INTERNATIONAL JOURNAL OF INNOVATIVE RESEARCH & DEVELOPMENT

ISSN: 2278 – 0211 (Online)

Pollination Biology Of Citrus Aurantiifolia (Christm.) Swingle: A Medicinally Important Fruit Plant

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Abstract:
While studying the pollination biology of Citrus aurantiifolia (Christm.) Swingle, it has been found that the plant produces 4-5 day flowers and flowering pattern is of cornucopia type. Altogether 8 species of insects belonging to the orders Hymenoptera, Diptera and Lepidoptera have been observed to visit the flower regularly. Pollen grains and nectar are the primary nutritive rewards supplied by the plants to the visitors. Here visitors play a significant role during pollination. The plant shows both autogamous and allogamous (primarily geitonogamous) type of pollination.

Key words*: Allogamous, Autogamous, Citrus aurantiifolia, Floral Biology, Insect*

1.Introduction

Citrus aurantiifolia (Christm.) Swingle (known as 'Patilebu' in Bengali, 'Kaghzinimbu' in Hindi, 'Nimbuk' in Sanskrite, 'Nimma' in Tamil and 'Limbe' in Kanner vernaculars), a small, densely and irregularly branched, evergreen large shrub sometimes small tree belongs to the family Rutaceae. Flowers small, white or creamish-white and are borne in axillary cymes. Numerous oil glands (monoterpenes with limonene) are present in the leaves, sepals and petals giving the plant a pleasant smell. The plant has originated in south-east Asia and spread during middle ages and later to become establish in almost all the countries. The plants grow and produce fruits under rather varied climatic condition, ranging in latitude from over 40° N (Japan) to almost 40° S (Newzeland); from equatorial, low-humid climates through warm-subtropical and even cooler maritime climates (Spiegel-Roy and Goldschmidt, 1996). In India, the plant grows widely in hotter parts found up to an altitude of 1000m above sea level. The fruit has a sour sharp taste; appetizer, antioxidant, stomachic, anthelmintic, cures abdominal complaints; removes loss of appetite, constipation, fatigue; good in biliousness, abdominal pain and foul breath; relieves vomiting; good for the eyes (Kritikar and Basu, 1933; Colker and Kalman 1999; Roger and Short, 2000; Preuss et al., 2002; Boshtam et al., 2011).
Despite, the immense medicinal properties of Citrus aurantiifolia no attention has been given on its pollination biology. The plant reproduced sexually through seeds. In order to formulate the strategy for conservation and successful propagation of this valuable medicinal plant, studies in its floral biology have been under taken.

2.Materials And Methods

The study conducted in the flowering seasons for two consecutive years starting from 2011 on the cultivated variety of C. aurantiifolia in an around Midnapore town and Vidyasagar University campus (2 km from Midnapore town) of Paschim Medinipur District, West Bengal, India. Floral organization and construction recorded regularly throughout the flowering season. Observation based on at least 05 plants. Floral organization was worked out in detail, under stereo binocular microscope taking 10 flowers from 5 different plants.
Flower longevity (from flower opening to complete senescence) has observed in natural habitat. Here observation was made in two different sets; 1. Open pollinated flowers (control) and 2. Bagged flower before opening without manipulation. To study flowering phenology, flower parts that would open the next day tagged and kept under observation (from the1st day to the day of senescence) to record time of anthesis, anther dehiscence and structural changes associated with aging of flowers.
Pollen morphological studies done under bright field microscope after palynological preparation of the slide following Erdtman's acetolysis technique (1960).
Stigma receptivity was determined based on pollen germination following manual pollination. Simultaneously stigma receptivity also verified through H_2O_2 test.
The frequency of visitors and foraging time were continuously recorded from 6 to 18 hours for three days. Their diversity, abundance, pattern of visitation and behaviour were also studied throughout the flowering season. Insects those are visiting the flowers collected by using an insect net. Collected insects were stored in borosil glass vials with 70% alcohol and identified by the scientists, Zoological Survey of India, Govt. of India, Kolkata, India.
To assess the pollination success, observation regarding pollen deposition over stigma (stigmatic load) during anthesis was done by collecting stigmas at regular intervals for two days. The numbers of pollen grains over stigma was counted and simultaneously

