Hinthada University Research Journal, Vol. 2, No. 1, 2010

# Pollination Biology of *Clerodendrum serratum* Spreng. (Yin-bya)

Kyaw Zay Moe[1] and Soe Min Aye[2]

## Abstract

*Clerodendrum serratum* Spreng. which belongs to the family Verbenaceae was an observed plant species for this study. This plant was located in the garden of Botany Department, University of Yangon and observation on pollination processes of this plant was done to examine the visitors and a significance pollinator, floral traits and phonological changes, and target pollen and non-target pollens from the body of significant pollinator. For visitor and a significance pollinator observation, 10 hours observation was done for this species. From this observation, a total of visits per flower per day by all visitors (bees, flies, moths and butterflies) were 22.763 and a total of visits per flower per day by a significance pollinator (*Amegilla* sp.) were 22.6. In phenological studies, average longevity of a flower to visit and pollinate it for a significance pollinator was 8.4 hours. In target pollen observation, a number of 10 pollen samples were observed from the whole body of a significance pollinator.

**Key words:** Significance pollinator, visitors, floral longevity, target pollen, non-target pollen.

## Introduction

*Clerodendrum serratum* Spreng. which belongs to the family-Verbenaceae is a useful medicinal plant. The leaves and roots are used for fever, asthma, cough, rheumatism and are also used as tonic and vermifuge (Myint Myint Aye, 2002).

In the diagnostic features of *C. serratum* the plant is shrub, erect sparsely branched, solid; the stems glabrescent; leaves opposite and decussate, elliptic-obovate with serrate margins; terminal and axillary dichasial cyme; flowers purple, pedicels flattened, reddish purple, puberulent; calyx 5-toothed, the tubes campanulate; corolla 5; stamens 4, the anthers 4, ovoid, pale purple, the bases hispidulous; ovary ovoid; globoide fruits (Myint Myint Aye, 2002).

A comparative study on the morphology, taxonomy and anatomy of *C. serratum* was undertaken by Myint Myint Aye (2002). But no one has done in pollination study for this species.

This observation was the first attempt to point out the role of mutualistic pathways of a flowering plant species and its pollinators among other botanical fields. Moreover, this field of pollination study is very useful to express the interactions of plant and pollinator in nature. And also, it may be the best guideline for biodiversity conservation projects in Myanmar.

## Aims of Study

This research was carried out with the following objectives.

- To determine a significant pollinator of *Clerodendrum serratum* Spreng. among visitors.
- To record the floral traits and floral phenology of *C. serratum.*.
- To examine the target pollen from a significant pollinator.

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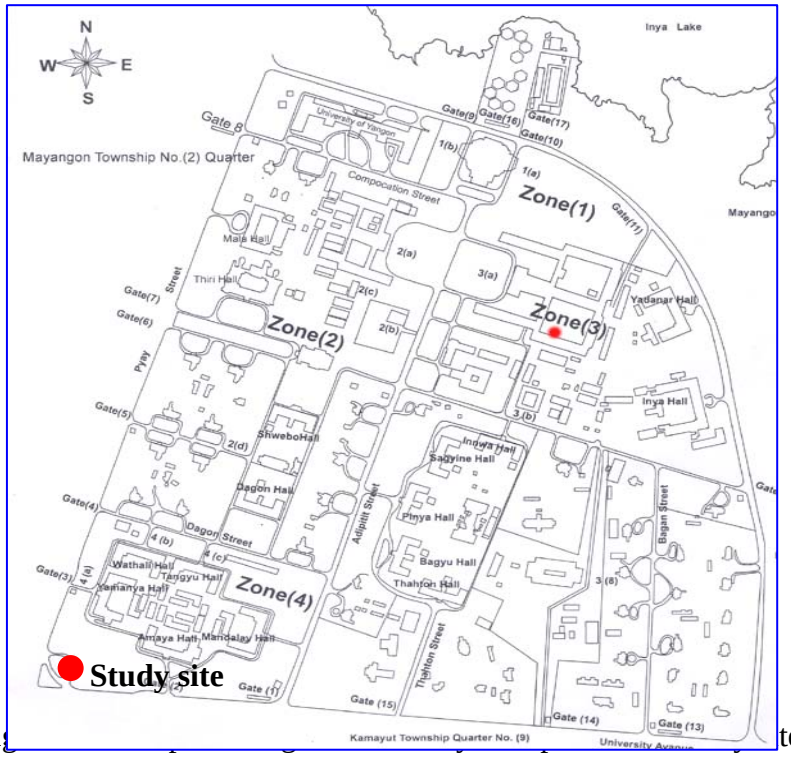
## Field Methodology

