Diversity of bee flora and pollination efficacy to crop yields of native honeybees and stingless bees in Thailand

Orawan Duangphadee, Preecha Rodim and Punpaporn Kongkaew

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ABSTRACT

This project focus on the study of the pollination potential of using have honeybees and stingless bees on the economic crops. The 2 honeybee species, *Apis cerana* and *Apis florea* and 2 stingless bee species, *Tetragonula laeviceps* and *Tetragonula pagdeni* have been targeted for this evaluation. To archive projects goals, following scope has been destinated. 1) The investigation and identification of bee flora in relative to pollination efficacy and the signatured honey properties. 2) Pollination efficacy targeted 4 plant species, *Selenicereus undatus (Dragon fruit), Passiflora edulis* Sims (Passion fruits), *Cucurbita moschata* (pumpkin) and *Solanum lycopersicum* (tomatoes).

A total of 35 commonly bee flora species were identified and recorded with their local name. Twenty one species are source both nectar and pollen. Whereas, 9 species are source of pollen. The others 5 species are either source of nectar or honey dew

Pollination and fruit set in pumpkin (Cucurbita *moschata* Duch.) by honey bees and stingless bees. The number of visit significantly affect the number of fruiting at 81%, 88%, 100 and 100% in 1, 3, and 9 and unlimited bee visits on stigmas in *A. cerana*. The number of visit significantly affect the number of fruiting at 93%, 98%, 100 and 100% in 1, 3, and 9 and unlimited bee visits on stigmas in *A. florea*. The number of visit significantly affect the number of fruiting at 73%, 88%, 95 and 100% in 1, 3, and 9 and unlimited bee visits on stigmas in *T. pagdeni*. The This result was corresponding to the other previous studies. This study indicating that pumpkins required at least 9 visits to transfer enough pollen for 100% pollination results. However, in the natural situation that allow unlimited visit on female flowers, the number of bee visit stigma was 18±1.5 visits per flowers.

The pollination efficiency of a stingless bee of the species *Tetragonula pagdeni* Schwarz was investigated in tomato (*Solanum lycopersicum* L.) cultivated in different growth conditions in greenhouse environment. In greenhouse with stingless bees presented 82% more fruit set than in greenhouse with mechanical vibration (78%) and greenhouse without stingless bees (17%). In addition, fruit produced in greenhouse with stingless bees showed higher quality compare to the mechanical vibration and greenhouse with stingless bees .

We investigated the fruit set and quality of purple passion fruit subjected to pollination by *A. cerana*. The bee pollinated versus control flowers had significantly greater fruit set (10/0, 10/0 and 20/0). The fruit weight, transverse diameter, longitudinal diameter, peel weight, number of seeds, and juice yield compare to control group. Bees started visiting the passion fruit flowers from 7:00 AM to 5:00 PM, with peak visitation at around 11:00 PM. The greatest mean duration of flower visits was observed at 11:30 AM (470 s), which indicated the peak hour of flower opening. We conclude that passion fruit farmers can manage cavity nesting honeybees. *A. cerana* to increase yield. *A. cerana* is also found naturally that could support sufficient pollination services on the passion fruit in the fields.

The study demonstrates the technique of a classification model for hissing behavior in Asian cavity-nesting bees, *Apis cerana,* under different circumstances. The monitoring devices were installed to collect hissing signals related to their defensive behavior. The best model was selected based on two competing objectives: the minimum number of parameters and 95% baseline accuracy. The results revealed that the one-dimensional neural network trained with the temporal domain spectrogram that consists of 2 hidden layers, 32 nodes for each layer, and a minimum of 3,737 trainable parameters could provide the best accuracy.

The multidiscipline uses of native bees will be presented both beekeeping and pollination aspects. Beekeepers and farmers can collaborate and manage for the multilevel benefits such as use the bee colonies to increase a crop yield and harvest a monofloral signature honey for the highest return of investments.

บทคัดย่อ

โครงการนี้ศึกษาศักยภาพของผึ้งและชันโรงในด้านการช่วยผสมเกสรในพืชเศรษฐกิจ ในผึ้ง 2 ชนิด ผึ้งโพรง *Apis cerana* และ ผึ้งมิ้ม *Apis florea* และชันโรง 2 ชนิด *Tetragonula laeviceps* และ *Tetragonula pagdeni* โดยทำภายใต้กรอบงานวิจัย 2 กรอบคือ 1) การสำรวจศึกษาและการระบุชื่อวิทยาศาสตร์พืชอาหาร เพื่อเป็นข้อมูลประกอบการวิเคราะห์ผสมเกสรและการผลิตน้ำผึ้งเอกลักษณ์เฉพาะ 2) ประสิทธิภาพในการผสมเกสรในพืช 4 ชนิด คือ *Selenicereus undatus* (แก้วมังกร), *Passiflora edulis* Sims (แพสชั่นฟรุ๊ต), *Cucurbita moschata* (ฟักทอง) and *Solanum lycopersicum* (มะเขือเทศ)

ในงานวิจัยพบพืชอาหารผึ้งทั้งหมด 35 ชนิด โดยเป็นพืชที่ให้ทั้งน้ำหวานและเกสร 21 ชนิด ให้เกสรอย่างเดียว 9 ชนิด และอีก 5 ชนิดให้น้ำหวานเพียงอย่างเดียว

ประสิทธิภาพการผสมเกสรของฟักทองโดยผึ้งและชันโรง พบว่าจำนวนผึ้งที่เข้าตอมดอกฟักทองมีผงต่อการติดผลของฟักทอง โดยในผึ้งโพงพบร้อยละการติดผลที่ 81%, 88%, 100 และ100% ในจำนวนครั้งการเข้าตอมของผึ้ง 1, 3, 9 และไม่จำกัดครั้ง ในผึ้งมิ้ม พบร้อยละการติดผลที่ 93%, 98%, 100 และ 100% ในจำนวนครั้งการเข้าตอมของผึ้ง 1, 3, 9 และไม่จำกัดครั้ง ในชันโรงพบร้อยละการติดผลที่ 73%, 88%, 95 and 100% ในจำนวนครั้งการเข้าตอมของผึ้ง 1, 3, 9 และไม่จำกัดครั้ง ในผึ้งที่ใช้ทุกชนิดให้การติดผล 100% ที่การเข้าตอมดอกประมาณ 9 ครั้ง อย่างไรก็ตามในสภาวะธรรมชาติทั้งผึ้งและชันโรงเข้าตอมดดอกฟักทองเฉลี่ยที่ 18±1.5 ครั้ง ซึ่งสามารถคาดหวังผลลัพธ์การผสมเกสรที่ 100 % ได้

ประสิทธิภาพการผสมเกสรมะเขือเทศราชินีโดยชันโรงชนิด *T. pagdeni* ในโรงเรือน พบว่าชันโรงให้ประสิทธิภาพการติดผลอยู่ที่ร้อยละ 82% ซึ่งมากกว่าการใช้มือเขย่าที่มีประสิทธิภาพ 78% และโรงเรือนที่ไม่มีชันโรง ติดผลที่ 17% นอกจากนี้ยังพบว่าคุณภาพของผลดีกว่าในการผสมโดยชันโรงเมื่อเปรียบเทียบกับการใช้มือผสมและการไม่มีชันโรงช่วยผสม

ประสิทธิภาพการผสมเกสรแพสชั่นฟรุ๊ตสีม่วงของผึ้งโพรง *A. cerana* พบว่าผึ้งโพรงช่วยในการติดผลของแพสชั่นฟรุ๊ตสีม่วง 100% เมื่อเปรียบเทียบกับกลุ่มควบคุมที่ไม่ได้รับการผสม คุณสมบัติของผลเช่นน้ำหนัก เส้นผ่านศูนย์กลางแนวขวาง แนวยาว น้ำหนักเนื้อ จำนวนเมล็ดและปริมาณเนื้อก็ดีกว่าเมื่อเปรียบเทียบกับกลุ่มควบคุม โดยผึ้งเข้าตอมดอกแพสชั่นฟรุ๊ตสีม่วงตั้งแต่ 7:00 -17:00 น โดยมีช่วงที่เข้าตอมมากที่สุดคือ 11:00 น. ช่วงเวลาที่ผึ้งเข้าตอมดอกนานที่สุดคือ 11:30 น (470 วินาที) ซึ่งบ่งบอกถึงช่วงที่ดอกไม้เปิดมากที่สุด ผลการทดลองสรุปได้ว่าเกษตรกรสามารถใช้ผึ้งโพรงช่วยเพิ่มแพสชั่นฟรุ๊ตสีม่วงได้อย่างมีประสิทธิภาพ

ในการศึกษาเทคนิคในการสกัดคุณลักษณะจากสัญญาณเสียงและสร้างแบบจำลองการเรียนรู้ของเครื่องในการแยกแยะสภาวะรังในผึ้งโพรงไทย (*Apis cerena*) จากผลการทดลองพบว่าแบบจำลองการเรียนรู้เชิงลึกแบบคอนโวลูชัน แบบ 1 มิติ ที่มีโครงสร้างจำนวนโหนด 32 โหนด, 2 เลเยอร์, จำนวนพารามิเตอร์ในการเรียนรู้ทั้งหมด 3,737 ตัว และใช้สเปตโตรแกรมเป็นคุณสมบัติในการเรียนรู้ สามารถให้ค่าความแม่นยำเฉลี่ยสูงสุด

ผลการดำเนินงานของโครงการสร้างแนวทางการใช้ประโยชน์ผึ้งไทยในหลายระดับโดยเฉพาะศักยภาพในการเลี้ยงและการผสมเกสร เกษตรกรสามารถรวบรวมและจัดการให้ได้ประโยชน์ในหลายระดับ เช่นนำผึ้งไปผสมเกสร และเก็บน้ำผึ้งเอกลักษณ์เฉพาะพืชชนิดนั้นไปด้วย ซึ่งจะทำให้เกิดการให้ผลตอบแทนเชิงเศรษฐกิจสูงสุด

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CHAPTER 1

INTRODUCTION

1.1 Rational

The honeybees and stingless bees has been long used for the pollination services. The estimation ecological impact ahs been report at 12.88 billion dollar in Canada (Scott-Dupee et al., 1995) 26.96 billion dollar in in Australia (Gordon and Devis, 2003, Oldroyd and Wongsiri, 2006) Eventhogh, the economic impact has not yet been evaluated in Asia countries, the use of honeybees and stingless bees for pollination services has been wide spread use.

The introduced species, *Apis mellifera* are the most domesticated in beekeeping industry in Thailand accounting for 85% of the total domesticated colonies. However, since 2006, *A. mellifera* population face the global problem with Colony Collapse Disorder (CCD). This symptom cause the lost of A. mellifera population for 33.8 per year consecutively, 18.5% in Europe, 25 % in Japan and 10-80 % in middle east. Additionally, using A. mellifera colonies for pollination sometimes facing the problematic due to their unsuitable characters of the introduce species such as low resistant to pest, parasite and disease or intolerance on the high humidity climates. In the small plantation, the use of *A. mellifera* often start with high capital compare to using the native species.

Therefore, this project focus on the study of the pollination potential of using have bees and stingless bees on the ecological services. The 4 honeybee species and 2 stingless bee species have been targeted for this evaluation. A long with the pollination potential evaluation, the beekeeping potential and managements was also evaluated. The advantage of using native species are well promised as following

1) Low capital is expected because the stock could be collected in the nature and the low cost or recycle material could be wisely integrated.

2) Native honeybees and stingless bees show high resistant to pests, parasites and diseases. The management cost was expected low. The beekeeper can start easily with the small scale beekeeping with the high return of investments.

3) High diversity of bee flora can be expected both regarding to the pollination services and food resources.

4) High quality of honey can be expected. The prices of honey in the existence market is higher than of the A. mellifera.

To archive projects goals, following scope has been destinated.

1. The investigation and identification of bee flora in relative to pollination efficacy and the signatured honey properties in four targeted plant species, *Selenicereus undatus*

(Dragon fruit), Passiflora edulis Sims (Passion fruits), *Cucurbita moschata* (pumpkin) and *Solanum lycopersicum* (tomatoes)

2. The observation of bee behaviour as a direction of evaluation of pollinator quality and honey production

We expect this project will provide a new professional with promising revenues from native beekeeping in Thailand. The multidiscipline uses of nativebees will be presented both beekeeping and pollination aspects.

Beekeepers and farmers can collaborate and manage their lands/farms in sustainable ways for current and future generations. For example, in each village, farmers and beekeepers can help each other, i.e., beekeepers can provide bee colonies to pollinate crops of farmers, which beekeepers can gain honey from colony placement in a farmer's cropland, while farmers need to cease pesticide application to allow managed bees to pollinate their crops and eventually increase their crop yields. Hence, this research will encourage people to do sustainable farming and increase awareness of pesticide usage.

1.2 *Apis florea* as a pollinator in ecosystems (Rod-im and Duangphakdee 2020)

The majority of flowering plants are pollinated by insects and other animals. The estimation, based on 352,000 species of angiosperms, is that 67 to 96 % of flowering plants need pollination. The dependence of plants on animals increases from temperate to tropical zones from 78 to 94 %, respectively (Fig. 17.11). Based on an estimation of 352,000 plant species of angiosperms Kier *et al*. (2005) and Ollerton *et al*. (2011) calculated that 50 % of the species are distributed in the tropics, 27.7 % in the subtropical, and 21.8 % in the temperate regions. Around 85 % of flowering plants, or 299,200 species, are biotically pollinated (Fig. 17.11).

Apis florea play a vital role in the pollination of cultivated crops and plants growing in their natural habitats in Thailand. As an endemic species, they have co-evolved with the native flora which has led to specific associations between the plant species and the bees. Because *A. florea* are able to adapt to various habitats, they are regarded as one of the most suitable bee species to co-exist with man. *Apis florea* play a critical role in maintaining the vital ecological process of cross pollination in agricultural, disturbed and fragmented areas (Roubik *et al*., 2005). *Apis florea* migrate in response to the availability or scarcity of flowering plants. Neupane and Thapa (2005) reported that foraging activity is greatly influenced by weather conditions and the availability of nectar and floral resources. In relation to the behavior of a short flowering period in tropical plants, they have developed the ability to establish and expand the colony rapidly in response to available resources to ensure survival (Itioka *et al*., 2001).

The sharp decline in the number of native honeybee colonies in Thailand, due to the extension of agricultural areas and urbanization, has been reported over the past four decades. This may be attributable to extensive deforestation between 1976 and 1989 due to the increase of urbanization and the construction of roads. Although no systematic study has been conducted, the number of the larger bee species, such as *A. dorsata* and *A. cerana*, have declined more rapidly than that of *A. florea* (pers. obs.). The giant honeybees, *A. dorsata*, migrate annually and form large colony aggregation areas; Ruttner (1998) reported more than 100 colonies in a single large tree. Large aggregations, such as this, require a substantial amount of floral resources, and it is this reward that drives the annual *A. dorsata* migrations (Oldroyd *et al*., 2000).

