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Nototriby, melittophily and polychory in three medicinally valuable tropical herbs, *Leucas aspera* (Willd.) Link, *Leucas biflora* (Vahl) and the endemic, *Leucas lavandulifolia* var. *nagalapuramiana* Chandrab. & Sriniv. (Lamiaceae)

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ABSTRACT

Leucas aspera and *L. lavandulifolia* var. *nagalapuramiana* are annual erect herbs while *L. biflora* is a perennial procumbent herb. These species show profuse flowering and fruiting occurs during winter season. The flowers are sessile, small, white, slightly fragrant, nectariferous, zygomorphic and bisexual, and borne in whorled verticils. The corolla is gullet-shaped and bilabiate with the essential organs placed inside the concave upper lip and the 3-4 lobed spreading lower lip providing landing place for probing foragers. In these species, anther dehiscence occurs in mature bud, anthesis before sunrise and stigma receptivity after anthesis. The flowers are pollinated nototribically by honey bees and digger bees but *L. lavandulifolia* var. *nagalapuramiana* is additionally pollinated by a Papilionid butterfly, *Papilio demoleus*. All modes of breeding system are functional but fruit and seed set rate is maximum in cross-pollination. In open-pollination also, fruit and seed set rate is the highest indicating that the plant has the ability to set seed with or without vector-mediated pollination. But, in *L. lavandulifolia* var. *nagalapuramiana*, seeds formed from autogamy are small/partly un-filled and their germination is most unlikely. In all the three species, seed dispersal is polychorous involving autochory, hydrochory and anemochory. Polychory is advantageous for these plant species to establish new plants/populations at or far away from parental sites. But, in *L. lavandulifolia* var. *nagalapuramiana*, hydrochory and anemochory are not very effective due to rocky habitat. As a result, the plant tends to produce new individuals mostly in the surroundings of parental sites making it as an endemic species.

Keywords: *Leucas aspera, Leucas biflora, Leucas lavandulifolia* var. *nagalapuramiana*, nototriby, melittophily, polychory.

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1. INTRODUCTION

The genus *Leucas* belongs to Lamiaceae family. The number of species assigned to it has been reported variously by different authors, 98 species by Sebald (1980), 80 by Hedge (1990) and Ryding (1998) and about 100 by Harley et al. (2004). It is distributed in dry or disturbed habitats in tropical countries from southern Africa, Arabian Peninsula, Iran to South China, Taiwan, Japan and Southeast Asia (Harley et al. 2004) with highest species diversity in East Africa (Ryding 1998). It has high adaptability to grow well in plains and also at higher altitudes of hilly regions of India (Chouhan and Singh 2011). Each species in this genus is widely used in traditional medicine to cure many diseases in several countries of its distribution including India (Hedge 1990; Chouhan and Singh 2011). In India, the number of *Leucas* species has been variously reported by successive authors. Hooker (1885) documented that 34 species and 12 varieties of *Leucas* occur in India. Mukerjee (1940) reported that *Leucas* genus has eastern tropical Africa and Asia as main centers of its distribution with 43 species and 11 infra-specific taxa occurring in India. Successive authors documented differently on the number of *Leucas* species distributed in India, 43 species by Majumdas and Datta (2011) 50 species by Singh (2001) and 40 by Sunojkumar (2005). The last author also noted that 32 species are distributed in Southern India and of these species, 14 are endemics.

Leucas aspera (Willd.) Link occurs in India, the Philippines, Mauritius and Java (Prajapati et al. 2010). Its leaves are used for treating psoriasis, chronic skin eruption, rheumatism and painful swellings (Kirtikar Basu 1918; Chopra et al. 1996) while its flowers mixed with honey are used orally for cough and cold in children (Caius 1998). *L. biflora* (Vahl) R. Brown is distributed throughout the mainland of India, Andaman and Nicobar Islands, Bangladesh and Sri Lanka (Boro and Sarma 2012). It is used for the treatment of conjunctivitis, nose bleeding and white discharge in women (Chakole et al. 2020). *Leucas lavandulifolia* var. *nagalapuramiana* Chandrab. & Sriniv. (=*Leucas indica* var. *nagalapuramiana* (Chandrab. & Sriniv.) D.A. Moulali & T. Pullaiah) is endemic to Nagalapuram hills of Chittoor district, Andhra Pradesh, India (BSI 2020). It is used for appetite promotion, skin diseases, sores, swellings, cold, fever, snake bite and scorpion sting (Kamala Pranoothi et al. 2014).