germinated pollen grains over stigma were recorded under bright field microscope. Pollen ovule ratio, flower fruit ratio and fruit seed ratio were also determined.
Leica DML1000 (Germany) trinocular optical microscope with 10x, 40x, 100x (oil) apochromatic objectives were used for bright field microscopic study. Photomicrographs were taken with a Leica, Digital photo micrographic attachment. For morphological studies, Leica Stereo-binocular microscope with photo micrographic attachment has been used.

3.Observation
The shape of the flower (Kugler 1970; Faegri and Van der Pijl 1979) is open disk type (Plate-1a). The flowers have a mass of stamens (22-25) in two whorls positioned centrally (encircling the gynoecium) within the disk and have shallow exposed nectaries, so that both pollen and nectar are easily available to the visitors.

3.1.Flowering Phenology
Phenology is the study of the periodicity or timing of recurring biological events in relation to short-term climatic change (Sakai et al. 2005). In the case of plants, phenological events involve flowering, fruiting, leaf flushing and seed germination (Leith, 1974). It has been demonstrated that particular angiosperm families flower at distinct times of the year (Kochmer and Handel, 1986). C. aurantiifolia flowers every year during first week of January to the 2nd week of March. The number of flowers gradually increases with the number of days and reaches its peak during early February (Diagram 1). Therefore, the pattern of flowering (Gentry 1974; Opler et al. 1980; Bawa 1983) is of cornucopia type. Before flowering, flower buds appear and it takes 4-5 days from initiation to complete maturation of the bud. Flower opening is gradual. The flowers start opening between 8.00 am to 14.00 pm of the 1st day and completed during 7.00 am to 10.00 am on the second day. During the process, at first a slit is formed by loosening the limbs. The slit gradually widens leading to its complete opening and the time required 20 – 23 hrs.
C. aurantiifolia produces 4-5 day flowers. In open pollinated condition, after opening the flower lasts up to 5th day afternoon. However, the reduced lustrous look of the flower together with browning of stigma and decreased pollinator activity were noticed from 3rd day afternoon. Abscission of petals and stamens starts from 4th day of flower opening and completed during 5th day.

3.2.Anther Dehiscence And Pollen Presentation
In C. aurantiifolia, anther dehiscence starts by 8.00 am – 9.00 am on the second day of flower opening. Initiation of anther dehiscence takes place by the appearance of thecal slit. At first, small number of pollen grains was oozing out from the thecal slit and gradually all the pollen mass from each anther locule comes out within 4-5 hrs. Simultaneously insect visitation starts. In natural condition, the pollen foragers have consumed almost all the pollen grains on the 2nd day and if present, that too a little amount left within the anthers for third day. In some flowers, anther dehiscence starts when the corolla yet to open and all the pollen comes out within the closed corolla. The pollen grains were bright yellow in colour. Pollen grains are radially symmetrical, oblate-spheroidal, amb circular, tetra to pentazonocolporate, colpi narrowly elliptic, ends acute, endoaperture lalongately oval, exine 2.5 µm thick, sexine tegillate, as thick as nexine, surface reticulate (Plate-1k).