In a study conducted at the Thai Department of Agriculture's Chanthaburi Tropical Fruit Research Centre (CTFRC), 95 colonies of honeybees/km^2 were recorded. Of these, the dwarf bees, *A. florea* and *A. andreniformis*, were the most commonly occurring native species, 12 colonies of each, 5 colonies of *A. cerana* and 2 colonies of *A. dorsata*. The mean distance from an *A. florea*, colony to the next nearest *A. florea* colony was 187 m, and the mean distance from that colony to the next nearest colony was 105 m (Rinderer *et al*., 2002). Liow *et al*. (2001) reported that the size and quality of habitats significantly impact the abundance of bee species to adjacent agricultural landscapes. In the presence of small-sized, fragmented patches of different habitats as a result of urbanization, the abundance of *A. florea* colonies signifies its role as a key pollinator species in maintaining plant diversity in these disjointed areas. Moreover, because of *A. floreas* short foraging range, it may be least affected by pesticide usage and the destruction of natural habitats (Kremen *et al*., 2002). *Apis florea* have already been reported as one of the most efficient pollinators of various crops in Pakistan (e.g. canola, *Brassica napus* (Ali *et al*., 2011); onion, *Allium cepa* (Saeed *et al*., 2008; Sajjad *et al*., 2008); fodder, *Sesbania sesban* (Sajjad *et al*. 2009); fodder alfalfa, *Medicago sativa* (Ahmad, 1976) and bitter gourd, *Momordica charantia* (Saeed *et al*., 2012).

In a study of bee diversity and pollen sources of Apidae in Thung Salaeng Luang, the third largest National Park in Thailand, which comprises four forest types (deciduous dipterocarp, deciduous with bamboo, seasonal evergreen and dipterocarp-pine forests), it was found that *A. florea* were the main pollinators of 46 of the 62 plant species recorded in the Park (Maxwell, 2004). Rod-im (2015 a / b), in a similar study conducted in the Tanassirim mountain range of western Thailand, found over 35 species of wild plants which were utilized by *A. florea* as a source of pollen and nectar. The morphology of this bee plays an important part in pollination because of its smaller size and widespread distribution across mainland Asia, occurring at elevations ranging from sea level to approximately 2000 m (Hepburn and Radloff, 2011). *Apis florea* has been found to be a regular visitor and pollinator in many crops i.e. *Jatropha curcas* (Inson and Malaipan, 2011), hog plum, lychee, mango, pomelo, tomato, and is reportedly an important visitor to cucurbits (Grewal and Sidhu, 1978). The same is true of crops being propagated for seed; *A. florea* was the most frequent visitor to cauliflower and fennel (81 %), and radish. Some observations from the 1940s recorded *A. florea* as the only species of honeybee found in sarson and toria (Rahman, 1940). *Apis mellifera* pollination of onion requires between 12-15 hives/ha as foragers tend to avoid the flowers. However, *A. florea* appears to be the most frequent *Apis* visitor on onion and was recommended for further consideration as a pollinator (Crane, 1991). *Apis florea* also play a leading role pollination of macadamias in northern Thailand (Kongpitak *et al.*, 2004), the nocturnally dehiscent king palm (*Archontophoenix alexandrae*) (Oldroyd *et al*., 1992), and as a potential pollinator of *Muntingia calabura* (Hawkeswood and Sommung, 2016).

Pollination by wild animals is a key ecosystem service which benefits man. A large proportion of the world's major crops are pollinated by animals (Richards, 2001; Ghazoul, 2005), or comes indirectly from animals fed on staple crops. Klein *et al*. (2007) collected primary data from 200 major food producing countries and found 87 species of plants are dependent on animal pollination, while 28 species are not. If production volume is considered, 60 % of global production does not depend on animal pollination, whereas 35 % does and 5 % is unknown / not evaluated (Fig. 17.12). Pollination is essential for 13 crops, of high importance for 30 crops, moderately important for 27, marginally important for 21, and 7 do not rely on pollination. The remaining 9 crops remained unknown (Fig. 17.13).

The decline of pollinator species can lead to a serious reduction in the quantity and number of plant species, biodiversity and essential nutrients in ecosystems, thus affecting man and other living creatures. According to Williams (1994) 70% of tropical crops are likely to have at least one variety that is improved by animal pollination). Studies on honeybees have mainly focused on *A. mellifera* and *A. cerana*, the most economically valuable pollinators of crops grown in monocultures worldwide (McGregor, 1976; Watanabe, 1994; Roubik, 2002). In Thailand, *A. mellifera* are introduced species and often do not visit some of the native plants. The indigenous bees therefore have an important role in ensuring pollination. Compared to other wild bees, *A. florea* are adaptable, versatile, cheap, effective, and a convenient choice as pollinators. Implementation of bee pollination must be locally managed, taking into consideration farming methods, crops cultivated, honeybee traits and local customs. Additionally, the strong turnover in the quality and quantity of floral resources are known to influence the behavior and foraging strategy of pollinator species (Aluri and Subba Reddi, 1994). High humidity, heavy rainfall, wind, and low temperatures have a negative effect on visits to sunflower inflorescence. Different floral nectar compositions are preferred by different species (Abrol, 2011). *Apis florea* prefer flowers with low caloric rewards (Sihag and Rathi, 1992), whereas, *A. dorsata* prefer flowers with high caloric rewards. However, this could be a result of resource partitioning by honeybees. In a competition, *A. florea* will lose the battle against *A. dorsata* and *A. cerana*. Although it has been reported that *A. florea* prefer flowers with low caloric rewards, this might be a disputed claim as *A. florea* is often found in high resource-rich flowers such as pumpkin and king palm.

The economic benefits of bees and other insect pollination has been discussed at both local and global levels . In considering the maintenance of honeybee populations, the conservation and management of resources within agricultural and surrounding natural landscapes needs to be addressed (Zhang *et al*., 2007), as well as the suitability of nesting habitats (Ali *et al*., 2017). The information on host plant species, temporal dynamics in providing floral resources, foraging preference, the behavioral interaction between honeybees and flowers, the effect of foraging competition on crops between pollinators, will serve as baseline studies for an ecosystem service. This would result in new beekeeping strategies being developed, providing a livelihood for local peoples (Devy and Davidar, 2006) and the conservation of natural habitats (Kovács-Hostyánszki *et al*., 2017).

1.3 Potential bee flora and pollination capacities (Duangphakdee et al., 2022)

The giant honey bee, *A. dorsata*, is considered an important pollinator of crops and wild plants. It is one of the major pollinators in Southeast Asian lowland dipterocarp forests, which pollinate at least 15 species of emergent and canopy trees in Lambir Hills National Parks, Sarawak, Malaysia (Momose *et al.*, 1998). In Thailand, giant honeybees visit several plant species. Suwannapong *et al.* (2013) reported the bee flora utilized by *A. dorsata* in Nan province, northern Thailand, by identifying pollen grains from their pollen loads and midguts. The number of bee flora found from the pollen loads and in the midgut of *A. dorsata* were 6 (Table 3) and 11 species (Table 4), respectively (Suwannapong *et al*., 2013). Stewart *et al.* (2018) studied pollination networks in 52 green areas throughout Bangkok, Thailand and found that *A. dorsata* visited 17 plant species. Wayo *et al.* (2018) revealed that *A. dorsata* appears to be the most legitimate and effective insect pollinator of durian (‘Monthong’ cultivar) in Southern Thailand since it commonly and consistently visited durian flowers at dusk (Fig. 11), which was also found in previous studies (Aziz *et al*., 2017, Bumrungsri *et al.*, 2009, Sritongchuay *et al.*, 2016). It should be noted that *A. dorsata* likely visited durian for nectar sources because the evidence indicates that bats play an important role in durian fruit setting. Moreover, *A. dorsata* is an important floral visitor of mangoes (Sritongchuay *et al.*, 2022) and a vital pollinator of longan in Thailand (Sritongchuay *et al.*, 2021).

Table 3 Pollen source plants identified from pollen loads of *A. dorsata* (Nan province, northern Thailand).

No.	Family	Plant species
1	Asteraceae	*Melampodium divaricatum* (Pers.) DC.
2	Asteraceae	*Wedelia trilobata* (L.) Hitchc.
3	Mimosaceae	*Mimosa pudica* L.
4	Mimosaceae	*Mimosa pigra* L.
5	Legumimosae	*Tamarindus indica* L.
6	Poaceae	*Zea mays* L.

Table 4 Bee plants identified from the midguts of *A. dorsata* (Nan province, northern Thailand)..

No.	Family	Plant species
1	Cucurbitaceae	*Cucurbita citrullus* L.
2	Poaceae	*Zea mays* L.
3	Cucurbitaceae	*Momordica charantia* L.
4	Palmae	*Cocos nucifera* L.
5	Amaranthaceae	*Celosia argentea* L.
6	Asteraceae	*Wedelia trilobata* L.
7	Sapindaceae	*Dimocarpus longan* Lour.
8	Myrtaceae	*Syzygium malaccense* L.
9	Asteraceae	*Ageratum conyzoides* L.
10	Mimosaceae	*Mimosa pigra* L.
11	Mimosaceae	*Mimosa pudica* L.

Figure 1.1 Durian flowers visited by *A. dorsata* at dusk. (Photo credit: K. Wayo)

A. dorsata is one of the species that well adaptive to new plants including the introduced species. The observation in the Queen Sirikit Botanical Park No. 9 (Rama Park 9) in Bangkok, Thailand disclosed that *A. dorsata* pollinate the introduced plant *Ruellia simplex* C. Wright (Acanthaceae) (Hawkeswood and Sommung, 2016). The introduced species, *Tecoma stans* (L.) Kunth (Bignoniaceae), which have now become a common ornamental plant in Thailand, is also beneficial for *A. dorsata* (Fig. 1.2).

Figure 1.2 *Tecoma stans* flowers were visited by *A. dorsata.* (Photo credit: P. Rod-im)

1.4 Stingless bees as a pollinator in ecosystems (Rattanawannee and Duangphakdee et al., 2020)

In subtropical and tropical regions, stingless bees (Apidae: Meliponini) are very diverse, consisting of nearly 600 species (Michener 2013) and play an important role in providing pollination services. They are considered as effective pollinators of 18 crops and contribute to pollination of more than 60 agricultural plant species (Heard 1999; Ramirez et al. 2002; Ramírez et al. 2018; Slaa et al. 2006). Stingless bee beekeeping or meliponiculture are generally undertaken by traditional communities and has local characteristics according to regional and traditional knowledge (Cortopassi-Laurino et al. 2006). Due to the lack of stinging behavior in stingless bees, it is much easier to handle stingless bees than most honeybees. Currently, many stingless bee species are maintained and managed for crop pollination and honey production.

To use stingless bees for pollination services has been apply by the traditional methods. The selection of the most suitable species and best management practices during pollination has not been studied yet. To date, thirty-five species in ten genera of tribe Meliponini has described. The one of the high potential group to use in pollination services in Thailand are Tetragonula genus. Unfortunately, this group has been the most problematic in taxonomic status and description. Incorrection species identification occur often among the stingless bee group. Because there is no complete reference in description of species, species identification key, DNA barcoding and geographic distribution. The mis match species will create a genetic population bottle neck and inbreeding which is a major obstacle in further research and development in this group. This problem creates a long chain affect in the stingless bee conservation and farming utilization.

Given that the wide diversity and different body size of stingless bee species, selection of the most appropriate species for both field and greenhouse crops is important. For example, in large farms, farmers need to ensure that their managed bees are able to pollinate all individuals of target crops. As the maximum distance from which bees return after displacement is believed to be a good indicator for a species' maximum foraging range (Gathmann and Tscharntke 2002; van Nieuwstadt and Iraheta 1996), translocation experiments (i.e., bees are caught and released at increasing distances from the colony) can be used to obtain the percentage of bees that return from each distance to estimate the flight/foraging range in stingless bees as shown in previous studies e.g., Campbell et al. 2019; Leonhardt et al. 2016; van Nieuwstadt and Ruano Iraheta 1996; Smith et al. 2017; Wayo et al. 2022.

Moreover, since each flowering plant species produces different concentrations of nectar to attract different types of pollinators. A previous study has revealed that hummingbirds are attracted to flowers that produced 35 - 45% sugar nectar concentration, whereas bees prefer 20 - 60% of sugar concentration and ants prefer 60% (Kim et al. 2011). Knowledge on preference of nectar resources in stingless bee species thus is crucial for beekeepers, for example, they need to ensure to provide suitable and sufficient floral resources in or close to fields (i.e. within maximum homing range).

1.5 Tomatoes pollination biology (Wongsa et al., 2022 and Wongsa 2022)

Tomato (*Solanum lycopersicum* L.) flower has self-compatible; Nevertheless, releasing the pollen grains through the apical must be vibrated on poricidal anthers (Moura-Moraes et al. 2021). In open-field, the wind action and insect visitation can encourage the essential vibration to release the pollen grains (Gaglianone et al. 2018). However the use of pollinating agents is required under greenhouse conditions for sufficient fruit set as well as fruit quality (Morandin et al. 2001, Palma et al. 2008). Mechanical vibration of the tomato flowers is the first option to pollinate in greenhouse. However, this procedure is expensive, intensive of labor, and can destruction the flowers (Ilbi and Boztok 1994). Another option for pollination of tomatoes cultivated in greenhouse is the used of bees. Previously, the attempt to compared effectiveness of the traditional vibration (Mechanical vibration) with pollination by honey bee (*Apis mellifera* L.) including bumble bee (*Bombus terrestris*) in greenhouse tomato. The result illustrate that bumble bees had effectiveness on tomatoes in greenhouse , whereas honey bees had not (Banda and Paxton 1991, Sabara and Winston 2003). This possible due to the little nectar secretion of tomato flowers is not deserve for honey bee visitors (dos Santos et al. 2009). Moreover, the pollination effectiveness of the stingless bee (*Melipona quadrifasciata*) and honey bee (*Apis mellifera*) was tested and compared in tomato greenhouse. The previous results were found stingless bee was significantly more efficient than honey bees in pollinating greenhouse tomatoes. In addition, under greenhouse condition, the quality of tomato fruits can be increased by the use of stingless bee species with different flower-visiting behaviors. (dos Santos et al. 2009, Moura-Moraes et al. 2021). In temperate regions, many species of bumble bees are successfully used for greenhouse tomato pollination that they exhibit the buzz-pollination behavior (Cauich et al. 2004). However, the usefulness of bumble bee is restricted because they are not perennial. Therefore, more colonies are needed to replace a number of times of year that raised the economic cost.

In tropical areas temperature inside greenhouse usually exceed that the outside environment (Bartelli et al. 2014). The microclimate may thus induce heat stress to bumble bees, which native to temperate areas. Among of numerous native bee species in the tropic, stingless bees have gained awareness as potential pollinators of both open and greenhouse cultivars (Slaa et al. 2000).