Selvarathinam et al. (2008) reported that *L. aspera* is visited by honey bees, carpenter bees, digger bees, leaf-cutter bees, flies, butterflies and sunbirds in dry deciduous forest of Sathyamangalam Forest Division in southern Eastern Ghats. All these foragers act as pollinating agents except butterflies and sunbirds which utilize the flowers as a transient nectar source. Kulloli et al. (2011) reported that *L. aspera* flowers open early in the morning and are foraged during day time by honey bees, carpenter bees, digger bees, ants, flies, butterflies, and sunbirds; hawk moths and also ants forage during night time in Kalakkad Mundanthurai Tiger Reserve, Tamil Nadu. Bhaskara Rao (2014) reported that *L. aspera* is pollinated by hymenopterans and lepidopterans in agricultural fields of Addanki, Prakasam District, Andhra Pradesh; but many species reported have been wrongly identified. Prasad and Sunojkumar (2013) reported that *Oecophylla* ant robs nectar from the calyx tube of *L. aspera* growing in Waynad, Munnar and Kudajadri hills of Karnataka. Prasad and Sunojkumar (2014a) reported that *L. aspera* is pollinated by honey bees, small carpenter bees and digger bees in sylvan Northern high ranges in Wayanad district of Kerala. Varma et al. (2020) reported that the short-tongued bee, *Hoplonomia* sp. mutilates the lower petal of *L. aspera* while robbing nectar. The mutilated lower petal alters floral morphology which in turn reduces visitation rate of pollinators and also alters the pollinator community visiting the robbed flowers. Prasad and Sunojkumar (2014b,c) reported that *L. chinensis, L. ciliata, L. angularis, L. sivadasaniana* and *L. biflora* are hermaphroditic nectariferous flowers which open during early morning hours. *L. sivadasaniana* is visited by honey bees, carpenter bees and the diurnal hawk moth, *Macroglossum lepidum* with the latter acting as a potential pollinator. All other *Leucas* species observed are visited frequently by hymenopterans. *L. biflora* is foraged by the digger bee, *Amegilla* and the honey bee *Apis cerana* for pollen collection in southern Western Ghats. This state of information indicates that only a few species of *Leucas* genus have been investigated for their reproductive ecology despite the aromatic and medicinal value of the genus. Further, the available information on *L. aspera* and *L. biflora* is incompletely studied while there is absolutely no information on the endemic species, *L. lavandulifolia* var. *nagalapuramiana.* Therefore, the present study is contemplated to describe pollination ecology of *L. aspera, L. biflora* and *L. lavandulifolia* var. *nagalapuramiana* and discuss the same in the light of relevant literature.

2. MATERIALS AND METHODS

Leucas aspera growing in a semi-agricultural site (15.8107°N, 79.9724°E) at Addanki area, Ongole District, *Leucas biflora* in Galikonda area (17.9008°N, 82.0965°E) situated between Borra Caves and Araku Valley in Visakhapatnam District, and *Leucas lavandulifolia* var. *nagalapuramiana* in Sahasralingalakona site which is typically rocky undulating terrain with grass and small herbs growing in rocky outcrops (13.2172°N, 79.1003°E), in Chittoor District, Andhra Pradesh, India were used for study during January 2019 to January 2020. The aspects investigated included the plant phenology, flowering time, flower morphology, floral structural and functional aspects, forage collection schedule and activity of insects involved in pollination, breeding systems, fruiting ecology and seed

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dispersal modes. The daily schedule of anthesis, anther dehiscence and stigma receptivity were observed in the field. The methods described in the book of Dafni et al. (2005) were used for nectar analysis and stigma receptivity duration. Hand-pollination tests for conducted for assessing the functional breeding systems. Number of flowers used for each mode - apomixis, autogamy (spontaneous and manual), geitonogamy and xenogamy were 50. The same number of flowers were used for each plant species. The number of flowers used for open-pollinations is 125 in *L. aspera*, 75 in *L. biflora* and 50 in *L. lavandulifolia* var. *nagalapuramiana*. Fruit and seed set rates in each mode of hand-pollination and in open-pollination were recorded. Flower visitors collecting pollen and/or nectar from all the three plant species included in the study were carefully examined to note their forage collection schedule, forage collected and probing behavior effecting pollination. The duration of fruit development, seed production and dispersal modes were also observed in the field.

3. RESULTS

The plant and flowering phenology: *L. aspera* (Figure 1a) and *L. lavandulifolia* var. *nagalapuramiana* are annual erect herbs (Figure 4a,b) while *L. biflora* is a perennial procumbent herb (Figure 3a). All three species form many branches from the stem but *L. aspera* shows diffuse shrubby habit in damp sites. *L. biflora* has woody rootstock that produces many branches of which the ones that grow on soil surface form roots from nodes which subsequently produce lateral branches. The lateral branches that grow on the soil surface again form roots and produce new branches to extend their occupancy area. *L. aspera* shows vegetative growth during late rainy season from August to November. *L. biflora* grows throughout the year if the soil is wet but prolific growth occurs during wet season from early July to late September. *L. lavandulifolia* var. *nagalapuramiana* appears during wet season with vegetative growth and during winter season with flowering and fruiting. In all the three species, the stem is quadrangular but it has prominent furrows, nodes and internodes in *L. aspera*, it is pubescent with deflexed hair at the ribs in *L. biflora* and it is finely pubescent in *L. lavandulifolia* var. *nagalapuramiana*. In all the three species, the leaves are petiolate simple and borne opposite to each other; they are finely pubescent, linear-lanceolate with acute apex in *L. aspera* and *L. lavandulifolia* var. *nagalapuramiana* while they are ovate-oblong in *L. biflora*. The flowering occurs during late October-early February with peak occurrence during late November-early January in *L. aspera* and *L. biflora* while it occurs during winter months in *L. lavandulifolia* var. *nagalapuramiana*. The inflorescence is an axillary or terminal verticillaster in *L. aspera* and *L. biflora* while verticils are borne at the terminal portion of the branch or stem in *L. lavandulifolia* var. *nagalapuramiana*. In *L. aspera*, each verticil produces numerous flowers over a period of three weeks but there is no definite anthesis pattern as mature buds open at random from the basal and apical portion on the same day (Figure 1b). In *L. biflora* and *L. lavandulifolia* var. *nagalapuramiana*, each verticil is few-flowered (Figure 3b, 4c) and but the verticils are lax and distant from each other in the latter species. In all the three species, the flowers are sessile and attached directly to the axis of the verticil.