An observed plant species, *Clerodendrum serratum* Spreng. was located in the garden of Botany Department, Zone III area of Yangon University Campus (Figure 1). To understand the common methods of field of pollination, visitors and significant pollinator observation, phenological studies and pollen extraction from the significant pollinator were carried out according to Parrish (2004).

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### Visitors and a significant pollinator observation

According to Parrish (2004), measuring insect visitation rate with 10 hours observation was carried out to determine the visitors and a significant pollinator. In this observation, a pollinator which possessed the highest visitation rates was determined as a significant pollinator. In this procedure, a total of 60 flowers were randomly selected from three inflorescences. Watch and note the pollinators' visits on 60-flowers from 0800 h to 1800 h (two-hour blocks – 0800 to 1000, 1000 to 1200, 1200 to 1400, 1400 to 1600, and 1600 to 1800 h). And then, calculate the average number of visits per 10 minutes observation period from data sheet and calculate the total visit per flower per day by all visitors(V), and total visit per flower per day by a significant pollinator (Vb) using the following equation.

$$\mathbf{V = Sum\ from\ i=1\ to\ n\ (v \times b/p\ ) / F}$$
$$\mathbf{V\ b= Sum\ from\ i=1\ to\ n\ (v \times b/p\ ) / F}$$

i = each time interval in which there were observations, 2 hours in this example

n = number of time intervals observed, 5 in this example (0700-0900, etc.)

v = average number of visits observed during observation period (10 minutes)

b = number of minutes in the time interval (120 per two-hour interval)

p = number of minutes in an observation period (10)

F = total number of flowers observed (60 as counted)

A logical framework of observation on *C. serratum* Spreng. was shown in Figure 2.

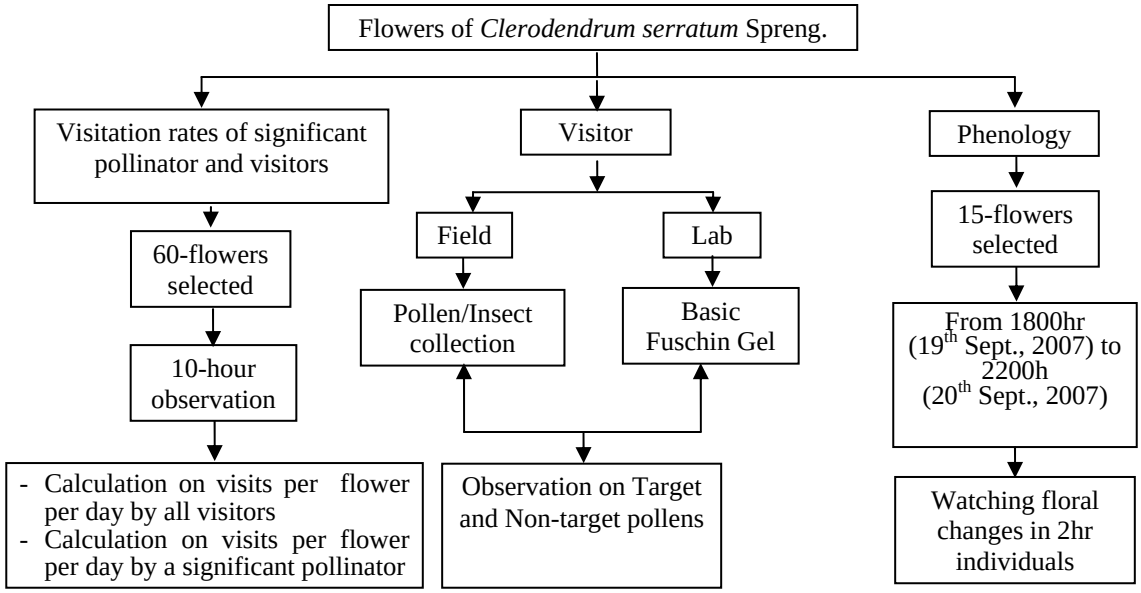


Figure 2. A logical framework of observation on *Clerodendrum serratum* Spreng.

