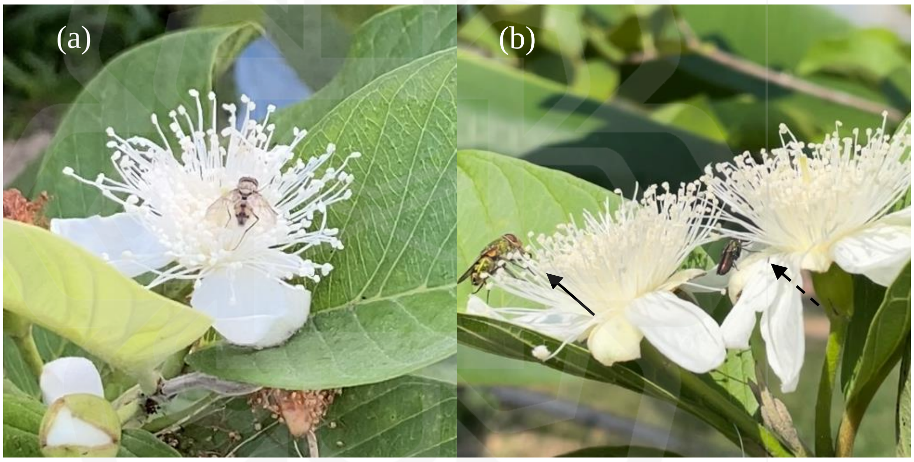


Figure 4.19 Dipterans that forage on lohan guava flowers; (a) *Stomorhina discolor*, (b) *Lucilia* sp. (full arrow) and *Stomorhina* sp. (dashed arrow).

4.3.2 Pollen Carrying Capacity by Pollinators

Pollen, nectar and other plant exudates that are commonly linked with flowers are the primary sources of nutrition for adults of many insect species (Jones & Jones, 2001). As insect visitors landed on the flowers, their body brushes up against the anthers in search of food. This behavior resulted in their body to be covered with pollen. Pollen usually adheres to insect's body to be carried and transmitted when they visit other flowers which resulted in pollination.

To understand the influence of pollinators visit on successful of pollination, the pollen loads on insect's body were counted and evaluated in relation to their behavior and body sizes. In this study, 19 species of pollinators were collected and observed for their pollen loads. Table 4.7 showed the estimated pollen abundance on the body of insects foraged on guava flowers. The number of observed insects varied per species as it depends on the availability of the insect in the orchard during the collection.

Table 4.7 Estimated pollen abundance (mean ± SE) on ♂ = male and ♀ = female insects visiting guava flowers.

Insects	Sex	Number of insects	Estimated Pollen Abundance	Presence of Foreign Pollen
Lasioglossum delicense	♂	29	114911.91 ± 9928.940	✓
	♀	12	112425.93 ± 18754.871	✓
	Total	41	114184.31 ± 8871.259	
Lasioglossum sp.	♂	1	1114222.22 ± 0.000	✓
	♀	27	250485.60 ± 18393.470	✓
	Total	28	281333.33 ± 24979.917	
Nomia strigata	♀	4	110055.55 ± 27886.550	✓
Halictus sp.	♀	1	259555.56 ± 0.000	✓
Heterotrigona itama	♂	1	154000.00 ± 0.000	✓
	♀	31	315752.94 ± 0.000	✓
	Total	32	310698.16 ± 29827.946	
Ceratina lieftincki	♂	2	83777.78 ± 23255.107	×
	♀	12	66425.93 ± 8947.958	✓
	Total	14	68904.76 ± 8300.943	
Xylocopa aestuans	♀	5	518000.00 ± 210653.952	✓
Apis dorsata	♀	3	723483.15 ± 134066.666	✓
Apis cerana	♀	5	333511.11 ± 54357.292	✓
Braunsapis sp.	♀	2	108777.78 ± 44771.049	×
Tetragonula atripes	♀	3	281629.63 ± 117590.108	×
Hyaleus sp.	♀	1	16222.22 ± 0.000	✓
Stomorhina discolor	♀	7	1936.51 ± 520.286	×
Stomorhina sp.	♀	12	1337.00 ± 237.976	×
Chrysomya sp.	♀	1	3111.11 ± 0.000	×
Lucilia sp.	♀	1	1555.56 ± 0.000	×
Prosena sp.	♀	5	1733.33 ± 468.251	×
Hoshihananomia sp.	♀	1	5777.78 ± 0.000	×
Ixorida pseudoregia	♀	1	34666.67 ± 0.000	×

In general, the highest mean of pollen load on the body was observed in Hymenoptera (227858.61 ± 13950.710) followed by Coleoptera (20222.23 ± 6459.752) and least in Diptera (1651.26 ± 201.100). There is a significant difference of pollen carrying capacity on insect's body between species observed at $p < 0.05$ (χ^2 =268.129, df =18). However, the estimated pollen abundance between sexes was found not significant under Mann-Whitney test at $p < 0.05$.

Among wild bees collected, *Apis dorsata* had the highest pollen loads on its body, followed by *Xylocopa aestuans* and *Apis cerana*. Beetle *Ixorida pseudoregia* also was recorded to have a lot of guava pollen adhered to the body although not as much as hymenopterans but still more than dipterans. Availability of the pollen load on this insect body provides strong evidence of its involvement in guava pollination. According to Kirmse and Chaboo (2020), beetles that visit flower can either be herbivores and pollinators or they can predators looking for prey.

Meanwhile, the least pollen load were observed on the body of flies particularly *Stomorhina* sp.. In comparison to honeybees, fly foraging behavior may be less likely to result in pollination, however, they could potentially supplement bee pollination by proving to be more resilient to changing environmental factors that could affect their activity (e.g., remaining active across a larger temperature range) (Cook *et al.*, 2020). However, this statement is partially in agreement with finding by Huda *et al.* (2015) which stated that, flies are more important pollinators than bees on mango as their visit flowers in high abundance but carried less pollen compared to wild bee due to their active grooming behavior and lack of hairy body.

