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ชื่อโครงการ ชีพลักษณ์การออกดอกและการถ่ายเรณูของ *Hoya verticillata* (Vahl) G. Don

Flowering phenology and pollination of *Hoya verticillata* (Vahl) G. Don

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ชีพลักษณ์การออกดอกและการถ่ายเรณูของ *Hoya verticillata* (Vahl) G. Don

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โครงงานนี้เป็นส่วนหนึ่งของหลักสูตรปริญญาวิทยาศาสตรบัณฑิต

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Flowering phenology and pollination of *Hoya verticillata* (Vahl) G. Don

Miss Pakwan Sa-nguanpong

This Senior Project Submitted in Partial Fulfillment of the Requirement

for the Degree of Bachelor of Science

Botany Program, Department of Botany

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บทคัดย่อ

พืชสกุล Hoya R. Brown เป็นไม้เลื้อยอิงอาศัยหรือขึ้นบนหิน มีดอกสวยงาม มีกลิ่นหอม จัดอยู่ในวงศ์ย่อย Asclepiadoideae วงศ์ Apocynaceae ปัจจุบันมีการศึกษาเกี่ยวกับการถ่ายเรณูของพืชสกุล Hoya น้อยมาก ขณะที่จำนวนประชากรพืชกำลังลดลงเพราะถิ่นอาศัยธรรมชาติถูกทำลาย และการลักลอบค้าพืชป่าผิดกฎหมาย Hoya verticillata เป็นชนิดที่มีการกระจายพันธุ์กว้าง คลอบคลุมทุกภาคของไทย ในงานนี้ได้ทำการศึกษาชีววิทยาการถ่ายเรณูของพืชชนิดนี้ในสวนพฤกศาสตร์บ้านเพ จ. ระยอง ผลการศึกษาพบว่าดอกของ H. verticillata บานในช่วงกลางคืน แต่ละดอกใช้เวลาเฉลี่ย 21.97 วัน ในการพัฒนาจากดอกตูมจนกระทั่งดอกร่วง โดยบานอยู่นาน 1-4 วัน เมื่อดอกบานจะส่งกลิ่นหอมหวานแรง โดยกลิ่นนี้ถูกปลดปล่อยจาก osmophore ที่ผิวด้านใกล้แกนของแฉกกลีบดอก น้ำต้อยจำนวนมากถูกกหลั่งจากต่อมน้ำต้อยที่อยู่ใต้ guide rails และสะสมที่บริเวณฐานของ gynostegium แมลงกลุ่มหลักที่เข้ามามีปฏิสัมพันธ์กับดอกคือผีเสื้อกลางคืน ในอันดับ Lepidoptera โดยผีเสื้อกลางคืนในวงศ์ Erebidae และ วงศ์ Geometridae มีโอกาสมากที่สุดที่จะเป็นพาหะถ่ายเรณูที่มีประสิทธิภาพของ H. verticillata เนื่องจากเข้ามามีปฏิสัมพันธ์กับดอกอย่างสม่ำเสมอ และพฤติกรรมเกาะบนกระบังรอบ และเดินวนไปที่ดอกหลายดอกในช่อขณะหาน้ำต้อย ซึ่งขาของผีเสื้อเมื่อสัมผัสกับโครงสร้างสำคัญของการสืบพันธุ์ของดอก มีความเป็นไปได้สูงมากที่เกิดการถ่ายเรณู โดยค่าประสิทธิภาพการถ่ายเรณู (pollen transfer efficiency; PTE) ของประชากรมีค่า 0.0% - 3.60%

คำสำคัญ: Lepidoptera, ดอกไม้บานกลางคืน, ค่าประสิทธิภาพการถ่ายเรณู, กลิ่นหอมหวานแรง, น้ำต้อยปริมาณมาก

Senior project title	Flowering phenology and pollination of Hoya verticillata (Vahl) G. Don
Student name	Pakwan Sa-nguanpong
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Department	Botany
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Abstract

Hoya R. Brown is genus of climbing epiphytes or lithophytes with beautiful and fragrance flowers, belonging to the subfamily Asclepiadoideae, family Apocynaceae. There were a few studies of pollination of *Hoya*, while the number of the plants in natural populations are degrading in Thailand because of threats like habitat destruction and illegal trade. *Hoya verticillata* is the most common species occurring widely throughout the country. In this study, its pollination biology at Ban Phe Botanic Garden, Rayong was revealed. The results showed that *H. verticillata* is a nocturnal plant, with night-blooming flowers. The flowers took an average of 21.97 days to develop from floral bud to the end of anthesis, with blooming period lasted 1-4 days. During anthesis, a strong sweet fragrance was emitted from osmophore located on the adaxial surface of the corolla lobes. Copious nectar was secreted from the nectariferous tissue hidden behind the guide rails and collected at the base of gynostegium to reward the pollinators. The main visitors were insects in order Lepidoptera. Moths from the families Erebidae and Geometridae were likely to be the most effective potential pollinators for *H. verticillata*. They visited regularly the flowers and while probing for nectar, perched on and walked on the corona of different flowers. This contact of essential organs of flowers on the legs of moths would most likely lead to pollination. The range of pollen transfer efficiency (PTE) in this population was between 0.0% and 3.60%.

Keywords: Lepidoptera, nocturnal flower, pollen transfer efficiency, strong sweet fragrance, copious nectar

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Chapter 1

Introduction

Pollination is the transfer of pollen from an anther or male reproductive organ to a receptive stigma or female reproductive organ. It is an important process in sexual reproduction of seed plants. Majority of plants prefer cross-pollination (transfer of pollen from one plant to another plant) than self-pollination (transfer of pollen within the same flower). Since two different plants are involved and two sets of parental characters are mixed, it results in higher levels of genetic variations that make offspring gets more chances to survive in several environments (Morton and Rafferty, 2017). However, like their parent plants, pollen grains are immovable and thus need a vector to reach another flower of the same species. The pollination can be abiotic (the pollen being freely dispersed by wind or water), or biotic (the pollen being transferred by animal vectors, called zoophily). The zoophily can contribute to a high pollination success because the process is more directed (Mitchell et al., 2009). Animal-pollinated flowers have adapted to attract pollinators by producing different attractants such as floral shapes, colours, fragrance and nectar. In general, plants attract a high diversity of pollinators, called generalized pollination (Bawa, 1990). Several plants' pollinations rely on a few species of pollinators because of their coevolution in terms of structures and behaviours, called specialized pollination (Johnson and Steiner, 2000; Krakos and Fabricant, 2014).

Hoya R. Brown is a genus of climbing epiphytes in the subfamily Asclepiadoideae, family Apocynaceae. In Thailand, the distribution of *Hoya* extends throughout the country with 49 species reported (Kidyoo, 2018). This plant group is characterized by an umbelliform (sciadioidal) inflorescence and pentamerous flower consisting of a gynostegium, formed by the fusion of androecium and gynoecium, with five lateral stigmatic surfaces (Kidyoo, 2018). The pollen grains are united into a waxy mass termed a pollinium, and paired pollinia are in turn united to a corpusculum by translator arms, forming a pollinarium. In this way, transfer of pollen within the same flower is obstructed by strict spatial separation of sexual organs (herkogamy) (Faegri and van der Pijl, 1979). This complex floral structure makes *Hoya* species require pollination by specialized pollinators.

With the beautiful and fragrant flowers, *Hoya* species become ones of the most popular ornamental plants. Nowadays, *Hoya* plants face a high risk of extinction in the wild due to the overharvesting and degradation of their natural habitats by land development and economic growth. Despite growing interest in the reproductive biology and having high economic importance, aspects of their pollination are very poorly known. Only a few studies have been conducted in the past few years, e.g. Forster (1992) reported Ocybadistes walker as pollinators of *H. australis*. Mochizuki et al. (2017) provided an evidence of pollinia transfer on moth legs in *H. carnosa*. In this study, the flowering phenology and pollination of *H. verticillata* (Vahl) G. Don will be investigated. This species is the most common species in Thailand and several plant populations can be found throughout the country (Kidyue et al, 2007). The result of the study will provide the basic knowledge on reproductive biology of *Hoya* species that is essential for supporting the planning and implementation of their *in situ* conservation in Thailand as well as plant propagation for commercial purpose.

Objective

To study the flowering phenology and pollination of *Hoya verticillata* (Vahl) G. Don in Thailand.

Chapter 2

Literature Review

Family Apocynaceae is one of the twelve largest families of angiosperm plants. The plants can be found in every biome of tropical and temperate regions except arctic tundra region, especially in tropical environment. This family consists of varied types of plant habits such as climber, scramblers, shrubs, herbs, caudiciform, epiphytes, succulent, and trees. Flowers are bisexual, mostly pentamerous, rarely tetramerous, except pistil, which of two ovaries or one (Li et al, 1995; Ollerton et al, 2018). The floral synorganization and fusion of androecium and gynoecium in flower of the family are shown in the different level including the pollinaria. These factors effect to their pollination mechanism. Comparing between different subfamilies, the pollination mechanism of Rauvolfioids and Apocynoids are not as complicated as the other three subfamilies known as Asclepiads, i.e. Periplocoideae, Secamonoideae, and Asclepiadoideae (Ollerton et al, 2018). The reproductive organs of plants in the subfamily Asclepiadoideae are distinct from those of the others. Anthers commonly adnate to stigma head, pollen grains fuse into pollinium and two of pollinia unite into a pollinarium by translator arms. These complex characters make plants normally require specialized pollinators to transfer the pollinarium from one plant to another by inserting the pollinia between the guide rail (Demarco, 2017; Endress, 2016; Kunze, 1994; Wyatt, and Broyles, 1994)

There are at least 5350 recognized species in Apocynaceae with worldwide distribution (Ollerton et al, 2018). The pollination biology was studied in at least 10% of them (Ollerton et al, 2018). The evidences showed that most of plants in the family were pollinated by insect and approximately 75% of the plants required insect specialists. The pollination relies on a few species of insects (especially higher taxon, e.g. flies or moths), whereas almost all the remaining are insect generalists, a high diversity of insects can be their pollinators (Ollerton et al, 2018; Sugiura, and Yamazaki, 2005; Ward, and Johnson, 2012, Auttama, McKey, and Kidyoo, 2018; Coombs, Dold, and Peter, 2011).

Hoya R. Brown is a genus of climbing epiphytes or lithophytes in the subfamily Asclepiadoideae. In Thailand, the distribution of *Hoya* extends throughout the country with 50 species reported (Obchant, Kidyoo, and Kidyoo, 2018). There are wide variations of leaf and flower morphology which could be related to the plant environment (Kidyue et al, 2005; Kidyoo, 2018). This plant group is characterized by milky sap often found in whole plant. Its leaves are usually fleshy or thick and has the opposite phyllotaxy. Inflorescence is umbelliform (sciadioidal) constituted of pentamerous flowers with a gynostegium, formed by the fusion of androecium and gynoecium, on which the five lateral stigmatic surfaces are found (Kidyoo, 2018). The pollen grains are united into a waxy mass termed a pollinium, and paired pollinia are in turn united to a corpusculum by translator arms, forming a pollinarium. In this way, transfer of pollen within the same flower is obstructed by strict spatial separation of sexual organs (herkogamy) (Faegri and van der Pijl, 1979). This complex floral structure makes *Hoya* species require pollination by specialized pollinators.

The remarkable, beautiful and fragrant flowers of *Hoya* interest people and made it become ones of the most popular ornamental plants. Hoya plants face a high risk of extinction in the wild due to the overharvesting and degradation of their natural habitats by land development and economic growth. Despite growing interest in the reproductive biology and having high economic importance, aspects of their pollination are very poorly known. Over the recent years, there were few studies on the pollination of this genus. Forster (1992) reported the butterfly *Ocybadistes walkeri sothis* as a pollinator of *Hoya australis* R. Br. ex Traill cultivated in Indooroopilly, Australia with a pollination efficiency of 0.70. The study of *H. carnosa* (L.) R. Br. in Japan (Mochizuki et al, 2017) showed that the pollinia was transferred by the medium to large moths, i.e. *Erebus ephesperis* (Noctuidae), *Bastilla arcuata* (Crambidae) and *Cleora injectaria* (Geometridae). The pollinia were attached only on the moth legs while they were walking on the flower to search for the nectar. Abrahamczyk *et al*. (2016, supplementary data, S2) suggested that moth were pollinators of *H. bella* Hook. and *H. imperialis* Lindl. Moreover, in the same study, bees and wasps were indicated as pollinator group of *H. kentiana* C.M. Burton, *H. kerrii* Craib, *H. lacunosa* Blume, *H. longifolia* Wall. ex Wight, *H. multiflora* Blume, *H. pentaphlebia* Merr., *H. serpens* Hook. f., and *H. thomsonii* Hook. f.

Hoya verticillata (Vahl) G. Don is the most common species of *Hoya* in Thailand and many populations are found throughout the country (Kidyue et al, 2007). The result of the study will provide the basic knowledge on reproductive biology of *Hoya* species which is necessary for supporting the planning and implementation of their *in situ* conservation in Thailand and plant propagation for commercial purpose as well.

Chapter 3

Materials and Methods

Tools

1. Compound Light Microscope
2. Dissecting Light Microscope
3. Scanning Electron Microscope (SEM)
4. Camera
5. VDO Camera
6. Needle
7. Vacuum Pump
8. Rotary Microtome
9. Data logger
10. Altimeter
11. Paraffin Embedding Plate
12. GPS tracker
13. Slide warming plate
14. Dynamic Headspace extraction system
15. 30 mg Porapak Q adsorbent tube
16. Vials, inserts and screw caps
17. Alcohol Burner
18. Laminar air-flow cabinet
19. 60˚C Oven
20. 40˚C Oven
21. -40˚C Refrigerator
22. Nylon mesh Net
23. Bladder
24. Microscopic slide and cover slip

25. 2ml Microcentrifuge Tube
26. Aluminium Foil
27. Filter Paper
28. Hot Plate

Chemicals

1. Clove oil
2. 3% Formalin
3. Formalin-acetic acid-alcohol (FAA) (37 % formalin – glacial acetic acid – 95 % ethanol [2:1:10])
4. Dehydrant Series
5. Paraffin
6. Safranin O
7. Fast Green
8. Neutral Red
9. Ethanol
10. Xylene
11. Distilled Water
12. Permount mounting medium
13. Hexane

Methods

A. Study Plan

1. Literature review

2. Field survey

Survey the field site at Ban Phe Botanic Garden, Royal Forest Department, Ban Phe, Rayong province. Gather information about the study area by measuring the altitude above mean sea level with an altimeter, installing a HOBO pendant temp/relative light two-channel data loggers (Onset Computer Corporation, Bourne, MA, USA) to record hourly air temperature and light intensity in the study period, and asking for local climate records (annual precipitation and mean daily temperature) from the nearest local meteorological station. Investigate the plant population, such as habitat and population size. Make a map showing approximate locations of the individual plants in the population.