Stingless bees are eusocial insects that widely distributed across subtropical and tropical regions (Quezada-Euán 2018, Rattanawannee and Duangphakdee 2019). These groups of eusocial bees with approximately 500 valid species (Quezada-Euan 2018), is divers in external morphology, body size, colony size, and foraging strategies (Hrncir and Maia-Silva 2013, da Silva et al. 2017) Stingless bees are the major group of pollinators in many native and cultivated plant species in the tropics (Momose 1998, Heard 1999, Michener 2007). The advantage of stingless bee that considered promising for use as pollinator are floral constancy, populous and maintenance of perennial colonies, non-functional sting, easy to handle, present a marked worker recruitment behavior (Bartelli et al. 2014, da Silva et al. 2017). Recently, the management of various agricultural plants in greenhouse has been tested with the introduction of difference species of stingless bees. The investigation of two stingless bee species (*Scaptotrigona* aff. *Depilis* and *Nannotrigona testaceicornis*) were found success in pollinating strawberries greenhouses in Brasil (Roselino et al. 2009). Moreover, in Malaysia the use of the stingless bee *Tetragonula iridipennis* was found successfully to be the pollinator in cucumber greenhouses (Mitta et al. 2017). Until, in recent year, the effectiveness of stingless bee to be a pollinator was found that may depend on suitability between plants and body sizes of each species (Moura-Moraes et al. 2021). Furthermore, Atmowidi T. et al. (2022) investigated the pollination effectiveness of stingless bees in two genera , *Tetragonula* in strawberry and *Heterotrigona* in melon in the greenhouse. The stingless bees, *Tetragonula* increased the number of fruits and qualities of strawberry whereas, the number of fruits and qualities of melon were increased by *Heterotrigona*. The possibility of domestication has therefore suggested stingless bees to be an alternative to honey bees and bumble bees as pollinators of economic crops in the tropical areas.

At least 33 species of stingless bees have been reported from Thailand (Schwarz 1939, Sakagami et al. 1985, Michener and Boongird 2004, Klakasikorn et al. 2005, Rasmussen 2008, Engel and Rasmussen 2017, Attasopa et al. 2018) and at least six species are commonly managed for commercial meliponiculture in Thailand (Rattanawannee and Duangphakdee 2019). Of these, *Tetragonula pagdeni* is one of the small size species with most easily transferred and multiplying colonies to artificial wooden hive boxes. It has been qualified by the National Bureau of Agricultural Commodity and Food Standards, Thailand to be one of two species that successfully domesticated in farm scales. Colony populations of this species have a several thousand individual female worker headed by a single female queen (Fatima et al. 2018). Additionally, the species are not only effective pollinator for economic crops and native plants, but also show high yield of honey production (Attasopa et al. 2018, Fatima et al. 2018, Rattanawannee and Duangphakdee 2019). Commercial cultivations in greenhouses, including tomato, is rapidly increase in the tropic areas; therefore, there is a great potential for the use of native bees that need to be examined. In this study, we aimed to evaluate *T. pagdeni* efficiency in pollination and consequent fruit production of tomatoes of different greenhouse conditions.

1.6 Pumpkin Pollination Biology

Pumpkins and squashes are the important vegetable in Cubicuta group. There are one of the most economical value crop worldwide which grown in 177 countries worldwide with production yield 26,486,616 ton produced (FAOSTAT, 2016). Although, the round cucurbita fruits are usually called pumpkins and those that are not round called squashes (Paris, 2005), *C. moschata* with a flattened and round shape accounts for the majority of cultivated pumpkins in Thailand.

Pumpkin in Thailand has been used as a staple food and been incorporated in various food recipes. All part of Fruits of Pumpkins are consumed in different ways and described as a socioeconomic and nutritional important (BLANKet al., 2013). It is an annual species with monoic that pistillate and staminate flowers are in the some plants. The pumpkin flowers are incomplete sexual flowers that have only one of the sexual parts, either the stamen or the pistil. The pumpkin flowers need a vector for pollen grain transferring from male to the stigma of female flowers during their pollination. Several types of insects are considered as a potential pollinators for pumpkin species. However, in our study, we test the pollination efficacy of different species of bees as a pollen grain vectors of on Thai pumpkin (Cucurbita moschata Duch.). Three species of insect vectors are the eastern cavity nesting honeybees, *A. cerana*, the red dwarf honeybees, *Apis florea* and stingless bee, *Tetragonula pagdeni*.

1.7 Passion fruit pollination biology

In recent years, passion fruit (P. edulis) interest has been increased in human consumption due to the eating quality of it's fruits, juiciness, attractive nutritional values, health benefits and a recipes of traditional used for medicine and cosmetic (Cazarin et al., 2016; Lima et al., 2016; Pereira et al., 2019). Accordingly, a production of passion has been extended to support a growth market. Pollination therefore plays a major role, in passion fruits and the success of pollination factors have studies focus. Like other flowering plants, pollination is crucial in the reproductive stage of passion fruits. The fruit setting rely on the trigger of pollen to extent their ovary maturation (Das et al., 2013). This suggests that fruit set in passion fruits highly rely on pollination services. Eventhough the requirement of flowers due to physiology still influence on of passion fruit setting.

The distance between the dehiscent side of the anthers and corolla, shape of anthers and their versaltility that easily moved upon the touch. The stigma of passion fruit resist to the movement to facilitated the adhesion of the pollen when touched by the pollinators (Bruckner et al., 1995; Silveira et al., 2012). The characteristics flowers of purple fruits seem to adapt to suit pollination by larger bees, such as the carpenter bees, which give a appreciated results in pollination services (Barrera et al., 2021). However, for pollination purpose, the carpenter bees are somehow not convenience for colony management. Thus, this study investigated the fruit setting of passion fruit and observed the foraging behavior of the cavity nesting bees, *Apis cerana* as well as the foraging pattern, time of visit, and length of stay to provide explanation about the importance of pollinators and hand pollination in fruit setting.

1.8 Hissing signal emissions of hive during predator disturbances (Boonmarueng, 2022)

Honeybees communicate with their nestmates while engaging in their various activities by using symbolic language, chemical pheromones, and a variety of sound signals. The vibroacoustic signals emitted by honeybees are produced from gross body movements, wing movements, high-frequency muscle contractions without wing movements, and pressing the thorax against the substrates or another bee (Nolasco et al., 2019; Hunt and Richard, 2013). In general, honeybees usually communicate in the range of 300–600 Hz (Hrncir et al, 2005).

Hissing is one of the acoustic signals emitted by multiple worker bees in response to disturbances such as the colony being knocked or poked, the branch supporting the colony being tapped (Hrncir et al, 2005) or attacking the colony by natural enemies (*Vespa velutina, Vespa mandarinia*) as shown in Figure 2.1. This hissing is sometimes called a Shimmering signal (Hrncir et al, 2005). Historically, hissing behavior was firstly observed in *Apis dorsata* by Butler in 1954 (Butler, 1954). Moreover, hissing behavior has also been observed in different honeybee species, including *Apis cerana (*Sakagami, 1960), *Apis florea* (Sarma, et al., 2002) , and *Apis mellifera* (Papachristoforou et al., 2008). The hissing signal is produced by several worker bees moving their abdomens dorsoventrally to create a ripple effect that is synchronously harmonized (Duangphakdee et al., 2020). In response to the hissing from the first honeybee group, the other worker bees are encouraged to join in and perform the hissing signal together. Finally, when all of the honeybees in the colony jointly produce this sound simultaneously, it creates a short sound (0.5-1.0 s) with the broadband frequency of 500–5,000Hz in *Apis florea* or 300-3,600Hz in *Apis cerana* (Hrncir et al, 2005) (Fig. 1.3).

Figure 1.3 *Vespa velutina* and *Vespa mandarinia*

However, Kawakita, et al. (Kawakita et al., 2018) demonstrated that *Apis cerana japonica* can hiss even without the presence of any obvious threatening stimulus, such as hornets or mammals near the colony. The authors installed mini microphones connected with IC recorders located inside 6 different beehives and recorded the hives 24 hours a day for 14 days. The sampling rate and resolution of the recordings were 44.1kHz and 16 bits, respectively. The audio data were processed using the Adobe Audition CC. The researchers defined a hissing signal as an acoustic signal within a frequency range of 300-3,600Hz and with a continuous duration greater than 0.5 seconds. The results from Figure 2.2 (a) and (b) showed that *Apis cerana japonica* hissed only during the daytime but more 100 times every day at a mean frequency of 402.7 and SD of 223.6. The mean dominant frequency of hissing was 755.1 Hz (SD: 236.7 Hz). The mean duration of the hissing was 1.51 s (SD: 0.63 s) (Fig 1.4).

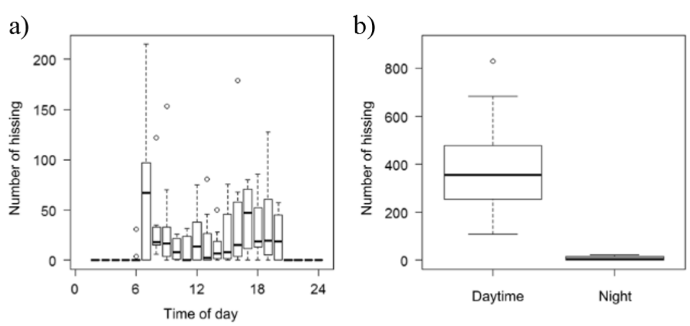

Figure 1.4 Temporal occurrence of hissing signals of *Apis cerana jabonica*;
(a) temporal occurrence of hissing signals during time of day,
(b) temporal occurrence of hissing signals during daytime and night

CHAPTER 2

MATERIALS AND METHODS

2.1 Availability of Bee Flora (adaptatipn from Teklay, 2011)

Availability of bee flora has been observed. Area within 1 km radius of each bee colony sites has been inspected for honeybee flora. The percentage of bee flora was coordinated using the GPS (Figure 2.1).

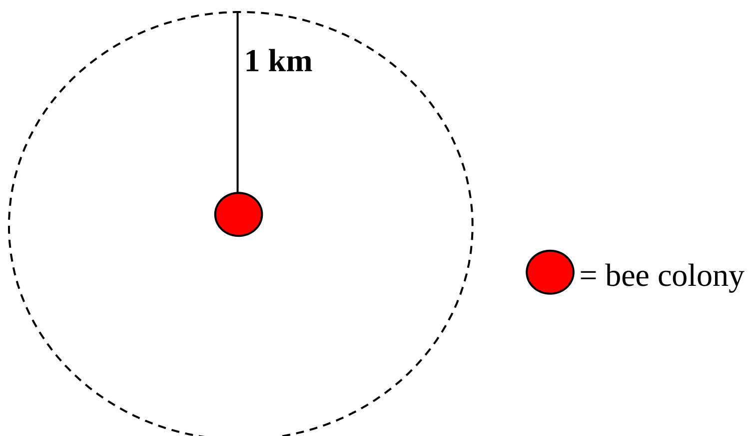

Figure 2.1 Diagram the transect for observe the availability of bee flora

Whenever bees are found on the flowers of such plants or on flowers near the bee tree, their foraging behavior was observed. Such plants was identified immediately using the key to plants by Gamble (1967), Rao (1973) and Basavarajappa and Raghunandan (2013) and/or with help of taxonomist at Royal Forest Department. If a plant was recorded as bee foraging species at a particular site and later encountered in subsequent surveys on other sites, it is only scored for presence (observations for bee foraging attempts were not repeated on them). Samples of plants that could not be identified in the field was collected using a sharp penknife; where a small twig or portion of a branch of the plant with the full complement of its leaves and flowers was cut, placed and pressed in-between the pages of old newspaper (Kongkanda, 2002), packaged in a properly labeled brown envelopes and placed horizontally in specimen box, and we returned to the transect to finish observations to its end mark. All collected samples have sent for proper identification by a taxonomist.

2.1.1 Bee flora and Landscape bee Index

In this research, we use the bee flora and Landscape bee index to compared the differences of bee flora in each areas. For bee flora index, we calculate by using the quality of bee flora (the data of quality come from Pyraman and Wongsiri, 1986, Adhikari and Ranabhat, 2011 and Rod-im, P., 2014) with this following formula.

$$\text{Bee flora index} = \frac{\text{Sources and status of nectar, pollen or honeydew of bee flora}}{9}$$

To calculate the landscape bee index, using the quality of bee flora, bee flora's density and %cover areas of bee flora's type multiply with areas (ha) for each site,

Landscape bee index = Bee flora index x Bee flora's density x %cover areas x Area (ha)

2.2 Pollination Efficiency of Stingless Bees, *Tetragonula pagdeni* Schwarz (Apidae: Meliponini), on Greenhouse Tomatoes (*Solanum lycopersicum* L.) (Comply from Wongsa et al., 2022 and Wongsa 2022)

Study site

This study was performed in Lam Sonthi district, Lopburi province, Thailand (15° 18' 6" N and 101° 21' 48" E). The climate is tropical with average precipitation of 553.92 mm and average temperature ranged from 26 - 30 °C. The experiments were conducted between August 2020 through March 2021. Ten colonies of *T. pagdeni* were provided from different commercial beekeepers, and kept at study area for four weeks before start experiment. All colonies were in standard wooden hive boxes (L x W x H = 30 x 20 x 15 cm^3), conventionally used for meliponiculture in Thailand. In addition, all colonies are queen-right and contained similar quantities of honey and pollen storage-pots at the beginning of the experiments (da Silva 2017, da Silva et al. 2017). The tomato plant (*S. lycopersicum*) used was a hybrid (Cherry tomato: F1-Hybrid) with a growing period of up to 120 days.

First experiment

Three greenhouses with three treatments were used in this experiment. The treatments were greenhouse with stingless bees (WSB), greenhouse without stingless bees (WoSB), and Greenhouse without stingless bee but hand-vibration (WoSBHV: each cordon of plants was vibrated by hand for 5 s between 08:00 – 10:00 am twice a week(Cauich et al. 2004)) with 60 replicates (plants). The greenhouses were flat-arch roof (size: 3 × 6 m, lateral height: 3 m, height at the top: 4.5 m; transparent low density polyethylene cover with a sheet thickness of 0.1 mm).

The tomato (*Solanum lycopersicum*) was used in experiment, which have period of flower blooming 45 - 50 days after seeding. Each inflorescence approximately has 6 flowers, and it has a period of fruit produce 80 – 120 days after seeding. The thirty days old of seedling were placed in each 5 liters-plastic pot (one plant per pot) that was contained 5 kg of substate. The substrate used in each pot consisted of five shares of forest soil and one of cattle manure supplement, with 20 g of NPK 4-14-8 (Silva-Neto et al., 2018). Thirty pots were used in each greenhouse, and each pot was considered a plot (Silva-Neto et al., 2018). Manual irrigation and 20 g of NPK 4-14-8 were treated cover fertilization 30 day after transplantation. Additionally, manual evaluation of pest and diseases were performed in this study, and pesticide application was not used throughout the experiment.

Stingless bee (*T. pagdeni*) colony was used in the greenhouse of WSB treatment. One colony (≈ 500 - 890 adult worker bees per colony (Del Sarto et al. 2005, dos Santos et al. 2009)) of *T. pagdeni* in standard wooden box was introduced into greenhouse as soon as flowers are visible (about 30 days after transplantation) and removed 30 days after. At least two different colonies were used in this experiment section. For WoSBHV treatment, each cordon of plants was vibrated by hand for 5 s at 08.00 and 10.00 am twice a week for four weeks (Cauich et al., 2004). For WoSB treatment, no pollinating activities of tomato plants were not conducted throughout the experiment.