3.3.Floral Visitors
Altogether 8 species of insects have been observed to visit the flower regularly (Table – 1). The visitors are belonging to the orders Hymenoptera, Diptera and Lepidoptera. Among the Hymenopteran members, Apis dorsata, A. cerana, A. florea and Trigona sp. visit the flower for both pollen grains and nectar while Halictus sp. visits for pollen only. Among the Lepidopteran members, butterflies (Papilio dravidarum and Parantica sp.) visit the flower for nectar. The Dipteran visitors of C. aurantiifolia are Helophilus sp. and hover fly (Episyrphus sp.). Helophilus acts on nectar disc for the collection of nectar whereas Episyrphus gathers only pollen grains.
Among all the visitors Apis dorsata come first. It visits the plant in two phases. In the 1st phase workers of A. dorsata start their foraging (Plate-1. c, h) over the flowers for both pollen grains and nectar at 8.00 am. and the visitation continues for 4 to 5 hrs (12.00 - 13.00 pm). After a gap of 3 hrs, again the visitation of A. dorsata starts. This time the bees are very less in number compared to that of previous one and their sporadic visitation restricted only to nectar. Apis cerana (Plate-1. i, j) and A. florea visit next sometimes during 8.30 am and continued their visitation up to 13.00 pm. The visitors together forage over the plant for both pollen grains and nectar. Trigona and Halictus started their visitation during 9.00 am. Though Halictus is strictly a pollen forager, Trigona visits the plant for both. Halictus (Plate-1g) continued its visitation up to 11.30 am while Trigona maintained its foraging activity up to 13.00 pm. The Dipteran member Helophilus (Plate-1. d, e) visit the flower at 9.15 am and acts upon the nectar disc situated at the base of the ovary to collect it. Episyrphus sp. (Plate-1a)) visits the flower during 9.15 am acts as pollen feeder. The Lepidopteran members, butterflies (Papilio dravidarum and Parantica sp.) collect nectar by their sporadic visit after complete opening of the flower. The entire visitor mentioned above forage the plant for the 2nd day flowers. The visitation reaches its zenith during 10.00 am -11.30 am on the 2nd day of flower opening when the rewards (nectar and pollen) were maximum. Most of the nectar visitors visit the flowers on 2nd day though a very lesser number of visitors (A. dorsata and members of Lepidoptera) visit the plant on the 3rd day for the leftover part of nectar. Visitors for pollen are totally absent on the 3rd day. After considering the arrangement of anthers and stigma within the flower, time of anthesis, visitors' frequency, their activity over the flower and the amount of adhered pollen grains of their body parts together with the possibility of such pollens deposited over the stigma (stigmatic load), it was found that Apis dorsata is the principal pollinator of the plant. The other important pollinators are A. cerana, A. florea, Trigona, Halictus, Helophilus and Episyrphus. All the pollinators pollinate the flower sternotribically (pollen grains adhered to the ventral side of the visitors). Members of Lepidoptera played very insignificant role in pollination of the plant, rather they performed as nectar robber.

3.4.Floral Rewards

Floral rewards cater to the essential need of the pollinator to ensure repeated visitation. In C. aurantiifolia pollen grains and nectar are the primary nutritive rewards. Active floral nectaries can be located by staining inflorescences with neutral red (Meeuse 1978). Here, the nectaries (Plate-1m) are present on nectar disc situated at the base of the ovary. Secretion starts early morning on the 2nd day of flower opening and continues up to 3rd day. Halictus sp., Apis dorsata, A. cerana, A. florea and Trigona sp. visits the flower for pollen grains while Apis dorsata, A. cerana, A. florae, Trigona sp., butterflies (Papilio dravidarum and Parantica sp.) and the Lepidopteran members (Helophilus sp. and Episyrphus sp.) visit the flowers for nectar. All these insects play some roles in the pollination of the flower. Besides, there is also sugar secreting very small glandular hairs present all over the surface of the stigmatic head.

3.5.Stigma Receptivity

Mature stigmas can be categorized as either wet or dry (Heslop-Harrison and Shivanna 1977). In C. aurantiifolia, the stigma is of wet type (Plate-1b). After performing bagging experiment it was found that, the receptivity of the stigma starts 3.00 am in the morning on the 2nd day prior to anther dehiscence and maintains its receptivity till the 4th day afternoon (±58hrs). However, in open pollinated condition the stigma lose its receptivity in the 3rd day afternoon. During this phase the stigmatic load exceeds the threshold value ensuring fertilization. During the receptive phase, stigma appears to be shiny, bright yellowish and juicy. The lustrous, juicy look continues till the end of the 2nd day and in the 3rd day afternoon stigma starts browning, dull looking leading to lose its receptivity. Receptivity of stigma is supported by H_2O_2 test (Zeisler1938; Galen & Plowright, 1987) and in-vivo pollen germination test over stigma (Plate-1l).