3. Study of flowering phenology

Observe growth and development of at least ten inflorescences (each from different plant individual) from the earliest stage of flower bud (when the first cluster of buds could be seen by the naked eye) to the post anthesis stage. This study will be carried out on the plants grown in the greenhouse of Department of Botany, Chulalongkorn University. A HOBO pendant temp/relative light two-channel data loggers will also be installed to record hourly air temperature and light intensity in the study period.

4. Study of floral visiting insects

To obtain data on the floral visitors and their behaviors while visiting flowers, continuous video recording will be made on at least five inflorescences during day time (6 a.m. to 6 p.m.) and on at least ten inflorescences during night time (6 p.m. to 6 a.m.). When possible, the floral visitors will be caught by using net and funnel traps, preserved by pinning technique and sent to the entomologist for identification. After these observations, the monitored flowers will be collected and preserved in 70% ethanol for subsequent examination of pollination success, i.e. removal of pollinaria and insertion of pollinia, effected by insects.

5. Study of floral attractants

5.1 Floral scent

The floral volatiles will be collected by dynamic headspace technique (Meekijjaroenroj et al., 2007) from at least 3 samples (each sample correspond to an inflorescence gathered from a different plant individual on the first day of anthesis). All samplings of floral volatiles will be conducted under the same conditions, with a blank simultaneously collected from an empty bag for each extraction. The volatile organic compounds (VOCs) will be adsorbed in a small adsorbent tube filled with 30 mg Porapak Q (ARS Inc., Gainesville, Florida, USA). The VOCs trapped in the adsorbent tubes will then be eluted with hexane in laboratory and stored at low temperature until analysis by Gas chromatography/Mass spectrometry technique (GC/MS) at Scientific and Technological Research Equipment Centre, Chulalongkorn University.

5.2 Nectar

Choose at least three inflorescences of fully developed flower buds for collecting the nectar. Cover the inflorescences with nylon mesh bags to protect them from visitors. After the flowers open, collect the nectar from the base of gynotegium by filter paper or capillary tube, depending on nectar's volume secreted from flowers, and keep it in low temperature until analysis by High-performance liquid chromatography (HPLC) at Scientific and Technological Research Equipment Centre, Chulalongkorn University.

5.3 Secretory Structures

To localize the osmophore (scent-secreting area) and nectary, stain the freshly opened flower in 0.01% neutral red solution for 30 minutes, wash in water three times (Effmert et al., 2005). Selective staining of intact tissue with neutral red indicates metabolically active sites and is a characteristic feature of osmophores or nectaries (Vogel, 1990; Kearns and Inouye, 1993).

For studying the anatomical features of osmophore and nectary, fix the flowers in FAA solution. The floral parts found to be stained with neutral red, as well as the gynostegium will be removed and processed for observation under light microscope (LM) and scanning electron microscope (SEM). A part of samples will be used to prepare permanent slides using paraffin embedding technique (Ruzin, 1999) and serial sectioning. The remaining samples will be processed for observation under SEM.

6. Morphological study of flowers and insect visitors

6.1 Study the floral morphology qualitatively and quantitatively in at least ten flowers preserved in 70% ethanol (from 4).

6.2 Check the number of pollinaria removed from the anthers and the number of pollinia inserted between guide rails (stigmatic surfaces) under dissecting microscope. And calculate the pollen-transfer efficiency (PTE) to assess the pollination success rate.

6.3 The floral visitors will be observed under dissecting microscope to consider whether they are potential pollinators by checking for the presence of pollinaria attached to their body parts, legs and proboscis, in particular.

7. Analysis of the results and writing of the report

Chapter 4

Results

4.1 Geography and climate of study site

Ban Phe Botanic Garden, Royal Forest Department is located in Klaeng district, Rayong province, eastern Thailand, 1 m a.s.l. The territory of the garden lies between latitudes 12°37’54’’ and 12°38’9’’, and longitude 101°27’7’’ and 101°28’41’’. It is on the east coast of the Gulf of Thailand, spanning approximately 1 km^2, covering beach and beach ridge of 2.7 km along the coast. The area is between sea and dry evergreen forest. The vegetation consists of halophyte of about 134 species from 68 families. The climate is tropical savanna, with wet season lasts from May to October and dry season lasts from November to April (Ban Phe Botanic Garden Office, 2018 [unpublished data]). At the Rayong meteorological station, the closest weather stations to the study area, mean temperature over the 30 years was approximately 28.8 °C. Mean annual rainfall is about 115.3 mm (Fig. 1), peaking in September and October and with a relatively dry season from December to February (Thai Meteorological Department, 2018 [online]). This study was carried out during a March to July, 2018 when rainfall was moderate (approximately 30.0 °C).

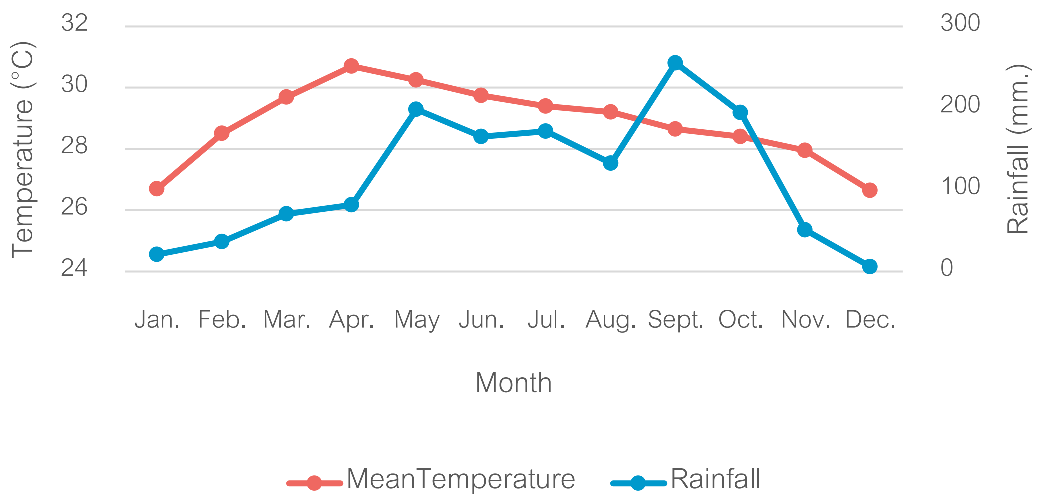

Figure 1. Mean monthly temperature and rainfall in Ban Phe, Rayong 30-year period: 1981-2010 (the data was obtained from Thai Meteorological Department, 2018 [online])

From data from data logger, mean temperature in 2018 (January to October, 2018) was 28.4 °C., the highest was in March to May and mean relative humidity was 79.7% (Fig.2) and the lowest was in February.

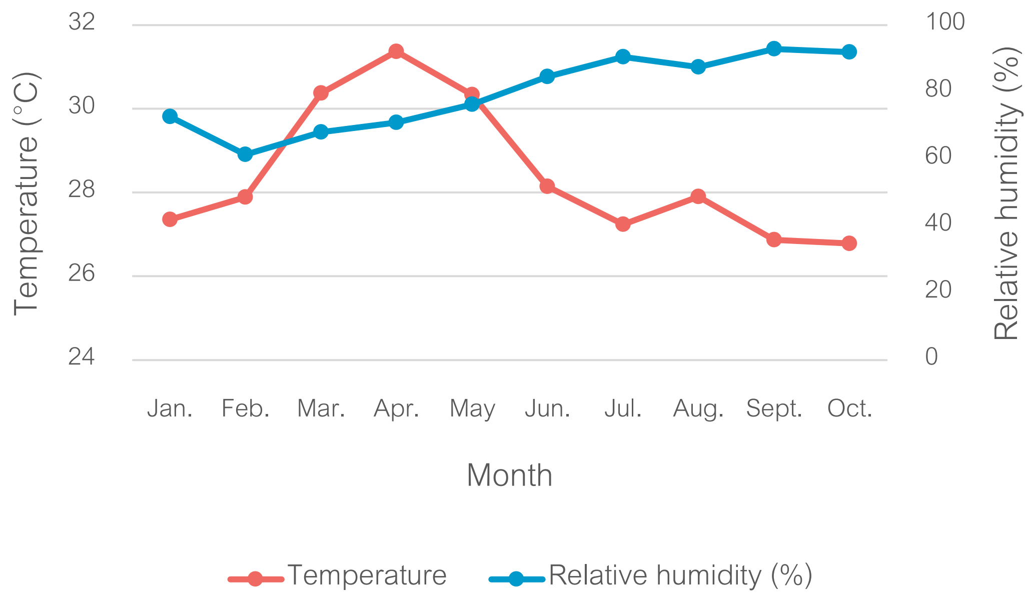

Figure 2. Mean monthly temperature and relative humidity (%) in Ban Phe, Rayong in 2018

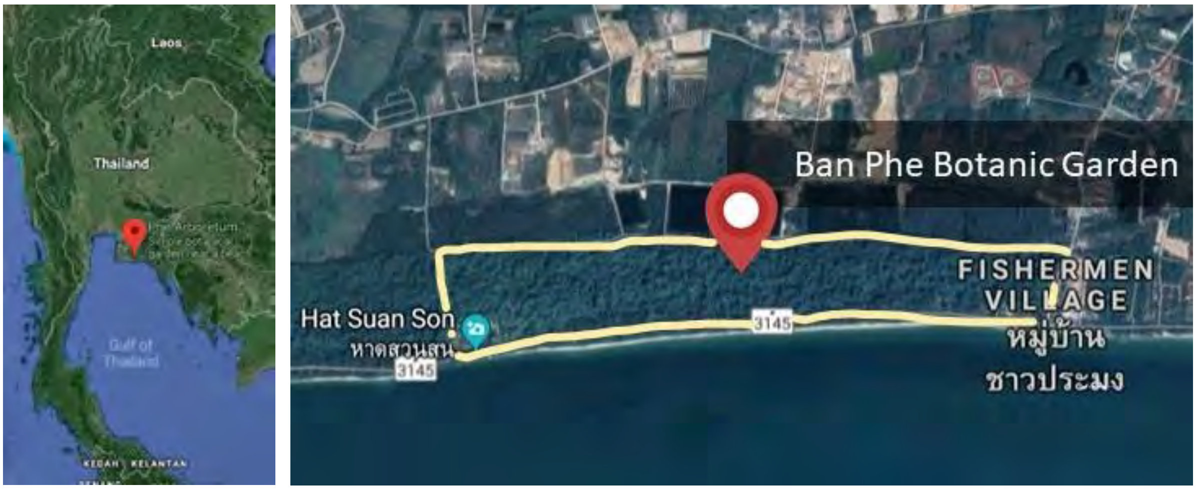

Figure 3. Location and territory of Ban Phe Botanic Garden (the figure was adapted from http://www.google.co.th/maps)

4.2 Study Species

Hoya vericillata (Vahl) G. Don is an epiphyte, twiner, with milky sap. Leaves are varying in shape and size with 2 lateral veins extending from the base to about halfway to the apex. Corolla is rotate, with reflexed corolla lobes and five ovate-lanceolate corona scales, the outer angle of which being erect and acute. The distribution of this species in Thailand extends throughout the country. (Kidyue et al, 2005; Thaithong et al, 2018). In Ban Phe Botanic Garden, during the study period, more than 100 individual plants could be found climbing on the trunks of the perennial trees (Fig.4 and 5). The study population consists of 25 accessible plants. This natural population of *H. verticillata* is threaten because the area around the botanic garden is destroying by land development (Fig. 6).

Figure 4. Population of *H. verticillata* at Ban Phe Botanic Garden, △showed location of *H. verticillata* (the figure was adapted from http://www.google.co.th/maps)

Figure 5. Habitat and habit of *H. verticillata*

Figure 6. Land development area beside the botanic garden

Figure 7. Human activity in the botanic garden

4.3 Flowering Phenology

The flowering phenology of *H. verticillata* was studied from 25 plants in greenhouse during March to May 2018. The 70 inflorescences were observed and divided into 11 stages (Fig. 8), which were defined as follow:

Stage 1	Very small floral buds appeared and pedicel did not develop yet.
Stage 2	Small floral buds were with noticeable elongated pedicels.
Stage 3	Five calyx lobes were obvious.
Stage 4	Floral bud was globular, with corolla bursting from the apex of the calyx.
Stage 5	Floral bud became pentagonal in shape with obvious corolla. The length of the calyx was equal or more than that of the corolla tube.
Stage 6	Floral bud was nearly in its full size. The length of the calyx was less than that of the corolla tube.
Stage 7	Floral bud was in its full-sized and star-shaped with a convex apex.
Stage 8	Flower was in full bloom with completely open corolla, producing strong fragrance and nectar.
Stage 9	Flower was at the end of anthesis stage. Pedicel wilted and corolla lobes were dry and started to close.
Stage 10	Flowers was at post-anthesis stage. Corolla lobes were entirely close.
Stage 11	Flower fell out or small fruit appeared