To evaluate pollination efficiency of *T. pagdeni* on tomatoes, 10 flowers of each of 10 tomato plants per treatment (WSB, WoSB, and WoSBHV) were randomly tagged. The numbers of fruit set of tagged-flowers were determined to evaluate the percentage of fruit set of each treatment (Cauich et al. 2004, dos Santos et al. 2009). The fruit production of each treatment were individually examined the weight, width and height to evaluate the fruit quality (Cauich et al., 2004). Thirty fruit per treatment were randomly dissected to count seed number. The pollination efficiency of *T. pagdeni* (WSB) against the other treatments (WoSB, and WoSBHV) were investigated. An analysis of variance (ANOVA) followed by a multiple comparison Tukey test ($p < 0.05$) was performed to compare the numbers of fruit set, number of seeds, fruit weight, fruit diameter, and fruit height. A multiple regression analysis was performed to investigated the relationship between treatments and the number of seeds, fruit weight, fruit diameter, and fruit height. The statistical analyses were performed using the R-program (Team 2018).

To examined the foraging activity of *T. pagdeni* in greenhouse condition, the numbers of returning foragers with and without pollen to the nest were recorded for 5 min every hour during 05.30 to 18.00 hr for 5 days To do this, the nest entrance was blocked, and returning foragers with and without pollen load attach on their corbicula were counted (Cauich et al., 2004). Additionally, the physical factors including temperature and relative humidity inside the greenhouse were also recorded. The differences between hours for each type of returning foragers were analyzed using the residuals of a chi-square test (Zar 1999). A Pearson's correlations was performed to investigated the relationship between foraging activity of the bees and the physical factors (Cauich et al., 2004; Zar, 1999).

Second experiment

The experiment was performed in the same greenhouses as the previous experiment. Unlike the first experiment, the treatments were applied in the same tomato plant to prevent the varying of fruit production among the different plants (Silva-Neto et al. 2013). The treatments were therefore performed in greenhouses with and without stingless bees. In each greenhouse, 20 inflorescences of tomato were randomly bagged to prevent foragers from visiting the flowers and another 20 inflorescences were tagged without being bagged. One colony of *T. pagdeni* was introduced into the greenhouse, following the same management procedures performed in the first experiment. The number of flowers in each tagged inflorescence was recorded for comparison with the number of fruits set, which is the fruiting rate per inflorescence. After the senescence of the tagged flowers, the stingless bee colony was removed from the greenhouse. Twenty tomato fruit were collected from each greenhouse treatments to investigate fruit weight, number of seeds per fruit, fruit height, and fruit diameter, according to the procedure of the first experiment. An analysis of variance (ANOVA) followed by a multiple comparison Tukey test ($p < 0.05$) was carried out to compare the fruiting rate, fruit weight, longitudinal and transverse diameter of fruit, and the number of seeds from bagged and unbagged flowers, in greenhouse with and without stingless bees (Silva-Neto et al. 2018). The statistical analyses were performed using the R-program (Team 2018).

2.3 Pollination Efficiency of *Apis florea*, Fabricius, Apis cerana, Fabricius (Apidae: Apinae) and *Tetragonula pagdeni* on Thai pumpkin (*Cucurbita moschata* Duch.) (Comply from Rodim et al, 2022)

2.3.1 Study Area

Experiment were carried out at Chongsarika, Phathananikom, Lopburi, Thailand. The spacing was 3.00 m between rows by 1 m between plants, giving a density of 3,344 plants per hectare. The pumpkin plantation were 6.97 hectares (43.59 rai) with the 75 meter width and 930 meter length. The area had followed standard cultural practices for pumpkin (Egel et al., 2003).

Before planting the N-P-K with 20-0-0 were applied to the plot in 150 kg per hectare. Plots were side-dresses with 13-13-13 with 150 Kg per hectare after 6 weeks ages of planting. The pest control consisted of spraying a mixture of permethrin after 45 day age before flowering. Weeds were controlled subsequent mechanical or hand cultivation.

2.3.2 Bag and unbag experiment

A honey bee colony was placed at the edge of the field. Flowers were randomly selected before anthesis and bagged with a net to prevent visits from honey bees and other insects. At anthesis, they were uncovered and the studies described below were performed.

2.3.3 Pollen removed from anther by bees

The collection protocol was followed GRAÇAS Vidal et al. (2010). In this experiment the number of pollen grains removed from anthers by honeybees in different numbers of bee visits was determined on different days, according to Vidal et al. (2006). Male flowers were bagged prior to anthesis and uncovered after anthesis to allow bee visits. Six treatments (number of visits) were evaluated: no visit (remained bagged after anthesis = control), 1, 2, 3, 6 visits in three replications (flowers) per treatment. After visiting, the anthers were cut off and placed in vials with 70% ethanol. The anthers were washed with ethanol until all pollen grains were removed. The pollen grains were decanted and the supernatant was removed by micropipet. Glycerol 50% was added to the remaining pollen in a graduated vial to make up 5 mL. The vials were shaken by a vortex mixer in order to get a uniform pollen suspension. Five samples of 50 uL were taken from the pollen suspension and all pollen grains were counted under 60X magnification. Total number of pollen grains per anther (in 5 mL) was estimated based on the amount of pollen counted in all subsamples (50 µL × 5 = 250 µL) (GRAÇAS Vidal et al., 2010).

2.3.4 Pollen on bee hair body

The collection protocol was followed GRAÇAS Vidal et al. (2010). The amount of pollen adhering to the body of honeybees was evaluated. Each flower was bagged before anthesis and uncovered after anthesis to allow just one visit of the collected bees. Five forager bees were collected in the field right after they finished their visit inside flowers. The collected bees were put in tubes with 70% ethanol and washed until all the pollen was removed from the bees' bodies and counted under the 10X magnifier without staining on countable microscope.

2.3.5 Pollen deposition on stigma

The collection protocol was followed GRAÇAS Vidal et al. (2010). Three treatments were evaluated (1, 3, and unlimited bee visits on stigmas). Fifteen female flowers were bagged prior to anthesis (five flowers per treatment) and uncovered after anthesis to allow bee visits according to treatments, in the period from 6h00 to 12h00 am. After the different number of visits, the uncovered flowers were cut off and placed in individual vials and frozen. The stigmas were thawed and the pollen grains washed and counted under a 10X magnifier without staining on countable microscope. Two set of experiment was conducted. One set was cut to count the pollen grain deposition while another one left for fruiting setting count. After each visit the flowers were bagged, labeled, and fruit set was evaluated 7 days after pollination when differences in ovary swelling or abscission were obvious. Fruits were follow up for 14 days and removed after fruit set evaluation).

2.3.6 Opening of nectaries and attractiveness

The collection protocol was followed GRAÇAS Vidal et al. (2010). This experiment evaluated 30 staminate flowers and pistillate flower during 3 days (15 flowers per day). On each day four flowers were bag from before anthesis until 12 hrs of the end period of flowering. The micropipette has been used to suck nectar and measure the volume. Nectar of each flower was placed with pedicel in a vial. Degree brix was immediately measure. The number of honey bee visits and the duration of each visit were recorded and determined using a video camera.

2.3.7 Real field experiments

A strong honey bee colony of either *Apis cerana* (about 25,000 bees) or *Apis florea* (about 8,000 bees) was placed in the treated plots at first flower of the batch, about July 6. All experiments were blocked east-to-west approximately. There experiments have been conducted. The *A. cerana* alone, *A. florea* alone and *A. cerana* and *A. florea*. The colony was placed near the middle of the test site along the north -south side. Before the experiment start, the natural colony of the bees have been inspected and removed, eventhough only one *A. florea* colony has been found during the whole experimental time periods.

Double control system were implemented. The control flowers of the same pumpkin trees and the control plots that was about 1.2 km between control and honey bee treatments.

Pollinator activity was recorded twice (before and after experiments) during each growing season in both treatment areas. At each evaluation for pollinator activity, about four to 5 pistillate flowers in close proximity to each other were observed near the center of the test site with pollinator type and visit number per flower recorded during peak flowering time (from 700 to 1100 HR); and, each time a pollinator landed on a flower (regardless of the amount of time the pollinator remained on the flower), it was counted as a visit. Honey bee pollinator visited was compared to honey bee colony absence for influence on pumpkin yields and seed characters at the Chong Sarika Model, Lopburi, Thailand. The experiment was set up as split-plot treatment arrangement in a randomized complete block design, with honey bee treatment (visited or non-visited of honey bee visitors) as the main plot and pumpkin cultivar as the subplot. The experiment had 3 replication per crop. Numbers and weights of pumpkins were measured after harvest on 1 hectare scale.

2.4 Pollination by *Apis cerana*, Fabricius in *Passiflora edulis* (purple passion fruit) (Comply from Rodim et al, 2022)

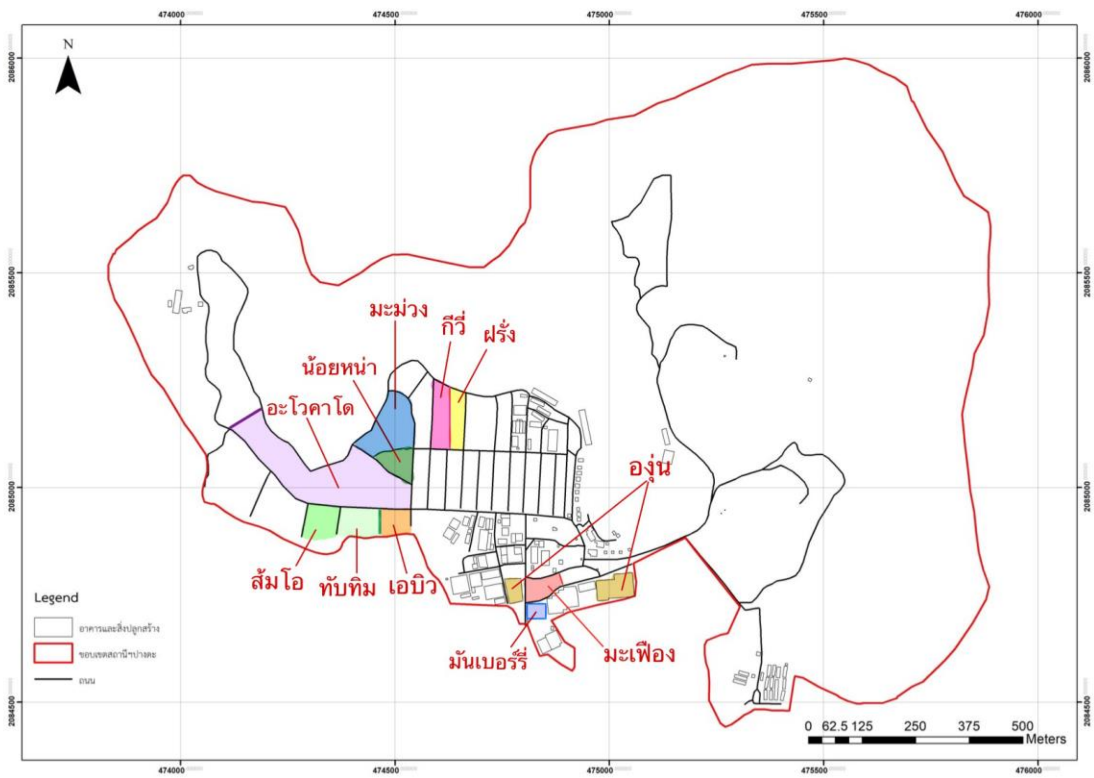

Figure 2.2 Experimental site of The Royal Agricultural Station PANG DA Samoeng, Chiang Mai

(a) (b)

Figure 2.3 (a) *Apis cerana* forage on passion fruit flowers (b) Stingless bees on Hemp

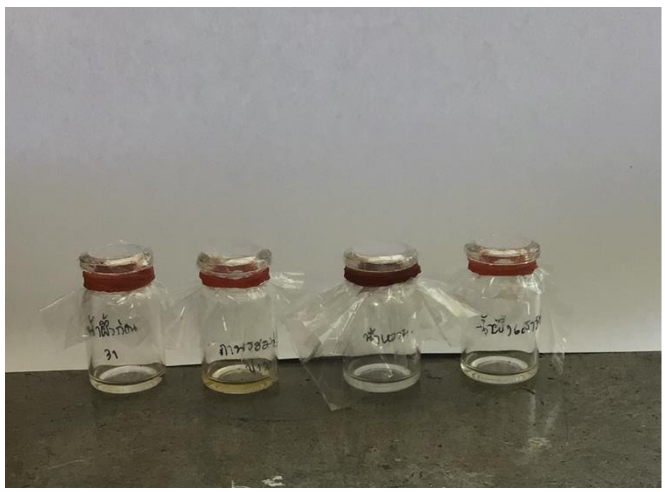

Figure 2.4 Honey Samples collected in this study

The study site is located at The Royal Agricultural Station PANG DA Samoeng, Chiang Mai is approximately at over 1100 meters above sea level. The climate is classified as a tropical forest climate. The passion fruit plantation has a total area of approximately0.2 ha. An experimental plot measuring 40 m x 20 m was established in the study site under green house condition. It was divided horizontally into 10, and vertically into 2, making 20 sub-plots with 2 m x 20 m dimension. Foraging pattern of *A. cerana* has observed on a quadrat measuring 1 m x 1 x 1 m along the plant trellis, 1.5 m above the ground was established.

The honeybee colony was placed in the middle of the plots of the experimental area. The observation was done after day 3 of colony set up to observe the foraging activity in terms of foraging pattern, time of visitation, and handling time. During the ocular observation, 2 observers conducted the experiments at the same tine from 7 AM to 5 PM. After observation, the plot will be marked and moved to the new plot. No repeat plot area were observed in the same day of experimental periods.

A video camera was used for documentation of the length of stay of the forager bee in one flower. However, the majority of the observations were based on ocular observation. The length of stay was measured using a stopwatch. The stopwatch was switched on as soon as the bee started to forage on the flower and then switched off just after the bee left the flower (Gautam et al., 2018). The selected bagged and unbagged flowers were on the same plant and opened in the same day. Flowers were divided into 2 treatments including *A. cerana* bee pollination and control. The flowers for both methods were covered with fine mesh, 0.44 mm in diameter, after brushing with pollen to prevent unwanted visitation of insect pollinators. Flowers from natural pollination were left open to facilitate natural pollination.

2.5 Behavioral signal in *Apis cerana* (Boonmarueng, 2022)

2.5.1 Audio Data

The audio data used in our work were gathered at the Native Honeybee Laboratory, King Mongkut's University of Technology Thonburi Ratchaburi Campus from 26th December 2017 to 10th January 2018 under the guidance of entomologists. All the audio data for this investigation were recorded from native honeybee (*Apis cerana*) by using the Beeconnex device, a multi-sensor board that we designed with a 16-bit resolution and a 48 kHz sampling rate. The microphone from the Beeconnex device was installed at the corner inside a Langstroth beehive. To determine the stimulus that motivated the hissing behavior, a video camera was also installed to record images from an elevated position over the beehive entrance.

For analyzing the audio data, we trimmed the long duration audio files into three-second audio files which covered the duration of the occurrence of hissing signals that mostly occur continuously for 1.0-1.5 seconds. We then listened carefully to each file and distinguished them into one of three categories: 1) normal class, 2) hissing class and 3) highly active class.

2.5.2 Proposed Method

Based on the numerous publications on non-invasive monitoring of beehives reviewed in the previous chapter, we selected both feature extraction and machine learning models. In this section, we provide in-depth detail about the various parameters as well as an assessment of the performance of the techniques and models used throughout the experiment.