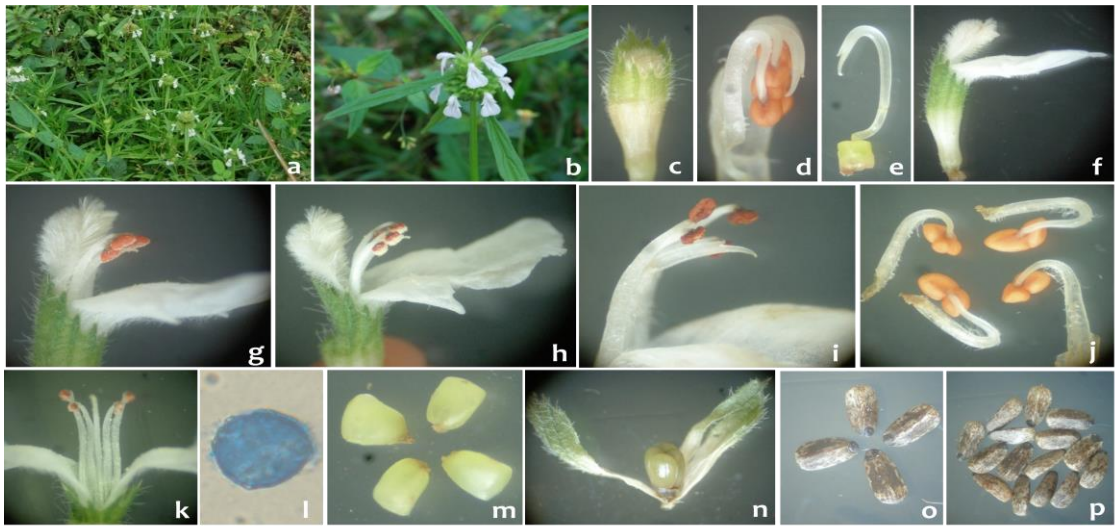

Figure 1. *Leucas aspera*: a. Habit – plant in flowering, b. Flowering verticil, c. Maturing bud, d. Position of stamens and stigma in bud, e. Position of style and stigma in bud, f-i. Corolla upper lip containing stamens and stigma inside, j. Stamens, k. Didynamous stamens, l. Pollen grain, m. & n. Ovules, o. & p. Seeds.

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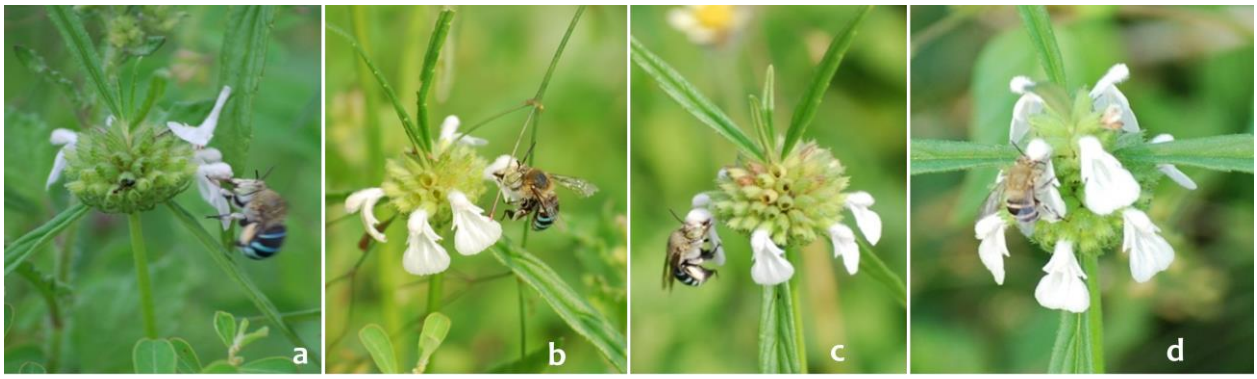

Figure 2. *Leucas aspera*: a. & b. *Anthophora cingulata* collecting nectar, c. & d. *Anthophora bicincta* collecting pollen.

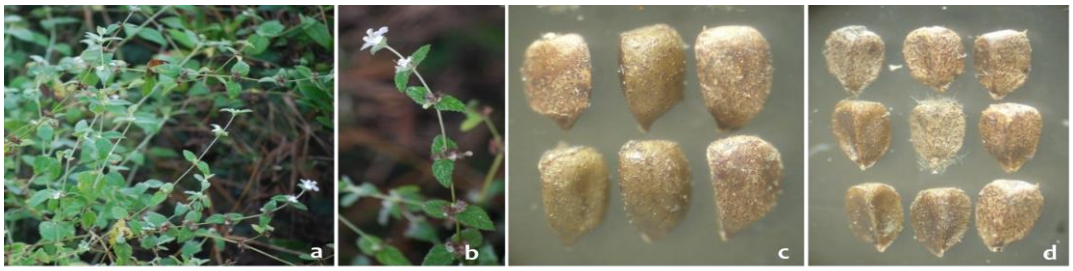

Figure 3. *Leucas biflora*: a. Habit, b. Twig with verticilled flowers, c. & d. Seeds.