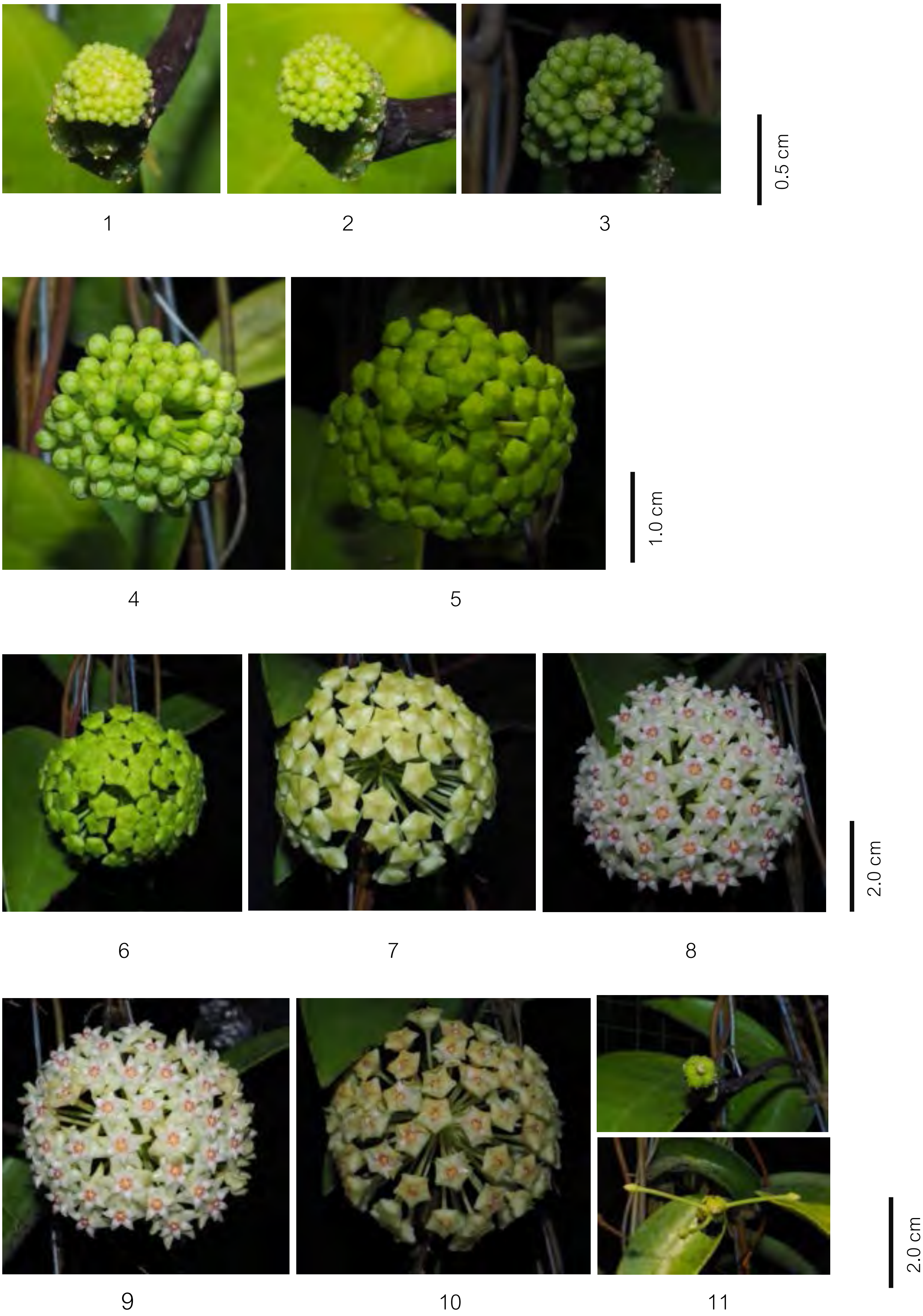

Figure 8. Flowering phenological stages of *Hoya verticillata*

Hoya verticillata took an average of 21.97 days to develop from stage 1, very small bud without pedicel, into stage 10, flower at the end of anthesis. The time required for each stage of development were shown in table1 and figure 9. The stage that took the longest time to develope was stage 3 (mean 3.80±0.18 days) and the shortest stage was stage 10 (mean 0.71±0.090 days). While the floral buds were developing, some of them wilted and did not develop to next stage. It could happen when floral bud was at stage 1 to stage 6, especially when it was at stage 3, more than 30% of floral buds did not develop to stage 4. At stage 10. after the flower closed, it could fall out easily.

Table 1. Average duration in days (mean±standard error) of the 10 flowering phenological stages of *Hoya verticillata*.

Stage	Length of time (days)
1	1.73±0.19 (n=11 inflorescences)
2	2.04±0.15 (n=24 inflorescences)
3	3.80±0.18 (n=30 inflorescences)
4	3.09±0.15 (n=33 inflorescences)
5	1.67±0.080 (n=36 inflorescences)
6	3.37±0.092 (n=35 inflorescences)
7	3.03±0.096 (n=38 inflorescences)
8	1.11±0.045 (n=52 inflorescences)
9	1.42±0.11 (n=38 inflorescences)
10	0.71±0.090 (n=34 inflorescences)

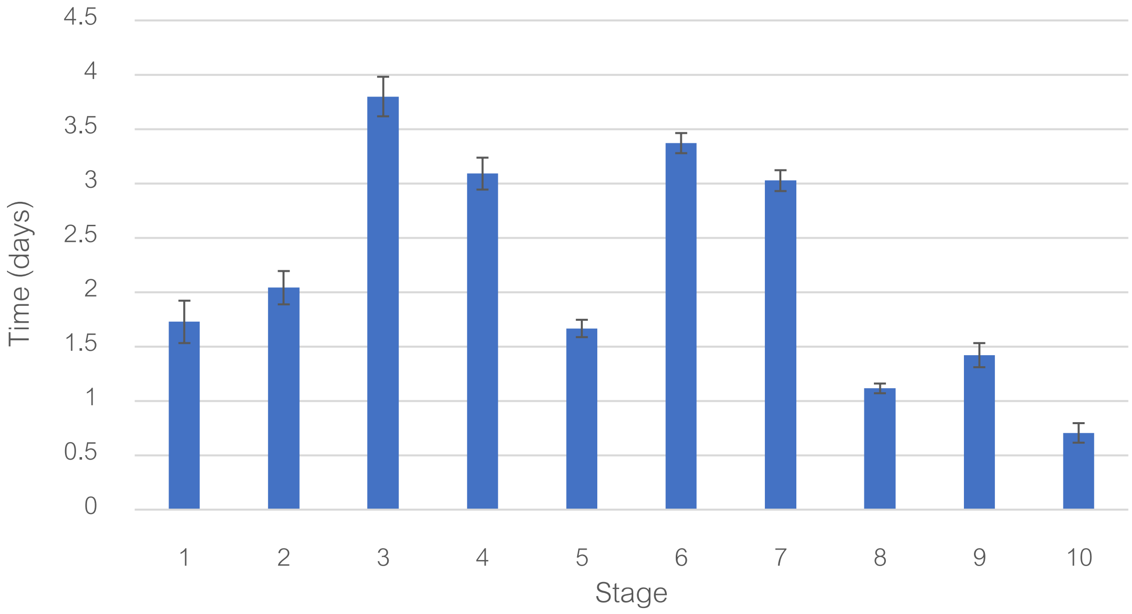

Figure 9. A bar graph showing the mean ± standard error of the 10 flowering phenological stages of *Hoya verticillata*

4.4 Blooming phenology

During the studied period of 61 consecutive days, blooming phenology were observed from the beginning of anthesis to the end of anthesis (from stage 8 to stage 9) (Fig. 10). Twenty-one plants bore 38 inflorescences in anthesis (1 to 4 plants per day). For 9 plants, only one inflorescence reached anthesis stage during this observation period, but 12 plants could bear 2 to 8 inflorescences in anthesis. However, the next inflorescence opened only 1 to 26 days after wilting of the first one, except for 8 plants on which two blooming inflorescences could be found in the same day. The duration of anthesis for each inflorescence of *H. verticillate* varied from 1 to 4 days. There was a single inflorescence that bloomed only 1 day, while 19, 15 and 4 inflorescences remained in anthesis 2, 3 and 4 days, respectively. All flowers in each inflorescence mostly (36 of 38 inflorescences) opened in the same day between 6 pm to 8 pm. There were 7 days during the studied period that 4-5 blooming inflorescences of 4 different plants were observed, hence there were most chance that cross-pollination could achieve.

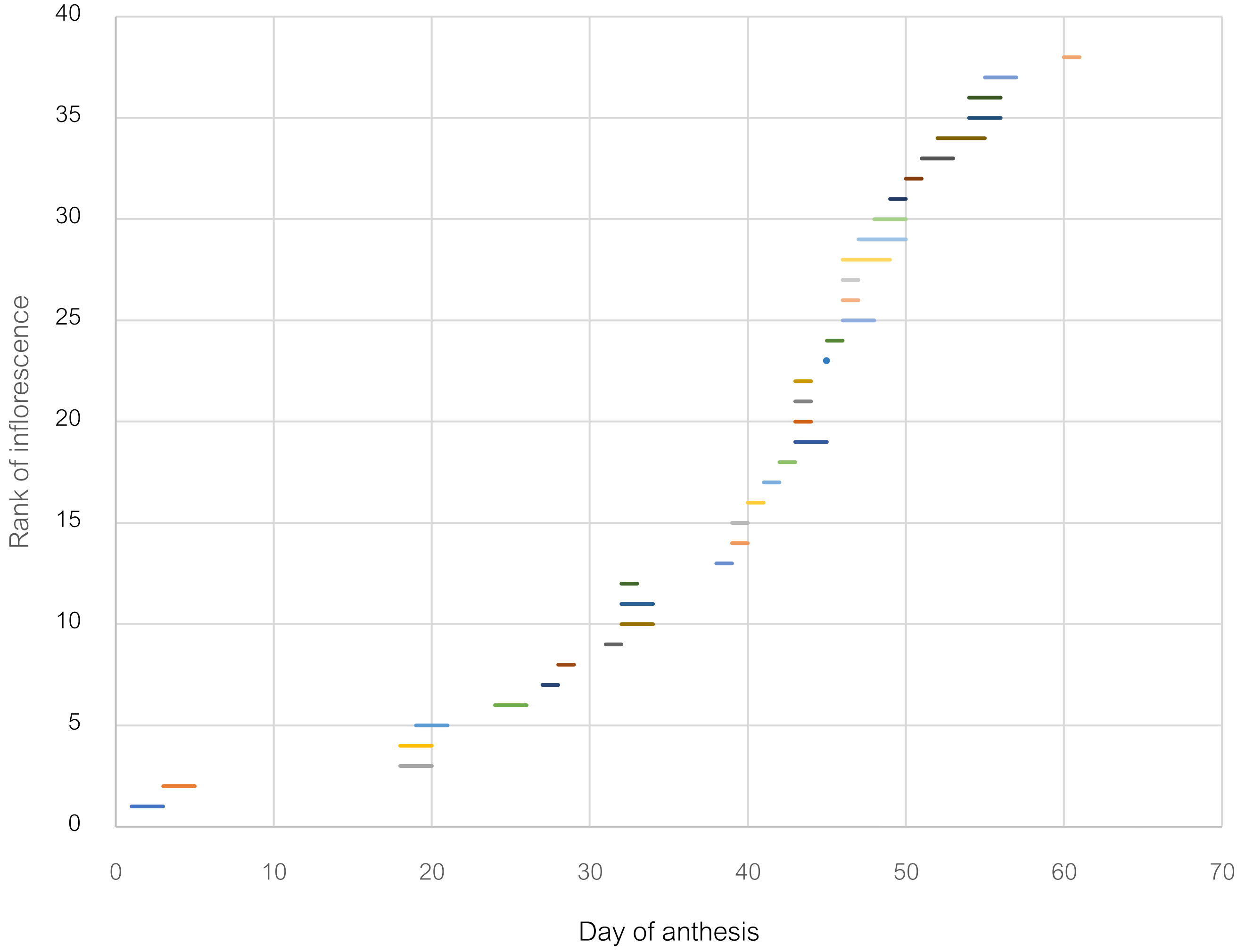

Figure 10. Duration of anthesis (x axis) for each inflorescence of each plant (y axis). Thirty-eight inflorescences from nineteen plants were scored. Each dot or line represents an inflorescence. The length of each line shows the duration of anthesis (days). The inflorescences were ranked according to their first day of anthesis.

4.5 Insect visitation

During the study period (March 10 and 23-25, 2018), 30 inflorescences from 24 plants (1-3 inflorescences per plant) were observed at anthesis stage. There were at least 5 orders of insects visiting flowers: Lepidoptera (moths, hawkmoths and butterfly), Diptera (flies), Hymenoptera (wasps, ants), Araneae (spider) and Blattodea (cockroach). These visitors had different behaviour while visiting flowers.

There were 3 groups of insects of the order Lepidoptera; moths, hawkmoths and butterfly. There were 3 sizes of moths (comparative to the size of flower), small, medium and large size (Fig. 11A, 11B and 11C, respectively). These 3 groups of moths had different visiting behavior. Most of small-sized and medium-sized moths landed on corolla lobes, put their legs on the corolla lobes and did not move while drinking nectar. However, there were some of medium-sized moths that put their legs on the top of corona while visiting flower. For large moths, they always put their legs on the top of corona while walking around the inflorescences or standing when they drinking nectar.

For hawkmoths (Fig. 12A), when they visited the flowers, they flew over the inflorescence, use their long proboscis to drink nectar and the other parts of their body did not touch the flower.

From observation, the butterfly (Fig. 12B) visited the inflorescence only once and take about 7 minutes for probing. It landed on the top of corona and walked on 4-5 flowers.

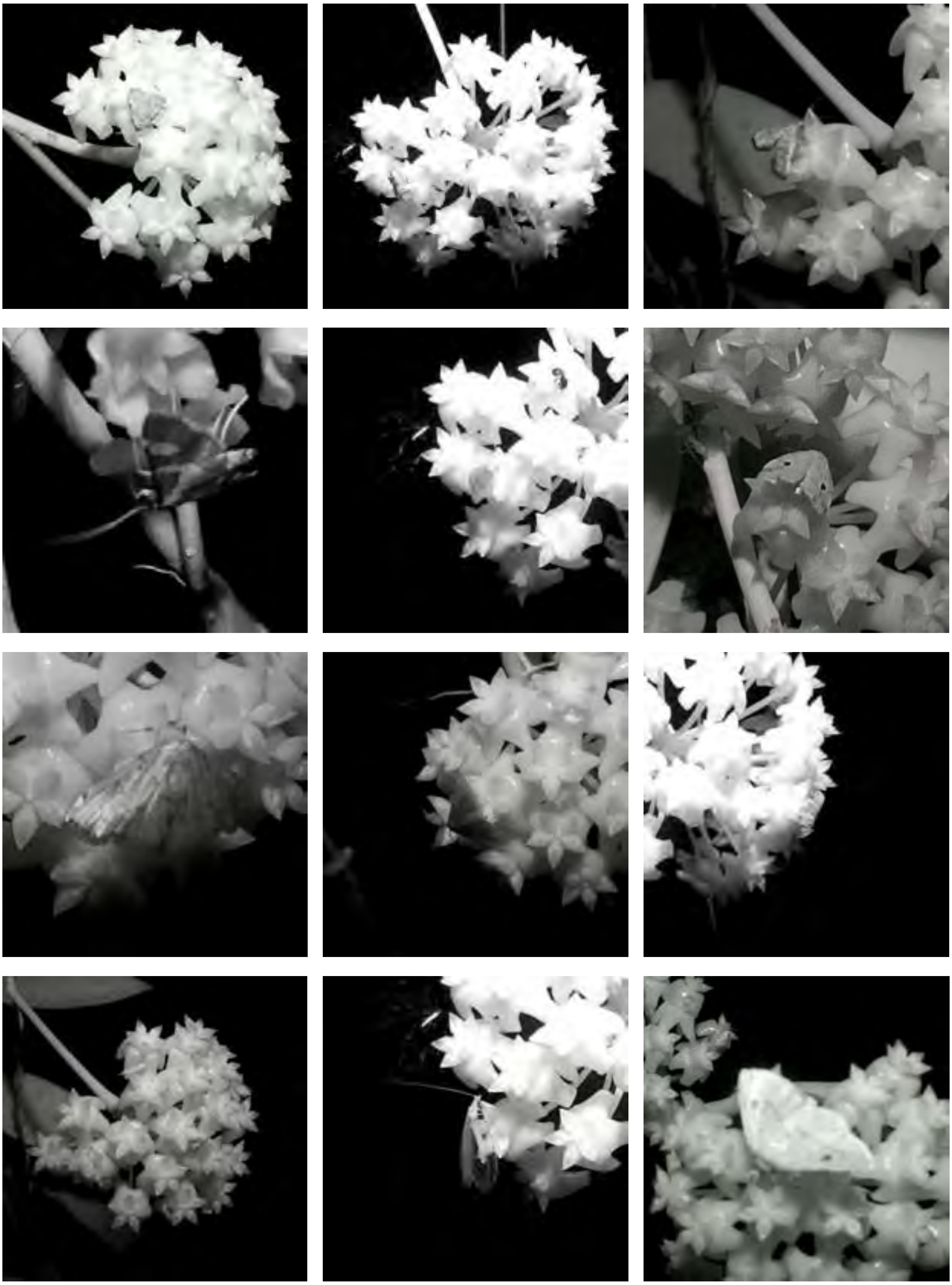

Figure 11A. Variations of visitors of small-sized moths (order Lepidoptera)

Figure 11B. Variations of visitors of medium-sized moths (order Lepidoptera)

Figure 11C. Variations of visitors of large-sized moths (order Lepidoptera)

Figure 12. Variations of visitors of order Lepidoptera A. Hawkmoths B. Butterfly

For flies (Fig. 13A), they always landed on corolla lobes, drank nectar and did not walk on the top of corona. Only one wasp (order Hymenoptera) (Fig. 13B) visited the flower. It walked on corolla lobes. On some inflorescences, ants (Fig. 13C) were found always stayed at the corona base where nectar was collected, walked on top of corona but did not put their legs near the guide rails or corpusculum. Sometimes they disturbed other visitors, for example moths could not land or stand on flowers. There were only 2 spiders (Fig. 13D) that visited flower. They did not walk around the inflorescence but only stay and put their legs on corolla lobes of 3-4 flowers. Cockroaches (Fig. 13E) always walked on the lower side of the inflorescence while probing nectar.