Calculations of the three-second audio signals of temporal domain data were made by using short-time energy with the RMSE technique and the results were not transformed into frequency domain but were instead converted by using the SciPy library from Python. We considered the frequency in the range 50-6,000 Hz as this covers the frequency range of the hissing signals and their harmonics (Papachristoforou et al., 2008). For the Mel filter-bank, audio data were analyzed through STFT with a 0.1 second frame size which is 10% of the hissing occurrence time and provides a value that is close to two cycles of the human perceived frequency at 20 Hz. After that, we applied the Hanning window to each interval, with 50% overlapping to minimize the effect of leakage caused by the window function. However, all frequencies were converted into the Mel frequency scale, arranged in 12-bin filter banks which is the least common parameter used in acoustical research (12-20 bins in general). We then calculated the energy for each filter bank. Finally, all Mel filter-bank matrixes were saved in math file form for the training and testing classification model.

The Mel filter-bank matrixes were visualized in Mel filter-bank spectrograms. Initially, this was in RGB form, with the size decreased by 75%. The dimensions of the data were then reduced by converting the RGB to grayscale. Finally, two different types of Mel filter-bank spectrogram were used for presenting the effects of rotation including: 1) temporal domain (vertical) spectrogram and 2) frequency domain (horizontal) spectrogram. Both vertical and horizontal spectrograms were used for training and testing the convolutional neural network.

We applied a variety of machine learning models, both traditional and deep learning-based models, to determine the best-performing model from the different features. The three traditional classification models consisted of a support vector machine, decision tree and random forest. Both one-dimensional and two-dimensional convolutional neural networks were applied as a deep learning-based classification model. The data were then divided into two parts for training (75% of the whole data) and testing the model's performance (25% of the whole data). The different extracted features were used for different training classification models. As shown in Table 3.1, short-time energy, spectral centroid, and Mel filter-bank matrix were performed on the support vector machine, decision tree, and random forest model, respectively. Both horizontal and vertical Mel filter-bank spectrograms were applied with one-dimensional and two dimensional convolutional neural networks (1DCNNs and 2DCNNs). Referring to Table 3.2, the controlled parameters in the 1DCNNs and 2DNCCs consisted of using the Adam optimizer, 100 epochs, early stop with 10 patience based on valuation accuracy, and learning rate reduction with 5 patience (0.1 factor, 0.00001 minimum). The regularization was set to 1 in the SVM model. In addition, the entropy function was used to measure the performance of the split data in both the decision tree and random forest. For both the 1DCNNs and 2DCNNs model,s we decided to use only one kernel because the results from the RawConvet model in Kulyukin's study demonstrated that using only 1 kernel (n=80) was able to provide 95.21% accuracy (Kulyukin et al., 2018) . The size of the kernel, number of nodes, and number of layers were modified to present the impact on the model's performance. In SVM experiments, four different filters were tested for comparison. Finally, the number of estimators was modified in the RF model to present the relationship between the number of estimators and the model's performance (Tab. 2.1).

Table 2.1 The features and classification models in the experiment

Feature	Classification model
Short-time energy	Support vector machine
	Decision Tree
	Random forest
Spectral centroid	Support vector machine
	Decision Tree
	Random forest
Mel frequency matrix	Support vector machine
	Decision Tree

	Random forest
Horizontal Mel spectrogram	One-dimensional convolutional neural networks
	Two-dimensional convolutional neural networks
Vertical Mel spectrogram	One-dimensional convolutional neural networks
	Two-dimensional convolutional neural networks

Table2.2 The independent and controlled parameters in different classification models

Classification Model	**Independent Parameter**	**Controlled Parameter**
One-dimensional convolutional neural networks	1) Number of nodes for each layer [8, 16, 32, 64, 128] 2) Number of layers (1- and 2-layers of the fully connected layer 3) Mel spectrogram form [horizontal, vertical]	1) Adam optimizer 2) 1 kernel 3) Early stop with 10 patience based on valuation accuracy. 4) Learning rate reduction with 5 patience (0.1 factor, 0.00001 minimum)
Two-dimensional convolutional neural networks	1) Kernel size [3x3, 5x5, 7x7, 9x9, and 11x11	1) Adam optimizer 2) 1 kernel 3) Early stop with 10 patience based on valuation accuracy. 4) Learning rate reduction with 5 patience (0.1 factor, 0.00001 minimum)
Support vector machine	Kernel filter [linear, poly, sigmoid and rbf]	Regularization is 1
Decision tree	-	Entropy
Random forest	Number of estimators [1, 9, 19, 29, …, 99]	Entropy

For selecting the best-performing model, we experimented by adjusting various parameters in the five classification models with two competing objectives: the minimum number of trainable parameters and 95% baseline accuracy. The model with the highest accuracy performance was then selected to be deployed on the smart hive system as shown in Figure 2.5.

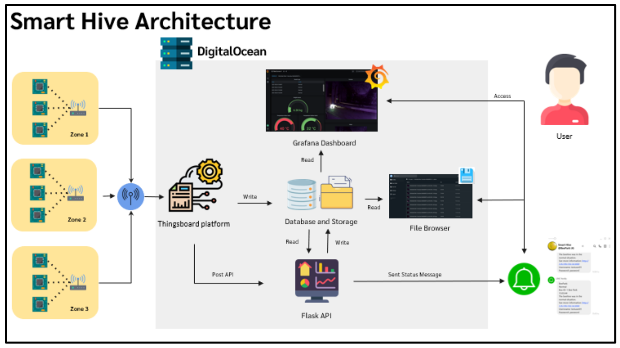

Figure 2.5 Smart hive architecture

CHAPTER 3

RESULTS AND DISCUSSION

3.1 Bee flora

3.1.1 The Common Bee Flora Species in The Royal Agricultural Station PANG DA Samoeng, Chiang Mai

A total of 35 commonly bee flora species were identified and recorded with their local name (Table 4.2).

Twenty one species are source both nectar and pollen as *Mangifera indica, Citrus maxima, Aegle marmelos, Actinidia deliciosa, Persea americana Mill, Passiflora edulis, Fragaria × ananassa, Solanum Torvum Swartz, Averrhoa carambola L, Punica granatum L., Pouteria caimito, Cannabis sativa L. subsp. Sativa, Physalis peruviana L., Cucurbita moschata, Vitis vinifera L., Litchi chinensis, Psidium guajava, Rubus idaeus, Rubus subg. Rubus, Hippeastrum johnsonii, Polianthes tuberosa, Eucalyptus globulus, Eucomis* spp.

Whereas, 9 species as *Morus alba Linn, Allamanda cathartica L., Oryza sativa L., Zea mays Linn, Solanum lycopersicum var. cerasiforme, Crotalaria juncea, Ananas comosus, Ipomoea batatas* are source of pollen.

The others 5 species *Curcuma sessilis, Curcuma alismatifolia Gagnep, Annona squamosa, Dendrobium* spp are either source of nectar or honey dew

Table 3.1 Bee flora calendar in The Royal Agricultural Station PANG DA Samoeng, Chiang Mai

No.	ชนิดพืชอาหาร	Scientific name	Month												Types	
			JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC	Pollen	Nectar
1	มะม่วง	*Mangifera indica*													+	+
2	ส้มโอ	*Citrus maxima*													+	+
3	มะตูม	*Aegle marmelos*													+	+
4	กีวี่	*Actinidia deliciosa*													+	+
5	อะโวคาโด	*Persea americana Mill*													+	+
6	เสาวรส	*Passiflora edulis*													+	+
7	สตรอเบอร์รี่	*Fragaria × ananassa*													+	+
8	มะเขือพวง	*Solanum Torvum Swartz*													+	+
9	ดอกกระเจียว	*Curcuma sessilis*													-	+
10	ดอกปทุมมา	*Curcuma alismatifolia Gagnep*													-	+
11	มะเฟือง	*Averrhoa carambola L.*													+	+

	หวาน															
12	มัลเบอร์รี่	*Morus alba Linn*													+	-
13	ดอก บานบุรี	*Allamanda cathartica L.*													+	-
14	ข้าว	*Oryza sativa L.*													+	-
15	ข้าวโพด	*Zea mays Linn*													+	-
16	ทับทิม	*Punica granatum L.*													+	+
17	เอบิ้ว	*Pouteria caimito*													+	+
18	กัญชง	*Cannabis sativa L. subsp. Sativa*													+	+
19	เคพกูส เบอร์รี่	*Physalis peruviana L.*													+	+
20	ฟักบัต เตอร์นัต	*Cucurbita moschata*													+	+
21	มะเขือ เทศเชอร์รี่	*Solanum lycopersicum var. cerasiforme*													+	-
22	ดอกปอ เทือง	*Crotalaria juncea*													+	-
23	องุ่น	*Vitis vinifera L.*													+	+

24	ลิ้นจี่	*Litchi chinensis*													+	+
25	สับปะรด	*Ananas comosus*													+	-
26	ฝรั่ง	*Psidium guajava*													+	+
27	ราสเบอร์รี่	*Rubus idaeus*													+	+
28	แบล็คเบอร์รี่	*Rubus subg. Rubus*													+	+
29	ว่านสี่ทิศ	*Hippeastrum johnsonii*													+	+
30	ซ่อนกลิ่น	*Polianthes tuberosa*													+	+
31	ยูคาลิปตัส	*Eucalyptus globulus*													+	+
32	น้อยหน่า	*Annona squamosa*													-	+
33	มันเทศ	*Ipomoea batatas*													+	-
34	ยูโคมีส	*Eucomis*													+	+
35	กล้วยไม้	*Dendrobium spp*													-	+

Table 3.2 Natural pollinator that found on the plots

Thai name	Plant species	Species (number of individual)				
		A. cerana	***A. mellifera***	***A. florea***	***A. dorsata***	**Stinglessbees**
ดอกปอเทือง	*Crotalaria juncea*	2	2	0	1	3
ข้าวโพด	*Zea mays*	2	0	0	2	3
ดอกปืนนกไส้	*Bidens pilosa*	1	1	1	1	1
ดอกฟักบัตเตอร์นัต	*Cucurbita moschata*	1	1	0	2	0
ดอกเสาวรส	*Passiflora edulis*	6	0	0	0	2
ดอกฝรั่ง	*Psidium guajava*	0	0	0	1	0
ดอกไมยราบ	*Mimosa pudica*	1	0	0	0	0

ดอกกัญชง	*Cannabis sativa*	1	0	0	0	4
ดอกตีนตุ๊กแก	*Tridax procumbens*	1	0	1	0	1

3.2 Pollination in Tomatoes by stingless bees, *T. pagdeni*

3.2.1 *Foraging activity of T. pagdeni in greenhouse condition*

After introduced *T. pagdeni* colony into greenhouse, we found the forager start their activities as soon as sunrise (around 6:00 am) and stop to forage after sunset (around 6:00 pm)(Department 2020) . According to video-recordings of foraging activity in front of the hive, the number of incoming and outgoing forager bees were not homogeneous (Table 1), and the highest number of forager bees that showed incoming with and without pollen loads occurred at 10:00 am (Incoming with pollen was 19 ± 2.43 and was 44 ± 3.49 Incoming without pollen) (Fig. 3.1). The temperature and humidity inside the greenhouse were ranged from 30.5 – 32.0 °C and 64.13 – 73.84%, respectively. After the temperature inside greenhouse was higher than 31 °C (at noon), the number of incoming and outgoing of foragers were significantly decreased (p<0.05; Fig. 1). Pearson's correlations of temperature and humidity in greenhouse with the foraging activity of *T. pagdeni* are showed in figure 1. The results showed that the number of incoming bees (both with and without pollen load) was negatively correlated with the temperature (r^2= -0.191, n=

130; $p<0.05$) (Fig. 1A) and humidity (r^2= -0.526, n= 130; $p<0.01$) (Fig. 1B). Similarly, the number of outgoing bees were negatively correlated with the temperature (r^2= -0.208, n= 130; $p<0.01$) (Fig. 31A) and humidity (r^2= -0.517, n= 130; $p<0.01$) (Fig. 3.1B).

First experiment

We found that *T. pagdeni* foragers visited the tomato flowers in greenhouse condition (Fig. 3.2). No significant different of percentage of fruit set between the treatment WSB (85±4.24) and WoSBHV (79.5±2.12) (Fig. 3A) was found. Interestingly, pollination by *T. pagdeni* and mechanical vibration significant increased fruit set compared to the treatment WoSB (without pollination agents) (Fig. 3A). The tomatoes produced in treatment WSB showed significant higher in all fruit parameters compared to treatment WoSBHV and WoSB (fruit weight: 4.43±1.03 and 3.20±0.97, $F_{(2,147)}$ = 86.519 ; $p<0.05$; seed number: 17.75±2.39 and 13.42±2.60, $F_{(2,147)}$ = 48.489 ; $p<0.05$; fruit longitudinal diameter: 27±3.31 and 23.3±3.24, $F_{(2,147)}$ = 92.374 ; $p<0.05$; and fruit transvers diameter: 16.82±2.43 and 14.28±2.25, $F_{(2,147)}$ = 13.558; $p<0.05$) (Fig 3 B-E). Additionally, we found that the fruit weight was positive correlation with number of seeds (r =0.455; p < 0.001), fruit longitudinal diameter (r = 0.809; p < 0.001), and fruit transvers diameter (r = 0.654; p < 0.001).

Second experiment

In greenhouse with stingless bees, the fruit set of the unbagged flowers (90%) were significantly higher than bagged flowers (20%) and flowers in greenhouse without stingless bees (unbagged flowers and bagged flowers were 9% and 2% respectively) ($F_{(3, 76)}$ = 20.832; $p<0.05$)

(Table 3.3). We also found that all fruit parameters of the unbagged flowers in greenhouse with stingless bee were higher than that form bagged flowers and flowers in greenhouse without stingless bees, except the fruit transvers diameter (Table 3.4). However, no significant different in seeds number, fruit weight, and fruit diameters between bagged flowers of the greenhouse with stingless bees and unbagged flowers of greenhouse without stingless bees ($p \geq 0.05$).

The obtained results of this study indicated that *T. pagdeni* increases the quality and production of tomatoes in greenhouse similar to those obtained by hand vibration. However, the pollination by mechanical vibration is considered as labor intensive (Ilbi and Boztok 1994).

We therefore suggest that the used of *T. pagdeni* might be more cost efficient. Additionally, the colony of this stingless bee can potentially acclimate to the condition inside greenhouse. We found that the foraging activity of *T. pagdeni* increased reaching its peak between 09:00 and 11:00 hours. The highest number of forager bees that showed incoming with and without pollen loads occurred at 10:00 am Then, the activity gradually decreased after 11:00 am. Similar results have been demonstrated by Cauich et al. (2004) investigating foraging behavior, and pollination efficiency of stingless bees (*Nannotrigona perilampoides*). They reported that the number of foraging bees returning to hive were high between 08:00 am – 11:00 am. Whereas, Del Sarto et al. (2005) also found *Melipona quadrifasciata* foraged on tomato from 08:00 am through 11:00 am. On the genus *Tetragonula*, the foraging activity of *Tetragonula iridipennis* Smith were observed with high peak at 10:00 am (8.17bees/5plant/5min), then the activity was decreasing after 11:00 am (Painkra and Malllaiah 2019). This declining in foraging activity pattern indicates that foraging of stingless bees could be relative with anthesis in tomato flowers. The previous result illustrate that the corolla of tomato (*S. lycopersicum*) was opened between 06:00 – 10:00 am (Teppner 2005). For this reason, the foraging activity of stingless bees were peaked in the morning, then decreased in the afternoon. However, Palma et al. (2008) using *Nannotrigona perilampoides* for tomato greenhouse pollination. They showed that a decreasing of the foraging activity was noticeable at 30 °C. Interestingly, this gradually decreasing is corresponded with the decreasing of fertile tomato pollen with an average daily temperature over 30 °C (Peet et al. 1998, Hikawa and Miyanaga 2009). However, the significant decrease of foraging activity of *T. pagdeni* did not affect the pollination efficiency when compared with hand vibration (Fig. 3). The fertilization may successfully carried during the morning visits of foragers.