Figure 4. *Leucas indica* var. *nagalapuramiana*: a. Habitat of the plant, b. Habit, c. Flowering axillary verticils, d-g. Different stages of anthesis, h. Arrangement of stamens and stigma, i. Dehisced anthers, j. Pollen grain, k. Pistil, l. Style and stigma, m. & n. Ovules, o. & p. Ventral and dorsal view of seeds.

Flower morphology: In all the three species, the flowers are small, white, slightly fragrant, flag-shaped, zygomorphic and hermaphroditic. The calyx is green, tubular with 10 unequal lanceolate teeth apically. It is 8 mm long in both *L. aspera* and *L. biflora* while it is 6 mm long in *L. lavandulifolia* var. *nagalapuramiana*; sparse to thick hairy structures are present outside and inside the calyx. The corolla is white, tubular and distally bilabiate but the tube length varied in each species, it is 7 mm long in *L. aspera*, 11 mm long in *L. biflora* and 10-13 mm long in *L. lavandulifolia* var. *nagalapuramiana*. The distal bilabiate portion consists of woolly upper lip enclosing the stamens and stigma and elaborate lower lip with sparsely minute hairy growth. The lower lip is tri-fid consisting of a large spreading middle lobe and two small sub-acute lateral lobes in *L. aspera*, 4-lobed, spreading and pubescent in *L. biflora,* and 3-lobed consisting of a large pubescent spreading middle lobe and two small lateral lobes in *L. lavandulifolia* var. *nagalapuramiana.* The stamens are 4, epipetalous, didynamous and ascending with hairy filaments, connivent and divaricate light orange to scarlet colored dithecous anthers (Figure 1j,k). In all the three species, the ovary has 2 united carpels, each with 2 locules

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and each locule with one ovule on axile placentation (Figure 1m,n; 4k-n). The style is gynobasic, filiform and extended into a stigma with one minute upper lobe and one prominent and extended lower lobe. The terminal portion of the style together with the stigmatic lobes is a bit away from the anthers and slightly incurved (Figure 4h).

Floral biology and pollination: Mature buds open during 0230-0430 h in *L. aspera* (Figure 1c), 0400-0600 h in *L. biflora* and 0530-0600 h in *L. lavandulifolia* var. *nagalapuramiana* (Figure 4d-g). In all the three species, flower-opening is indicated by the unfolding of the lower corolla lip while the hooded and concave-shaped upper corolla lip holds the stamens and stigma within (Figure 1d-i). The anthers dehisce by longitudinal slits about 30 minutes before flower-opening (Figure 4i). Within the upper lip, the curved stigma stands below the height of stamens and slightly protrudes out from the anthers. Such a placement enables the probing insect to strike the stigma first and then the anthers in quick succession to result in cross-pollination if the probing insect had the pollen of previously visited flowers on its head portion and in self-pollination if the insect had no pollen of other flowers on its head portion. The flowers secrete nectar in the tubular portion of the corolla by the time of sunrise; it is secreted in minute volume which varied from 0.4 to 0.7 µl with 25-30% sugar concentration in *L. aspera*, 0.2 to 0.4 µl with 24-29% sugar concentration in *L. biflora* and 0.3 to 0.5 µl with 23-31% sugar concentration in *L. lavandulifolia* var. *nagalapuramiana*. In all the three species, the nectar consists of sucrose, glucose an fructose in that order of dominance. The pollen output is 875 ± 25.7 per anther and 3,500 per flower in *L. aspera*, 504 ± 32.4 per anther and 2,016 per flower in *L. biflora* and 864 ± 23.8 per flower and 3,456 per flower in *L. lavandulifolia* var. *nagalapuramiana.* In all the three species, the pollen-ovule ratio is equal to the pollen output/anther due to the production of 4 stamens and 4 ovules per flower. The pollen grains are powdery, dry, spheroidal, prolate-tricolpate with thick exine and psilate ornamentation but the size varies with each species, it is 24.4 µm on polar axis and 18 µm on equatorial axis in *L. aspera* (Figure 1l), 28.74 µm on polar axis, 16 µm on equatorial axis in *L. biflora* and 27.54 µm on polar axis, 18 µm on equatorial axis in *L. lavandulifolia* var. *nagalapuramiana* (Figure 4j). The stigma is receptive soon after anthesis and ceases by late evening with maximum receptivity around noon time in all the three species.

In *L. aspera* and *L. biflora*, the flowers were foraged by honey bees, *Apis cerana* and *A. florea,* and digger bees, *Anthophora cingulata* (Figure 2a,b, 5a) and *A. bicincta* (Figure 2c,d) but in *L. lavandulifolia* var. *nagalapuramiana*, the flowers were foraged by the first three bee species only. These bees began their foraging activity from 0730 h onwards, gradually increased their activity towards noon, then onwards, gradually decreased their activity towards evening and ceased finally by 16:30 h. They were regular and consistent foragers and foraged for both nectar and pollen either in the same visit or in different successive visits to the same or different verticils on the same or different conspecific individuals. These bees struck the stigma and stamens with the dorsal side of their head portion in quick succession effecting pollen transfer resulting in nototribic pollination. All bees loaded pollen into their corbiculae or pollen baskets which are part of the tibia on their hind legs indicating that this plant is an important pollen source for them. Among these bees, digger bees were swift fliers as they tended to spend little time on individual flowers which enabled them to visit several flowers of different verticils in a minute time and promote cross-pollination rate. The honey bees tended to spend more time at each flower which enabled them to visit only a few flowers, mostly of the same plant in a minute time effecting mostly self-pollination rate through geitonogamy and/or autogamy. Further, the Papilionid butterfly, *Papilio demoleus* (Figure 5b) visited the flowers of *L. lavandulifolia* var. *nagalapuramiana* consistently and regularly for nectar collection. It hovered at the front side of the flower, inserted its long proboscis into the corolla tube and collected the nectar. In this probing act, the proboscis invariably contacted the stigma and the anthers effecting cross- or self-pollination.