3.6.Pollen-Ovule Ratio

The average number of pollen grains produced per flower 7500 ± 500. Mean number of ovules per flower are 9. So; the pollen-ovule ratio is 833 : 1 in *C. aurantiifolia*.

3.7.Seed-Ovule Ratio

The seed-ovule ratio is 1: 1.8.

4.Discussion

C. aurantiifolia flowers every year during first week of January to the second week of March and the flowering (cornucopia type) reaches its zenith during early February. Flower opening is gradual. The flowers start opening (through formation of a slit) in between 8.00 am to 14.00 pm of the 1st day and completed during 7.00 am to 10.00 am on the 2nd day. Though the plant produces 4-5 day flowers, however, the reduced lustrous look of the flower together with browning of stigma and decreased pollinator activity were noticed from 3rd day afternoon. Abscission of petals and stamens starts from 4th day of flower opening and completed during 5th day.

In C. aurantiifolia, pollen grains and nectar are the primary nutritive rewards. Here, the nectaries are present on nectar disc situated at the base of the ovary and the secretion starts early morning on the 2nd day of flower opening and continues up to 3rd day. Anther dehiscence together with oozing out of pollen grains starts by 8.00 am – 9.00 am on the second day of flower opening and gradually all the pollen mass from each anther locule comes out within 4-5 hrs. Concomitantly insect visitation starts. Altogether 8 species of insects have been observed to visit the flower regularly. The visitors are belonging to the orders Hymenoptera, Diptera and Lepidoptera. Among the Hymenopteran members, Apis dorsata, A. cerana, A. florea and Trigona sp. visit the flower for both pollen grains and nectar while Halictus sp. visits for pollen only. Among the Lepidopteran members, butterflies (Papilio dravidarum and Parantica sp.) visit the flower for nectar. The Dipteran visitors of the plant are Helophilus sp. and hover fly (Episyrphus sp.). Helophilus visits the flower for the collection of nectar whereas Episyrphus gathers only pollen grains.

The visitors forage the plant mainly on the 2nd day flowers during 10.00 am - 11.30 am when the rewards (nectar and pollen) were maximum. Most of the nectar visitors visit the flowers on 2nd day though a very lesser number of visitors (A. dorsata and members of Lepidoptera) visit the plant on the 3rd day for the leftover part of nectar. Visitors for pollen are totally absent on the 3rd day. After considering the arrangement of anthers and stigma within the flower, time of anthesis, visitors' frequency, their activity over the flower and the amount of adhered pollen grains of their body parts together with the possibility of such pollens deposited over the stigma (stigmatic load), it was found that A. dorsata is the principal pollinator of the plant. The other important pollinators are A. cerana, A. florea, Trigona, Halictus, Helophilus and Episyrphus. All the pollinators pollinate the flower sternotribically. Members of Lepidoptera is less significant regarding pollination of the plant, rather they performed as nectar robber. In C. aurantiifolia, the stigma is of wet type. After performing bagging experiment it was found that, the receptivity of the stigma starts 3.00 am in the morning on the 2nd day prior to anther dehiscence and maintains its receptivity till the 4th day afternoon (±58hrs). However, in open pollinated condition the stigma lose its receptivity in the 3rd day afternoon as the stigma saturated with the pollen loads ensuring fertilization. In C. aurantiifolia the pollen-ovule ratio and seed-ovule ratio is 833 : 1 and 1: 1.8 respectively.