Considering the fruit produced quality, *T. pagdeni* performed significantly greater than those produced by without stingless bees. In addition, the weight and number of seeds of the fruits produced in WSB (5.92 ± 0.67, 19.83±2.84, 85 ±4.24) was higher than in WoSB (3.20 ± 0.97, 13.42 ± 2.59, 15 ± 1.41) and WoSBHV (4.34 ± 1.03, 17.75 ± 2.39, 79.5 ± 2.12). The pollination performance of *T. pagdeni* was confirmed by our second experiment. We found that the tomatoes in greenhouse with stingless bees showed number of fruit set and fruit quality of the unbagged flowers almost twice as high as the found in bagged flowers (Table 3.3). Similar positive results were obtained using stingless bee as insect pollinator for some economic crops in a greenhouse or protected environment. For instance, Cauich et al. (2004) demonstrated that the used of *N. perilampoides* and hand vibration for pollinating greenhouse tomatoes in tropical climates showed significantly higher in fruit set compared no pollination. They also reported that the total productivity in kilograms of fruit per square meter was higher in greenhouse with stingless bees compare with hand vibration and no pollination. Same results were demonstrated by using *Melipona quadrifasciata* as pollination agent in greenhouse. Silva-Neto et al. (2018) found that tomato produced in greenhouse with a colony of *M. quadrifasciata* showed 15% more fresh mass and 41% more seeds than those produced in an open environment. The species *M. quadrifasciata* has also been successfully used for other crops pollination in greenhouse, for instance eggplants (*Solanum melongena* L.) and sweet peppers (*Capsicum* spp.) (Cardoso et al. 2015, Freitas et al. 2015).

Another important question for testing the pollination efficiency of *T. pagdeni* in tropical greenhouse climate is the effective transfer of pollen among flowers. This study did not directly determine the removal rate of pollen grains. However, we found that the number of seed per tomato fruit produced in greenhouse with *T. pagdeni* was significantly higher than greenhouse with hand vibration and greenhouse without pollination (Fig. 3). Another evidence for pollen transferring efficiency of *T. pagdeni* was the visible pollen load attached on corbicular of the incoming foragers. These results therefore support that *T. pagdeni* show more efficacy for removing and transferring of the pollen grains among flowers in greenhouse condition.

Although *T. pagdeni* could be increased tomato production in greenhouse, some disadvantages of keeping colonies of this species in proximity is presented. For example, the colony of stingless bee need resin as construction material. This might be more severe in greenhouse environment. This may be equipoised by provide excessive cerumen for the used colony inside greenhouse (Slaa et al., 2000) or using a healthy colony with encapsulated cerumen before introduce into greenhouse. Another disadvantage of the small in size of *T. pagdeni* foragers might be that they have short of foraging range as well as visit low number of flower to obtain a enough pollen load (Slaa et al., 2000; Cauich et al., 2004). However, this might be counterbalanced by provide more colonies to cover number of plants in greenhouse. Del Sarto et al. (2005) determined that a single colony of around 1,200 workers could be enough to pollinate 800-1,500 plants in confinement area like greenhouse. And a normal queen right colony of *T. pagdeni*, the population number of adult workers are more than 800 individuals (AR, unpublished data).

We suggest that the used of *T. pagdeni* could be a beneficial candidate as an insect pollinator of greenhouse tomatoes especially in tropical regions, where the use of honey bees and bumblebee could be restricted. The advantages of keeping colonies of *T. pagdeni* is that safe for famers because they do not sting. The species is commonly found in disturbed and undisturbed habitats. Furthermore, *T. pagdeni* is easily managed to artificial hive boxes and propagation (Rattanawannee and Duangphakdee 2019). Additionally, because of the colony of *T. pagdeni* is a perennial, it is thus not necessary to replace the hive as necessary for the bumblebees. However, more research is needed on some foraging behavioral biology of this stingless bee species and a rigorous investigation as pollinator against currently used bee species.

Table 3.3 The number of the incoming bees and outgoing bees in difference time between 5:00 am – 7:00 am (VDO recorded 5 min/ hour).

Time	incoming bee	outgoing bees
5:00 AM	0	0
6:00 AM	5±1.34	5±1.96
7:00 AM	26±4.76	61±5.59
8:00 AM	37±6.13	71±3.52
9:00 AM	54±4.44	71±5.03
10:00 AM	63±4.71	77±4.32
11:00 AM	53±17.07	62±5.20
12:00 PM	25±12.47	60±5.08
1:00 PM	17±3.94	53±6.71
2:00 PM	13±2.63	29±9.11
3:00 PM	7±2.20	17±3.58
4:00 PM	9±3.37	10±3.93

5:00 PM	8±1.46	8±2.06
6:00 PM	5±1.87	4±1.65
7:00 PM	0	0

Table 3.4 The percentage of fruit, seeds number, fresh mass (g), fruit diameter (mm) and fruit height (mm) of tomato plants (*Solanum lycopersicum* L.) of the second experiment.

Parameters	**Greenhouse with stingless bees**		**Greenhouse without stingless bees**	
	Bagged (n=20)	**Without bagged (n=20)**	**Bagged (n=20)**	**Without bagged (n=20)**
Number of Fruit set (%)	4 (20)a	18 (90)b	2 (10)a	9 (45)c
Number of Seeds	13.45±0.41a	17.72±0.87b	5.50±2.83c	12.48±1.05a
Fresh mass (g)	3.33±1.17a	5.30±0.06a	2.68±0.60a	5.27±2.01a
Fruit diameter (mm)	16.08±2.36a	17.32±0.34a	15.63±1.94a	16.82±0.69a
Fruit height (mm)	22.29±3.48a	29.21±3.27b	19.75±0.35a	24.56±0.94a

Mean (±SD) followed the same letter in any given row are not significantly different from one another ($p < 0.05$) by Tukey’s pairwise comparison test.

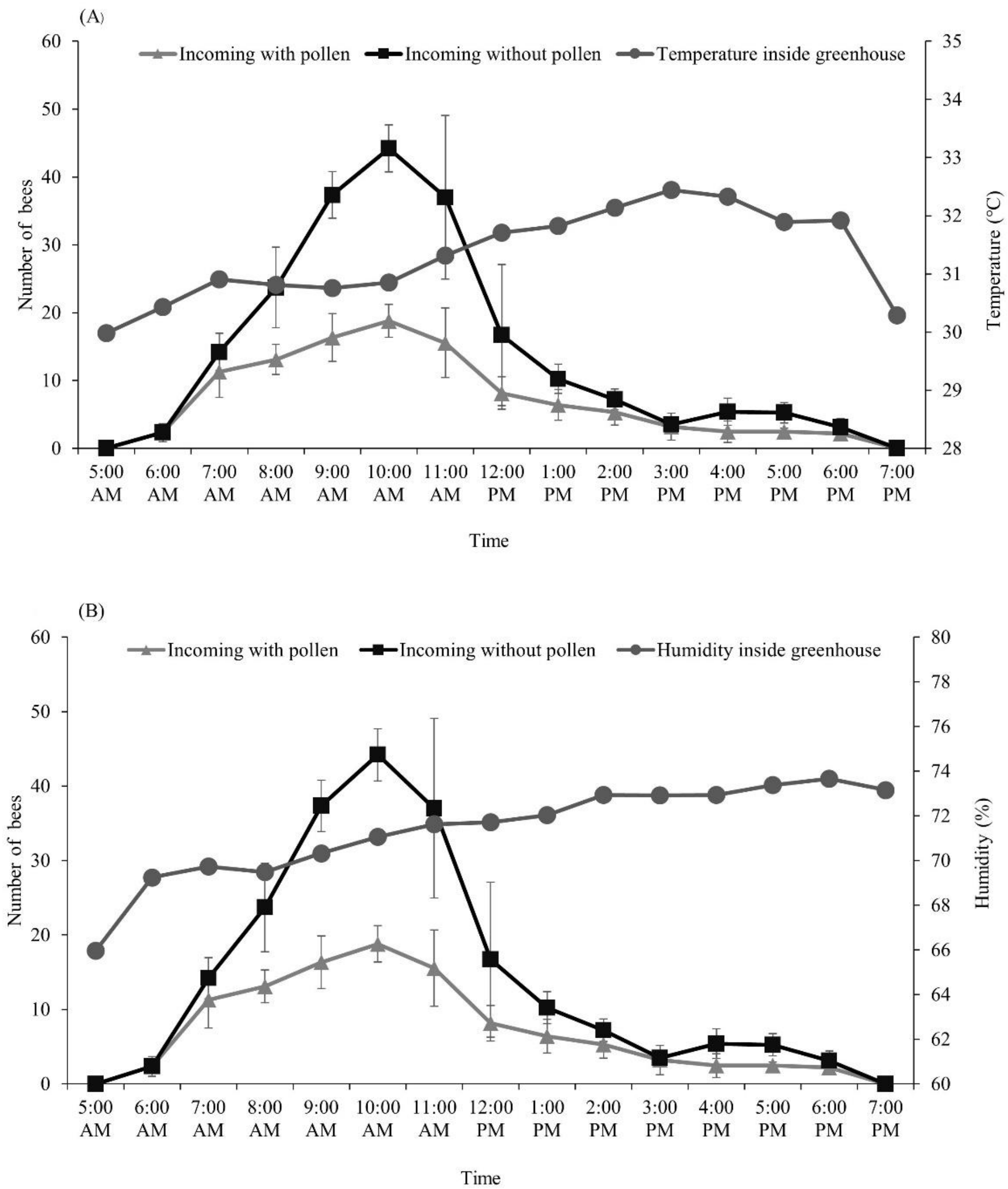

Figure 3.1 Average number of *Tetragonula pagdeni* foragers entering the hive with and without pollens in greenhouse condition corresponded with (A) temperature and (B) humidity during 5 min every hour.

Figure 3.2 *Tetragonula pagdeni* workers are visiting tomato flower in greenhouse.

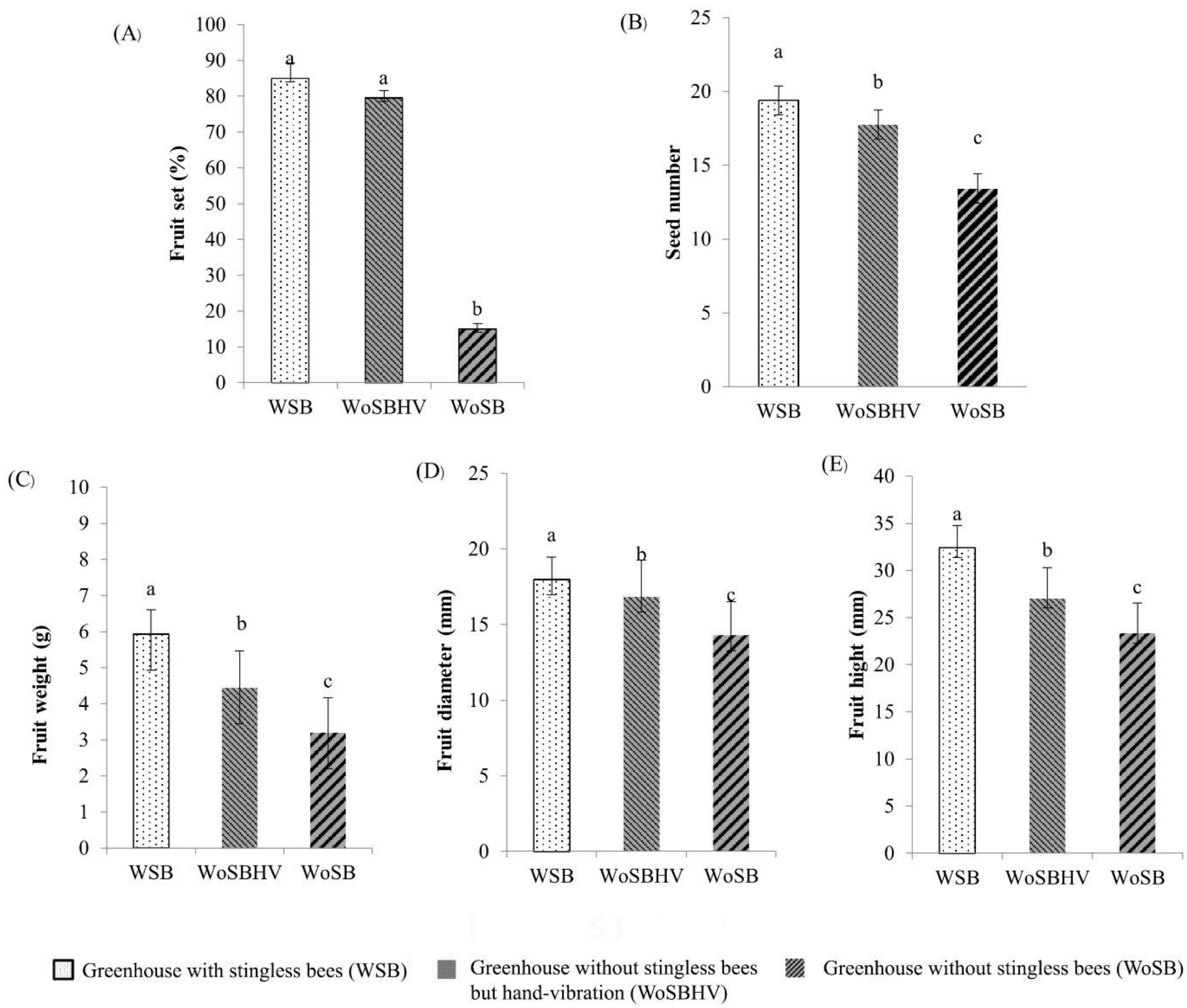

Figure 3.3 Fruit quality of tomato were produced in three greenhouse condition of first experiment; (A) percentage of fruit set, (B) seed number, (C) fruit weight, (D) fruit diameter, and (E) fruit height. Values are given by mean ± standard deviation. Different letters above each bar indicate the significantly different ($p < 0.05$) by Tukey's pairwise comparison test.

3.3 Pollination in Pumpkin

Pollen removal from anthers by honey bees – Of the nearly 38,500 ±2700 pollen grains produced by male pumpkin flowers, honey bees removed nearly two thirds with the first two visits and with sixth visits, honey bees had taken away nearly 80% of the total pollen (table 1). Considering the short period of anthesis in pumpkin (6 to 12 h), the efficient pollen removal in the first visits is advantageous for pumpkin and the most effective. As only one pollen grain is need in pollination, the effective fertilization might enough already at the first set of pollen removal. The flower morphology and position are effected the pollen removal is more efficient when anthers are well exposed similar with the study of Harder (1990).

