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Pollination Biology of critically endangered *Leucas sivadasaniana*

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Abstract
Pollination studies on critically endaged *Leucas sivadasaniana.* The *L. sivadasaniana* is endemic to southern Western Ghat, from a population of less than 100 plants known from a single locality in the Kudachadri Hills of Udupi District in Karnataka. Detailed studies were carried out on the Phenology, floral biology, pollination and breeding system of *L. sivadasaniana.* The flowering starts in January and ends in March. The pollen grains were. The size of the pollen (spherical and tricolpate) is 31.80±0 and 85% of pollen viable at 12 am. The stigma is dry type and receptive between 9-10.00hrs pm. In the open pollination in natural conditions resulted in 15% fruit set and self-pollination resulted 0% fruit set But manual pollinations using pollen from other flowers of the same plant resulted in 30% fruit set and manual cross pollinations using pollen from flowers of different plant resulted in 65% fruit set. The percentage of fruit set was lower in natural conditions and higher in manual pollination. The higher altitude; fragmented population also limited distribution of species in the wild. Flowers were visited by several insects and butterflies. The potential pollinators were identified as *Macroglossum lepidum, Apis cerana* and *Apis* sp.

Keywords: *Leucas sivadasaniana*, Pollination Biology.

1. Introduction
Worldwide, the genus *Leucas* R. Brown (Lamiaceae) comprises about 100 species and is found in the tropical regions of Africa, Arabia, and Asia[6]. In India, there are about 41 species—mostly distributed in the south—of which 23 are endemic. Two additional endemic species from this area were described recently by Sunojkumar and Mathew [11] and Sunojkumar [10].
Lamiaceae is well known for its floral specialties and pollination mechanisms. Pollination in family Lamiaceae has been well studied by many biologists. A summary on pollination biology of Lamiaceae was published by Huck [1]. In his observation he described about general out-crossing patterns of the family Lamiaceae. Recently the nectar dynamics and floral visitors of *L. aspera* studied by Srishali K. Kullolil [8] and insect diversity and pollination biology of *L. chinensis* documented by Prasad.ER & Sunojkumar[3]. The previous studies of *Leucas* mentioned that majority of insects' guests belong to the order Hymenopterans. They were the potential pollinators of *Leucas* species [3, 8]. The pollination studies of *Leonotis* sp previously done by Solomon Raju [9]. He also documented the pollination biology of *Hyptis saveolens* [4] and pollination studies of *Anisomeles* indica [5].

1.1 *Leucas sivadasaniana*
IUCN Red List category. Its occurrence in the Kudachadri Hills, inside the Mookambika Wildlife Sanctuary guarantees the preservation of this species in the near future. Based on IUCN Red List criteria [2].
Leucas sivadasaniana Sunojkumar [12], sp. nov. TYPE: India. Karnataka: Udupi Dist., Kudachadri Hills, 1200–1400 m, 12 Feb. 2003, P. Sunojkumar CU88126 (holotype, CALI; isotypes, K, MH, MO).

2. Materials and Methods
2.1 Study area
Field work was conducted during 2011-2014 in Kudajadri hills. Kudajadri (3°51′39″N 74°52′29″E) is a mountain peak with dense forests (elevation - 1,343 meters above

sea level) in the Western Ghahts in South India (Karnataka State). It has been declared as natural heritage site by Government of Karnataka.

2.2 Methodology

To study flowering phenology and 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. Identified 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 [7]. Pollen viability was estimated by tetrazolium test. Stigma receptivity analyzed by α-naphthayl acetate. In this test excised stigma dipped in to 2 drops of α- naphthyl solution. Incubate the preparations in a humid chamber for 10-20 min. Observe the preparations under the microscope.

2.3 Pollination biology

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 and Discussion

The flowering of *L. sivadasaniana* was started in January and reduced in March. The flowers of *L. sivadasaniana* are typical Lamiacean bilabiate. The flower opening was observed at early morning 5.30-6.30 hrs. After that anther dehiscence was occurred through longitudinal slit.

3.1 Pollen morphology

Pollen grains were spherical, tricolpate, the equatorial outline was rounded triangular or circular. The average diameter of pollen grain was recorded as 31.80±0 µm.