Breeding systems: Hand-pollination tests indicated that apomixis is totally absent while all modes of pollination are functional. Fruit set and seed set rates are the lowest in spontaneous autogamy and the highest in xenogamy. In *L. aspera*, fruit set is 36% in spontaneous autogamy, 50% in manipulated autogamy, 56% in geitonogamy, 84% in xenogamy and 82% in open-pollinations. Seed set 25% in spontaneous autogamy, 40% in manipulated autogamy, 51% in geitonogamy, 88% in xenogamy and 75% in open-pollinations. In *L. biflora*, fruit set is 32% in spontaneous autogamy, 54% in manipulated autogamy, 60% in geitonogamy, 88% in xenogamy and 83% in open-pollinations. Seed set 26% in spontaneous autogamy, 45% in manipulated autogamy, 56% in geitonogamy, 90% in xenogamy and 78% in open-pollinations. In *L. lavandulifolia* var. *nagalapuramiana*, fruit set is 34% in spontaneous autogamy, 46% in manipulated autogamy, 62% in geitonogamy, 90% in xenogamy and 78% in open-pollinations. Seed set 23% in spontaneous autogamy, 36% in manipulated autogamy, 54% in geitonogamy, 85% in xenogamy and 74% in open-pollinations (Table 1).

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Table 1. Results of hand-pollination tests in *Leucas aspera*, *L. biflora* and *L. lavandulifolia* var. *nagalapuramiana*

Pollination mode	*Leucas aspera*			*Leucas biflora*			*L. lavandulifolia* var. *nagalapuramiana*		
	No. of fruits produced	Fruit set (%)	Seed set (%)	No. of fruits produced	Fruit set (%)	Seed set (%)	No. of fruits produced	Fruit set (%)	Seed set (%)
Apomixis	0	0	0	0	0	0	0	0	0
Autogamy (spontaneous)	18	36	25	16	32	26	17	34	23
Autogamy (manual)	25	50	40	27	54	45	23	46	36
Geitonogamy	28	56	51	30	60	56	31	62	54
Xenogamy	42	84	88	44	88	90	45	90	85
Open-pollinations	102	82	75	62	83	78	39	78	74

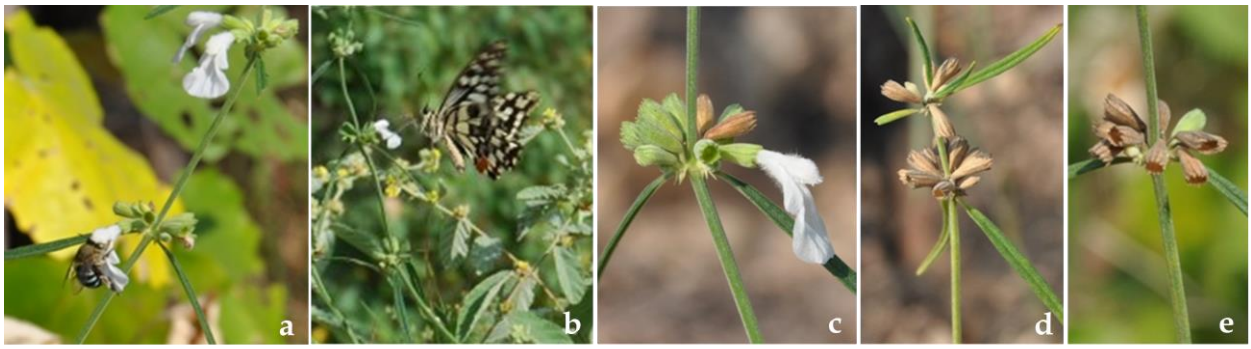

Figure 5. *Leucas indica* var. *nagalapuramiana*: a. *Anthophora cingulata* collecting nectar, b. Papilionid butterfly, *Papilio demoleus* collecting nectar, c. Inflorescence with flower, maturing and mature fruits, d. Mature fruits with seeds, e. Fruiting calyx remained attached after self-seed dispersal.

Fruit set, seed set and seed dispersal: Fruits mature after a time lapse of 15-20 days. Fruiting calyx is light to dark brown consisting of 2-4 ash-grey or light to darkish brown, erect, smooth, trigonous obovoid-oblong nut lets with rounded dorsal face and angular inner face (Figure 5c-e); but nut let size varies with each species, it is 2 x 1 mm in *L. aspera*, 1.5 x 1 mm in *L. biflora* (Figure 3c,d) and 3 x 1 mm in *L. lavandulifolia* var. *nagalapuramiana* (Figure 4o,p). In all the three species, mature and dry nut lets fall from the downwardly oriented fruiting calyx by gravity indicating the function of autochory. The nut lets fallen to the ground are subsequently dispersed by rain water during wet season and by wind during dry season indicating that hydrochory and anemochory are also functional. Seeds germinate immediately in damp sites to produce new plants while they are dormant in dry sites until the onset of rainy season. In *L. lavandulifolia* var. *nagalapuramiana*, the rocky habitat where the plant is growing is not favorable for long distance dispersal. Seeds germinate during rainy season to produce new plants and flowering and fruiting during winter season.