Additionally, the presence of other pollen grains apart from guava is also evident on the insect's body. Table 4.7 also recorded insects with foreign pollen grains. All Hallictidae and Collectidae species recorded a presence of foreign pollen. Apidae species except *Ceratina lieftincki*, *Braunsapis* sp. and *Tetraonula atripes* also have foreign pollen grains on their body. On the other hand, all dipterans and coleopterans recorded the absence of foreign pollen grains. Figure 4.20 depicted different shape and size of foreign pollen grains in comparison with guava pollen of triangular amb. This proved that insects that visited guava flowers may also visit other flowers available near to them. For example, bees can carry far larger amounts of honey and pollen than are typically found in flowers, they frequently visit a variety of flowers and plants while foraging (Raju & Reddi, 2000). According to Abrol (2012) insects which flit from flowers of one plant species to those of another will cause little fertilization. In order to ensure successful fertilization, flower fidelity (constancy) shown by pollinator is very important. However, too much specialization may limit the usefulness of the insect. Inconstancy may happen due to a lack of available guava flowers blooming continuously in the orchard.

Equally important, the insects' body size may affect the pollen loads on its body. The pollinator's size, amount of hair, cleaning habits, and foraging style all have an impact on how much pollen is carried over (Eswaran *et al.*, 2016). For instance, most small smooth-bodied insects carry little or no pollen, whereas large 'hairy' insects carry a lot. Huda *et al.* (2015), stated that on average, larger pollinators' bodies contained more pollen in comparison to smaller pollinators. In this study, the insects' body sizes were measured for its body length, body width, head length and head width (Figure 4.21) and recorded in Table 4.8.

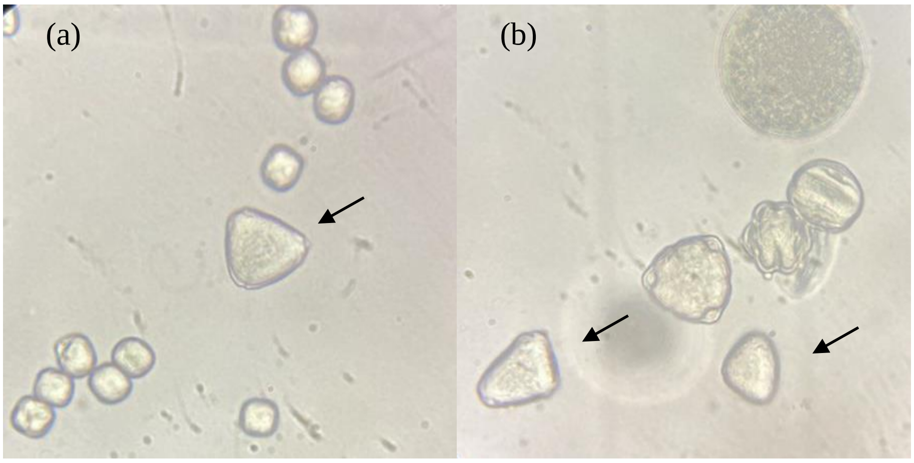


Figure 4.20 The presences of foreign pollen in comparison to guava pollen (arrow) on insect body under light microscope at 100x magnification; (a) *Lasioglossum delicense* and (b) *Nomia strigata*.

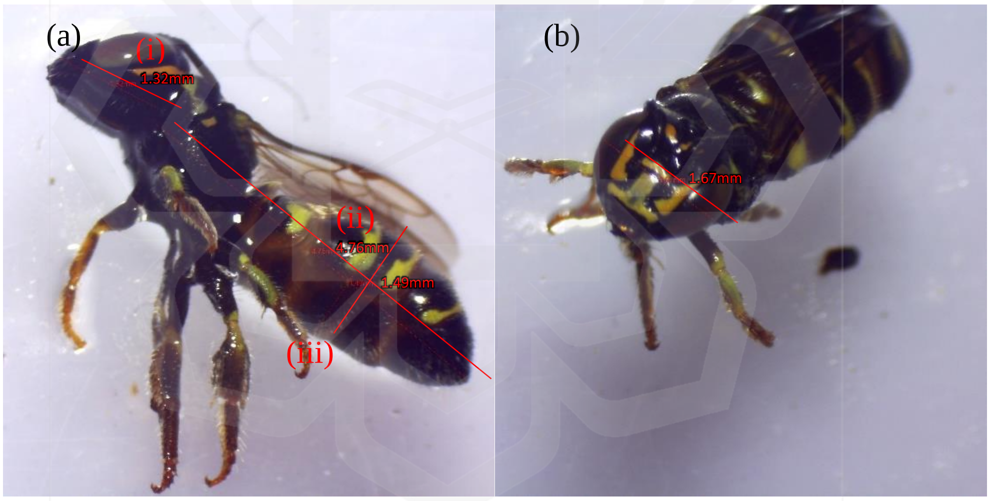


Figure 4.21 *Ceratina lieftincki* under dissecting microscope with the measurement for its (a) (i) head length, (ii) body length, (iii) body width and (b) head width.

Table 4.8 Measurement of insect body sizes (mean ± SE) in mm of ♂ = male and ♀ = female insect pollinators.