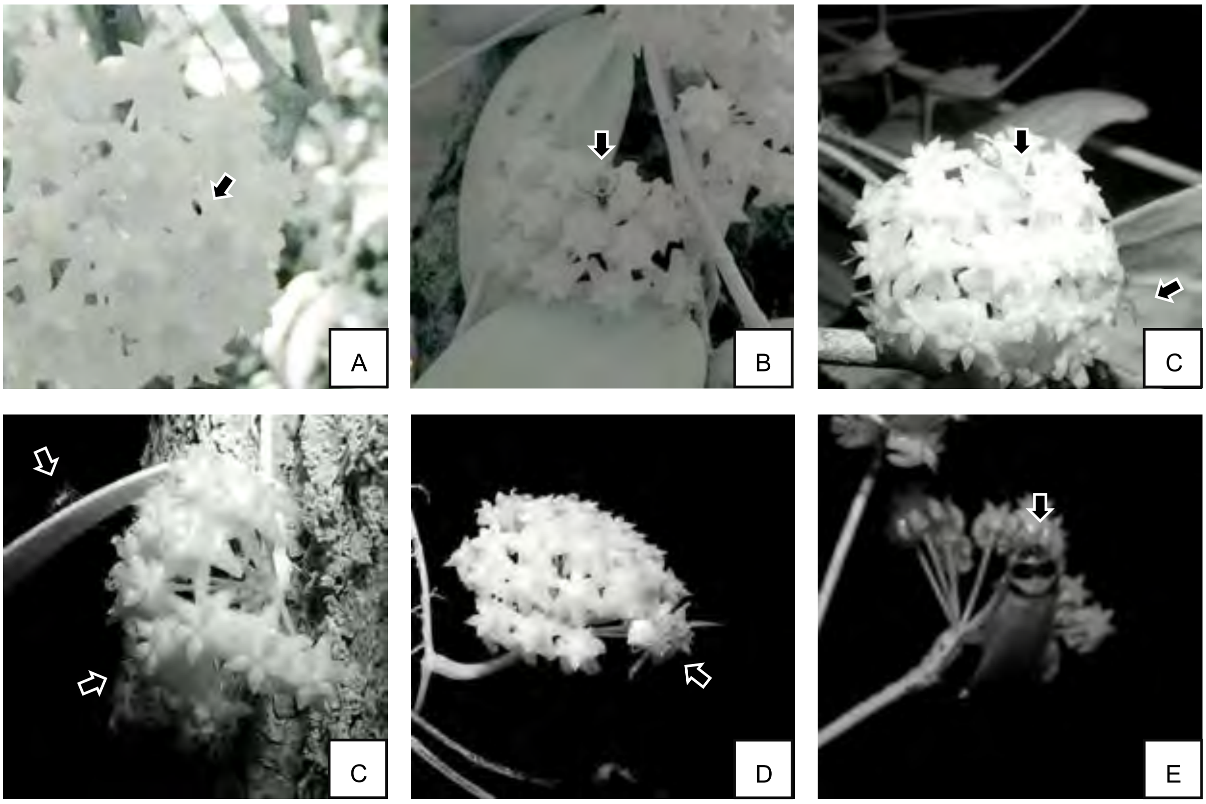

Figure 13. Variations of visitors A. order Diptera (fly), B. order Hymenoptera (wasp), C. order Hymenoptera (ant), D. order Araneae (spider), E. order Blattodea (cockroach)

Table 2. Number of insects visiting *Hoya verticillata* flowers

Date	Plant number - Inflorescence number	Potential pollinator group		Visitor group								
		Order Lepidoptera		Order Lepidoptera			Order Diptera	Order Hymenoptera		Order Araneae (Spider)	Order Blattodea (Cockroach)	Unidentified insect
		Medium moth	Large moth	Hawkmoth	Moth	Butterfly		Genus *Ceriana*	Ant			
10-Mar-18	1-1	-	-	-	-	-	-	-	14	-	-	-
	2-1	-	-	-	15	-	-	-	-	-	3	2
	3-1	-	-	-	2	-	-	-	-	-	7	1
	3-2	-	1	-	2	-	-	-	-	-	-	-
	4-1	-	-	-	20	-	-	-	-	-	-	-
	5-1	-	-	-	9	-	-	-	-	-	-	2
	6-1	-	-	1	-	-	-	-	-	1	-	6
	7-1	-	-	1	31	-	-	-	-	-	-	1
	8-1	1	-	-	9	1	2	-				3
	9-1	-	-	3	8	-	-	-	-	-	-	1
	10-1	-	3	-	33	-	-	-	-	-	-	-
	10-2	-	2	-	13	-	-	-	-	-	-	-

Table 2. Number of insects visiting *Hoya verticillata* flowers (continued)

Date	Plant Number - Inflorescence Number	Potential Pollinator Group		Visitor Group								
		Order Lepidoptera		Order Lepidoptera			Order Diptera	Order Hymenoptera		Order Araneae (Spider)	Order Blattodea (Cockroach)	Unidentified Insect
		Medium moth	Large moth	Hawkmoth	Moth	Butterfly		Genus Ceriana	Ant			
23-Mar-18	11-1	-	-	-	5	-	-	-	-	-	-	1
	11-2	-	-	-	3	-	-	-	-	-	-	1
	11-3	-	-	-	-	-	-	2	-	-	-	-
	12-1	-	-	-	-	-	8	-	3	-	-	-
	13-1	-	-	-	11	-	-	-	30	1	-	-
	14-1	-	-	-	-	-	-	-	-	-	-	1
	14-2	-	-	-	1	-	-	-	-	-	-	3
	15-1	-	-	-	-	-	-	-	5	-	1	-
	16-1	-	1	-	6	-	-	-	5	-	-	1
	17-1	-	-	-	3	-	-	-	4	-	-	-

Table 2. Number of insects visiting *Hoya verticillata* flowers (continued)

Date	Plant Number - Inflorescence Number	Potential Pollinator Group		Visitor Group								
		Order Lepidoptera		Order Lepidoptera			Order Diptera	Order Hymenoptera		Order Araneae (Spider)	Order Blattodea (Cockroach)	Unidentified Insect
		Medium moth	Large moth	Hawkmoth	Moth	Butterfly		Genus Ceriana	Ant			
24-Mar-18	18-1	-	-	-	-	-	-	-	-	-	-	3
	19-1	-	-	-	1	-	-	-	-	-	-	2
	21-1	-	-	-	6	-	-	-	-	-	-	-
	21-2	-	-	-	11	-	-	-	-	-	-	1
	22-1	-	-	-	22	-	-	-	-	-	2	6
	27-1	-	-	-	-	-	-	-	8	-	-	-
	28-1	-	-	-	1	-	-	-	-	-	-	-
	28-2	-	-	-	1	-	-	-	-	-	-	-

4.6 Morphology and size of pollinarium

Pollinarium of *H. verticillata* is formed by two pollinia and each side connect to corpusculum by translator arms. The pollinium is transparent yellow, oblong-shape, about 580-590 µm in length and 186-203 µm (Fig. 14) in width. Pollen tubes germinated from the pellucid margin located on the outer margin of the pollinium (Fig. 15).

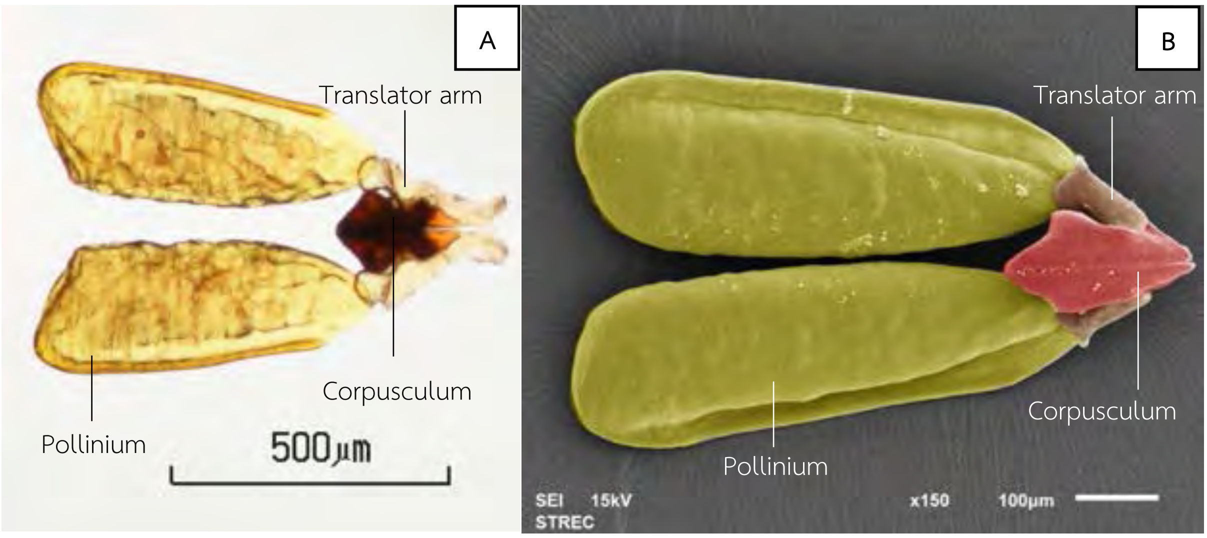

Figure 14. A. Micrograph of a pollinarium from light microscope;
B. SEM micrograph of a pollinarium

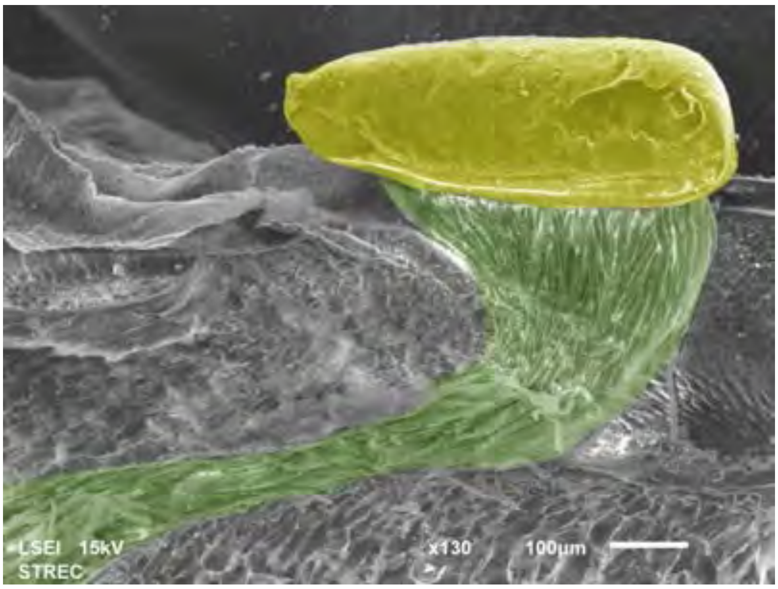

Figure 15. SEM micrograph of the germinated pollen tubes

4.7 Floral Attractants

4.7.1 Floral Scent

Floral volatile compounds (VOCs) were collected 2 times: at day time during 1410h to 1610h i.e. sample 1D, 2D and 3D and night time during 2200h to 2400h i.e. sample 2N, 3N and 4N, from 4 inflorescences at anthesis using two types of adsorbance, i.e. HayeSep Q and Porapaq Q. The VOCs were analysed by Gas Chromatography/Mass Spectrometry (GC-MS). The example of chromatograms of the VOCs detected was illustrated in Fig. 16. The result showed that the average number of VOCs detected when collecting by Porapaq Q during daytime and nighttime were 25, which was much higher than 11 compounds detected using HayeSep Q (Table 3). Porapraq Q is thus more suitable for studying the VOCs of *Hoya verticillata* and therefore, only the components collected using Porapaq Q were further taken into consideration.

Table 3. The number and amount (ng per 15 min per flower) of VOCs emitted by the inflorescences of *Hoya veritcillata* during anthesis.

Compound name	RI	Number of Sample (type of adsorbance)					
		Day			Night		
		1D (H)	2D (H)	3D (P)	4N (P)	2N (H)	3N (P)
3-Methylbutanol	725	1.652	1.522	16.091	93.403	47.197	44.485
4-Methylpentanol	831				2.398		
3-Methylpentanol	839				5.705		0.964
(*Z*)-3-Methylbutanal oxime	852				29.116		9.01
Unknown1 (m/z: 73, 55, 59, 56, 41, 86)	854				3.712		3.199
Unknown2 (m/z: 55, 73, 56, 54, 59, 41)	860				2.081		1.57
Unknown3 (m/z: 59,43, 41, 70,55, 56)	873				29.478		6.789

Table 3. The number and amount (ng per 15 min per flower) of VOCs emitted by the inflorescences of *Hoya veritcillata* during anthesis (continued).

