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Original Article

Insect diversity and pollination biology of *Leucas chinensis* (Lamiaceae)

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Abstract: Detailed studies were carried out on the Phenology, floral biology, pollination and breeding system of *Leucas chinensis.* The flowers were found throughout the year. The flower is typical Lamiacean bilabiate. The inflorescence is verticillaster. Inflorescence initials are generally activated in the leaf axils. Anther dehiscence mode is longitudinal. The pollen grains were spherical and tricolpate. The size of the pollen is 26.55 ± 3.0 and 85% of pollen viable at 12am. The stigma is dry type and receptive between 12am-1.00pm. The fruit is nutlet and seed ovule ratio is 4:4. In breeding experiment we found that apomixis not present in this *Leucas* member maximum fruit set occur in the open pollination and xenogamy (95% and 90% respectively). The flower offer nectar and pollen as rewards for floral visitors. Flowers were visited by several insects and butterflies. The potential pollinators were *Apis cerana*, *Amegilla sp.*, *Ceratina sp.* and an ant species, *Componotus parius*.

Keywords: *Leucas chinensis, Pollination, Diversity, Nototriby, Amegilla*

Introduction

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 in bat pollination or wind pollination in Lamiaceae (Huck, 1992). The main pollination system of the family includes nototriby and sternotriby. Bees, by far, most commonly observed pollinators of this family. The family is known for volatile oil glands that may stimulate the responses from pollinators (Beker et al., 1989). Previous studies on *Leucas* species (Shrihail K. Kulloli et al., 2011) *confirm* that ants were very common flower visitor.

Materials and Methods

The plant was collected from Munnar (Idukki district). The plant were multiplied and grown in the Calicut university Botantical garden for detailed study.

Floral biology:

Phenology is defined as the timing of biological events. Plants in the experimental plot were observed daily to study the flowering phenology; special attention was given to identify the flowering season, 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.

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The number of pollen grains per flower was calculated as suggested by Shivanna & Rangaswami (1992). Pollen fertility was assessed by staining them in 1% acetocarmine. Pollen viability was estimated by tetrazolium test. Histochemical analyses were carried out in stigmas to study the presence of primary metabolites like starch, protein and lipids. For histochemical analysis thin hand sections were taken. Stigma receptivity analyzed by α- naphthayl acetate. In this test excised stigma dipped in 2 drops of α- naphthyl acetate solution for 30 minutes, after that, the stigma washed with phosphate buffer and observe the colour changes.

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 visit calculated. After each visit stigma were observed by hand lens and confirmed the transfer of pollens by each 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.

Result

Leucas chinensis produce flowers throughout the year. Inflorescence initials are

generally activated in the leaf axils. Flower opening in *L. chinensis* begins around 4.00 am and was completes around 5.00 am. Anther dehiscence begans with a longitudinal slit appears on the anther lobes at 4.30am after opening the flower and the process was completed between 4.30-5.00am, this result in the release of the masses of pollen grains around 5.00am. The average duration taken from inflorescence initiation to opening of the first flower was 10 days and average duration taken from the opening of the first flower to the opening of the last flower was 3-4 weeks. Pollen productivity of plants depends upon anther length, pollen grains and mode of anther dehiscence (Linskens 1992 & Reddi, 1986). The stigma receptivity maximum at 12am to1.00pm.

Pollen morphology:

Pollen grains were spherical, tricolpate, the equatorial outline is usually rounded triangular or circular. They average diameter of pollen grain is 26.55 ± 0µm.

Pollen–Ovule Ratio:

Floral analysis of the two species indicated that, the flower has didynamous stamen and are 4 in number. An anther contains 334 pollen grains. They ovaries are 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 is 1960:4.

Table.1: Floral phenology of L*eucas chinensis*

Floral characters	Observation
Flowering period	August –December
Inflorescence type	Verticillaster
Flower type	Regular, Bisexual
Flower colour	White
Odour	Absent
Nectar	Present
Anthesis time	4-5am
Anther dehiscence time	4.30-5am
Anther dehiscence mode	Longitudinal
Number of anthers per flower	4
Mean number of ovules per flower	4
Mean number of pollen per anther	334
Pollen ovule ratio	1960:4
Pollen shape	Spherical
Pollen type	Tricolpate
Pollen size	26.55 ± 3.0
Sigma type	Dry
Stigma receptivity	12am-1.00pm
Fruit type	Nutlet
Ovule seed ratio	4:4

Pollen viability:

Pollen viability was tested by using tetrazolium solution. They test revealed that, only 10% and 20% of pollen grains were viable soon after anthesis 80% pollen grains were viable at 12pm.. At 12pm the pollen viability is also 80% and gradually decreases (Table 2).