Insect	**Sex**	**N**	**body length (mm**	**body width**	**head length**	**head width**
Lasioglossum delicense	♂	29	3.43 ± 1.288	1.29 ± 0.181	1.11 ± 0.27	1.47 ± 0.11
	♀	12	3.88 ± 0.185	1.34 ± 0.036	1.29 ± 0.27	1.44 ± 0.18
	Total	41	3.56 ± 0.098	1.30 ± 0.017	1.14 ± 0.023	1.50 ± 0.019
Lasioglossum sp	♂	1	3.94 ± 0.023	1.54 ± 0.009	1.08 ± 0.027	2.09 ± 0.147
	♀	27	3.75 ± 0.048	1.50 ± 0.017	1.27 ± 0.028	1.80 ± 0.011
	Total	28	3.76 ± 0.016	1.50 ± 0.016	1.26 ± 0.028	1.81 ± 0.013
Nomia Strigata	♀	4	5.32 ± 0.178	2.09 ± 0.089	1.55 ± 0.050	2.39 ± 0.053
Halictus sp	♀	1	4.10 ± 0.032	1.61 ± 0.012	1.63 ± 0.025	2.02 ± 0.006
Heterotrigona itama	♂	1	4.57 ± 0.066	1.61 ± 0.012	1.06 ± 0.007	2.25 ± 0.015
	♀	31	4.08 ± 0.045	1.56 ± 0.020	1.19 ± 0.023	2.05 ± 0.020
	Total	32	4.10 ± 0.045	1.56 ± 0.019	1.18 ± 0.023	2.06 ± 0.019
Ceratina lieftincki	♂	2	4.74 ± 0.211	1.60 ± 0.114	1.22 ± 0.144	1.68 ± 0.113
	♀	12	4.68 ± 0.098	1.61 ± 0.041	1.40 ± 0.063	1.80 ± 0.035
	Total	14	4.69 ± 0.088	1.61 ± 0.039	1.37 ± 0.058	1.78 ± 0.034
Xylocopa aestuans	♀	5	17.00 ± 0.088	7.40 ± 0.220	2.83 ± 0.190	5.35 ± 0.106
Apis dorsata	♀	3	10.07 ± 0.496	3.04 ± 0.127	2.58 ± 0.078	3.08 ± 0.048
Apis cerana	♀	5	7.19 ± 0.138	2.24 ± 0.053	1.68 ± 0.113	2.60 ± 0.023
Braunsapis sp.	♀	2	4.02 ± 0.321	1.25 ± 0.092	1.22 ± 0.083	1.17 ± 0.039
Tetragonula atripes	♀	3	3.93 ± 0.073	1.46 ± 0.065	1.01 ± 0.077	1.77 ± 0.010
Hyaleus sp	♀	1	5.10 ± 0.111	1.48 ± 0.12	4.87 ± 3.667	1.76 ± 0.003
Stomorhina discolor	♀	7	3.42 ± 0.092	1.05 ± 0.031	0.78 ± 0.026	1.46 ± 0.036
Stomorhina sp.	♀	12	4.36 ± 0.060	1.04 ± 0.029	1.02 ± 0.038	1.59 ± 0.047
Chrysomya sp.	♀	1	5.85 ± 0.012	2.08 ± 0.020	1.17 ± 0.019	2.95 ± 0.006
Lucilia sp.	♀	1	6.21 ± 0.015	2.00 ± 0.007	1.30 ± 0.010	2.76 ± 0.012
Prosena sp.	♀	5	4.86 ± 0.149	1.42 ± 0.045	1.03 ± 0.047	1.83 ± 0.056
Hoshihananomis sp.	♀	1	2.43 ± 0.006	0.85 ± 0.015	0.44 ± 0.015	0.80 ± 0.003
Ixorida pseudoregia	♀	1	8.96 ± 0.010	4.63 ± 0.015	1.23 ± 0.016	2.29 ± 0.009

Variations in body size parameters; body length, body width, head length and head width were significantly different among insect species at $p < 0.05$ (body length, $\chi^2 = 273.79$; body width, $\chi^2 = 325.21$; head length, $\chi^2 = 198.70$; head width, $\chi^2 = 371.91$; df = 18). Among them, *Xylocopa aestuans* is the largest, followed by *Apis dorsata*, *Ixorida pseudoregia* and *Apis cerana*. Meanwhile the smallest pollinator is *Hoshihananomia* sp. Besides, significant difference of body sizes between sexes were prove significant at $p < 0.05$ (body length, U = 12463.00, z = -5.763; body width, U = 11972.50, z = -6.144; head length, U = 15567.50, z = -3.357; head width, U = 7623.00, z = -9.515).

Influence on body size of pollinator and pollen carrying capacity was observed in this study. The insects' body sizes positively influence the pollen carrying capacity. Measurements of body length ($\rho = 0.27$), body width ($\rho = 0.29$), and head length ($\rho = 0.24$) had a weak influence on the pollen loads however head width ($\rho = 0.37$) had a moderate influence. According to Cullen *et al.* (2021), size and diversity of the pollen load were positively related with body size but negatively correlated with the ecological specialization of the insects. Besides, Solís-Montero and Vallejo-Marín (2017) stated that, in order for more pollen to reach the stigma, pollinators should be the same size as or larger than the distance between a flower's sexual organs. Pollen loads are highly influenced not just by pollinator size, but also by the flower's compatibility with pollinators.

CHAPTER FIVE

CONCLUSIONS

5.1 CONCLUSIONS

In this study, the morphological characteristics of *Psidium guajava* flower with comparison to *Psidium cattleianum* were analyzed and distinguished. Both species have flowers that are white colored with multiples of five petals. There are slight differences in morphology particularly in sizes and numbers of certain aspects among cultivars and species which may result of coevoluation with their pollinators. Guava flowers is hermaphrodite and self-fertile but tristyly floral polymorphism displayed was noted to encourage cross-pollination. Pollen grains of *Psidium* were classified as small and described as triangular amb, elliptic in equator for all *P. guajava* cultivars but irregular in *P. cattleianum*. The exine surface for all guava cultivars were granulate/scarbate pattern but rugulate in *Psidium cattleianum*. These characteristics also reported were favoured by pollen-gatherer.

Pollen viability and pollen deposition are reported to have direct influence on seed set. By comparing the viability of pollen grains for guava cultivars and species, it was concluded that there are differences in viability percentage under different staining methods even though it is from the same cultivars. The best flower stage to be used for staining is flower bud nearing anthesis as many viable pollens were detected under both staining methods. However, IKI staining is more preferred for pollen viability study in guava compared to TTC staining as it recorded high percentage of viable pollen. IKI has a high viability percentage (> 85%) for all *P. guajava* cultivars except in *P. cattleianum* (28.99%)

The result from field study in conventional guava orchard at Kg. Lebak Temerloh, recorded insect families of Apidae, Halictidae and Collectidae (Hymenoptera) and a few Rhiniidae, Tachinidae and Calliphoridae (Diptera) together with Mordellidae and Cetoniidae (Coleoptera) as important guava pollinators. Among them, 6 dominant species that visited guava flowers are *Xylocopa latipes, Xylocopa aestuans*, *Apis dorsata*, *Apis cerana*, *Heterotrigona itama* and *Ceratina lieftincki*. The highest visitation rate recorded by *X.latipes* while the lowest is *A. cerana*. Insects' behaviors often associated with activities to fully make use of flower benefits. *Xylocopa* species usually hover around the flower and quickly devour the pollen, *Apis* species and *Ceratina lieftincki* often buries themselves among stamen to forage on the pollen. Meanwhile, *H. itama*, *T. atripes* usually hover around the flower before landing for foraging activities. Similarly, Halictidae species and *Hyaleus* sp. also buries themselves in stamen but were seen to forage on middle parts of flower. Dipterans were often seen to forage onto the anthers directly, while Coleopterans spend most of their time in the flower to forage on either pollen or the flower itself.