Compound name	RI	Number of Sample (type of adsorbance)					
		Day			Night		
		1D (H)	2D (H)	3D (P)	4N (P)	2N (H)	3N (P)
Isopentyl acetate	874					1.135	3.973
Benzaldehyde	957			26.502	134.641	25.992	81.551
Benzyl alcohol	1033				118.367	31.369	90.427
Benzeneacetaldehyde	1042		5.437	48.807	121.019	124.768	292.506
Eucalyptol	1070				11.853		8.407
(*E*)-Linalool oxide (furanoid)	1087				39.339	31.182	52.673
Methyl benzoate	1092	4.863	2.012	117.647	55.084	17.576	99.328
Linalool	1100				56.559		9.241
Unknown4 (m/z: 68, 67, 110, 82, 43, 55)	1106				1.553		
2-Phenylethanol	1110	1.504	0.862	3.998	6.781	26.804	61.053
Benzyl acetate	1163				8.871		
Epoxylinalool	1167						2.273
Ethyl benzoate	1168				8.876		
Linalool oxide (pyranoid)	1173				80.878	12.021	83.784
Methyl salicylate	1190	3.404		7.207	10.793	3.013	12.422
Eugenol	1355			2.462	14.943		38.995
Isopentyl benzoate	1434			6.793	7.145	3.755	17.038
Benzyl benzoate	1757			4.025	9.576		26.358

Table 3. The number and amount (ng per 15 min per flower) of VOCs emitted by the inflorescences of *Hoya veritcillata* during anthesis (continued).

Compound name	RI	Number of Sample (type of adsorbance)					
		Day			Night		
		1D (H)	2D (H)	3D (P)	4N (P)	2N (H)	3N (P)
Total amount of VOCs		11.423	9.833	233.532	852.171	324.812	946.046
Number of flowers per inflorescence		12	25	22	27	25	22
Period of collection (hours)		2	2	2	2	2	2
Number of compounds		4	4	9	23	11	21

***1, 2. 3 and 4 = number of inflorescences**

**H =Hayesep Q and P=Porapaq-Q*

The results of sample 3D (first day of anthesis) and sample 4N (first night of anthesis) and 3N (second night of anthesis) were presented in table 4. The collection was made on the flower that opened at about 1900h on 6 April 2018 and the floral volatile was collected at 1410h to1610h on 7 April 2018. There were 9 components detected from the floral scent during daytime, of which the three major compounds were methylbenzoate, benzeneacetaldehyde, and benzaldehyde (50.4, 20.9 and 11.3% of total amount volatile emitted, respectively).

For the floral fragrance emitted during nighttime. VOCs were collected from 2 inflorescences from different individual plants, one from an inflorescence on the first night (4N Table 4) and the other from an inflorescence on the second night of anthesis (3N, Table 4) at 2200h to 2400h on 7 April 2018. There were 25 components (Table 4). The major compositions of nighttime- VOCs were not different qualitatively, but quite different quantitatively from those of daytime-VOCs. The three major compounds were benzeneacetaldehyde, benzaldehyde, and benzyl alcohol, 22.6, 11.8 and 11.4% of total amount volatile emitted, respectively.

Generally, there was no great difference in chemical composition, neither qualitative nor quantitative, between the fragrance emitted from the inflorescence on the first and second night. Only four compounds detected in the first night fragrance that were not found in the second night fragrance, i.e. 4-methylpentanol, unknown 4, benzyl acetate and ethyl benzoate (0.281, 0.182, 1.041 and 1.042% of total amount volatile emitted, respectively). In contrast, isopentyl acetate and epoxylinalool (0.420 and 0.240% of total amount volatile emitted, respectively) were detected only in the second night fragrance.

Table 4. The amount (ng per 15 min per flower) of VOCs emitted from inflorescences of *Hoya veritcillata* analyzed from Porapaq Q

Compound name	Number of sample				Compound group
	Day	Night			
	3D	3N	4N	Average	
3-Methylbutanol	16.091	44.485	93.403	68.944	fatty acid derivative
4-Methylpentanol			2.398	2.398	fatty acid derivative
3-Methylpentanol		0.964	5.705	3.334	fatty acid derivative
(*1Z*)-3-Methylbutanal oxime		9.010	29.116	19.063	fatty acid derivative
Unknown1 (m/z: 73, 55, 59, 56, 41, 86)		3.199	3.712	3.455	-
Unknown2 (m/z: 55, 73, 56, 54, 59, 41)		1.570	2.081	1.825	-
Unknown3 (m/z: 59,43, 41, 70,55, 56)		6.789	29.478	18.133	-
Isopentyl acetate		3.973		3.973	fatty acid derivative
Benzaldehyde	26.502	81.551	134.641	108.096	benzenoid compound
Benzyl alcohol		90.427	118.367	104.397	benzenoid compound

Table 4. The amount (ng per 15 min per flower) of VOCs emitted from inflorescences of *Hoya veritcillata* analyzed from Porapaq Q (continued)

Compound name	Number of sample				Compound group
	Day	Night			
	3D	3N	4N	Average	
Benzeneacetaldehyde	48.807	292.506	121.019	206.762	benzenoid compound
Eucalyptol		8.407	11.853	10.130	terpenoid compound
Trans-Linalool oxide (furanoid)		52.673	39.339	46.006	terpenoid compound
Methylbenzoate	117.647	99.328	55.084	77.206	benzenoid compound
Linalool		9.241	56.559	32.900	terpenoid compound
Unknown4 (m/z: 68, 67, 110, 82, 43, 55)			1.553	1.553	-
2-Phenylethanol	3.998	61.053	6.781	33.917	benzenoid compound
Benzyl acetate			8.871	8.871	benzenoid compound
Epoxylinalool		2.273		2.273	terpenoid compound
Ethyl benzoate			8.876	8.876	benzenoid compound
Linalool oxide (pyranoid)		83.784	80.878	82.331	terpenoid compound
Methyl salicylate	7.207	12.422	10.793	11.608	benzenoid compound
M-Eugenol	2.462	38.995	14.943	26.969	terpenoid compound
Isopentyl benzoate	6.793	17.038	7.145	12.092	benzenoid compound
Benzyl benzoate	4.025	26.358	9.576	17.967	benzenoid compound

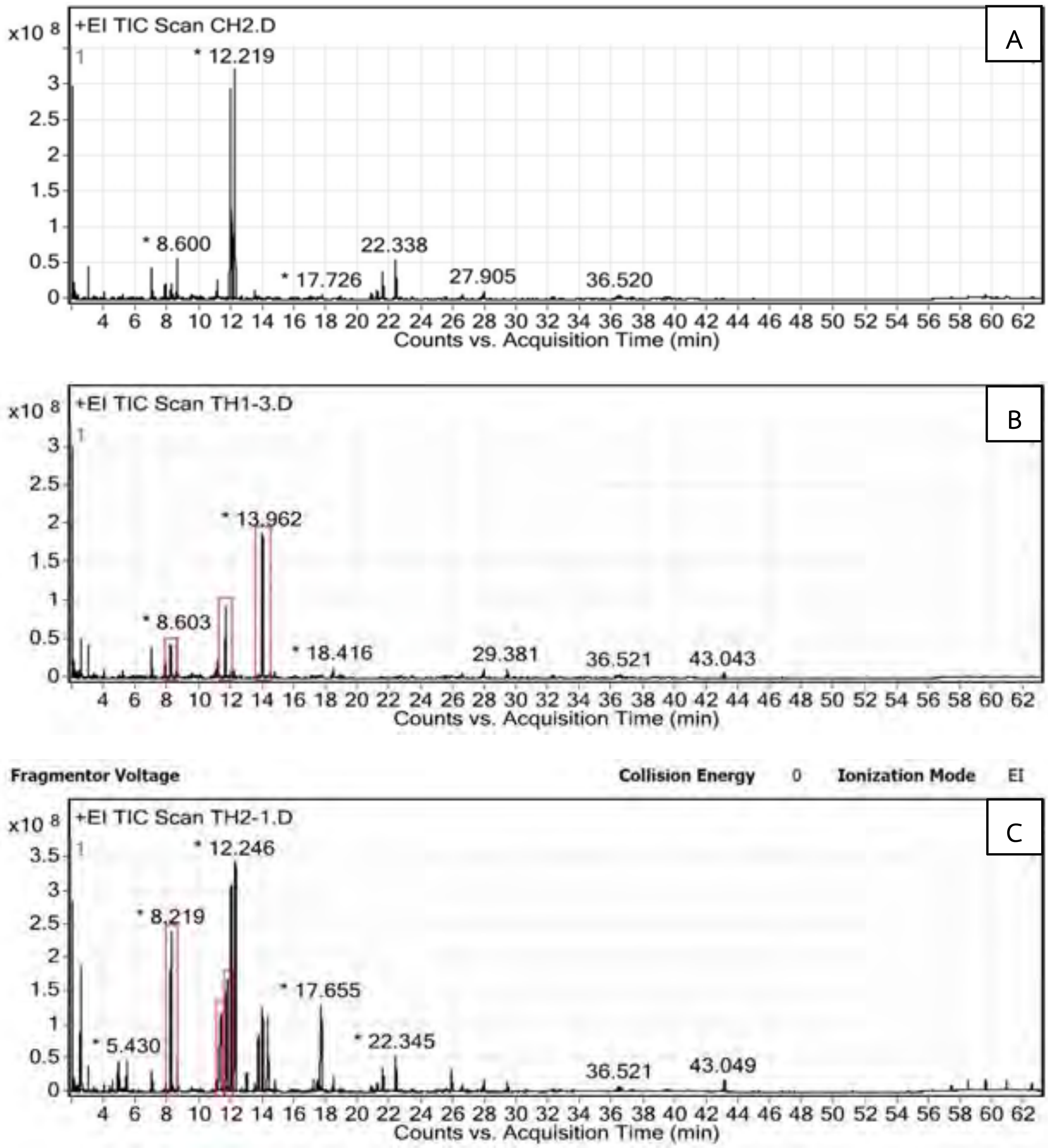

Figure 16. Example of chromatograms obtained from GC-MS analysis of floral volatiles of *Hoya verticillata*. A. Control (surrounding air), B. Floral volatiles emitted from inflorescence during daytime, C. Floral volatiles emitted from inflorescence during nighttime

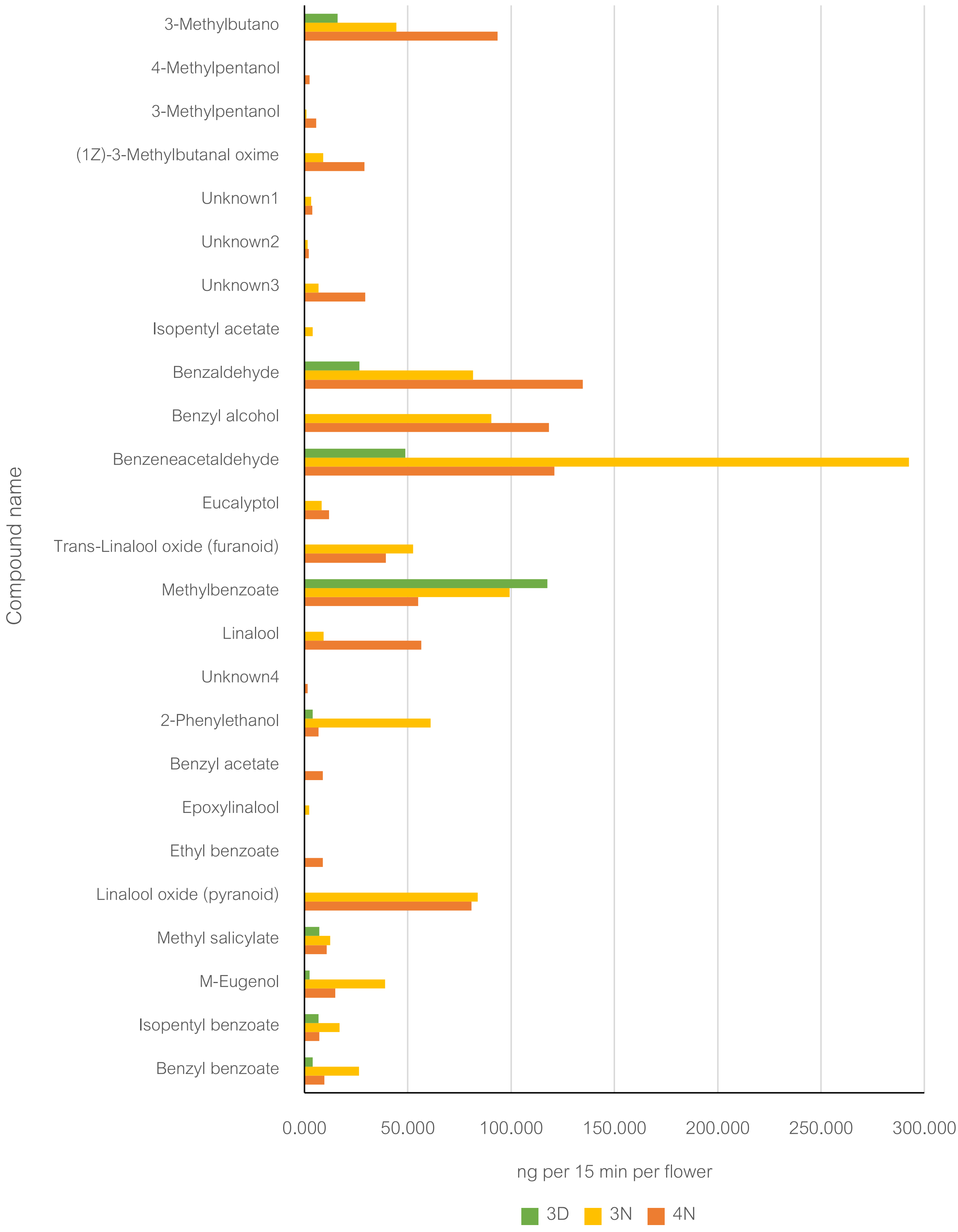

Figure 17. The amounts of components of VOCs from 2 individual plants during day and night

4.7.2 Osmophore

The fleshly opened flower was stained by neutral red to localize the high metabolic activity area and it was found that all parts of corolla, tissue under pollinia and the lower side of corona appeared in red (Fig.18).

The structure responsible for floral scent production/emission are the unicellular glandular trichomes which are epidermal outgrowths found on the adaxial surface of the corolla lobes. These trichomes are long cone-shaped. The adaxial epidermis consists of large of epidermal cells, which are (side-view) in pentagonal or hexagonal shape, subtended by mesophyll which consists of 3-4 layers of anticlinally arranged rectangular palisade parenchyma cells and 2-3 layers of loosely arranged spongy parenchyma cells. Cells in these layers are characterized by presence of a large nucleus and dense cytoplasm (Fig.19 and 23).