**Data collection of floral traits**

According to Machado & Lopes (2004), the floral traits such as floral symmetries, floral rewards, floral shapes, types of anther dehiscence, floral colours and sexual systems were recorded for this species in the field.

**Phenological study**

Observation was also carried out according to Parrish (2004). In this procedure, 15-buds were selected from three inflorescences. Watch the floral changes in two-hour intervals and floral changes were recorded. Depending on floral changes, phase keys were created. And then, calculate the average longevity of a flower to pollinate it.

**Target pollen observation**

According to Parrish (2004), a jelly coating stain to make a semi-permanent pollen slide was prepared in the Laboratory of Tissue Culture, Department of Botany, University of Yangon. In this observation, Basic Fuchsin Gelatin was used to extract pollens from insect body. A significant pollinator was caught by a hand net and placed into a small vial, and made pollinator to forget as a short time with the help of cigarette smoke. And then, pollen samples were extracted from the whole body of a significant pollinator (legs, head, thorax

and abdomen) and made semi-permanent pollen slides in field. Before this observation, a target pollen sample slide of *C. serratum* had already been made to compare the extracted semi-permanent pollens from the body of a significant pollinator. This comparative study was done by a DP-12 digital image microscope at the laboratory, University of Yangon.

### Plant and pollinator identifications

Plant specimens were collected for herbarium and verified by using available literature and collected pollinator was submitted to Department of Zoology, University of Yangon for identification.

## Results

### Observed plant species and visitors

Observation was made on 17th September, 2007 in the garden of Botany Department, University of Yangon. The plant *C. serratum* which belongs to the family Verbenaceae can attract 4 types of visitors (bee, fly, moth and butterfly) (Figure 3) and these visitors can be seen in Figure 4.

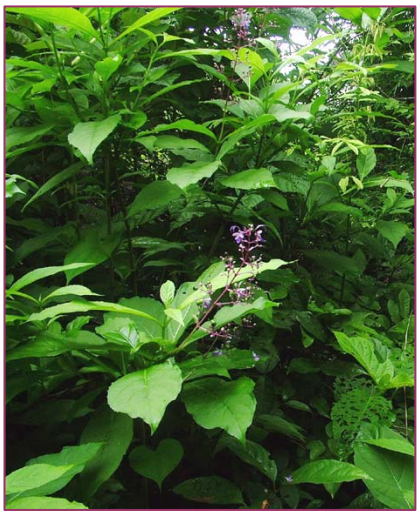

Figure 3. Habit of *Clerodendrum serratum*

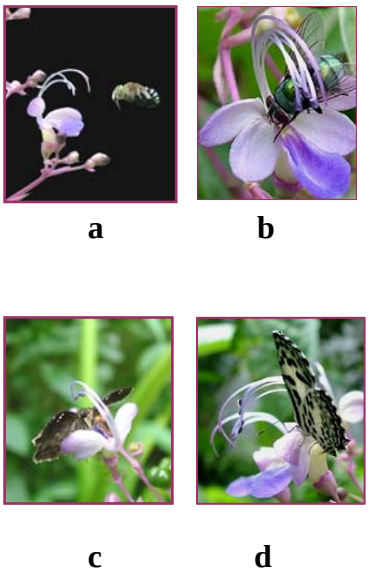


Figure 4. Visitors of *Clerodendrum serratum* (a) bee, *Amegilla* sp. (b) fly (c) moth (d) butterfly

### Visitors and a significant pollinator

To determine the significant pollinator and visitors, 10 hours observation was made in field according to Parrish (2004). The following table of 10 hours observation includes date, time, temperature, cloud condition, visitor groups, their total visits and average numbers of their visit per 10 minutes of field data (Table 1).

