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Pollination Biology of medicinally important plant *Leucas aspera*

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Abstract
Leucas aspera Willd. (Lamiaceae) is an important and widely used medicinal plant. *L. aspera* started flowering from Middle of June ending in February. Detailed studies were carried out on the Phenology, floral biology, pollination and breeding system of *L. aspera* during 2011-2013 at Vythiri, Wayanad. Inflorescence initials are generally activated in the leaf axils. Flower opening in *L. aspera* began around 2.00 am and was completed around 3.00 am. Anther dehiscence began with a longitudinal slit appearing on the anther lobes at 3.00 am after opening the flower and the process was completed between 3.30-4.00 am. The main floral visitors were observed as Hymenopterans. The potential pollinators were recorded and calculated the % of visits for 2012 and 2013 respectively, *A. dorsata* (21.64 & 19.87), *A. cerana* (14.43 & 12.32), *A. florea* (13.4 & 11.3), *Ceratina* sp (18.6 & 22.6) and *Ameigilla* sp (16.5 & 16.5). The breeding experiment shows that manual cross pollinations using pollen from flowers of different plant resulted in 65% fruit set. In the open pollination in natural conditions resulted in 56.67% fruit set. Apomixis, flower bud bagged by removing stamens and stigma, resulted no fruit set. Our study concluded that the *L. aspera* was a cross pollinated species but absence of vector self-pollination were also found. The study clearly shown that cross-pollinated species has been shifting in to self- pollination.

Keywords: *Leucas aspera*, Pollination.

1. Introduction
Leucas aspera has been better-known it's for medicative price. The plant has been the main ingredient for several ancient medicines and domestically called thumba. It has been used for wide applications, treatment of cold, headache and redness [3]. The leaves of *L. aspera* are applied on bites of serpents, poisonous insects and scorpion sting and also used as insecticide and mosquito repellent in rural area [8]. An overview on pollination biology of Lamiaceae was published by Huck [2]. The intricate methods by which cross pollination is accomplished in the Lamiaceae reflect a long history of co-evolution between plants and pollinators. In spectrum of pollination systems includes entomophily and ornithophily but no records are available regarding bat pollination or wind pollination in Lamiaceae [2]. Lamiaceae is well known for its floral specialties and pollination mechanisms. Among the adaptations liver mechanism (Salvia), weight –introduced trigger mechanism (Trichostema L., R.Br), and explosive pollen release (Hyptis) are already reported [2]. Recently Potgieter *et al* [5] studied the adaptation of long corolla tubed Plectranthus spp of South Africa. In their studies the long corolla tubed Plectranthus species were pollinated by long proboscid flies. Placement of stamens with in corolla is associated with two types of pollinating syndromes: flag flowers with sternotriby and gullet flowers with nototriby [11]. Bees, by far, are the most commonly observed pollinators of this family. The family is known for volatile oil glands that may stimulate the responses from pollinators [1]. Recently the nectar dynamics and floral visitors of *L. aspera* studied by Srishali K. Kullolil [10]. The pollination studies of *L. chinensis* were reported by Prasad. E.R and Sunojkumar [6]. Still Leucas is a big genus under Lamiaceae and many species have been reported as endemic. So the role of pollination studies holds importance in understanding the species distribution and endemism.

2. Materials and methods
2.1 Study area
The present investigation was carried out in Wayanad, Vythiri period of 2011-2013. Vythiri is

located in the Wayanad District of Kerala. It is situated in the sylvan Northern High Ranges of Kerala. Its geographical coordinates are N 11° 33' 0" and E 76° 2' 59" and it is located at an elevation of 700 meters above sea level.

2.2 Methodology

To study flowering phenology special attention was given to identify the flower initiation, development, anthesis, anther dehiscence etc. The floral parts were studied by using hand lens and stereomicroscope (Leica CM, 1100). The measurements of the floral parts were taken with the help of a plastic scale. Floral visitors and foraging mode of each insect were studied. The floral visitors were identified by the help of entomologists (ZSI, Calicut).

The number of pollen grains per flower was calculated as suggested by Shivanna & Rangaswami [9]. Pollen viability was estimated by tetrazolium test. The tetrazolium test is based on the reduction of a colourless soluble tetrazolium salt to a reddish insoluble substance called formazan, in the presence of dehydrogenases. Nitroblue tetrazolium and 2, 3, 5-triphenyl tetrazolium chloride are the most commonly used tetrazolium salts. Take a drop of TTC solution on a slide. Suspend a small amount of pollen in the TTC drop and distribute it uniformly in the drop and apply a cover glass. Transfer the preparation to a humidity chamber. Incubate the preparation in dark under laboratory temperature or at 30 ± 2 ^{0}C for 30-60 min. Observe under microscope.

Stigma receptivity was analyzed by α- naphthyl acetate. The excised stigma were dipped in to 2 drops of α- naphthyl solution. The preparations were incubated in a humid chamber for 10-20 min and observed under the microscope.