4. DISCUSSION

The phenology of *Leucas* species now studied indicate that they show prolific vegetative growth during wet season, and flowering and fruiting during winter season. However, *L. aspera* and *L. biflora* have the ability to grow and display sexual activity throughout the year in damp or wet areas while *L. lavandulifolia* var. *nagalapuramiana* being an endemic is characteristically a seasonal bloomer due to water stress as it grows in rocky outcrops and rocky habitat. *L. aspera* and *L. lavandulifolia* var. *nagalapuramiana* do not root from nodes because of their erect habit and hence this habit might have made them to acquire annual habit. On the contrary, *L. biflora* has the ability to produce many branches from the woody rootstock and lateral branches from the branches that grow on soil surface due to its procumbent habit which might have enabled this species to acquire perennial habit.

Prasad and Sunojkumar (2014a) reported that the zygomorphic flowers and well developed corolla upper lip to hold the stamens and stigma within and corolla lower lip as landing place for insects, and nectar concealed in the tubular portion of the corolla are adaptations of the members of Lamiaceae family. These authors also noted that bees are the most common pollinators of

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this family. van der Pijl (1972) reported that Lamiaceae flowers display two types of pollination syndromes according to the placement of stamens and the stigma within the corolla. Flag flowers with the essential organs placed close to the corolla lower lip exhibit sternotribic pollination because the probing insect strikes the stamens and stigma with their ventral side effecting pollination while probing the flowers for nectar. Gullet flowers with the essential organs placed within the concave upper lip exhibit nototribic pollination because the probing insect strikes the stamens and stigma with their dorsal side (notum) effecting pollination while probing the flowers for nectar. In the present study, all the three *Leucas* species produce zygomorphic flowers with sex organs placed within the woolly corolla upper lip and well developed corolla lower lip offering landing place for the probing insects.

These floral characters are indicative of gullet flowers adapted for nototribic pollination and also the bees probing the flowers while probing the flowers in upright position effect nototriby. As bees collect large quantities of pollen to feed their offspring, nototribic mode of pollination is efficient in effecting pollination. Because, pollen is deposited on the dorsal side of the head portion which serves as a safe site for pollen transfer by bees between flowers. Further, bees cannot groom pollen from the pollen deposited portion by their legs. Therefore, nototriby ensures pollen precision and minimization of pollen loss as a compensation by pollen collection activity of bees in all the three *Leucas* species now studied.

Kulloli et al. (2011) reported that anthesis occurs during early morning hours in *L. aspera*. Prasad and Sunojkumar (2014c) reported that *L. biflora* shows anthesis during early morning hours. In this study also, it is observed that anthesis of all the three *Leucas* species occurs before sunrise indicating they are adapted for pollination by day-time foragers. Kulloli et al. (2011) reported that *L. aspera* is pollinated by day and night flower visitors. The day visitors are honey bees, stingless bees, carpenter bees, digger bees, flies, butterflies and sunbirds while night visitors are hawk-moths. Bhaskara Rao (2014) reported that *L. aspera* is pollinated by hymenopterans and lepidopterans. Prasad and Sunojkumar (2014a) reported that *L. aspera* is pollinated by honey bees, small carpenter bees and digger bees. Prasad and Sunojkumar (2014c) reported that *L. biflora* is pollinated by hymenopterans, especially bees. In the present study, all the three *Leucas* species have been observed to be pollinated by honey bees and digger bees only. But, *L. lavandulifolia* var. *nagalapuramiana* is additionally pollinated by the Papilionid butterfly, *Papilio demoleus*. Since the flowers of all the three *Leucas* species are nectariferous by producing minute volume of nectar, they are important as pollen and nectar sources. Bir Bahadur et al. (1986) reported that *L. aspera* nectar is a source of sucrose, glucose and fructose, and also of α-amino acids. In this study, the nectars of all three *Leucas* species have been found to be source of sucrose, glucose and fructose in that order of dominance. *Leucas* species in principle are adapted for nototribic pollination by bees and all other foragers reported by different authors are only supplementary pollinators. Therefore, all the three *Leucas* species are typically melittophilous.

Prasad and Sunojkumar (2013) reported that the ant, *Oecophylla alba* steals nectar from the calyx tube of *L. aspera* but the picture depicting the ant collecting nectar included in their research paper shows that the ant is attempting to collect nectar from the calyx base after the fall of the corolla and hence it is not a case of nectar robbery since it is collecting the nectar after pollination is achieved and also after the fall of the corolla together with the sex organs. Varma et al. (2020) reported that a short-tongued halictid bee, *Hoplonomia* sp. robs nectar from *L. aspera* during which it mutilates the lower corolla lip; the lower lip mutilation does not affect nectar dynamics but alters flower morphology which in turn reduces pollinator visitation and also alters the pollinator community in successive visits to these robbed flowers. In this study, ants have never visited the flowers of all the three *Leucas* species and hence nectar stealing by ants is totally ruled out. Further, the bees foraging on these *Leucas* species have not resorted to the mutilation of lower corolla lip.