Table 1. Data sheet of 10 hrs observation on 17th September, 2007

| Datasheet of 10 hrs observation | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **0800-1000** | | **Temperature- 26°C** | | | | **Cloud cover- Completely overcast** | | | | | | | | |
| **Category** | 0800-0810 | 0810-0820 | 0820-0830 | 0830-0840 | 0840-0850 | 0850-0900 | 0900-0910 | 0910-0920 | 0920-0930 | 0930-0940 | 0940-0950 | 0950-1000 | **Total** | **/10minutes** |
| Bees | 5 | 0 | 9 | 0 | 14 | 7 | 20 | 7 | 13 | 23 | 19 | 9 | 126 | 10.5 |
| Small bees | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Flies | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0.083 |
| Butterflies | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 2 | 0.166 |
| Beetles | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Other | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **1000-1200** | | | | | | | | | | | | | | |
| **Category** | 1000-1010 | 1010-1020 | 1020-1030 | 1030-1040 | 1040-1050 | 1050-1100 | 1100-1110 | 1110-1120 | 1120-1130 | 1130-1140 | 1140-1150 | 1150-1200 | **Total** | **/10minutes** |
| Bees | 38 | 24 | 14 | 56 | 42 | 58 | 30 | 26 | 19 | 30 | 36 | 29 | 512 | 42.666 |
| Small bees | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Flies | 0 | 0 | 0 | 0 | | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0.083 |
| Butterflies | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0.083 |
| Beetles | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Other | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **1200-1400** | | | | | | | | | | | | | | |
| **Category** | 1200-1210 | 1210-1220 | 1220-1230 | 1230-1240 | 1240-1250 | 1250-1300 | 1300-1310 | 1310-1320 | 1320-1330 | 1330-1340 | 1340-1350 | 1350-1400 | **Total** | **/10minutes** |
| Bees | 35 | 8 | 23 | 21 | 11 | 12 | 39 | 36 | 33 | 59 | 67 | 5 | 290 | 24.166 |
| Small bees | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Flies | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Butterflies | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 2 | 0.166 |
| Beetles | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Other | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **1400-1600** | | | | | | | | | | | | | | |
| **Category** | 1400-1410 | 1410-1420 | 1420-1430 | 1430-1440 | 1440-1450 | 1450-1500 | 1500-1510 | 1510-1520 | 1520-1530 | 1530-1540 | 1540-1550 | 1550-1600 | **Total** | **/10minutes** |
| Bees | 0 | 3 | 2 | 6 | 2 | 0 | 31 | 12 | 44 | 66 | 93 | 33 | 292 | 24.333 |
| Small bees | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Flies | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0.083 |
| Butterflies | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Beetles | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Other | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **1600-1800** | | | | | | | | | | | | | | |
| **Category** | 1600-1610 | 1610-1620 | 1620-1630 | 1630-1640 | 1640-1650 | 1650-1700 | 1700-1710 | 1710-1720 | 1720-1730 | 1730-1740 | 1740-1750 | 1750-1800 | **Total** | **/10minutes** |
| Bees | 39 | 26 | 31 | 29 | 5 | 3 | 0 | 0 | 3 | 0 | 0 | 0 | 136 | 11.333 |
| Small bees | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Flies | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Butterflies | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Beetles | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Other | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 2 | 0.166 |

From the above table, the highest visitation rates were found in bee (*Amegilla* sp.) and others (fly, moth and butterfly) were recorded as visitors for this plant species.

Table 2. Average visitation rates of each visitor on numbers of 60 flowers per 10 minutes

| Time of Day | # Visits by Flies | Bees | Wasp | Other | Butterflies | Total visits |
|---|---|---|---|---|---|---|
| 0800-1000 h | 0.083 | 10.5 | 0 | 0 | 0.166 | 10.749 |
| 1000-1200 h | 0.083 | 42.666 | 0 | 0 | 0.083 | 42.832 |
| 1200-1400 h | 0 | 24.166 | 0 | 0 | 0.166 | 24.332 |
| 1400-1600 h | 0 | 24.333 | 0 | 0 | 0.083 | 24.416 |
| 1600-1800 h | 0 | 11.333 | 0 | 0.166 | 0 | 11.499 |