3.2 Pollen –Ovule Ratio

Floral analysis of *L. sivadasaniana* indicated that, the flower has didynamous stamen and were 4 in number. An anther contains 592 pollen grains. The ovaries were basically dimerous, but as each carpel is divided by a false wall, four rooms are formed, all containing one ovule which form 4 nutlets often fertilization. Hence the pollen ovule ratio was 2368:4 (Table .1).

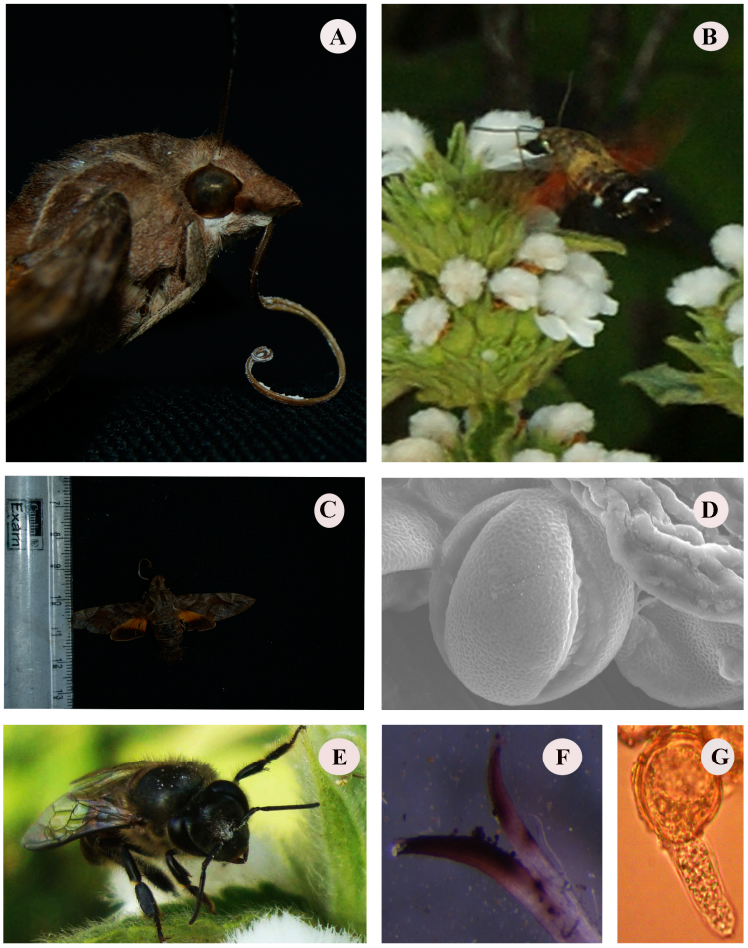

Plate 1: A, Pollen attachments on proboscis of *Macroglossum lepidum* B, foraging by *M. lepidum* on *Leucas sivadasaniana. C, M. lepidum* showing with measuring scale. E, pollen grains of *L. sivadasaniana* (SEM view) adhering on to the stigmatic surface. E, *Apis cerana* showing pollen attachments on head and proboscis. F, pollen germination on stigmatic surface. G, pollen tube growth after treatment of sucrose solution.

Table 1: Floral Morphology

Floral charaters	Observation
Flowering period	January-March
Inflorescence type	Verticillaster
Flower type	Regular, Bisexual
Flower colour	White
Odour	Absent
Nectar	Present
Anthesis time	5.30-6.30
Anther dehiscence time	6.00-6.30
Anther dehiscence mode	Longitudinal
Number of anthers per flower	4
Mean number of ovules per flower	4
Mean number of pollen per anther	592
Pollen ovule ratio	2368:4
Pollen shape	Spherical
Pollen type	Tricolpate
Pollen size	31.80±0
Sigma type	Dry
Stigma receptivity	9-10.00hrs
Fruit type	Nutlet
Ovule seed ratio	4:4

3.3 Pollen viability

Pollen viability was tested by using tetrazolium solution. They test revealed that, only 10-20% of pollen grains were viable soon after anthesis 84% pollen grains were viable at 12 pm and after that the rate of viability was gradually decreases. (Table 2).