5.Conclusion

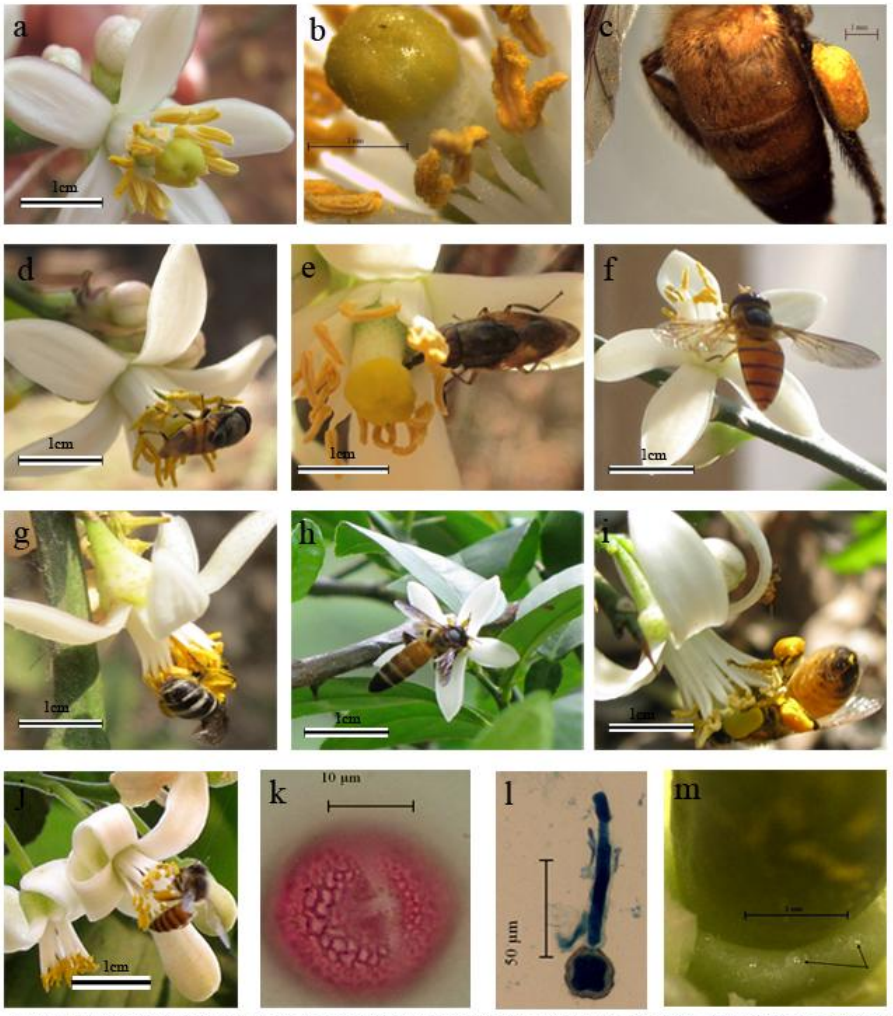
Controlled experiments showed that C. aurantiifolia is an allogamous species, mainly geitonogamous though the plant is self-compatible. Therefore, the plant shows both autogamous and allogamous type of pollination. However, bagging experiments showed that in absence of pollinators' flower-fruit ratio and fruit-seed ratio declines than open pollinated condition.

6.Acknowledgements
The author is very much thankful to Ms. Krishna Sikdar and Ms. Panirpiya Hansda for their active involvement during collection of field data. The author is highly grateful to Dr. P. K. Pal, Professor, Dept. of Botany, The University of Burdwan for helping in the preparation of the Manuscript. The author is also indebted to Mr. Bulganin Mitra, Scientist, ZSI for helping in identifying the insects. The present work is partly supported by UGC as SAP, Department of Botany and Forestry, VU.