Pollen carrying capacity is often related to insects' body sizes. It was determined that the pollen carrying capacity was moderately influenced by head width and very weakly influenced by body length, body width, and head length. *A. dorsata*, the second largest pollinator after *X. aestuans* was identified with the highest pollen deposit on the body. Higher pollen loads were also recorded on *X. aestuans* and *A. cerana*. The same is true of *Hoshihananomia* sp., which carries high guava pollen despite being the smallest beetle.

Up to this day, the role of pollinators to tropical agricultural production including guava usually is underappreciated. This study highlighted the important of pollinators in Malaysian guava industry. Although guava is self-fertile, cross-pollination is beneficial for improvement of the yield. Despite the efficiency of some species to carry and transfer pollen, these pollinators may not be sufficiently abundant in our conventionally managed orchard to have a strong effect on fruit mass. Thus, selection of the best cultivars together with manipulation of pollination services (i.e., introducing or enhancing population) by wild pollinator such as *Xylocopa latipes*, *Xylocopa aestuans*, *Apis dorsata*, *Apis cerana*, *Heterotrigona itama* and *Ceratina lieftincki* may result in improve quantity and quality of pollination of guava in Malaysia.

REFERENCES

Abdelgadir, H. A., Johnson, S. D., & Van Staden, J. (2012). Pollen viability, pollen germination and pollen tube growth in the biofuel seed crop *Jatropha curcas* (Euphorbiaceae). *South African Journal of Botany*, *79*, 132–139.

Abrol, D. P. (2012). *Pollination biology: biodiversity conservation and agricultural production* (p. 792). New York: Springer.

Aldahadha, A., Samarah, N., & Bataineh, A. (2020). Effect of storage temperature and duration on pollen viability and in vitro germination of seven pistachio cultivars. *Journal of Applied Horticulture*, *22*(3), 184–188.

Alves, E., & Magalh, B. (2006). Foraging behavior and pollination efficiency of five bee species on guava (*Psidium guajava* L.) flowers. *Revista Ciência Agronômica*, *37*(2), 216–220.

Amin, M. R., Khisa, S., Rahman, H., Shipa, A. S., Jannat, R., & Amin, M. R. (2019). Abundance and Diversity of Insects on Guava and Foraging Behavior of Pollinators. *Bangladesh Journal of Ecology*, *1*(1), 31–34.

Arnold, S. E. J., Faruq, S., Savolainen, V., McOwan, P. W., & Chittka, L. (2010). FReD: The Floral Reflectance Database — A Web Portal for Analyses of Flower Colour. *PLoS ONE*, *5*(12), e14287.

Arunkumar, R., Wang, W., Wright, S. I., & Barrett, S. C. H. (2017). The genetic architecture of tristyly and its breakdown to self-fertilization. *Molecular Ecology*, *26*(3), 752–765.

Bailes, E. J., Ollerton, J., Pattrick, J. G., & Glover, B. J. (2015). How can an understanding of plant-pollinator interactions contribute to global food security? *Current Opinion in Plant Biology*, *26*, 72–79.

Bapat, V. A., Jagtap, U. B., Ghag, S. B., & Ganapathi, T. R. (2020). Molecular

Approaches for the Improvement of Under-Researched Tropical Fruit Trees: Jackfruit, Guava, and Custard Apple. *International Journal of Fruit Science*, *20*(3), 233–281.

Barbalho, S. M., Farinazzi-Machado, F. M., de Alvares Goulart, R., Brunnati, A. C. S., Otoboni, A. M., & Ottoboni, B. J. M. A. P. (2012). *Psidium guajava* (Guava): A plant of multipurpose medicinal applications. *Med Aromat Plants*, *1*(4), 1-6.

Barrett, S. C. H. (2019). 'A most complex marriage arrangement': recent advances on heterostyly and unresolved questions. *New Phytologist*, *224*(3), 1051–1067.

Basari, N., Ramli, S. N., & Khairi, N. 'Aina S. M. (2018). Food reward and distance influence the foraging pattern of stingless bee, *Heterotrigona itama*. *Insects*, *9*(4), 1–10.

Benedick, S., Gansau, J. A., & Ahmad, A. H. (2021). Foraging Behaviour of *Heterotrigona itama* (Apidae: *Meliponini*) in Residential Areas. *Pertanika Journal of Tropical Agricultural Science*, *44*(2), 485–502.

Boora, R. S., Dhaliwal, H. S., & Arora, N. K. (2016). Crop regulation in guava-A review. *Agricultural Reviews*, *37*(1), 1-9.

Bose, S. K., Ahmed, S., Howlader, P., & Ali, M. (2019). Flowering, Fruiting Behavior and Nutritional Quality of Selected Guava Genotypes. *International Journal of Horticultural Science and Technology*, *6*(1), 11–25.

Bots, M., & Mariani, C. (2005). Pollen viability in the field. *COGEM report, Radboud Universiteit Nijmegen, Netherlands*.

Britannica, T. Editors of Encyclopaedia (2021, November 23). guava. Encyclopedia Britannica. https://www.britannica.com/plant/common-guava

Chambers, J. A. (2011). The Rise of Chinese Military Medicine: Opportunity for Mercy Ship, not Gunboat, Diplomacy. *Military Medicine*, *176*(9), 1043–1050.

Cook, D. F., Voss, S. C., Finch, J. T. D., Rader, R. C., Cook, J. M., & Spurr, C. J. (2020). The role of flies as pollinators of horticultural crops: An Australian case study with worldwide relevance. *Insects*, *11*(6), 23–31.

Coser, S. M., Fontes, M. M. P., & Ferreira, M. F. S. (2012). Assessment of pollen viability in guava genotypes. *Acta Horticulturae*, *959*, 141–144.

Cullen, N., Xia, J., Wei, N., Kaczorowski, R., Arceo-Gómez, G., O'Neill, E., … Ashman, T. L. (2021). Diversity and composition of pollen loads carried by pollinators are primarily driven by insect traits, not floral community characteristics. *Oecologia*, *196*(1), 131–143.

da Silva, S. N., Silva, M. A., Marçal, T. de S., Ferreira, A., Fontes, M. M. P., & Ferreira, M. F. da S. (2017). Genetic parameters of pollen viability in guava (*Psidium guajava* L.). *Australian Journal of Crop Science*, *11*(1), 1–8.