Table 2: Pollen Viability

Sl. No	Time	*Leucas sivadasaniana*
1	6.00am	15.67 ± 4.38
2	7.00am	18.19 ± 6.20
3	8.00am	30.32 ± 6.50
4	9.00am	48.59 ± 12.35
5	10.00am	57.45 ± 9.32
6	11.00am	66.16 ± 6.78
7	12.00pm	84.97 ± 3.31
8	1.00pm	66.02 ± 7.49
9	2.00pm	46.59 ± 12.66
10	3.00pm	29.86 ± 10.51
11	4.00pm	14.71 ± 4.94
12	5.00pm	5.93 ± 4.02
13	6.00pm	0

3.4 Stigma receptivity

Receptivity of stigma was analysed by α-Naphthyl acetate. If the stigma was more receptive, the stigma was stained in deep blue colour. In *L. sivadasaniana* the stigma was more receptive at 9.00 am-10.00 pm after that receptivity was gradually decreases. (Table. 3 & plate. 1).

3.5 Breeding analysis

Open pollination in natural conditions resulted in 15% fruit set. Apomixis, flower bud bagged by removing stamens and stigma resulted in no fruit set. Autogamy was carried out on around 30 flowers. Self-pollination resulted in 0% fruit set. Manual pollinations using pollen from other flowers of the same plant resulted in 30% fruit set. Manual cross pollinations using pollen from flowers of different plant resulted in 65% fruit set. (Table. 4).

Table 3: Stigma Receptivity of Flower on A-Naphthaline Acetate

S. No.	Time	Frequency of colour
		Leucas sivadasaniana
1	6-8.00 am	Light bluish black
2	8-10.00 am	Dark bluish black at the tip
3	10-12.00 pm	bluish black
4	12-2.00 pm	Bluish black
5	2-4.00 pm	blue
6	4-6.00 pm	Light blue

Table 4: Breeding system analysis.

S. No.	Breeding system analysis	No. of flowers pollinated	No. of flowers fruit set	Percentage of fruit set
1	Open pollination	60	9	15
2	Apomixis	20	0	0
3	Autogamy	30	0	0
4	Geitonogamy	60	18	30
5	Xenogamy	60	39	65

3.6 Floral visitors

Initial studies on floral visitors were made from dawn to dusk for 30 min every hour, on the basis of which subsequent observations were confined to the time frame of 06.00 h to 17.00 h. The number of floral visits made by an insect and time spent on each flower were recorded by using stop watch. Hymenopteran members were chief flower visitors. The main visitors were *Apis cerana, Xylocopa pubescens*, *Xylocopa latipes* and *Macroglossum lepidum*.

The potential pollinators were identified as *A. cerana*, *Apis* sp and *M. lepidum*. We collected pollen samples from those insect body parts. The foraging behavior were varies with Hyminopteran and Lepidopteran members. The *M. lepidum* was shown hovering nature during flower visitation. The long proboscid fly *M. lepidum* was inserted its proboscis deeply in to the corolla tube and suck nectar, and during those nectar collection proboscis dusted with pollen. The foraging behaviour of Hymenopteran members were shown entirely different strategy. They were landing on lower lip of flower and probed nectar. While probing nectar pollen grains were get dusted insects back, nototribic pollination. (Plate. 1).

The most frequent visitor was recorded as *M. lepidum.* Other visitors were observed as *A. cerana, X. pubescence*, and *X. latipes.* The *L. sivadasaniana* was a cross pollinated species and vectors were playing crucial role as a pollen carriers. The flower construction was favours the insect vectors. The inflorescences were containing very closely packed flowers. It might be helped vectors to reduce energy loss during visitation.

This is the first pollination study documentation on critically endangered *L. sivadasaniana*. The closely packed inflorescence visited regularly by hawk moth *M. lepidum* and we observed that this moth is the one of the main pollinators of the plant. The long proboscis of the insects and the corolla tube of the flower correlated each other. Again this is the first report on hawk moth pollination in any *Leucas* members. The pollen gets stuck on insects back while foraging, the pollination is nototribic in Hymenopteran members.

The percentage of fruit set in manually pollinated flowers (65%) is higher than that is resulting open pollination (In the open pollination in natural conditions resulted in 15% fruit set). in natural conditions. The higher altitude, fragmented population and narrow ecological niche could be the reason for its low distribution

and become critically endangered.

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