However, the influence of flower morphology can effect pollen removal more than pollen deposition on the stigma (Nilsson (1988) and Murcia (1990). The size of stamens and a abundant of pollen was also facilitated the pollen removal efficacy of *C. pepo* (Free 1993, Harder 1990, Murcia 1990). However, the amount of pollen removal between the bee species were range from *A. cerana*>*A. florea* > *T. pagdeni*. However, considering to the relative weight carrying by the bees were range *T. pagdeni*> *A. florea* >*A. cerana* respectively.

The duration the bee visit on staminate flowers were 2.8 min ±15 sec and pistillate flower 15.18 min ±55 sec. The time bees forage on one trip for only pollen or nectar is highly depending on the flower species and availability of its pollen and nectar. In this study the time spending on flowers were varied without pattern corresponding to the other experiment (Free, 1993). The average number of pollen grains adhering to the body of the bee was high early in the day at 4,200 grains right after the first visit male flowers and declining of pollen on body to 500-700 grains after visit female flowers . The bees visit begins soon after uncovering the male flowers of pumpkin within 12-25 minutes after uncovered flowers.

The number of pollen deposition on stigma were 47 to 311 per visits. The duration of each visit was 5-13 second on stigma and later on the nectary at the base of female flower. The average number of pollen grain on the second visits in was 95-716 pollen grains and increasing to 1100-1750 pollen grains on the unlimited visits at the end of flowering periods. In this observation showing that the number of pollen grain deposited on stigma was 3-3.5 times higher than of the first visit. The number of pollen grain deposited on stigma on first visit were higher than other pumpkin (215 pollen grain, Tepedino 1981), C. pepo (53 to 230 pollen grains, GRAÇAS Vidal et al. 2010) and cucumber, Cucumis sativus (129 pollen grains, (Collison & Martin, 1979). The number of pollen grain that been removed from the male flowers were much larger than the number of pollen grain deposited on the stigma .This could cause by the loss of pollen grain during their flight path and activities visiting each flowers. However, the pollen colling foragers were also monitored during the returning flight at their nest entrances, resulting that 8-12.3 % of forages were a pollen collection.

The number of visit significantly affect the number of fruiting at 81%, 88%, 100 and 100% in 1, 3, and 9 and unlimited bee visits on stigmas in *A. cerana*. The number of visit significantly affect the number of fruiting at 93%, 98%, 100 and 100% in 1, 3, and 9 and unlimited bee visits on stigmas in *A. florea*. The number of visit significantly affect the number of fruiting at 73%, 88%, 95 and 100% in 1, 3, and 9 and unlimited bee visits on stigmas in *T. pagdeni*. The This result was corresponding to the other previous studies (Vidal et al. 2006, GRAÇAS Vidal et al. 2010). This study indicating that pumpkins required at least 9 visits to tranfer enough pollen for 100% pollination results. However, in the natural situation that allow unlimited visit on female flowers, the number of bee visit stigma was 18±1.5 visits per flowers.

3.4 Pollination by *Apis cerana*, Fabricius in *Passiflora edulis* (purple passion fruit) (Comply from Rodim et al, 2022)

The *A. cerana* bees started to visit the farm at the colony at 6:30 AM and at 7:00 AM precent in the plots looking for open flowers. Surveying continued until 8:00 AM, even though some of the flowers were still partially open. The number of bees visiting the farm and the number of open flowers started to increase at 10:00 AM (Fig. 1). The highest number of the open flowers was observed at 11:30 noon which remained constant from 1:00 PM to 5:00 PM. On the other hand, the trend in the number of bees visiting the experimental area relative to the time of day was approximately symmetrical related to time of flower opened, wherein the peak in the number of bees was observed at 11:30 PM (Table 3).

There was no fruit set in self-pollinated (bagged) and unpollinated flowers. The self-pollinated flowers died off immediately 5-6 days after it was dusted with its own pollen. Bee pollination has a higher fruit set and quality than control and natural pollination. All thirty (30/30) bee pollinated flowers developed into fruit. Significant differences in the fruit characteristics per pollination type were determined statistically using the Student's t-test. Only bees and natural pollination were compared since no fruits were obtained (Table 1). Our results showed that the differences in fruit weight, transverse longitudinal diameter and peel weight were statistically significant at 0.01 while pulp weight, number of seeds, and seed weight were statistically significant at 0.05 (Figure 3.4-3.).

Table 3.5: Fruit set result

Bagged

Date	Fruiting (%)
31/10/63 (with bees)	91.66
01/11/63 (with bees)	83.33
03/11/63 (without bees)	20

Unbagged

Date	Fruiting (%)
31/10/63 (with bees)	8.3
01/11/63 (with bees)	0
03/11/63 (without bees)	10

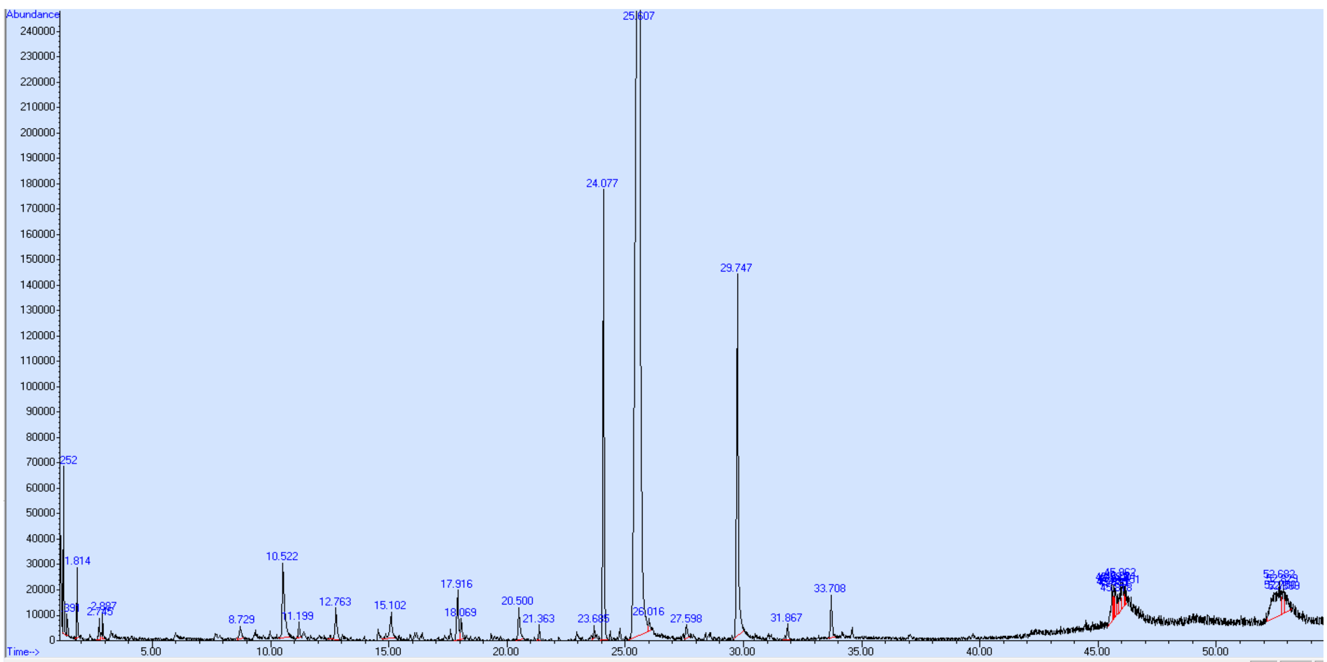

Figure 3.4 Chromatogram of passion fruit honey collected *A. cerana* colony

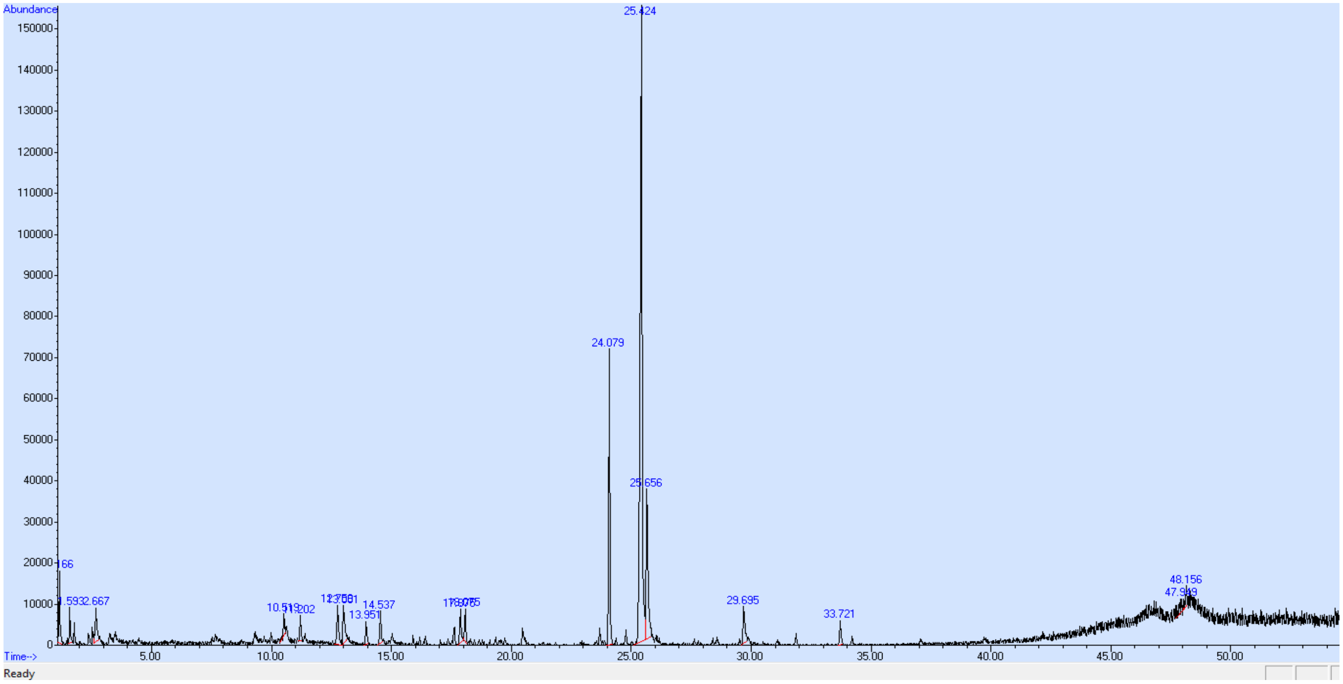

Figure 3.5 Chromatogram of passion fruit nectar before introduced *A. cerana* colony

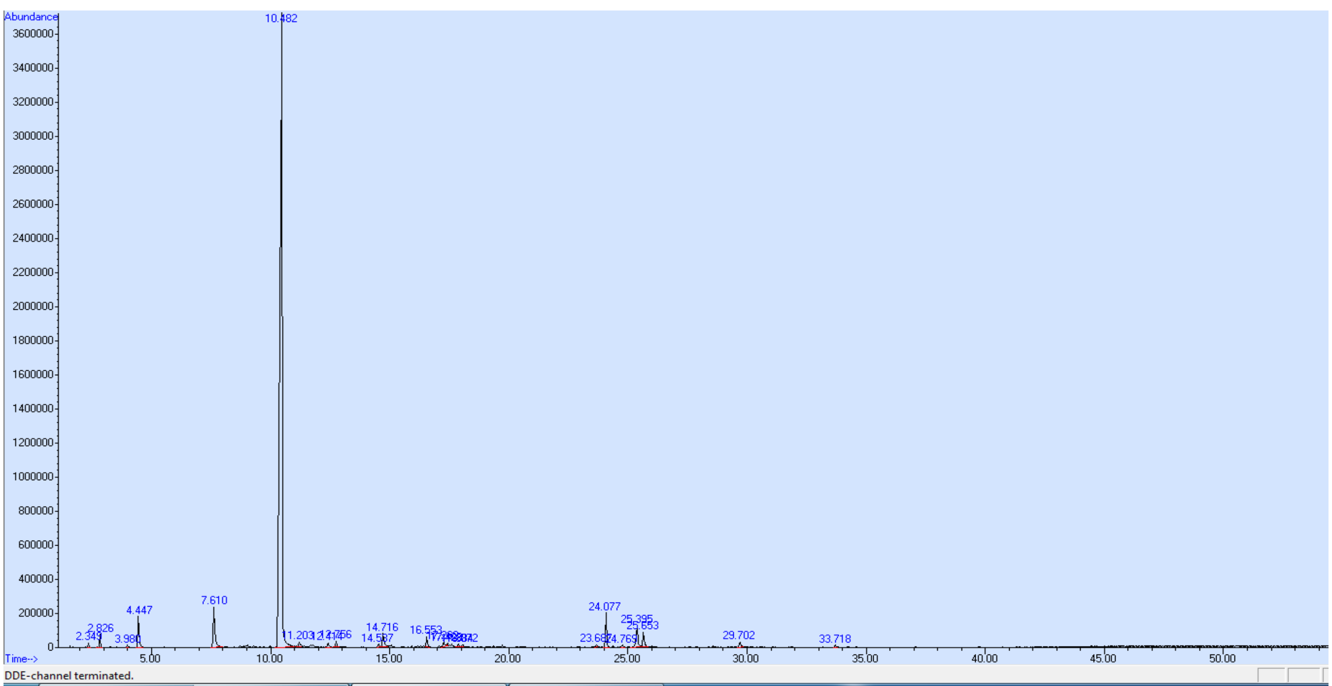

Figure 3.6 Chromatogram of passion fruit nectar before introduced *A. cerana* colony