The abaxial surface consists of large tetragonal, pentagonal or hexagonal epidermal cells (top-view) and stomata. No trichome was found on the abaxial surface of corolla (Fig.22).

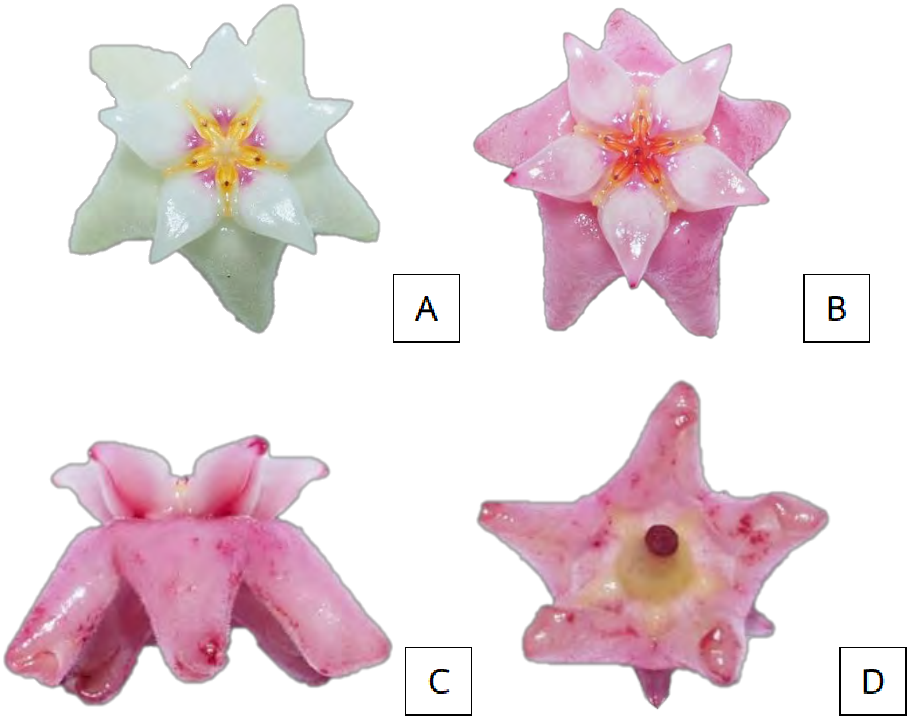

Figure 18. Localisation of osmophore of *Hoya verticillata* A. Newly opened flower before being stained with neutral red. B. and C. Top and side views of a stained flower, D. Lower side of a stained flower

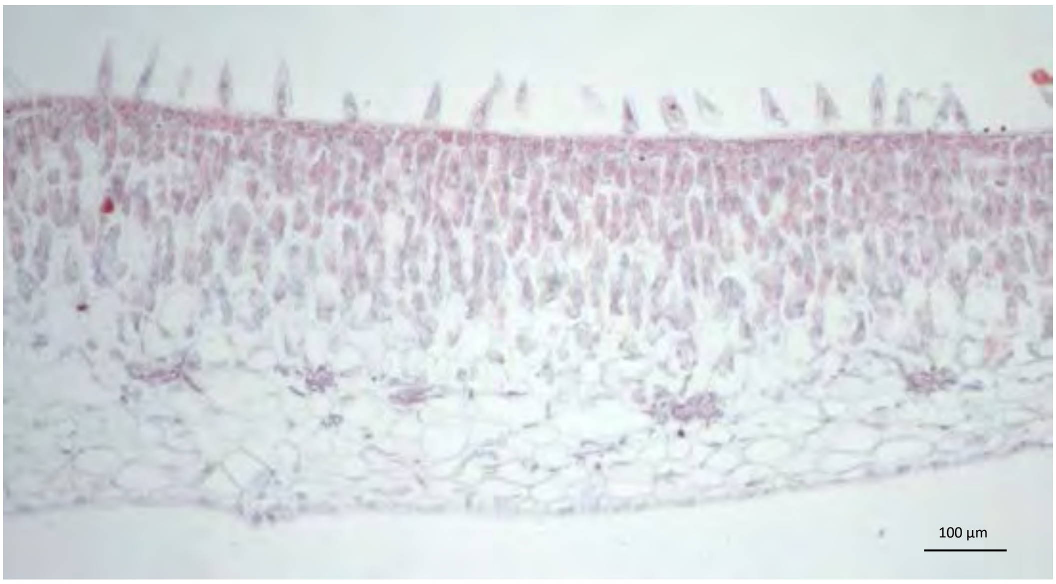

Figure 19. Cross section of the corolla lobe of *Hoya verticillata* stained by Safranin O and Fastgreen

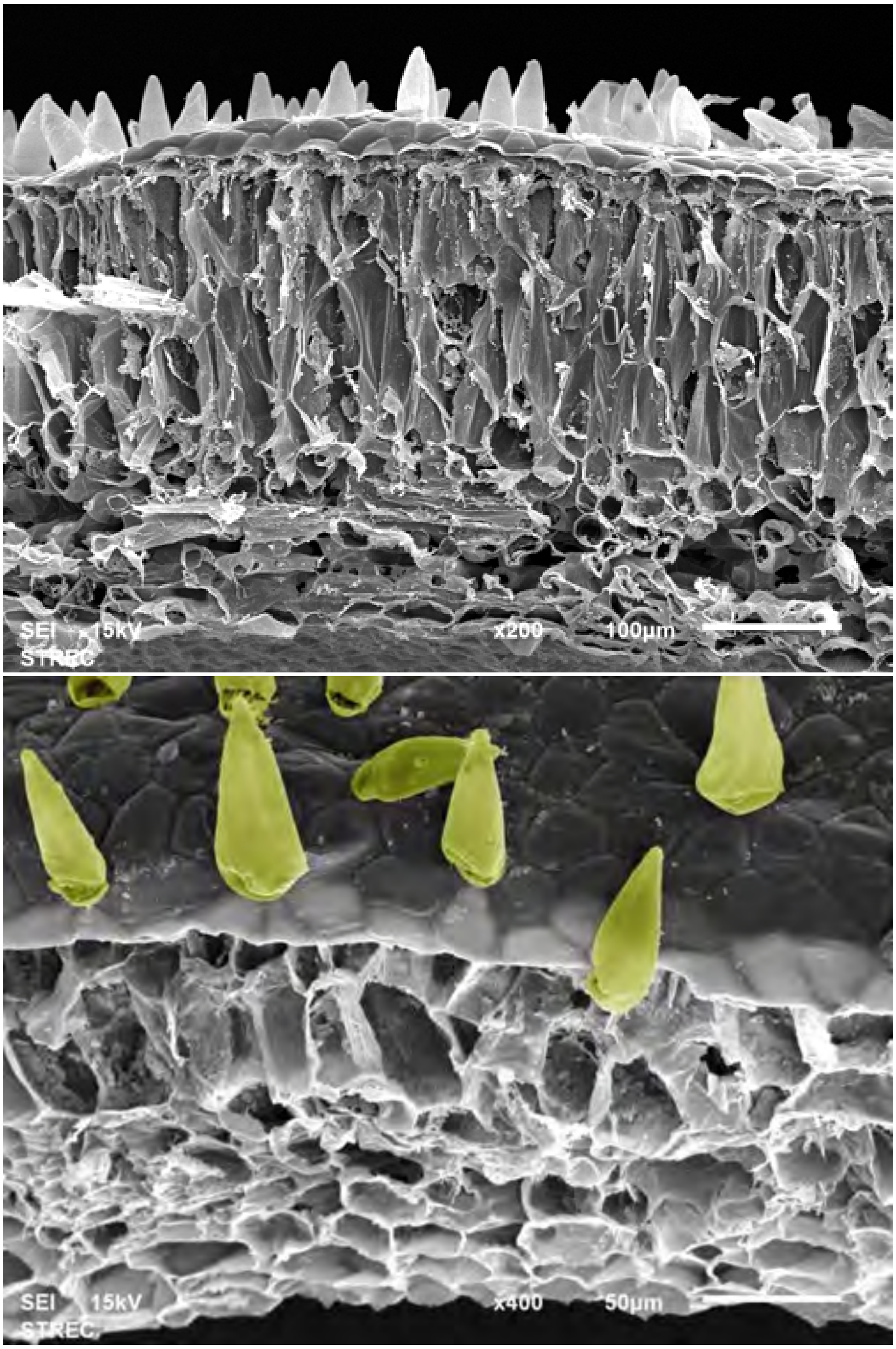

Figure 20. SEM micrographs of cross-section of the corolla lobes of *Hoya verticillata* A. side view,

B. Oblique view showing trichomes which were false-coloured in yellow

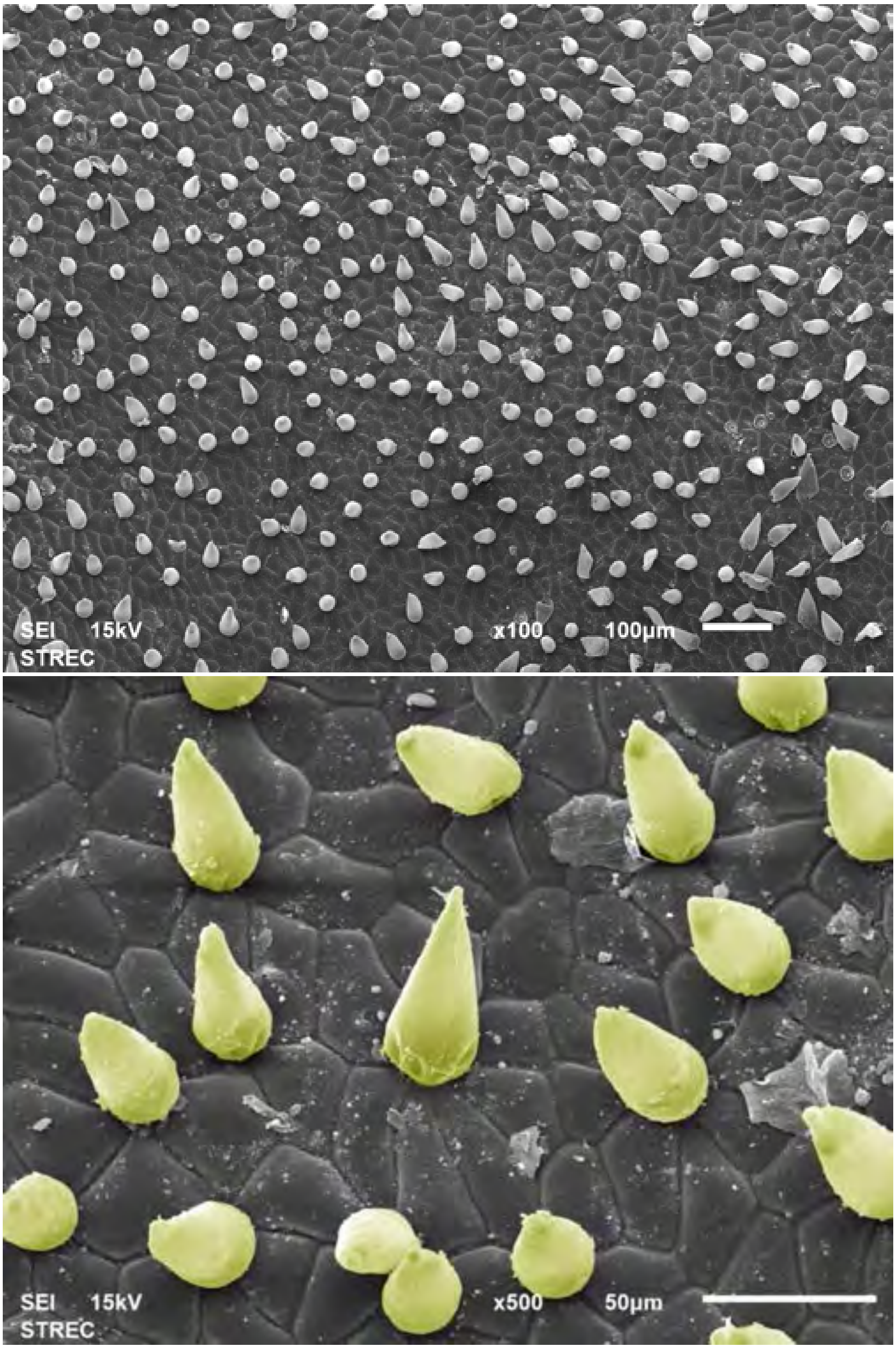

Figure 21. SEM micrographs of adaxial surface of the corolla lobes of *Hoya verticillata* A. Low magnification top view, B. High magniciation top view showing long cone-shaped trichomes which were false-coloured in yellow

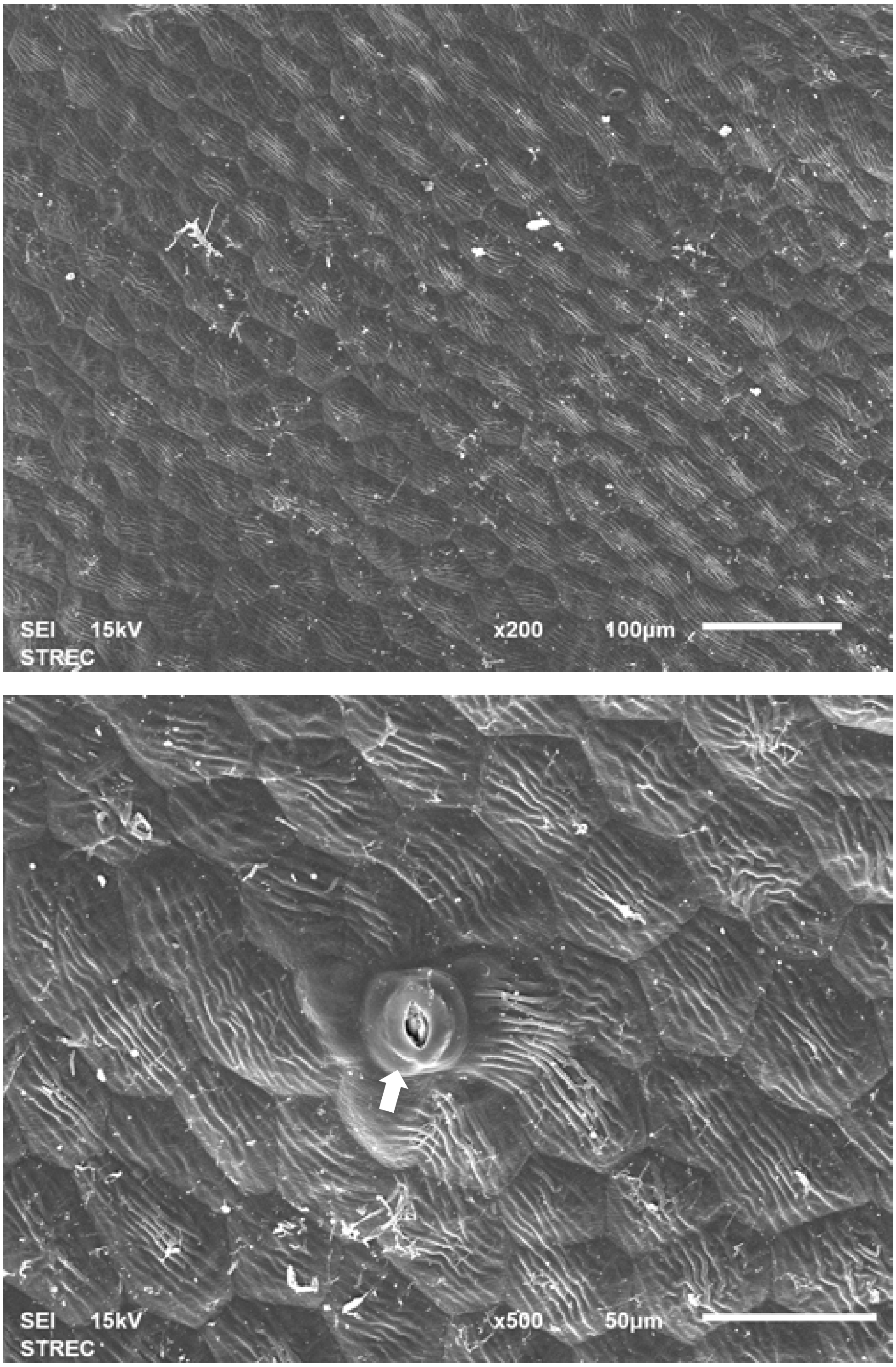

Figure 22. SEM micrographs of abaxial surface of the corolla lobe of *Hoya verticillata* A. Low magnification top view showing general feature of epidermis B. High magnification top view showing a stoma and guard cells (arrow)