Calculation of visits per flower per day by all visitors

$V$ = Sum from i=1 to n ( v x b/p ) / F

$V$ = ( 10.749 x 120/10 ) / 60) + ( 42.832 x 120/10 ) / 60 + ( 24.332 x 120/10 ) / 60 + ( 24.416 x 120/10 ) / 60 + (11.499 x 120/10 ) / 60

$V$ = 2.149 + 8.566 + 4.866 + 4.883 + 2.299

$V$ = 22.763visits per flower per day by all visitors

Calculation of visits per flower per day by a significant pollinator

Vb= Sum from i=1 to n ( v x b/p ) / F

Vb= 10.5 x 0.2 + 42.666 x 0.2 + 24.166 x 0.2 + 24.333 x 0.2 + 11.333 x 0.2
( v x b/p ) / F = (120/10) / 60) = 0.2

Vb= 2.1 + 8.533 + 4.833 + 4.867 + 2.267

Vb= 22.6 visits by bees per flower per day

## Floral traits of *Clerodendrum serratum* Spreng.

Floral traits to attract visitors and a significant pollinator were zygomorphic in floral symmetry, nectar major source in floral reward, gullet type in floral shape, anther longitudinal dehiscence, purple in floral colour and hermaphrodite with heterostylous in sexual systems.

## Phenological study

This observation was carried out from 2200h of 19th September, 2007 to 1800 h of 20th September, 2007. Phenological changes were mainly recorded by photos. A total of 15 flowers were selected to observe their floral changes. Six phase keys were included. The life span of flowers lasted for one day. Their average longevity was 8.4 hrs.

Table 3. Selected flowers with their phase keys from 2200h (19th September, 2007) to 1800h (20th September, 2007)

| Table . Sample data for diurnal flowering of the *Clerodendrum serratum* Spreng., 19 Sept. 2007. | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Time | Flower #1 | Flower #2 | Flower #3 | Flower #4 | Flower #5 | Flower #6 | Flower #7 | Flower #8 | Flower #9 | Flower #10 | Flower #11 | Flower #12 | Flower #13 | Flower #14 | Flower #15 |
| 2200 h | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A |
| | | | | | | | | | | | | | | | |
| Table . Sample data for diurnal flowering of the *Clerodendrum serratum* Spreng., 20 Sept. 2007. | | | | | | | | | | | | | | | |
| Time | Flower #1 | Flower #2 | Flower #3 | Flower #4 | Flower #5 | Flower #6 | Flower #7 | Flower #8 | Flower #9 | Flower #10 | Flower #11 | Flower #12 | Flower #13 | Flower #14 | Flower #15 |
| 0600 h | B | B | B | B | A | B | B | B | B | B | B | B | A | A | B |
| 0800 h | C | C | C | C | B | C | C | C | C | C | C | C | A | B | C |
| 1000 h | D | D | D | D | C | D | D | D | D | D | C | C | B | C | D |
| 1200 h | D | D | D | D | D | D | D | D | D | D | D | D | C | D | D |
| 1400 h | E | E | E | E | F | E | E | D | E | E | E | E | E | E | E |
| 1600 h | E | F | E | F | F | E | E | E | F | E | E | F | E | E | F |
| 1800 h | F | F | F | F | F | F | F | F | F | F | F | F | F | F | F |

Phase keys of *Clerodendrum serratum* Spreng.

| |
|---|
| A - Enclosed in bud, |
| B - Flower opens but not fully, style is straighten up, anthers dehiscent and light purple in color. |
| C - Flower opens fully, style downward over filaments a little, anthers with shedding pollens and purple in color. |
| D - Anther dark purple with a little amount of pollen, style more downward over the filaments |
| E - Petals pale color, and anthers dry and brown in color, pollens no visible |
| F - Petals no intact and fruit set |


Figure 5. Phenological phases mainly occurred in *Clerodendrum serratum* Spreng.

**Target pollen observation**

Target pollen sample was collected from observed plant species with the help of basic fuchsin gel before the collection of pollens from the significant pollinator (Figure 6a). According to Sawyer (1981), target pollen was identified and their morphological features of size, shape, aperture type and fresh pollen colour were recorded. Morphological features of target pollen were large in size (93 µm), oval flattened in shape, colporate in aperture type and white colour in fresh pollen (Figure 6 c).