Prasad and Sunojkumar (2014a) reported that *L. aspera* displays a breeding system that enables to fruit through autogamy and allogamy but it favors allogamy. But, these authors did not document whether the plant requires a vector for autogamy. In this study, all the three *Leucas* species exhibit both autogamy and allogamy with maximum fruit set rate in cross-pollination followed by geitonogamy and manipulated autogamy and autonomous autogamy. In open pollinations, fruit set rates exceed 80% in *L. aspera* and *L. biflora*, and nearly 80% in *L. lavandulifolia* var. *nagalapuramiana*. Seed set rates stand at or above 75% in open-pollinations indicating that all the three plant species have the ability to produce seed with or without vector-mediated self- and/or cross-pollination. Further, in *L. lavandulifolia* var. *nagalapuramiana*, seeds formed from spontaneous and/or autonomous autogamy are either small in size or partly un-filled indicating that the seed produced through autogamy is most unlikely to contribute to the new recruitment and build up of population, and hence this situation could be partly attributable to its endemic status.

In all the three *Leucas* species, seed dispersal occurs by autochory, hydrochory and anemochory constituting the function of polychory. Autochory involves seed fall by gravity from the horizontally oriented fruiting calyx and it is very effective when ambient conditions are dry with little humidity in the air. Hydrochory is functional only on rainy days while anemochory is effective during dry season. These three seed dispersal modes are advantageous for these plant species to establish new plants/populations at or far away from parental sites. But, in case of *L. lavandulifolia* var. *nagalapuramiana*, hydrochory and

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anemochory are not very effective due to blockage or minimization of seed dispersal by rocky habitat. As a result, the plant tends to produce new individuals mostly in the surroundings of parental sites making it as an endemic species.

L. aspera leaves are used for treating psoriasis, chronic skin eruption, rheumatism and painful swellings (Kirtikar Basu 1918; Chopra et al. 1996) while its flowers mixed with honey are used orally for cough and cold in children (Caius 1998). *L. biflora* is used for the treatment of conjunctivitis, nose bleeding and white discharge in women (Chakole et al. 2020). *L. lavandulifolia* var. *nagalapuramiana* is used for appetite promotion, skin diseases, sores, swellings, cold, fever, snake bite and scorpion sting (Kamala Pranoothi et al. 2014). Since these *Leucas* species are traditionally used for treating different diseases, they can be exploited not only in traditional medicine but also in allopathy after careful analysis of every part of these plant species. But, appropriate measures are necessary for the *in situ* conservation and management of *L. lavandulifolia* var. *nagalapuramiana* while exploring appropriate methods for the expansion of population size of this endemic plant species.

5. CONCLUSIONS

Leucas aspera and *L. lavandulifolia* var. *nagalapuramiana* are annual erect herbs while *L. biflora* is a perennial procumbent herb. In these species, profuse flowering and fruiting occurs during winter season. The flowers are sessile, small, white, slightly fragrant, nectariferous, zygomorphic and bisexual, and borne in whorled verticils. The corolla is gullet-shaped and bilabiate with the essential organs placed inside the concave upper lip and adapted for nototribic pollination, and the 3-4 lobed spreading lower lip providing landing place for probing foragers. In these species, anther dehiscence occurs in mature bud, anthesis before sunrise and stigma receptivity after anthesis. The flowers are pollinated by honey bees and digger bees but *L. lavandulifolia* var. *nagalapuramiana* is additionally pollinated by a Papilionid butterfly, *Papilio demoleus*. All modes of breeding system are functional but fruit and seed set rate is maximum in cross-pollination. In open-pollination also, fruit and seed set rate is highest indicating that the plant has the ability to set seed with or without vector-mediated pollination. But, in *L. lavandulifolia* var. *nagalapuramiana*, seeds formed from autogamy are small/partly un-filled and their germination is most unlikely. In all the three species, seed dispersal occurs by autochory, hydrochory and anemochory. These three seed dispersal modes are advantageous for these plant species to establish new plants/populations at or far away from parental sites. But, in *L. lavandulifolia* var. *nagalapuramiana*, hydrochory and anemochory are not very effective due to rocky habitat. As a result, the plant tends to produce new individuals mostly in the surroundings of parental sites making it as an endemic species.

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Authors' contributions

All authors contributed equally.

Conflict of Interest

The authors declare that there are no conflicts of interests.

Ethical approval

The ethical guidelines for plants & plant materials are followed in the study for species collection & identification.

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Data and materials availability

All data associated with this study are present in the paper.

REFERENCES AND NOTES

1. Bhaskara Rao, Ch., 2014. Reproductive ecology of *Leucas aspera* (Lamiaceae). Indian J. Res. Pharm. Biotech. 2: 1332-1338.
2. Bir Bahadur, Chaturvedi, A., Rama Swamy, N., 1986. Nectar types in Indian plants. Proc. Indian Acad. Sci. (Plant Sci.) 96: 41-48.