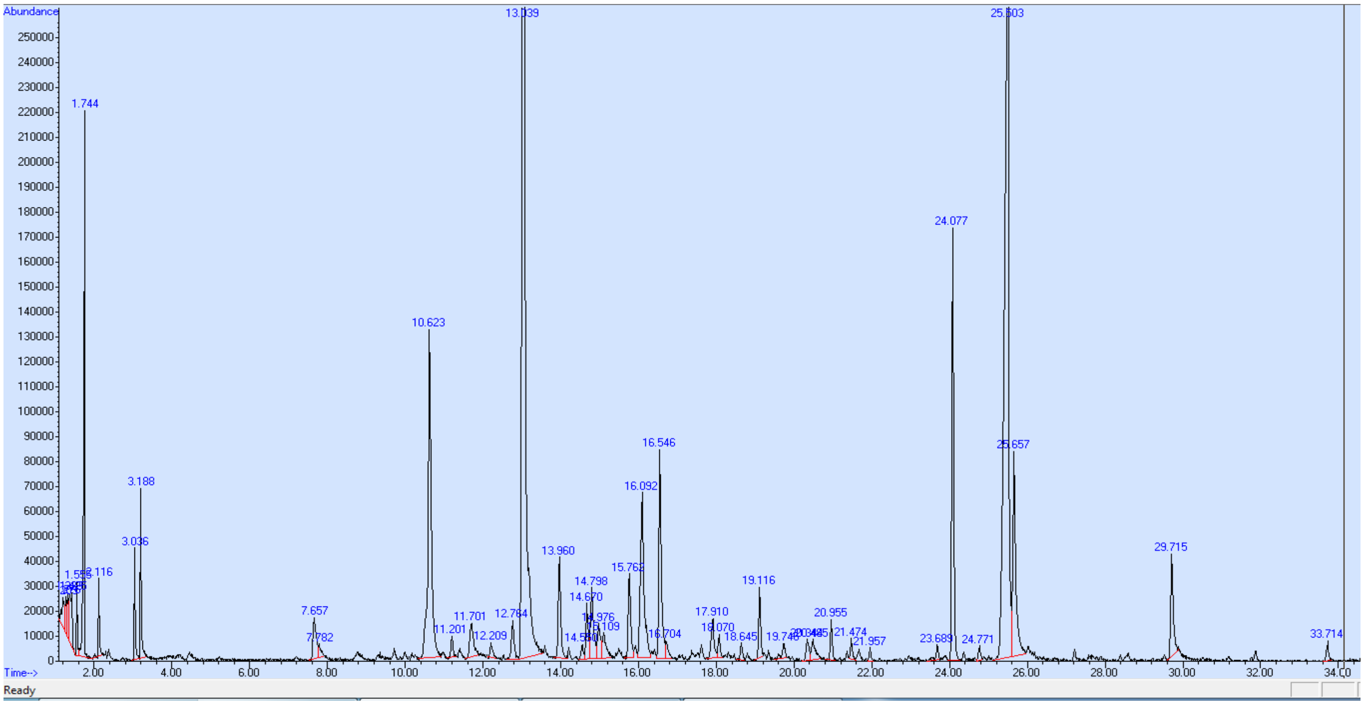

Figure 3.7 Chromatogram of multiflora honey collected *A. cerana* colony

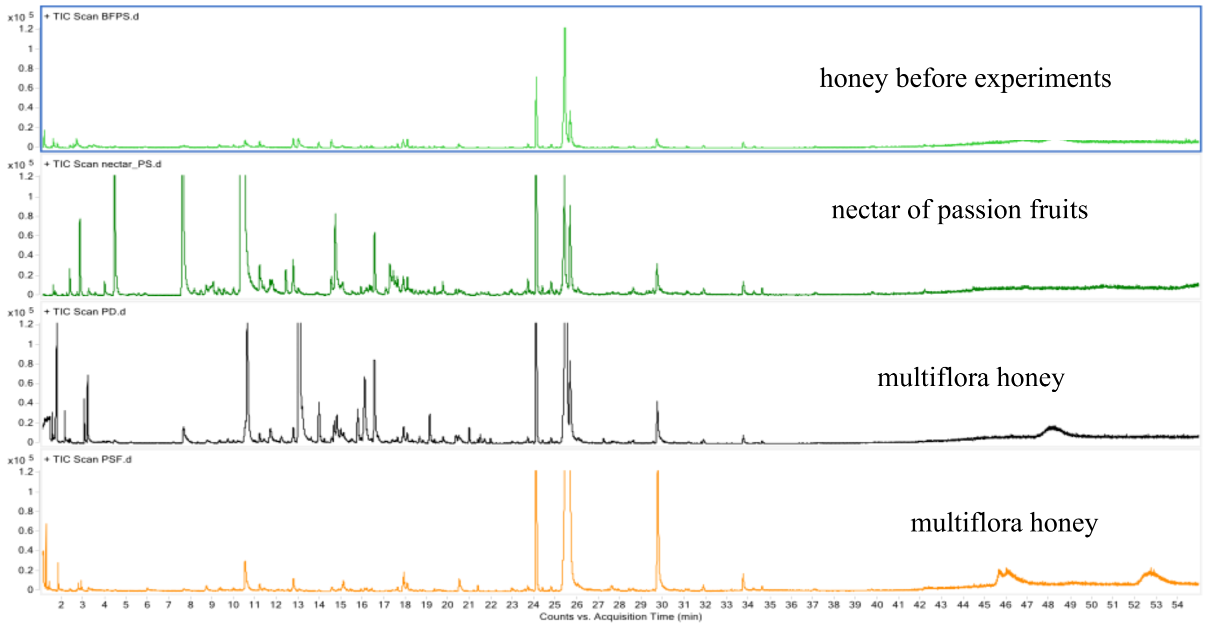

Figure 3.8 Chromatogram of honey before experiments (light green), nectar of passion fruits (dark green), multiflora honey (black) and passion fruit honey (yellow)

Table 3.5 The compounds found in each types of honey. Total of 112 compounds were found unknown with less than 60 confident percentages
RT = Retention Time, n.d. = not detectable

No.	RT (min)	Name	Peak Area (x10^6)			
			passion fruit honey	nectar of passion fruits	multiflora honey	honey before experiments
1	4.448	Cyclopentane, methyl-	n.d.	7.03	n.d.	n.d.
2	7.61	Benzenemethanimine	n.d.	12.25	n.d.	n.d.
3	10.523	3-Butenenitrile, 2-methyl-	1.93	n.d.	n.d.	n.d.
4	10.624	Butanedioic acid, 2,3-dihydroxy- [R-(R*,R*)]-, dimethyl ester	n.d.	n.d.	7.55	n.d.
5	14.715	3-Pyridinecarbonitrile	n.d.	4.04	n.d.	n.d.
6	24.076	2,5-di-tert-Butylaniline	5.79	6.74	5.76	n.d.
7	24.771	Eicosane, 10-methyl-	n.d.	0.6	n.d.	n.d.

3.5 The behavioral signal of *A. cerana* (Boonmarueng, 2022)

3.5.1 Data Exploration

From analyzing the 12-day long audio file recording of the honeybee behavior in our experiment, we discovered that the *A. cerana* honeybees being studied emitted the hissing signal 243 times at a frequency of approximately 20.25±29.69 times a day (mean±SD). The minimum and maximum number of hissing signals was 0 (Dec 27th and Jan 2nd, 3rd and 10th) and 96 (Jan 9th) times, respectively. We investigated the timing of the hissing occurrences from the video recording and found that 154 instances were caused by threatening stimuli such as disturbance by flies or encounters with ants. However, in 89 instances, no natural stimuli could be identified as the source of the interference, as shown in Table 3.6, because of the limitation of the video camera's perspective. For example, on Jan 5th, 2018, the stimuli were not defined for some instances, but as shown in Figure 3.9, there was a possibility that the disturbances were caused by the same flies that had triggered the previous disturbance. Regarding the pattern of hissing occurrences for each hour as presented in Figure 3.10, the results reveal that there was no correlation between the two parameters (Pearson correlation: r=0.043), with the hissing signals frequently occurring in the morning (10 a.m.) and afternoon (3 p.m.). However, these signals always happen during the daytime (tab. 3.6, Figure 3.11).

Table 3.6 The occurrence of hissing signals caused by different stimuli

Stimulus	Frequency
Ant	114
Fly	34
Human	6
Unknown	89
	243

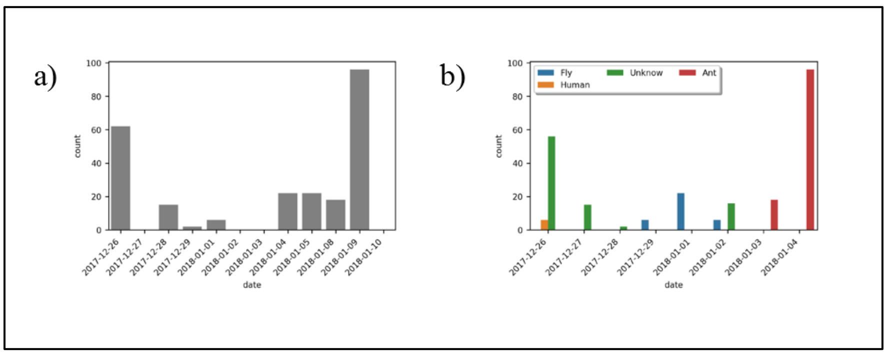

Figure 3.9 Occurrence of hissing signals;
a) occurrence of hissing signals during the period of observation
b) occurrence of hissing signals for each stimulus during the date

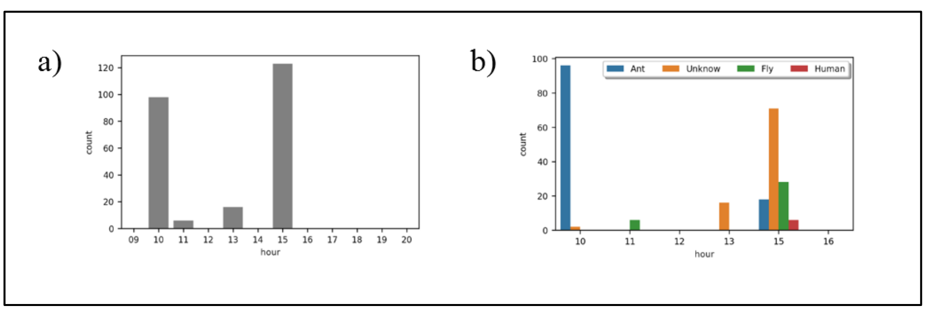

Figure 3.10 Occurrence of hissing signals;
a) occurrence of hissing signals for each hour
b) occurrence of hissing signals for each stimulus during the hour

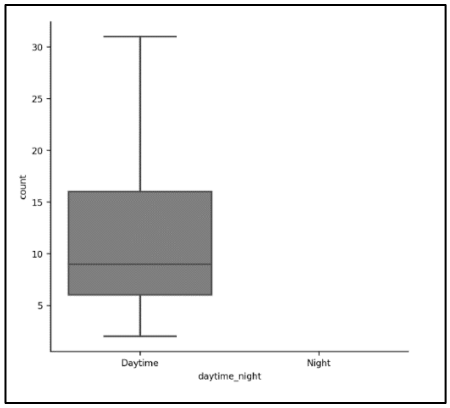

Figure 3.11 Occurrence of hissing signals during the daytime and night

CHAPTER 4

CONCLUSION

A total of 35 commonly bee flora species were identified and recorded with their local name. Twenty one species are source both nectar and pollen as *Mangifera indica, Citrus maxima, Aegle marmelos, Actinidia deliciosa, Persea americana Mill, Passiflora edulis, Fragaria × ananassa, Solanum Torvum Swartz, Averrhoa carambola L, Punica granatum L., Pouteria caimito, Cannabis sativa L. subsp. Sativa, Physalis peruviana L., Cucurbita moschata, Vitis vinifera L., Litchi chinensis, Psidium guajava, Rubus idaeus, Rubus subg. Rubus, Hippeastrum johnsonii, Polianthes tuberosa, Eucalyptus globulus, Eucomis* spp.

Whereas, 9 species as *Morus alba Linn, Allamanda cathartica L., Oryza sativa L., Zea mays Linn, Solanum lycopersicum var. cerasiforme, Crotalaria juncea, Ananas comosus, Ipomoea batatas* are source of pollen. The others 5 species *Curcuma sessilis, Curcuma alismatifolia Gagnep, Annona squamosa, Dendrobium* spp are either source of nectar or honey dew

Pollination and fruit set in pumpkin (*Cucurbita moschata Duch.)* by honey bees). Species of Cucurbitaceae are cultivated worldwide and are depend on bee pollination for fruit set. Field and lab experiments were conducted at Chongsarika, Phathananikom, Lopburi, Thailand. This projects aim to determine how Thai pumpkin pollinate by honeybees and stinglessbees and the comparison on the experimental plot and the real field efficacy. The number of visit significantly affect the number of fruiting at 81%, 88%, 100 and 100% in 1, 3, and 9 and unlimited bee visits on stigmas in *A. cerana*. The number of visit significantly affect the number of fruiting at 93%, 98%, 100 and 100% in 1, 3, and 9 and unlimited bee visits on stigmas in *A. florea*. The number of visit significantly affect the number of fruiting at 73%, 88%, 95 and 100% in 1, 3, and 9 and unlimited bee visits on stigmas in *T. pagdeni*. The This result was corresponding to the other previous studies (Vidal et al. 2006, GRAÇAS Vidal et al. 2010). This study indicating that pumpkins required at least 9 visits to transfer enough pollen for 100% pollination results. However, in the natural situation that allow unlimited visit on female flowers, the number of bee visit stigma was 18±1.5 visits per flowers.

The pollination efficiency of a stingless bee of the species *Tetragonula pagdeni* Schwarz was investigated in tomato (*Solanum lycopersicum* L.) cultivated in different growth conditions in greenhouse environment. Two experiments were carried out. In the first experiment, number of fruit set, number of seed, fresh weight, and longitudinal diameter of fruit were quantified in the greenhouse cultivation treatments with stingless bees, without stingless bees, and pollinated by mechanical vibration by hand. In the second experiment, the treatments were conducted with tomato of indeterminate growth in greenhouse cultivation treatments with and without stingless bees to prevent the varying among the different tomato plants. Additionally, foraging behavior was observed by using video recording in front of the hives. We found that the foragers showed visitation to tomato flowers. In greenhouse with stingless bees presented 82% more fruit set than in greenhouse with mechanical vibration (78%) and greenhouse without stingless bees (17%). In addition, fruit produced in greenhouse with stingless bees showed 30.75% and 8.41% more seeds than fruit produced in greenhouse without stingless bees and pollinated by mechanical vibration, respectively. Interestingly, the tomato produced in greenhouse with stingless bees presented 45.95% more weight than the tomato produced in greenhouse without stingless bees. In determined growth experiment, number of seed per tomato fruit was 41.99% higher when the flower was pollinated by stingless bee.These results demonstrate that *T. pagdeni* can be used as a satisfactory pollinator of tomatoes in greenhouse environment.

We investigated the fruit set and quality of purple passion fruit subjected to pollination by *Apis cerana* in Northern Thailand. We also recorded the foraging behavior of the bees. Twelve quadrats measuring 1 m x 1 m was marked in a purple passion fruit plantation for the investigation. Two groups of 30 flowers that had been either hand pollinated or visited by the bees were followed to determine fruit set and quality. The bee pollinated versus control flowers had significantly greater fruit set (10/0, 10/0 and 20/0). The fruit weight, transverse diameter, longitudinal diameter, peel weight, number of seeds, and juice yield compare to control group. Hundred percent of flowers that were bagged allow only self-pollinated produced no fruit. Bee flower visit times and patterns were also investigated. The carpenter bees started visiting the passion fruit flowers from 7:00 AM to 5:00 PM, with peak visitation at around 11:00 PM. The greatest mean duration of flower visits was observed at 11:30 AM (470 s), which indicated the peak hour of flower opening. We conclude that passion fruit farmers can manage cavity nesting honeybees. *A. cerana* to increase yield. *A. cerana* is also found naturally that could support sufficient pollination services on the passion fruit in the fields.

Our results from the FFT approach disclosed that using the dominant frequency of 300-3,600 Hz is insufficient to conclude whether an unknown signal is a hissing signal because the harmonics caused from other honey activities can also be within this range. As a result, both the Mel frequency matrix and Mel spectrogram were used as main features in the classification models. We investigated tuning the parameters and structures of the 1DCNNs, 2DCNNs, SVM, DT, and RF models. The 1DCNNs models learning from the temporal domain Mel spectrogram with the number of nodes between 32-128 in each of the two layers was able to distinguish three different classes, namely normal class, hissing class, and highly active class, with an average accuracy of greater than 99% based on the testing dataset.

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