A total of 10 target pollens and a total of 3 non-target pollens were collected from the whole body of significant pollinator in which 5 target pollens from thorax, 3 from head, 2 from upper abdomen, and a total of 1 non-target pollen from head and 2 from legs (Figure 6d,e,f) were presented. Most of the target pollens were carried by the upper body parts of the significant pollinator.

Table 4. The longevity of each flower and average longevity of a flower to pollinate it.

| No. of flowers | 19-9-2006 | | 20-9-2006 | | | | | | | | | Longevity of each flower (hrs) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | #2200 | #2400 | #0200 | #0400 | #0600 | #0800 | #1000 | #1200 | #1400 | #1600 | #1800 | |
| Flower#1 | | | | | | | | | | | | 10 hrs |
| Flower#2 | | | | | | | | | | | | 8hrs |
| Flower#3 | | | | | | | | | | | | 10 hrs |
| Flower#4 | | | | | | | | | | | | 8hrs |
| Flower#5 | | | | | | | | | | | | 4hrs |
| Flower#6 | | | | | | | | | | | | 10 hrs |
| Flower#7 | | | | | | | | | | | | 10 hrs |
| Flower#8 | | | | | | | | | | | | 10 hrs |
| Flower#9 | | | | | | | | | | | | 8hrs |
| Flower#10 | | | | | | | | | | | | 10 hrs |
| Flower#11 | | | | | | | | | | | | 8hrs |
| Flower#12 | | | | | | | | | | | | 8hrs |
| Flower#13 | | | | | | | | | | | | 6hrs |
| Flower#14 | | | | | | | | | | | | 8hrs |
| Flower#15 | | | | | | | | | | | | 8hrs |
| Total number of flower lifespan (hrs) | | | | | | | | | | | | **126 hrs** |
| Average longevity of a flower (hrs) to visit and pollinate for visitors | | | | | | | | | | | | **8.4 hrs** |