Das, A., Sau, S., Pandit, M. K., & Saha, K. (2018). A review on: Importance of pollinators in fruit and vegetable production and their collateral jeopardy from agro-chemicals. *Journal of Entomology and Zoology Studies*, *6*(4), 1586–1591.

Das, R., Jha, S., & Halder, A. (2019). Insect pollinators of litchi with special reference to foraging behaviour of honey bees. *Journal of Pharmacognosy and Phytochemistry*, *8*(4), 396-401.

Delaplane, K. S., Dag, A., Danka, R. G., Freitas, B. M., Garibaldi, L. A., Goodwin, R. M., & Hormaza, J. I. (2013). Standard methods for pollination research with *Apis mellifera*. *Journal of Apicultural Research*, *52*(4).

Deuri, A., Rahman, A., Gogoi, J., Borah, P., & Bathari, M. (2018). Pollinator diversity and effect of *Apis cerana* F. pollination on yield of mango (*Mangifera indica* L.). *Journal of Entomology and Zoology Studies*, *6*(5), 957-961.

dos Santos Pereira, E., Vinholes, J., Franzon, R. C., Dalmazo, G., Vizzotto, M., & Nora, L. (2018). *Psidium cattleianum* fruits: A review on its composition and bioactivity. *Food Chemistry*, *258*, 95-103.

Dötterl, S., & Vereecken, N. J. (2010). The chemical ecology and evolution of bee-flower interactions: A review and perspectives. *Canadian Journal of Zoology*, *88*(7), 668–697.

Dyer, A. G., Jentsch, A., Burd, M., Garcia, J. E., Giejsztowt, J., Camargo, M. G., ... & Shrestha, M. (2021). Fragmentary blue: Resolving the rarity paradox in flower colors. *Frontiers in Plant Science, 11*, 618203.

Edlund, A. F., Swanson, R., & Preuss, D. (2004). Pollen and stigma structure and function: the role of diversity in pollination. *The plant cell, 16*,

Erdtman, G. (1960). The Acetolysis Method. A Revised Description. Svensk Botanisk Tidskrift, 54, 561-564. S84-S97.

Erickson, M. F., & Pessoa, D. M. A. (2022). Determining factors of flower coloration. *Acta Botanica Brasilica*, *36*, 1–18.

Eswaran, R., Anand, K., Baskaran, S., & Pramod, P. (2016). Foraging dynamics and pollination efficiency of *Apis cerana indica* (Hymenoptera: Apidae) on eggplant, *Solanum Melongena* L. *Kongunadu Research Journal*, *3*(2), 104-112.

Evrenosoğlu, Y., & Misirli, A. (2009). Investigations on the pollen morphology of some fruit species. *Turkish Journal of Agriculture and Forestry*, *33*(2), 181–190.

Faheem, M., Aslam, M., & Razaq, M. (2004). Pollination ecology with special reference to insects a review. *J Res Sci*, *4*(1), 395-409.

Frankie, G. W., & Thorp, R. W. (2009). Pollination and pollinators. *Encyclopedia of Insects* (pp. 813-819). Academic Press.

Friedman, J., & Barrett, S. C. H. (2009). Wind of change: New insights on the ecology and evolution of pollination and mating in wind-pollinated plants. *Annals of Botany*, *103*(9), 1515–1527.

Gemmill-Herren, B., & Ochieng', A. O. (2008). Role of native bees and natural habitats in eggplant (*Solanum melongena*) pollination in Kenya. *Agriculture, Ecosystems and Environment*, *127*(1–2), 31–36.

Giannini, T. C., Costa, W. F., Cordeiro, G. D., Imperatriz-Fonseca, V. L., Saraiva, A. M., Biesmeijer, J., & Garibaldi, L. A. (2017). Projected climate change threatens pollinators and crop production in Brazil. *PLoS ONE*, *12*(8), 1–13.

Glaettli, M., & Barrett, S. C. H. (2008). Pollinator responses to variation in floral display and flower size in dioecious *Sagittaria latifolia* (Alismataceae). *New Phytologist*, *179*(4), 1193–1201.

Gupta, K. R., Reybroeck, W., Van Veen, J. W., & Gupta, A. (2014). *Beekeeping for Poverty Alleviation and Livelihood Security. Vol. 1: Technological Aspects of Beekeeping*. Dordrecht: Springer.

Gutiérrez, R. M. P., Mitchell, S., & Solis, R. V. (2008). *Psidium guajava*: A review of its traditional uses, phytochemistry and pharmacology. *Journal of Ethnopharmacology*, *117*(1), 1–27.

Halbritter, H., Ulrich, S., Grímsson, F., Weber, M., Zetter, R., Hesse, M., ... & Frosch-Radivo, A. (2018). Pollen morphology and ultrastructure. *Illustrated pollen terminology*, 37-65.

Halder, S., Ghosh, S., Khan, R., Khan, A. A., Perween, T., & Hasan, M. A. (2019). Role of pollination in fruit crops: A review. *The Pharma Innovation*, *8*(5), 695–702.

Hao, K., Tian, Z. X., Wang, Z. C., & Huang, S. Q. (2020). Pollen grain size associated with pollinator feeding strategy. *Proceedings of the Royal Society B: Biological Sciences*, *287*(1933).

Hiwale, S. (2015). Sustainable horticulture in semiarid dry lands. *Sustainable Horticulture in Semiarid Dry Lands*, 1–393.

Huang, Z., Zhu, J., Mu, X., & Lin, J. (2004). Pollen dispersion, pollen viability and pistil receptivity in *Leymus chinensis*. *Annals of Botany*, *93*(3), 295–301.

Huda, A. N., Salmah, M. R. C., Hassan, A. A., Hamdan, A., & Razak, M. N. A. (2015). Pollination Services of Mango Flower Pollinators. *Journal of Insect Science*, *15*(1), 113.

Jacobs, J. H., Clark, S. J., Denholm, I., Goulson, D., Stoate, C., & Osborne, J. L. (2010). Pollinator effectiveness and fruit set in common ivy, *Hedera helix* (Araliaceae), 19–28.

Jesson, L. (2016). Reproductive Strategies. *Encyclopedia of Applied Plant Sciences, 2*, 321–326.

Jones, G. D. (2012). Pollen analyses for pollination research, unacetolyzed pollen. *Journal of Pollination Ecology, 9*(13), 96–107.