ISSN 2319–5746 EISSN 2319–5754 | OPEN ACCESS

3. Boro, A., Sarma, G.C., 2012. *Leucas biflora* (Vahl) R. Brown (Lamiaceae): a new record for Assam, India. Pleione 6: 429-431.
4. BSI., 2020. State-wise distribution of endemic and threatened plant taxa of India. ENVIS Resource Partner on Biodiversity, Botanical Survey of India, Calcutta.
5. Caius, J.F., 1998. The medicinal and poisonous plants of India. Scientific Publishers, India.
6. Chakole, K.R., Chandak, K.K., Umekar, M.J., 2020. Phytochemical screening and TLC profile of ethyl acetate extract of *Leucas biflora* leaves. World J. Pharm. Res. 9: 845-855.
7. Chopra, R.N., Nayar, S.L., Chopra, I.C., 1996. Glossary of Indian Medicinal Plants. National Institute of Science Communication, New Delhi, India.
8. Chouhan, S.H., Singh, S.K., 2011. A review of plants of genus *Leucas*. J. Pharmacog. Phytotherapy 3: 13-26.
9. Dafni, A., Kevan, P.G., Husband, B.C., 2005. Practical Pollination Biology. Enviroquest Ltd., Cambridge, 590pp.
10. Harley, R.M., Atkins, S., Budantsev, A.L., Cantino, P.D., Conn, B.J., Greyer, R., Harley, M.M., De Kok, R., Krestovskaja, T., Morales, R., Paton, A.J., Ryding, O., Upson, T., 2004. Labiatae. In: Families and genera of flowering plants, Vol. 7, K. Kubitzki and J.W. Kadereit (Eds.), pp. 167-275, Springer, Berlin.
11. Hedge, I.C., 1990. Labiatae. In: Flora of Pakistan. S.I. Ali and Y.J. Nasir (Eds.), University of Karachi, Karachi, p. 192.
12. Hooker, J.D., 1885. The Flora of British India - 4. Reeve & Co., London, 680-691 pp.
13. Kamala Pranoothi, E., Narendra, K., Suman Joshi, D.S.D., Swathi, J., Sowjanya, K.M., Rathnakarreddi, K.V.N., Emmanuel, S.J., Padmavathi, Ch., Krishna Satya, A., 2014. Studies on qualitative, quantitative, phytochemical analysis and screening of in vitro biological activities of *Leucas indica* (L.) var. *nagalapuramiana*. Intl. J. Herbal Med. 2: 30-36.
14. Kirtikar, K.R., Basu, B.D., 1918. Indian Medicinal Plants 2: 1010-1049, Indian Press, Calcutta.
15. Kulloli, S.K., Chandore, A.N. and Aitawade, M.M. 2011. Nectar dynamics and pollination studies in three species of Lamiaceae. Curr. Sci. 100: 509-516.
16. Majumdar, K., Datta, B.K., 2011. *Leucas biflora* (Vahl) R.Br. (Lamiaceae): a new distributional record and its less known ethno-medicinal usage from Tripura. Indian J. Trad. Knowledge 10: 575-577.
17. Mukerjee, S.K., 1940. A revision of the Labiatae of the Indian Empire. Records of Botanical Survey of India 14: 1-205.
18. Prajapati, M.S., Patel, J.B., Modi, K., Shah, M.B., 2010. *Leucas aspera*: a review. Phcog. Rev. 4: 85-87.
19. Prasad, E.R., Sunojkumar, P., 2013. Nectar robbery in some South Indian Lamiaceae. Ann. Plant Sci. 2: 386-387.
20. Prasad, E.R., Sunojkumar, P., 2014a. Pollination biology of medicinally important plant *Leucas aspera*. J. Entomol. Zool. Stud. 2: 341-344.
21. Prasad, E.R., Sunojkumar, P., 2014b. Pollination biology of critically endangered *Leucas sivadasaniana*. J. Entomol. Zool. Stud. 2: 115-118.
22. Prasad, E.R., Sunojkumar, P., 2014c. Pollination biology of five *Leucas* spp. (Lamiaceae) in Southern Western Ghats. J. Entomol. Zool. Stud. 2: 250-254.
23. Ryding, O., 1998. Phylogeny of the *Leucas* Group (Lamiaceae). Syst. Bot. 23: 235-247.
24. Sebald, O., 1980. Die Gattung *Leucas* R.Br. (Labiatae) in Afrika und auf der Arabischen Halbinsel. Stuttgarter Beitr. Naturk. Ser. A. 341: 1-200.
25. Selvarathinam, T., Balasubramanian, P., Manikandan, P., Murugesan, M., 2008. Foraging behavior of flower visitors and pollination in *Leucas aspera* (Willd.) Link. Adv. Pollen-Spore Res. XXVI: 55-63.
26. Singh, V., 2001. Monograph on Indian *Leucas* R.Br. (Dronapushpi) Lamiaceae. J. Economic Taxonomic Bot. Add. Ser. 20. Scientific Publishers, Jodhpur.
27. Sunojkumar, P., 2005. *Leucas sebaldiana* Sunojk. (Lamiaceae), a new species from India. Candollea 60: 233-236.
28. van der Pijl, L., 1972. Functional considerations and observations on the flowers of some Labiate. Blumea 20: 93-103.
29. Varma, S., Rajesh, T.P., Manoj, K., Asha, G., Jobiraj, T., Sinu, P.A., 2020. Nectar robbers deter legitimate pollinators by mutilating flowers. Oikos 129: 868-878.

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