Nectar dynamics and pollination studies in three species of Lamiaceae

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The nectar dynamics and pollination study of three species, viz. *Leonotis nepetifolia* (L.) R. Br., *Leucas aspera* (Willd.) Link and *Orthosiphon thymiflorus* (Roth) Sleesen were studied in their natural habitat. Little information is available on the nectar dynamics and pollinator preference of these medicinally important plants of the family Lamiaceae. There is wide array of floral foragers comprising honey bees, butterflies, flies, ants, birds and hawk moths. The nectar is dominated by sucrose–glucose and fructose in all species. Insects accounted for a majority of the visits (96%). Their nectar shows marked temporal fluctuations in volume and concentration of total sugars. These changes are interrupted in terms of removal by visitors and evaporation or condensation. Foraging behaviour of most of the insects and birds indicated that they are genuine pollen-transferring agents. However, infrequent visits by butterflies and sunbirds show that they use these plants only as a transient source of food. Honey bees accounted for ~66% visits in all three species and thus could be the major pollinator of these species.

Keywords: Lamiaceae, *Leonotis nepetifolia*, *Leucas aspera*, nectar, *Orthosiphon thymiflorus.*

FLORAL nectar and pollen are offered as a reward for the biotic pollinating agents[1]. Pollination rewards differ in their composition, amount and accessibility, and are highly associated with specific functional groups of pollinations[2]. Nectar is the most common form of floral reward furnished by animal-pollinated plants to their mutualistic partners[3]. Considerable correlative evidences link the broad variation in energetic and nutritional content and chemical composition of angiosperm nectars to differences in the identity of pollinators and their energetic and nutritional needs[4,5]. This applies particularly to the sugars, which are the dominant chemical components of most nectars and whose variation has been thoroughly studied in relation to differences in pollinator composition[6–8]. The most common sugars in nectar are the disaccharides, sucrose and the hexose monosaccharides, glucose and fructose[9–11]. Data on the chemical composition of nectar may give significant clues to ascertain the principal pollinator groups[2]. Usually, the relative proportion of disaccharides and monosaccharides, i.e. sucrose to hexose ratio has been considered a distinctive compositional significance of nectars that could be predictably related to the type of pollinator.

Nectars have been traditionally classified as either sucrose- or hexose-rich[12,13], and a number of studies have indicated a relationship between sucrose : hexose ratio and pollinator type[14,15]. Production of nectar involves substantial cost[16,17] and animal-pollinated plants incur this cost to enhance pollinator visits[18]. Quantitative analysis of nectar sugars for the amount of energy produced has formed the basis for studies of ecological principles such as foraging strategies[19] and competition[20,21]. To pursue the ecological advances of energy derived from nectar sugar, it is necessary to know about the fluctuations in its availability in flowers in the field. The sugar composition and concentration of floral nectar vary widely among plant species[22–24].

Lamiaceae, known as the mint family, comprising ca. 210 genera and 3500 species is cosmopolitan in distribution with its greatest distribution in the Mediterranean[25,26]. The lower lip or libia is characteristic and responsible for the conserved family name, Labiatae[27]. The members grow under various habitat conditions at different altitudes and show a high degree of adaptability with their occurrence ranging from the Arctic to the Himalayas. The chief centres of development of the family are Southwest Asia to Hawaii and Australia, throughout Africa and in the new world from North to South. A few large groups are cosmopolitan. The family is represented in India by ca. 64 genera and 350 species, of which (60 species and 5 varieties) 18% is strictly endemic to peninsular India. These endemic taxa are spread over genera, of which *Anisochilus*, *Eusteralis*, *Leucas*, *Plectranthus* and *Pogostemon* have a large representation of endemic taxa. As many as 18 of these endemic plants are rare in their habitat.

A comprehensive study on nectar dynamics and pollinator preference at the species level in Lamiaceae has not been made so far. Study on pollination mechanism, reproductive ecology and breeding behaviour of *Leonotis*, *Leucas*, *Hyptis*, *Anisochilus*, *Oscimum*, *Scutellaria*[28–34]

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has been made, but a comprehensive study on the nectar dynamics is scarce. In this background, the present study was conducted on nectar dynamics and pollinator behaviour on three species of Lamiaceae, viz. *Leonotis nepetifolia* (L.) R. Br., *Leucas aspera* (Willd.) Link and *Orthosiphon thymiflorus* (Roth) Sleesen, important medicinal plants which are considered as weeds. The objectives were to assess the nectar dynamics (temporal fluctuations), foraging preferences and document floral visitors as well as identify the potential pollinators.