Table.2: Pollen viability

Sl. No	Time	*Leucas chinensis*
1	6.00am	17.67 ± 5.38
2	7.00am	20.19 ± 7.20
3	8.00am	31.32 ± 9.50
4	9.00am	42.59 ± 11.35
5	10.00am	46.45 ± 9.32
6	11.00am	62.16 ± 6.78
7	12.00pm	80.97 ± 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

Pollen fertility:

Pollen fertility was determined by using acetocarmine-glycerine stains. Acetocarmine-glycerine staining technique in *L. chinensis* revealed that 95% pollen grains were fertile at 11pmIn, soon after dehiscence pollen grains show lesser fertility.

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. chinensis* the stigma was more receptive at 12am-1.00pm receptivity is gradually decreases (Table. 3).

Table.3: Stigma receptivity of flower on α-naphthaline acetate

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

Breeding analysis:

In the open pollination in natural conditions resulted in 95% fruit set. Apomixis, flower bud bagged by removing stamens and stigma resulted no fruit set. Autogamy was carried out around 20 flowers. Self pollination resulted in 85% fruit set. Manual pollinations using pollen from other flowers of the same plant resulted in 80% fruit set. Manual cross pollinations using pollen from flowers of different plant resulted in 90% fruit set (Table 4).

Table.5: Breeding system analysis.

S. No.	Breeding system analysis	No.of flowers pollinated	No.of flowers fruit set	Percentage of fruit set
1	Open pollination	20	19	95
2	Apomixis	20	0	0
3	Autogamy	20	17	85
4	Geitonogamy /	20	16	80
5	Xenogamy	20	18	90

Pollinators of *L.chinenis*, a. ant *componotus parius,* b. *Amegilla sp 1,* c. *Apis cerana,* d. *Ceratina sp,* e&f *Amegilla sp,* g. stigma showing pollen growth, h. viable pollen grains, i. pollen tube growth.

Pollinators

Initial studies on floral visitors were made from dawn to dusk for 30 min every hour, on the basis of which subsequent observation 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. Hyminopterans members were chief flower visitors. *Apis cerana, Ceratina sp*, and *Apis florae* were visiting flower regularly. The solitary bees like *Amegilla sp.* was found throughout the day. The frequency of visits was high when the plants produce more flowers. The foraging activities of butterflies were influenced by weather. The butterflies were active on sunny days less active in the cloudy days.

Table.6: Visitors of *L. chinensis*.

PLANT SP	ORDER	FLOWER VISITORS	COMMON NAME	REWARD
L. chinenis	**Hymnoptera**	*Apis cerana*	Honey bee	POLLEN+NECTAR
		Apis florae	Honey bee	POLLEN+NECTAR
		Amegilla sp	Solitary bee	POLLEN+NECTAR
		Megachile sp	Cutila bee	POLLEN+NECTAR
		Trigona iridipennis	Bee	NECTAR
		Ceratina	Bee	POLLEN+NECTAR
		Vespa affinis	Paper wasp	NECTAR
		Componotus parius	Ant	POLLEN+NECTAR
	Lepidoptera	*Neptis hylas*	Common sailer	NECTAR
		Junonia atlites	Grey pansy	NECTAR
		Bibasis sena	Bibasis sena	NECTAR
		Ampittia discorides	Bush hopper	NECTAR
		Borbo cinnara	Rice swift	NECTAR
		Papilio helenus	Red Helen	NECTAR
		Delias eucharis	Common jezebel	NECTAR
		Papilio polytes	Common mormon	NECTAR
		Eurema hecabe	Common grassyellow	NECTAR
		Tirumala limniace	Blue tiger	NECTAR
		Hypolimnas	Danaid eggfly	NECTAR

The arrangement of androecial characters is a major concern in the pollination biology. In the *Leucas* sp essential characters were arranged in the upper lip and insects landing on the lower lip insert proboscis in to corolla tube, while probing nectar insect back touching the anther lobe and big chance to pollen grains stuck on the insect body (nototriby).

Discussion

In peak flowering season, *Leucas chinensis flowers were* visited by various insects. Amongst them, Lepidopteran members are highest (57.89%) in number, followed by Hymnopterans (42.10). This study shows that Hymnopterans are the main pollinators. The flowers are typical bilabiate,

having a dome shaped upper lip in which the essential whorls are situated and the lower lip with two side lobes act as a platform for the insects to alight on it. Hymnopterans body is well adapted to flower architecture and while sucking honey, pollen get dusted on insects back. The flowers are produced in loose clusters in verticillaster inflorescence. The flowers offer nectar and pollen as rewards for floral visitors. Amongst the insects, *Apis cerana*, *Amegilla sp., Ceratina sp.* are affective pollinators. It is found that an ant species *Componotus parius* were also a pollinator. Lamiaceae is well known for its floral specialties and pollination mechanisms. Above study reveals the nototribic pollination mechanism in *L. chinensis*, essential reproductive structures hide on upper lip and pollen deposition in dorsal side of the insects. The actual mechanism is insects produce pressure on lower lip of flower helps bending of reproductive structures, while probing nectar these structures touching insects back and pollen transfer occurs.

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