Continuous observations were made on the behavior of different floral visitors. The number of floral visitors, percentage of floral visit and stigma touch by insects were noted. Foraging period and foraging nature were observed. Frequency of visits was calculated. After each visit stigma were observed by hand lens and confirmed for the transfer of pollens by visitors. The visitors were captured using hand net, killed using ethyl acetate or ethanol and observed under stereo microscope for pollen load on the body parts. Pollination systems such as apomixis, autogamy, geitonogamy and xenogamy and open pollination were tested.

3. Results

The flowering in Leucas aspera began in middle of June and ending in February. Inflorescence initials were generally activated in the leaf axils. Flower opening in *L. aspera* began around 2.00 am and completed by 3.00 am. Anther dehiscence began with the appearance of a longitudinal slit on the anther lobes at 3.00 am after opening the flower and the process was completed between 3.30-4.00 am. The average duration from inflorescence initiation to opening of the first flower was 10 days. The average duration from the opening of the first flower to the opening of the last flower was 3-4 weeks.

3.1 Pollen morphology

Pollen grains were spherical, tricolpate, the equatorial outline were usually rounded triangular or circular. The average diameter of pollen grain was 24.90 ± 3.0 µm.

3.2 Pollen –Ovule Ratio

Floral analysis *L. aspera* indicated that, the flower had four didynamous stamens. An anther contains 284 pollen grains. The ovaries were basically dimerous, but as each carpel was divided by a false wall, four chambers were formed, all containing one ovule which form 4 nutlets fertilization. Hence the pollen ovule ratio was 1136:4 (Table 1).

Table 1: Floral phenology of *Leucas aspera* during the study period

Floral characters	Observation
Flowering period	June - February
Inflorescence type	c
Flower type	Regular, Bisexual
Flower colour	White
Odour	Absent
Nectar	Present
Anthesis time	2-3am
Anther dehiscence time	3-4am
Anther dehiscence mode	Longitudinal
Number of anthers per flower	4
Mean number of ovules per flower	4
Mean number of pollen per anther	1136
Pollen ovule ratio	1136:4
Pollen shape	Spherical
Pollen type	Tricolpate
Pollen size	24.90 ± 3.0
Sigma type	11am-12pm
Stigma receptivity	Dry
Fruit type	Nutlet
Ovule seed ratio	4:4

3.3 Pollen viability

Pollen viability was tested by using tetrazolium solution. The test revealed that, only 10- 20% of pollen grains were viable soon after anthesis. After that the viability rate increased, with the maximum viability recorded at 12.00 pm (84%). Soon after, the rate of viability was found to decrease.

3.4 Stigma receptivity

Receptivity of stigma was analysed by α-Naphthyl acetate test. The indication of receptivity was the pink blue colour of stigmatic surface. If the stigma was more receptive, the stigma was stained in deep blue colour. In *L. aspera* the stigma was more receptive at 11 am-12 pm.

Open pollination in natural conditions resulted in 56.67% fruit set. Apomixis, Flower bud bagged by removing stamens and stigma, resulted in no fruit set. Autogamy took place in around 60 flowers and self-pollination resulted in 40% fruit set. Manual pollinations using pollen from other flowers of the same plant resulted in55 % fruit set. Manual cross pollinations using pollen from flowers of different plant resulted in 65% fruit set. (Table 2).

Table 2: Breeding experiments

Sl. No.	Breeding system analysis	No. of flowers pollinated	No. of flowers fruit set	Percentage of fruit set
1	Open pollination	120	68	56.67
2	Apomixis	20	0	0
3	Autogamy	60	24	40
4	Geitonogamy	60	33	55
5	Xenogamy	60	39	65

3.5 Floral visitors

Many floral visitors were observed during flowering seasons.

Majority of the visitors belonged to the order Hymenoptera. Pollen attachments were found from flower visiting insects. But all flower visitors were not considered as pollinators. Many of the butterfly visitors were nectar robbers. While foraging insects landed on the lower lip of corolla to collect nectar. During that foraging strategy pollen got dusted as nototribic on insects back. (Plate. 1).