4.7.3 Nectar

The nectar was collected from 3 inflorescences of different individual plants, one was flowers on the first day of anthesis and the other was flower on the second day of anthesis. The nectar sample contained mainly three sugars: sucrose, glucose, and fructose. The proportion of these sugars present in nectar varied greatly from sample to sample (Fig. 23 A-C) i.e. 8-90% for sucrose, 5-57% for fructose, and 4-35% for glucose. Each sample was either dominated by fructose (Fig. 23A) or sucrose (Fig. 23B-C). Moreover, small amount of an unknown compound (1-2%) was found in two samples (Fig. 23B-C).

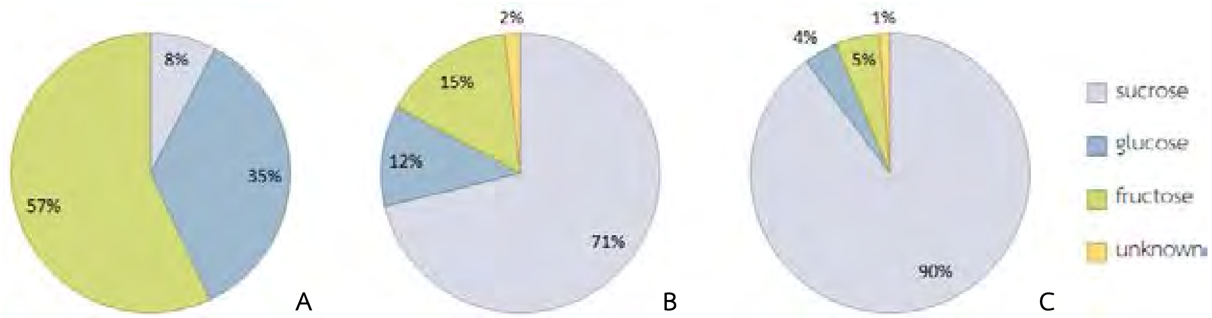

Figure 23. Nectar compositions from 3 inflorescences of different individual plants. Samples A and B were collected from the inflorescences on the second day of anthesis, and sample C from the inflorescence on the first day of anthesis.

4.7.4 Nectary

A nectary was found located behind each of the five guide rails, hence there are five nectaries around the gynostegium, that connects to the stigmatic chambers (Fig. 23A-B). The nectariferous tissue is formed by a layer of oval-shaped epidermal cells and 1-2 subepidermal layers of elongated parenchyma cells of filament tube facing stigmatic chamber. These cells were strongly stained in pink, and had dense cytoplasm.

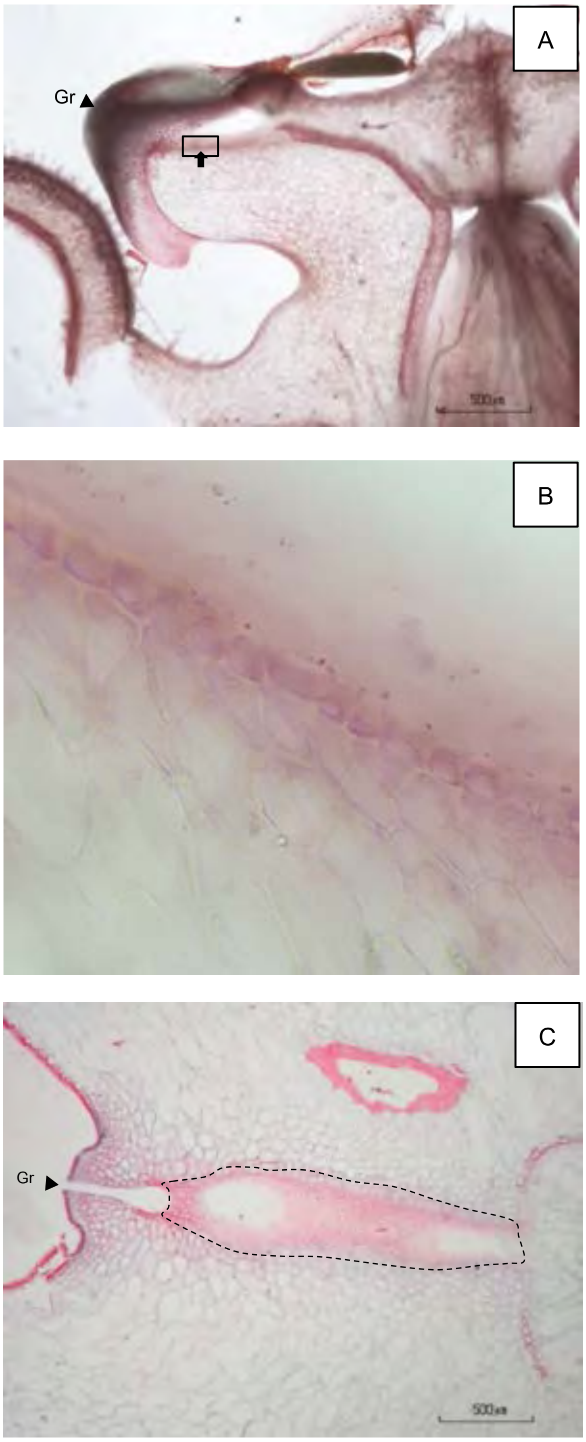

Figure 24. Longitudinal and cross sections of the gynostegium stained with Safranin O and Fastgreen A. Longitudinal section ofgynostegium through the middle of a guide rail showing a nectary (arrow) behind the guide rail. Gr = guide rail. The framed zone in A is enlarged in B. showing the detail of nectary C. Cross section of the lower part of gynostegium showing nectariferous tissue (dot line). Gr = guide rail.

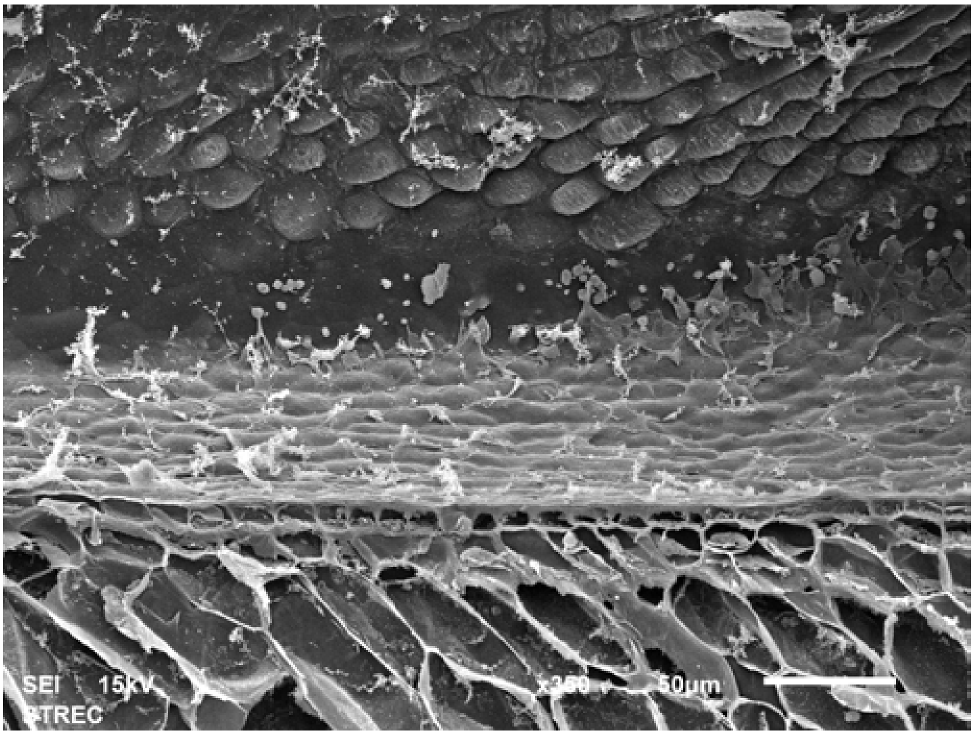

Figure 25. SEM micrograph of longitudinal section of gynostegium through the middle of a guide rail, showing nectariferous tissue facing stigmatic chamber

4.8 Environmental condition in relation to blooming phenology and insect visitation

Mean temperature, relative humidity and illuminance of every hour were showed in Fig 26 and 27. The highest temperature was about 40.0 ˚C and lowest relative humidity was 36.6% at 1400h. At 0600h, the temperature was lowest and the humidity was hightest (25.3 ˚C and 86.0%, respectively). The highest illuminance was 35631.1 lux at 1000h.

Considering the insect visitation rate during the study period (March 10-11 and 23-25, 2018), the number of visiting insects peaked during 1800h to 1900h. (Fig.28), which corresponded to the period of greatly decreased temperature, greatly increased relative humidity, and the surrounding area was becoming entirely dark (Fig.27).

Between 1100h and 1800h, no visitor was observed. During this period, the temperature was considerably higher and the relative humidity was significantly lower comparing to the other period of the day.

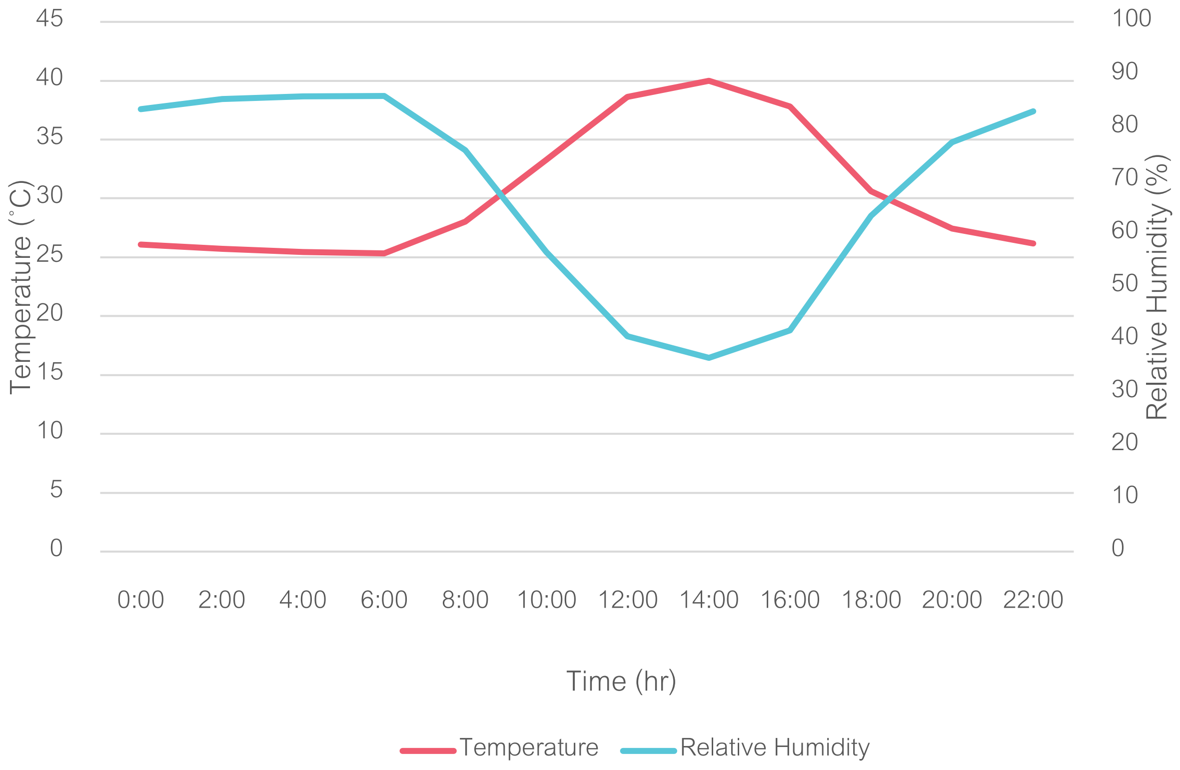

Figure 26. Mean hourly temperature (˚C) and relative humidity (percent) obtained from data logger installed at Ban Phe, Rayong Province in March 2018