Species	Family	Order	Duration of visit	Highest incidence	Forage material
Apis cerana	Apidae	Hymenoptera	8.30 am – 13.00 pm	9.30 am – 10.00 am	Nectar and pollen
Apis dorsata	Apidae	Hymenoptera	8.00 am – 16.00 pm	9.15 am – 10.15 am	Nectar and pollen
Apis florea	Apidae	Hymenoptera	8.30 am – 13.00 pm	9.15 am – 09.40 am	Nectar and pollen
Halictus	Halictidae	Hymenoptera	9.00 am – 11.30 am	9.35 am – 10.15 am	Pollen
Trigona	Apidae	Hymenoptera	9.00 am – 13.00 pm	10.45 am – 11.45 am	Nectar and pollen
Episyrphus	Syrphidae	Diptera	9.15 am – 14.45 pm	Stray individuals	Pollen
Helophilus	Syrphidae	Diptera	9.15 am – 13.30 pm	9.30 am – 10.30 pm	Nectar
Papilio dravidarum	Papilionidae	Lepidoptera	9.30 am – 15.25 pm	Stray individuals	Nectar
Parantica sp.	Nymphalidae	Lepidoptera	9.15 am – 15.00 pm	Stray individuals	Nectar

Table 1: Floral Visitors Of C. Aurantiifolia In Midnapore

PLATE - 1



a. Opened disc flower; b. Stigmatic head and dehisced anthers; c. Pollen loads of *A. dorsata*; d & e. *Helophilus* collecting pollen and nectar respectively; f. hoverfly visiting the flower; g. *Halictus* collecting pollen grains; h. *A. dorsata* collecting nectar; i & j. *A. cerana* visiting for pollen grains and nectar; k. Pollen grain of Citrus showing reticulate surface and colporate aperture; l. pollen with pollen tube; m. nectar droplets at ovarian base

Figure 1

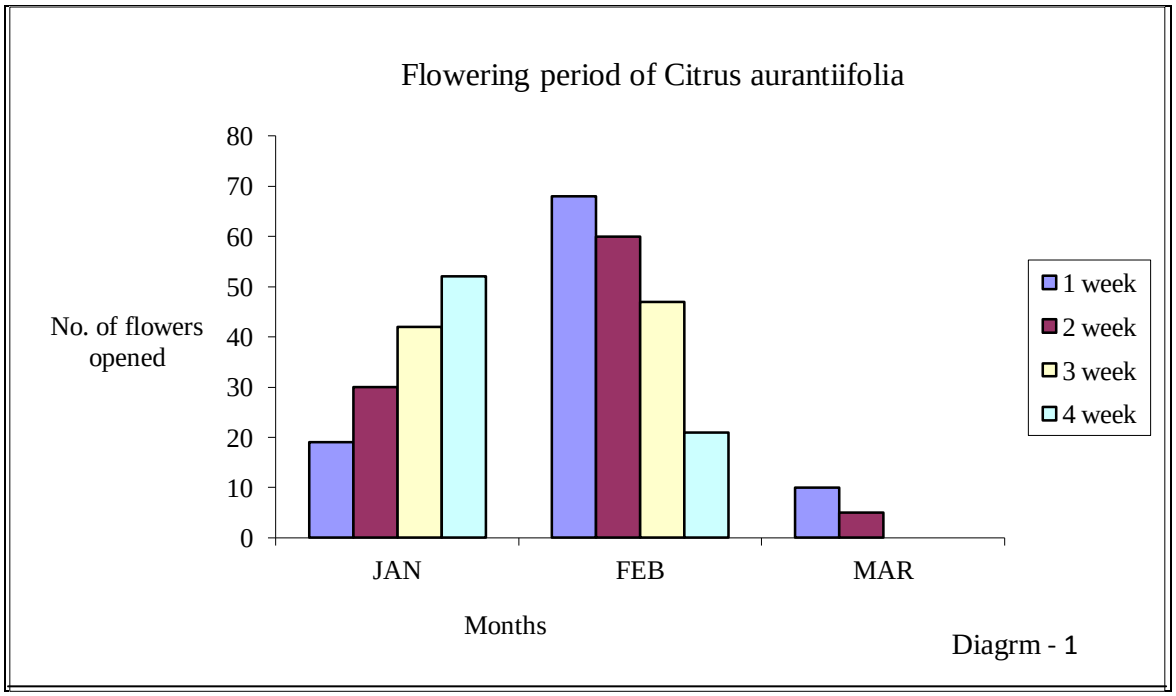


Figure 2

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