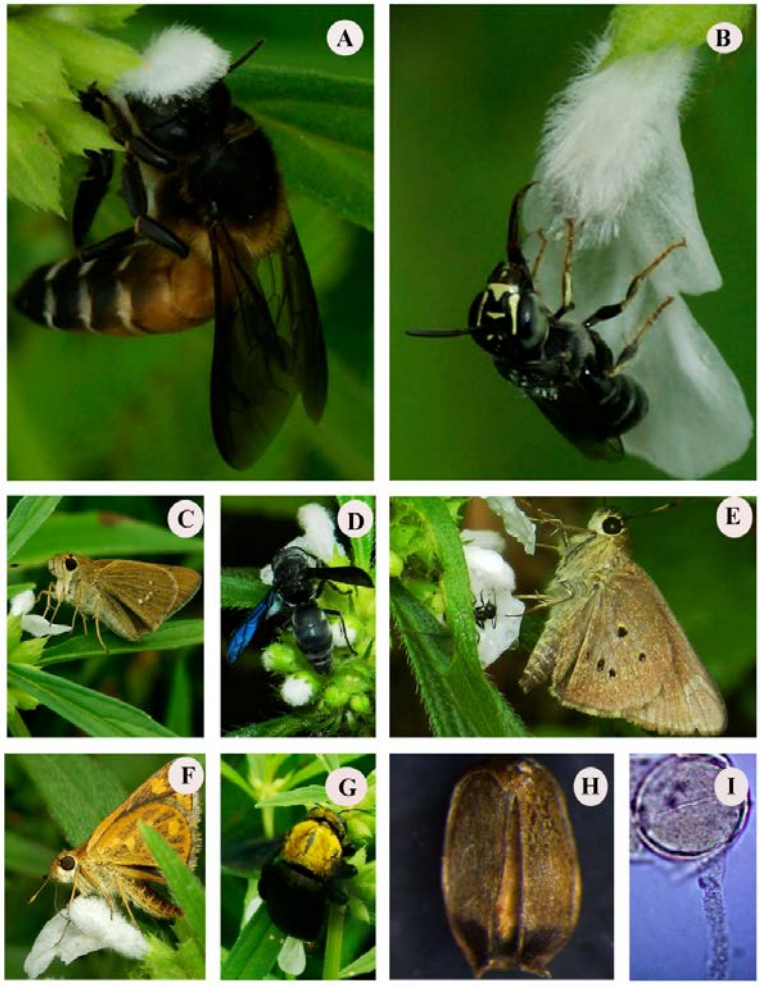

Plate 1. A, *A. dorsata* foraging on *L.aspera.* B,foraging by *Ceratina* on *L. aspera.* C,Nectar robbing by butterfly *Borbo cinnara.* D,Wasp. E,unidentified butterfly.
F, Necatar feeding by *Ampittia discorides.* G, *Xylocopa pubscence.* H, The seed of *L. aspera.* I, *In-vitro*pollen germination, pollen tube growth observed under microscope .

Table 3: The number and percent of dominant floral visitors of *L. aspera* during 2012 and 2013.

Year	Insect visitor	No. of visits	%	Year	Insect visitor	No. of visits	%
2012	*Apis dorsata*	63	21.64	2013	*Apis dorsata*	58	19.87
2012	*Apis cerana*	42	14.43	2013	*Apis cerana*	36	12.32
2012	*Apis florea*	39	13.4	2013	*Apis florea*	33	11.30
2012	*Amegilla* sp	48	16.5	2013	*Amegilla* sp	45	16.5
2012	*Ceratina* sp	54	18.6	2013	*Ceratina* sp	66	22.6
2012	*Wasp*	18	6.19	2013	*Wasp*	21	7.2
2012	*Xylocopa pubescens*	15	5.15	2013	*Xylocopa pubescence*	18	6.17
2012	*Xylocopa latipes*	12	4.12	2013	*Xylocopa latipes*	15	5.14
	Total visits	291		2013	Total visits	292	

4. Discussion

The *L. aspera* small whitish flower was opens at early morning (2 am-3 am). The average duration taken from inflorescence initiation to opening of the first flower was 10 days. The anther dehiscence was recorded between 3.30-4 am. Pollen productivity of plants were depends upon anther length, pollen grains and mode of anther dehiscence [4, 7]. The pollen viability and stigma receptivity both were maximum at 11-12 am.

The medicinal herb *L. aspera* cross was a pollinated species. The vectors were playing crucial role for pick up and delivering of pollen. The bilabiate corolla favours mainly for

Hymenopteran visitors. The essential reproductive structures of the flower were arranged in the upper lip and the lower lip acting as a landing place for insects. For accessing nectar the Hymenopteran visitors were sitting on the lower lip and inserted proboscis deeply in to corolla tube. While probing nectar from flower pollen grains were got stuck in the insects back. The breeding analysis was shown that the plant favours for cross pollination (65%) but autogamy (40%) was also found. The study concluded that even though the plant was cross-pollinated species but still self –pollination was occurred the absence of vector.

The results for insect visitation were shown in table 3. The most common visitors were recorded as Hymenopterans'. The dominated species were *A. dorsata*, 21.64 (2012) and *Ceratina* sp 22.6 (2013). The Lepidopteran (plate. 1) members were also observed as visitors but not included in the table. The butterflies were observed as nectar robbers they never landing on the flower and did not touch stigma or pollen. Here we found that the small herb *L. aspera* partially or fully benefited with many insect species. The study indicated that the Hymenopterans were playing crucial role as a pollen transfers. The insects and plant species were mutually interacted. The insects were getting floral rewards as a pollen and nectar. Our study concluded that the *L. aspera* was a cross pollinated species but absence of vector self-pollination were also found. The study clearly indicated that cross-pollinated species has been shifting in to self-pollination. It could be due to the evolutionary aspects (reduction of insect vectors).

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