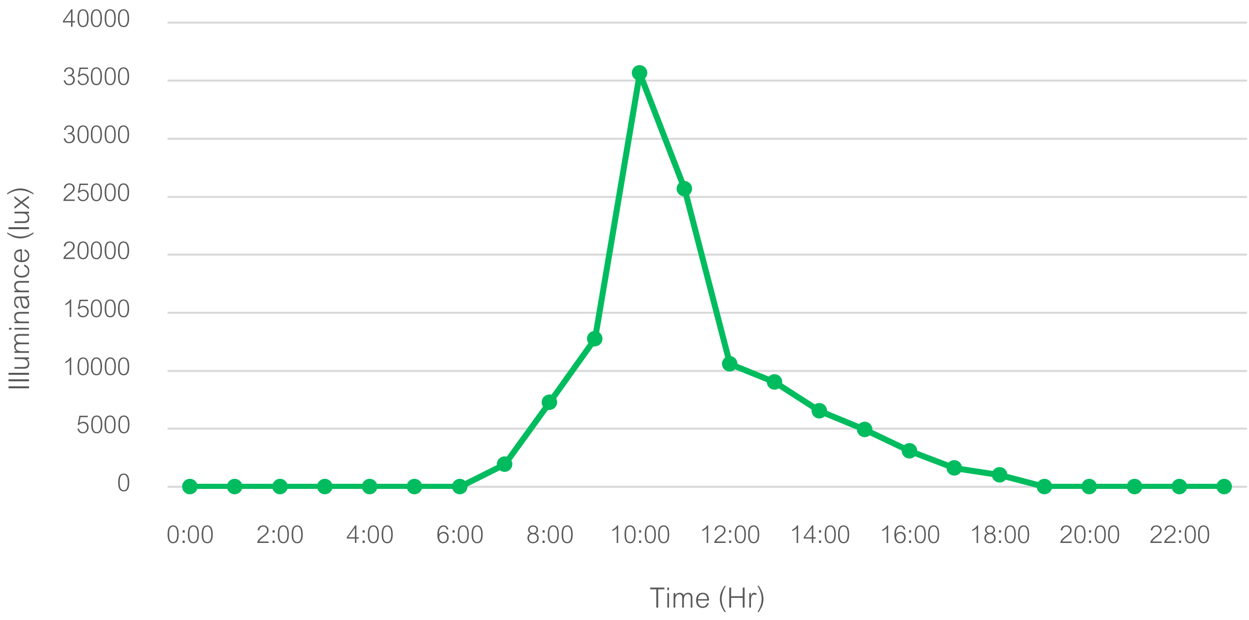

Figure 27. Mean hourly Illuminance (lux) obtained from data logger installed at Ban Phe, Rayong Province in March 2018

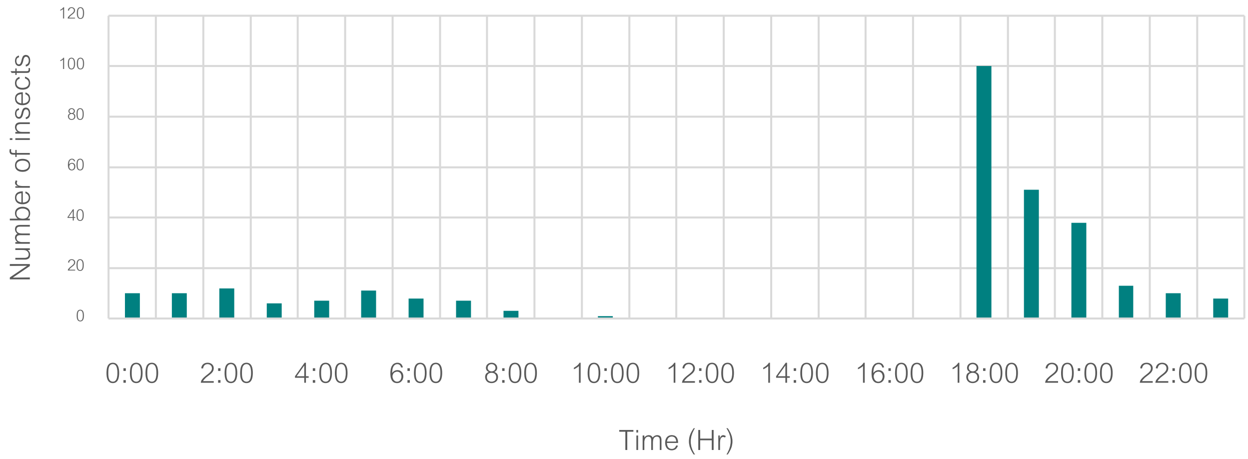

Figure 28. Number of visitors per hour during the study period

4.9 The pollen-transfer efficiency (PTE)

Estimation of pollination success in the population was made by calculating the pollen-transfer efficiency (PTE), which is the proportion of pollen grains removed from anthers that were deposited on the stigma. In *Hoya*, the pollen grains are packaged into a pair of pollinia. The PTE was determined as the percentage of removed pollinia that were inserted between guide rails (stigmatic slits) as shown below (Fig 29 and 30).

$$\text{Pollen-transfer efficiency} = \frac{\text{Number of inserted pollinium}}{2 \times \text{Number of removed pollinarium}}$$

Table 5. Pollen-transfer efficiencies calculated from 18 inflorescences, 284 flowers which were collected 3 times during March to July 2018.

Duration	PTE	PTE %
23 – 25 March 2018 (n = 108 flowers, 7 inflorescences)	0.036	3.60
5 – 8 April 2018 (n = 118 flowers, 8 inflorescences)	0.00	0.00
20 – 23 July 2018 (n = 58 flowers, 3 inflorescences)	0.0079	0.79

The PTE of different study periods were quite fluctuated, ranging from 0.00% to 3.60%. The highest PTE was obtained during 23 – 25 March 2018 and the lowest one was during 5 – 8 April 2018 (Table 5).

Figure 29. A flower of *Hoya verticillata* with inserted pollinium (➡) and removed pollinarium (▶)

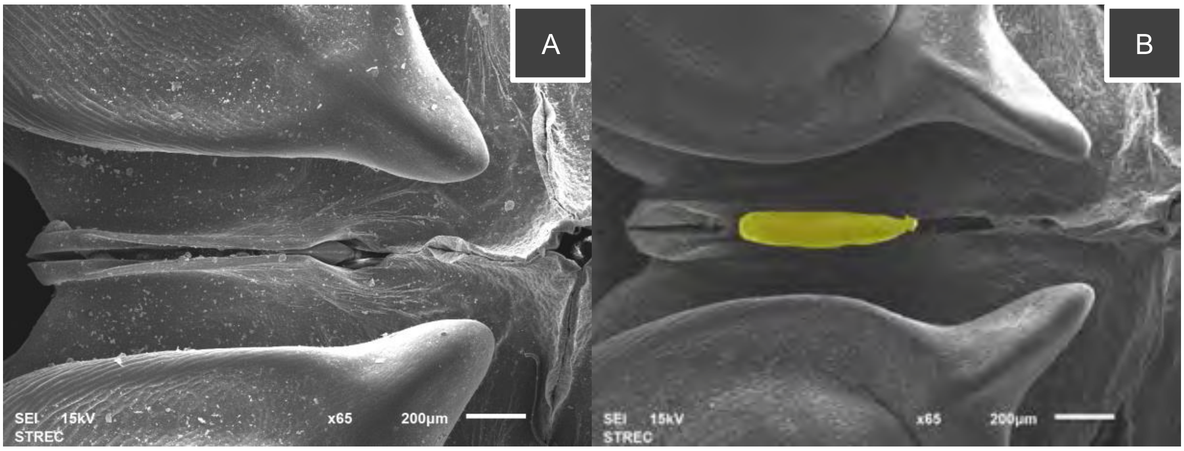

Figure 30. A. SEM micrograph showing guide rail without removing of pollinarium or insertion of a pollinium, B. SEM micrograph showing guide rail with an inserted pollinium

Chapter 5

Discussion

1. Effective pollinator

During the blooming period there were several groups of animals visited flowers of *Hoya verticillata*, including spider (order Araneae), cockroach (order Blattodea), and hawkmoth, moth and butterfly (order Lepidoptera). However, only insects that visited flowers regularly, stayed long enough time to provide pollination service and had suitable morphology and behaviour while visiting the flowers could be considered as potential pollinators. There were only large and medium moths from the families Erebidae (Fig.31) and Geometridae (Fig.32) that could be effective pollinators because of their behavior and their shape and size of legs and proboscis. When these moths visited the flowers, they put their legs on the top of corona, walked around the inflorescence and used their proboscis to probe nectar. Apart from these moths, the other animals could only be considered as floral visitors because of their unsuitable morphology, irregular visitation and visiting behaviour.

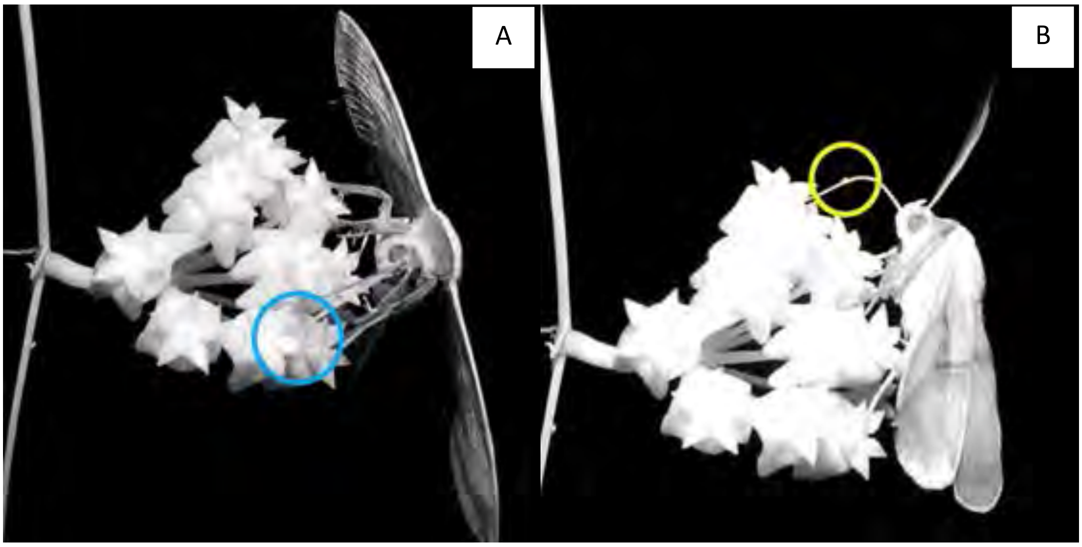

Figure 35. Potential Pollinators of family Erebidae (Large Moth) A. Moth's proboscis and leg touch on the top of corona B. Pollinarium attach on moth's proboscis

Figure 36. Potential Pollinators of family Geometridae (Large Moth)

2. Association between environmental conditions, insect visitation and blooming of flowers

The flowers of *H. verticillata* bloomed during 1800h to 2000h, the period when the temperature was close to lowest, the relative humidity was highest, and the surrounding area was in dark. The visitation rate peaked between 6 pm to 8 pm. During the daytime, many moths (Lepidoptera: Tortricidae) were inactive, to avoid the high temperature and low humidity, but they were active after sunset during the period of highest humidity and lowest temperature (Quiring, 1994; Pinault et al, 2012). The Geometridae and Pyralidae moths were found to be very active around midnight (Rydell, Entwistle and Racey, 1996). The foraging activity of moths in this study might occurred before the ovipositing and matting behaviour that was peak between 2200h to 0400h (Quiring, 1994; Pinault et al, 2012).

3. Pollinator-mediated selection on floral traits

Hoya verticillata produced fragrance as a long-distance attractant for its pollinator. Flowers can attract insects at short and long ranges (Huber et al, 2004; Valenta et al., 2016). In a dark environment, a short-distance attractant like visual attraction is almost useless (Pettersson, Ervik, and Knudsen, 2004) and flowers which have nocturnal pollinators use scent as a long-distance attraction (Huber et al, 2004). In *H. verticillata*, floral scent was emitted from the unicellular glandular trichomes found on the adaxial surface of the corolla lobes. This structure is similar to scent-emitting glandular papillose cells present on the adaxial surface of the petal of Milkweed (*Asclepias speciose*, Apocynaceae) (Poinar, and Poinar, 2018).

The sweet fragrance of *H. verticillata* was strong at night and the components were different from day scent. The major group of the volatile organic compounds (more than 64 percent) was benzenoid compound. It was consistent with the studies in the other plant groups that benzenoid compounds were major components of floral scents of moth-pollinated plants (Knudsen, and Tollsten, 1993; Dobson, 2006). The emission of strong scent at night and the blooming time (started about 1900h) of *H. verticillata* flowers were related to the nocturnal behaviour of its potential pollinators. The other attractant was nectar secreted during the night from nectariferous tissue behind the guide rails then collected at the base of corona as a reward for pollinators. The copious amount of nectar might extend the visit duration of the pollinators (Manetas, and Petropoulou, 2000). Its nectar was composed of sucrose as a major component. This was consistent with the other study reporting that moth preferred plants that had nectar with high proportion of sucrose (Nicolson, and Thornberg, 2007).

4. Pollen transfer efficiency

The pollination success of the population of *H. verticilla* in the botanic garden provided by insect pollinators was estimated as 0.00 – 0.036 of PTE. The lowest of PTE value was from April (0.0% of PTE), that might due to the low number of blooming inflorescences, only 9 blooming inflorescences from more than 100 individual plants, while there were more than 20 inflorescences bloomed in March or July. The PTE value (0.00-0.036) of *H. verticillata* were lower than that reported in 36 species of milkweeds (Asclepiadoideae), of which the mean PTE was 0.26 (Livshultz, Hochleitner, and Lakata, 2018) and very low when compared to the value of PTE of 0.70 provided by the butterfly *Ocybadistes walkeri sothis* in *H. australis* cultivated in Australia (Forster, 1992). Moreover, the number of the fruit set observed between March and July, 2018 were lower than that found in the previous years (Kidyoo, personal communication, March, 2018). This might be due to lower foraging activity of the insects because nowadays the natural area around Ban Phe Botanic Garden are degrading because of land development and human activity.

5. BREEDING SYSTEM

In this study, the breeding system of *H. verticilla* is still unclear. The male and female reproductive organs of this plant are strictly spatially separated within flowers. This spatial separation is known as herkogamy and this function occurs in both self-incompatible and self-compatible species (Medrano, Herrera and Barrett, 2005). Therefore, further study, such as hand-pollination, is required for clearly determine the breeding system of this plant.

Chapter 6

Conclusion

Flowers of *Hoya verticillata* took about 21.97 days to developed from very small bud to post-anthesis. They bloomed at night, between 1800h to 2000h, and remained open for 1-4 days. While opening, a strong sweet fragrance was emitted from unicellular glandular trichomes located on adaxial surface of corolla lobes and copious nectar was secreted from nectary behind each of guide rails and collected at the corona base. The dominant component of the scent was benzenoid compounds including benzeneacetaldehyde, benzaldehyde, and benzyl alcohol. The nectar was composed of three sugar and one unknown component (1-2%), the major component was sucrose. *Hoya verticillata* was a nocturnal and open access flower, the flower that attract various group of visitors and allow them to exploit flower's reward. Therefore, the visitation is generalized. However, not all visitors could provide pollination service but only the insects, large moths and medium moth of family Erebidae and Geometridae, that visited flowers regularly and have suitable morphology and behaviour while visiting flowers could be consider as potential pollinator. So, the pollination is specialized. The pollen transfer efficiency of 0.0% - 3.6% was very low comparing to those reported in other species of *Hoya* or other asclepiads. This might due to a low foraging activity of insects, affected by human activity and degradation of the natural habitat.

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