Jones, G. D., & Jones, S. D. (2001). The uses of pollen and its implication for entomology. *Neotropical Entomology*, *30*(3), 341–350.

Joshi, N. C., & Joshi, P. C. (2010). Foraging Behaviour of *Apis* spp. on Apple Flowers in a Subtropical Environment. *New York Science Journal, 3*(3), 71–76.

Kadam, D. M., Kaushik, P., & Kumar, R. (2012). Evaluation of guava products quality. *International Journal of Food Science and Nutrition Engineering*, *2*(1), 7-11.

Kamath, J. V., Rahul, N., Kumar, C. A., & Lakshmi, S. M. (2008). *Psidium guajava* L: A review. *International Journal of Green Pharmacy (IJGP)*, *2*(1).

Kevan, P., Giurfa, M., & Chittka, L. (1996). Why are there so many and so few white flowers? *Trends in Plant Science, 1*(8), 252–284.

Kirmse, S., & Chaboo, C. S. (2020). Flowers are essential to maintain high beetle diversity (Coleoptera) in a Neotropical rainforest canopy. *Journal of Natural History*, *54*(25–26), 1661–1696.

Koetz, A. (2013). Ecology, Behaviour and Control of *Apis cerana* with a Focus on Relevance to the Australian Incursion. *Insects, 4*(4), 558–592.

Koyuncu, F., & Tosun, F. (2008). Evaluation of pollen viability and germination capacity of some sweet cherry cultivars grown in isparta, Turkey. *Acta Horticulturae, 795 PART 1*, 71–74.

Krizek, B. A., & Anderson, J. T. (2013). Control of flower size. *Journal of Experimental Botany, 64*(6), 1427–1437.

Kumar, M., & Rai, C. P. (2020). Pollinator diversity and foraging behaviour of insect visitors on fennel (*Foeniculum vulgare* L.) bloom. *Journal of Entomology and*

Zoology Studies, *8*(5), 2545–2548.

Kumar, S., Joshi, P. C., Pashupati, Singh, V. K., & D.K., M. (2016). Role of Insects in Pollination of Mango Trees. *International Research Journal of Biological Sciences*, *5*(1), 64–67.

Kumari, N., Gautam, S., & Ashutosh, C. (2013). *Psidium guajava* A fruit or medicine- An overview. *The Pharma Innovation*, *2*(8, Part A), 63.

Lange, D., Calixto, E. S., & Del-Claro, K. (2017). Variation in extrafloral nectary productivity influences the ant foraging. *PloS one*, *12*(1), e0169492.

Les, D. H., Cleland, M. A., & Waycott, M. (1997). Phylogenetic Studies in Alismatidae, II: Evolution of Marine Angiosperms (Seagrasses) and Hydrophily. *Systematic Botany*, *22*(3), 443.

Li, J., Webster, M., Furuya, M., & Gilmartin, P. M. (2007). Identification and characterization of pin and thrum alleles of two genes that co-segregate with the Primula S locus. *Plant Journal*, *51*(1), 18–31.

Luo, E. Y., Ogilvie, J. E., & Thomson, J. D. (2014). Stimulation of flower nectar replenishment by removal: a survey of eleven animal-pollinated plant species. *Journal of Pollination Ecology*, *12*, 52-62.

Medina, N. N. R., & Herrero, J. V. I. (2016). Guava (*Psidium guajava* L.) cultivars: an important source of nutrients for human health. In *Nutritional composition of fruit cultivars* (pp. 287-315). Academic Press.

Melloni, M. L. G., Scarpari, M. S., de Mendonça, J. R., Perecin, D., de Andrade Landell, M. G., & Pinto, L. R. (2013). Comparison of two staining methods for pollen viability studies in sugarcane. *Sugar Tech*, *15*, 103-107.

Nascimento, A. S., & Carvalho, C. A. L. (2019). Pollen morphology of Myrtaceae visited by social bees. *Plant Science Today*, *6*(2), 98–105.

Norliyana, A., Ismariah, A., & Lim, H. F. (2019). Assessing economic benefits of pollination services by natural pollinators. *Plant Productivity and*

Environmental Conservation, *25*, 132.

Pacini, E. (2015). Pollination. *Encyclopedia of ecology*, 2857-2861.

Padilla-Ramirez, J. S., Gonzalez-Gaona, E., Perez-Barraza, M. H., Osuna-Garcia, J. A., De La Espindola-Barquera, M. C., & Reyes-Aleman, J. C. (2012). Phenological behavior of guava trees (*Psidium guajava* L.) under different climatic conditions of Mexico. *Acta Horticulturae*, *959*(1996), 97–102.

Panda, M., Lele, N., Samal, R. N., Dalai, D., Parida, S. P., & Murthy, T. V. R. (2019). Insect Flower Visitors and their Role in Mangrove Pollination: A Study from East Coast of India. *Journal of Tropical Forestry and Environment*, *9*(2), 78–92.

Partap, U. (2010). The pollination role of honeybees. In *Honeybees of Asia* (pp. 227-255). Berlin, Heidelberg: Springer Berlin Heidelberg.

Pashte, V. V, & Kulkarni, S. R. (2015). Role of Pollinators in Qualitative Fruit Crop Production: A Review. *Trends in Biosciences*, *8*(15), 3743–3749.

Patel, S. (2012). Exotic tropical plant *Psidium cattleianum*: A review on prospects and threats. *Reviews in Environmental Science and Biotechnology*, *11*(3), 243–248.

Pontikis, C. A. (1996). *Psidium guajava* L. (Guava). In *Biotechnology in Agriculture and Forestry* (Vol. 35, pp. 308–320).

Rai, M. K., Asthana, P., Jaiswal, V. S., & Jaiswal, U. (2010). Biotechnological advances in guava (*Psidium guajava* L.): recent developments and prospects for further research. *Trees*, *24*(1), 1–12.

Raju, A. J. S. & Reddi, C. S. (2000). Foraging behaviour of carpenter bees, Genus: *Xylocopa*: Xylocopidae: Hymenoptera, and the pollination in some Indian plants. *Journal of the Bombay Natural History Society*, 381-389.

Rudall, P. J. (2020). Colourful cones: how did flower colour first evolve? *Journal of Experimental Botany*, *71*(3), 759–767.

Sachin, A. J., Ramteke, V., & Bharath, K. R. (2015). Flower regulation in guava and pomegranate. *Popular Kheti*, *3*(4), 53-54.