Longevity of Bud  Longevity of open corolla  Fruit set period

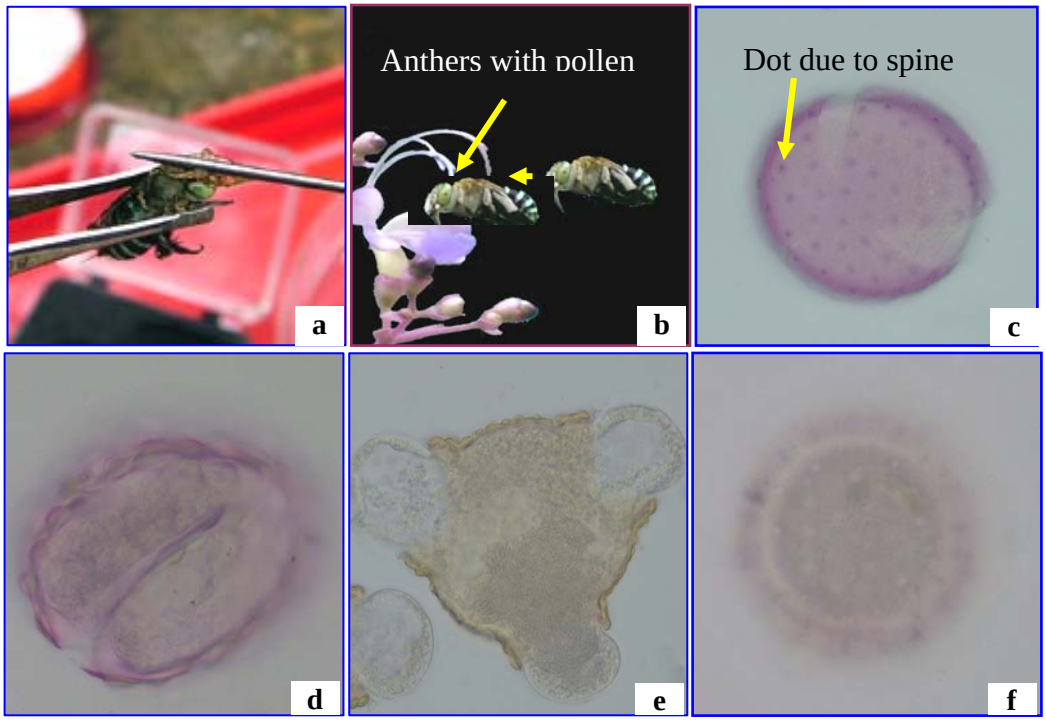


Figure 6. Pollens collection from the whole body of significant pollinator with basic fuchsin gel (a); Foraging pattern (b); Target pollen with spines (c); Non-target pollens from the significant pollinator (d, e, f).

## Discussion and Conclusion

Pollination study on *C. serratum* was a pioneer research among the pollination status of the flowering plant species in Myanmar. According to Parrish (2004), common methods of

pollination study were done for this species. Moreover, data collection of floral traits was also carried out according to Machado & Lopes (2004).

Fly, moth and butterfly were visitors and bees (*Amegilla* sp.) were significant pollinators for the plant species. A total visit of visitors per flower per day was 22.763 and a total visit of significant pollinators per flower per day was 22.6.

The floral traits of zygomorphic in floral symmetry, nectar major source in floral reward, gullet type in floral shape, anther longitudinal dehiscence, purple in floral colour and hermaphrodite with heterostylous in sexual systems were essential attractants for these visitors and significant pollinators. Zygomorphic flowers are largely known as a visual attractant for some groups of pollinators such as bee and humming-birds (Machado & Lopes, 2004). One of the floral traits of this flower was also zygomorphic, and it can therefore attract the significant pollinator (bee) with its visual attractant colour.

For plant species, a total number of floral life span of 15 flowers were 126 hours and average longevity of a single flower was 8.4 hrs. Floral reward, especially nectar was an essential reward of significant pollinators and visitors during this period. And also, this floral longevity can be destined as pollination time of a single flower for next generation of *C. serratum* Spreng. All of these facts were recorded from phenological study. Floral changes and common phase keys were also carried out and it can be seen in Figure 5.

Pollen is the major attractant for many pollinators, an important part of the diet of many flower visitors, and an essential component of sexual reproduction and gene flow (Kearns & Inouye, 1993). Therefore, morphological features of target pollen were also identified. Morphological features of the target pollen were large in size (93 µm), oval flattened in shape, colporate in aperture type and white colour in fresh pollen. Pollens from a significant pollinator were examined with target pollen by a DP-12 image microscope. Sexual organs of gullet flowers are upper side of the flower, and pollen is deposited on the back of the pollinator (Faegri & Pijl, 1971). From this observation, a total of 10 target pollens was found in the upper parts (head, thorax, upper abdomen) of the significant pollinator because the floral shape was gullet type and bees hit reproductive organs of the flower with upper parts of the body firstly when forage it (Figure 6b). Moreover, pollens can stick to the body of significant pollinators because spinous outgrowths were found in pollen surface. A total of 3 non-target pollens were not identified but mentioned it with photographs (Figure 6 d, e, f).

In conclusion, the flowers of *C. serratum* Spreng. were pollinated by *Amegilla* sp. in accordance with their floral traits, and its pollinator bee species were essential to pollinate them for next generation. On the other hand, nectar was also essential reward for *Amegilla* sp. in their life processes. Therefore, mutualisms occurring between plant *C. serratum* and its significant pollinator *Amegilla* sp. are needed to conserve for a long existence, especially for their invaluable resources in nature.

## Acknowledgements

We are very grateful to Dr. Tin Tun Myint, Acting Rector and Dr. Si Si Hla Bu, Pro-Rector, Hinthada University for their interest and encouragement in research works. Moreover, we would like to express our deepest gratitude to Dr. Khin Khin Sann, Professor and Head, Department of Botany, Hinthada University, for her advice, encouragement and permission to submit this research paper. We also like to express our gratitude to Dr. Maung Maung Gyi, Professor and Head, Department of Zoology, University of Yangon for his suggestion. Lastly we would like to convey our thanks to our teachers and colleagues, Department of Botany, Hinthada University who have given constant moral support for this research work.

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