Salazar, D. ., Melgarejo, P., Martinez, R., Martinez, J. ., Hernandez, F., & Burguera, M. (2006). Phenological stages of the guava tree (*Psidium guajava* L.), *108*, 157–161.

Schiestl, F. P., & Johnson, S. D. (2013). Pollinator-mediated evolution of floral signals. *Trends in Ecology and Evolution*, *28*(5), 307–315.

Sharma, A., Sehrawat, S. K., Singhrot, R. S., & Tele, A. (2010). Morphological and chemical characterization of *Psidium* species. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, *38*(1), 28–32.

Sharma, S., Sehrawat, S. K., & Sharma, K. D. (2017). Studies on Time and Duration of Flowering, Floral Bud Development and Morphology of Guava (*Psidium guajava* L.) under Semi-Arid Region of India. *Int. J. Curr. Microbiol. App. Sci*, *6*(12), 4176-4186.

Silva, M. J. R. da, Tecchio, M. A., Domiciano, S., Leonel, S., & Balestrero, R. I. (2016). Phenology, yield and fruit quality of "Paluma" guava tree at different pruning times. *Ciência e Agrotecnologia*, *40*(3), 317–325.

Singh, M. M. (2009). Foraging behaviour of the Himalayan honeybee (*Apis cerana* F.) on flowers of Fagopyrum esculentum M. and its impact on grain quality and yield. *Ecoprint: An International Journal of Ecology*, *15*, 37-46.

Singh, S. P. (2011). Guava (*Psidium guajava* L.). *Postharvest biology and technology of tropical and subtropical fruits*, 213-246e.

Singh, V. K., Ravishankar, H., Singh, A., & Soni, M. K. (2015). Pruning in guava (*Psidium guajava*) and appraisal of consequent flowering phenology using modified BBCH scale. *Indian Journal of Agricultural Sciences*, *85*(11), 1472-1476.

Soares, T. L., de Jesus, O. N., de Souza, E. H., dos Santos-Serejo, J. A., & de Oliveira, E. J. (2013). Morphology and viability of pollen grains from passion fruit species (*Passiflora* spp.). *Acta Botanica Brasilica*, *27*(4), 779–787.

Solís-Montero, L., & Vallejo-Marín, M. (2017). Does the morphological fit between flowers and pollinators affect pollen deposition? An experimental test in a buzz-pollinated species with anther dimorphism. *Ecology and Evolution*, *7*(8), 2706–2715.

Souza-Pérez, M., Mourelle, D., Trujillo, C., Borges, A., & Speroni, G. (2021). Pollen grain performance in *Psidium cattleyanum* (Myrtaceae): a pseudogamous polyploid species. *Flora*, *281*, 151863.

Sulusoglu, M., & Cavusoglu, A. (2014). In vitro pollen viability and pollen germination in cherry laurel (*Prunus laurocerasus* L.). *The Scientific World Journal*, *2014*.

Suneetha, T., & Raju, A. S. (2019). Foraging activity of Carpenter bees in relation to floral biology of the drumstick tree, *Moringa oleifera* Lamk.(Moringaceae). *Journal of Palynology*, *55*, 99-108.

Takano, A., Gisil, J., Yusoff, M., & Tachi, T. (2005). Floral and pollinator behaviour of flexistylous Bornean ginger, *Alpinia nieuwenhuizii* (Zingiberaceae). *Plant Systematics and Evolution*, *252*(3–4), 167–173.

Teixido, A. L., Barrio, M., & Valladares, F. (2016). Size Matters: Understanding the Conflict Faced by Large Flowers in Mediterranean Environments. *Botanical Review*, *82*(2), 204–228.

Thapa, R. (2006). Honeybees and other Insect Pollinators of Cultivated Plants: A Review. *Journal of the Institute of Agriculture and Animal Science*, *27*, 1–23.

Thornhill, A. H., & Crisp, M. D. (2012). Phylogenetic assessment of pollen characters in Myrtaceae. *Australian Systematic Botany*, *25*(3), 171–187.

Tuler, A. C., da Silva, T., Carrijo, T. T., Garbin, M. L., Mendonça, C. B., Peixoto, A. L., & Gonçalves-Esteves, V. (2016). Taxonomic significance of pollen morphology for species delimitation in *Psidium* (Myrtaceae). *Plant Systematics and Evolution*, *303*, 317-327.

Van der Kooi, C. J. (2021). How much pigment should flowers have? Flowers with moderate pigmentation have highest color contrast. *Frontiers in Ecology and Evolution*, *9*, 731626.

Vinod, M., & Sattagi, H. N. (2018). Pollinator Fauna and Their Relative Abundance in Guava. *Journal Experimental Zoology*, *21*(2), 1173–1179.

Vishwakarma, P. K., Vincent, L., Vasugi, C., & Rajasekharan, P. E. (2021). Effect of cryopreservation on pollen viability, fertility and morphology of different *Psidium* species. *Cryobiology*, *98*, 112-118.

Wang, H., Yu, W. B., Chen, J. Q., & Blackmore, S. (2009). Pollen morphology in relation to floral types and pollination syndromes in *Pedicularis* (Orobanchaceae). *Plant Systematics and Evolution*, *277*, 153-162.

Willmer, P., & Finlayson, K. (2014). Big bees do a better job: intraspecific size variation influences pollination effectiveness. *Journal of Pollination Ecology*, *14*, 244-254.

Woodcock, T. S., Larson, B. M. H., Kevan, P. G., Inouye, D. W., & Lunau, K. (2014). Flies and Flowers II: Floral Attractants and Rewards. *Journal of Pollination Ecology*, *12*, 63–94.

Yamasaki, E., & Sakai, S. (2013). Wind and insect pollination (ambophily) of *Mallotus* spp. (Euphorbiaceae) in tropical and temperate forests. *Australian Journal of Botany*, *61*(1), 60–66.

Yang, X. S. (2012, September). Flower pollination algorithm for global optimization. In *International conference on unconventional computing and natural computation* (pp. 240-249). Berlin, Heidelberg: Springer Berlin Heidelberg.

Zariman, N. A., Omar, N. A., & Huda, A. N. (2022). Plant Attractants and Rewards for Pollinators: Their Significant to Successful Crop Pollination. *International Journal of Life Sciences and Biotechnology*, *5*(2), 270-293

APPENDIX

PUBLICATION

2nd Applied Science Research International Conference 2022 (2ndASRIC 2022)

Evaluation of Pollen Viability by Colorimetric Analysis of Guava cultivars

Zariman, N.A*, Nurul Huda, A.

Department of Plant Science, Kulliyah of Science, International Islamic University Malaysia, Malaysia

**Corresponding author E-mail: nurathirahzariman@gmail.com (Zariman, N.A)*

1. INTRODUCTION

Psidium guajava belongs to the family of *Myrtaceae*. In Malaysia, guava are mostly grown for fresh consumption and also processed and exported for other purposes [1]. However, low fruit production remains even with good plant variety and best orchard management. Lack of pollination might be the reason for low food production. Therefore, study related to pollination biology is important as adequate pollination led to successful fertilization of gametes and pollen viability is essential for this process. Pollen quality is evaluated based on the viability, speed of germination of pollen grains and pollen tube growth [2]. Pollen viability is important for the sexual reproduction success in plants [3,4] and through this aspect, it is possible to increase the productivity and fruit quality by selecting the pollinating genotypes, however, it is necessary to have broader understanding of the characteristic of pollen viability in specific species [4]. Pollen viability can be assessed by different methods such as staining, in vitro and in vivo germination tests or analyse the final seed set [5]. Staining tests are faster and easier than pollen germination tests [2,3,6]. Several staining that were successfully studied on plant species by previous researcher includes, lugol's solution, acetic orcein, Alexander's solution, acetic carmine, 2,3,5-triphenyl tetrazolium chloride (TTC), lactophenol blue on guava, passion fruits and sugarcane, cherry laurel and *Polygala paniculata* [2,4,7–10]. In this study, the assessment of pollen viability was made on three different guava cultivars namely Lohan, Kim Choo Red and Pink heirloom collected from random number of trees under staining method with 2,3,5-triphenyl tetrazolium chloride (TTC) and Iodine Potassium Iodide (IKI) solution.

Review article

Omar, N.A., N.A. Zariman, and A.N. Huda, Pollination in the Tropics: Role of Pollinator in GuavaProduction. International Journal of Life Sciences and Biotechnology, 4(3): p. 623-639.

DOI: 10.38001/ijlsb.9076962021.

Pollination in the Tropics: Role of Pollinator in GuavaProduction

Nurul Alia Omar[1], Nur Athirah Zariman[1], A. Nurul Huda[1*]

ABSTRACT

Pollinators are the key point for successful pollination in most flowering plants where around 90% of plants depend entirely on them. Majority of the pollinating species are wild, comprising bees, certain species of flies, wasps, moths, butterflies, thrips, beetles, bats, birds and other vertebrates, and among them insect pollinators are the most crucial. Fruit crops benefit in an impressive way from insect pollination, where there is a remarkable improvement both in the productivity and the quality of self-fertilized, self- incompatible and cross-cropping crops worldwide.

The pollinators are responsible for assisting these flowering plants with their

reproduction. However, the crisis of the pollinator's decline (wild and managed pollinators) which could seriously disrupt pollination activities in the ecosystems has attracted the attention of the world. Despite the growing concern about the decline in pollinators worldwide, some issues remain uncertain as data are often limited and undermined. Guava, *Psidium guajava* is a marketable fruit in numerous tropical and subtropical regions around the world. There has been a growing interestin pollination studies on guava because of its great economic importance. In guava, self-pollination is evident, however, it benefits greatly from insect pollination. This article aims to provide an overview of tropical pollination and pollination problems that have occurred around the world with a focus on pollination activities in guava.

ARTICLE HISTORY

Received

01 April 2021

Accepted

17 June 2021

KEYWORDS

Psidium guajava, insects, fruit crops, pollination, reproduction

Review Article

Zariman, A. Z., N. A. Omar and A. Nurul Huda, Plant Attractants and Rewards for Pollinators: Their Significance to Successful Crop Pollination. International Journal of Life Sciences and Biotechnology, 2022. 5(2): p. 270-293. DOI: 10.38001/ijlsb.1069254

Plant Attractants and Rewards for Pollinators: Their Significance to Successful Crop Pollination

Nur Athirah Zariman[1], Nurul Alia Omar[1], A. Nurul Huda[1*]

ABSTRACT

Plant and pollination have a mutualistic relationship where both parties offer and gain benefits for each other. The plant-pollinator interactions resulted in successful crop pollination in which the plant received pollination services by animal pollinator to increase food production that eventually increase crop economic value. Overall, ecosystem is highly dependent on pollinator thus there is a need to review potential valuation method of crop production and analyse the current understanding of the value of pollination service towards the ecosystem as well as the traits plant offer and benefits that pollinator gain from the relationship. The attractant and rewards highly depending on each other. Plant often able to attract pollinators through traits like the shape, size and colours of flower, deception, scents as well as location. In the meantime, plant would provide a reward for pollinators that visited the flower which includes food from pollen and nectar that contains high nutritional value, energetic rewards to reduce energy cost of survival, protection and shelter against predator and not to forget breeding, oviposition and mating sites inside the flower plant. This review emphasizes the ecological relationship of plant and pollinator that resulting in effective crop pollination if the attractant and incentives are significantly reliant onone another. However, there could be flaws, such as modifications to plant or environmental factors, would affect the rewards supplied and resulting in decrease crop output. With this review and current technological advancements, optimistically deeper investigations in the interaction of pollinator and flowering plant can be conducted and best pollinator management approaches can be established to secure sustainable crops production.

ARTICLE HISTORY

Received

7 February 2022

Accepted

13 April 2022

KEYWORDS

Pollination, plant-pollinator relationship, attraction, rewards, fruit crop

GLOSSARY

Amb. The outline of pollen grains seen in polar view.

Ambophily. The process of pollinating with the aid of the wind and insects.

Anemophily. The process of pollinating with the aid of the wind

Brevicolpate, colpi that fail to meet at the end ofthe pole because of the exine layer.

Exine. The outer coating or surface of pollen grains

Herkogamy. Distance that separates stigma and stamen in hermaphroditic angiosperms.

Hermaphrodite flower. The flowers having both kinds of reproductive organs and can produce both gametes associated with male and female sexes.

Hydrophily. Pollination process with the help of water.

Parasyncolpate. Colpi that do not meet at the pole but form a triangularshape in the middle ofthe pole region known as the apocolpial field

Syncolpate. Colpi that meet at the pollen grain pole.