Materials and methods

Study area

The present study was carried out in the Kalakkad Mundanthurai Tiger Reserve (KMTR) during 2006–2007 by selecting three populations of each species. The study area lies between lat. 8°25′ and 8°53′N, and long. 77°10′ and 77°35′E. KMTR is situated in the southern Western Ghats, Tirunelveli District, Tamil Nadu, and is the second largest protected area in the state. It covers a range of 40–1800 m amsl. The rainfall ranges from 2000 to 5000 mm during the southwest monsoon and temperature ranges from 15°C to 23°C. The forest type is dry-deciduous to wet-evergreen with red loamy, acidic and ferruginous type of soil[35].

Study material

L. nepetifolia (L.) R. Br., *L. aspera* (Willd.) Link and *O. thymiflorus* (Roth) Sleesen, (Figure 1 *a–e*), grow in KMTR. *L. nepetifolia* is a large herb, reaching up to 6 ft along the road side and in wastelands (Figure 1 *b*). It has long internodes with spinous whorls of scarlet–orange coloured flowers. The upper lip of the flower is densely wooly in nature (Table 1 and Figure 1 *c*). *L. aspera* is an important plant in traditional medicine. It is a 20–35 cm long annual herb with coarse and diffusely branched stem. Flowers are white with lower lip longer than the hood (Table 1 and Figure 1 *e*). *O. thymiflorus* (Roth) Sleesen is an annual plant that grows in the deciduous forest at low elevations and throughout the plains. It reaches 70–90 cm in height and has less branched stem. The flowers are purple and borne in terminal racemes (Table 1 and Figure 1 *a*).

Phenology

Twenty healthy plants were selected from each population (20 populations) of each species, and observations were made on a day-to-day basis on flowering phenology, which includes season, flower development, anthesis and anther dehiscence. Floral morphology was studied with the help of a hand lens and dissection microscope. Fifty flower buds were selected from twenty populations of each species and observations were made to know the time of anthesis and anther dehiscence.

Pollinator behaviour

Continuous observations during 2006–2007 were made on the behaviour of different flower foragers. The observations were made during the day from 0600 to 1800 h. A total of 48 h was spent and a pair of binoculars was used for close observation of pollinators (Olympus, 7X21 PC III classic, Japan). During the extended observations, the night pollinators were observed with the aid of a portable night-vision device (Javelin Model 222 NVD, New York). The foraging period and the type of reward collected by different visitors on a daily basis were recorded by close observations. The flower-visiting insects were captured by hand net and identified using standard insect taxonomy manual as well as consulting experts from the Kerala Agricultural University (Thrissur), Kerala Forest Research Institute (Peechi) and Tropical Botanic Garden and Research Institute (TBGRI, Thiruvananthapuram).

Nectar analysis

The amount of nectar secreted per flower was measured and expressed in microlitres of nectar per flower. The nectar sugar concentration was measured using handheld sugar refractometer (Erma, Japan), following Dafni *et al.*[36]. Standing-crop nectar volume measurements were taken primarily from unbagged flowers in the field, which reflects both rates of secretion and depletion. Nectar volume is expected to be lower in unbagged versus bagged flowers, but sampling of nectar from unbagged flowers in the population being visited by visitors that confirmed pollinators in the field. To collect nectar, whole flowers ($N = 50$) were picked and nectar was withdrawn from the base of the corolla tube with 5 µl calibrated capillary tube (Drummond, UK) after separating the ovary from the perianth base. Every morning the flowers were bagged (0600 h) with pollination bags made of paper to prevent floral visits[37]. The volume of nectar for each species was measured at 2 h intervals, i.e. 0700–1700 h and recorded at each interval. Fifty ($N = 50$) flowers were selected at each measurement at each interval to minimize error. The dimensions of floral parts were measured from freshly excised flowers (fully opened). The temperature and relative humidity were also recorded at each time interval of measurement for analysis of nectar qualitatively and quantitatively.

Samples of collected nectar were immediately loaded on the chromatographic paper (Whatmann No. 1 55×45 cm, Whatmann International Ltc., Springfield Mill, UK) to assess the kind of nectar sugar[38], using *n*-butanol, aceticacid and water (4 : 5 : 1) as the running

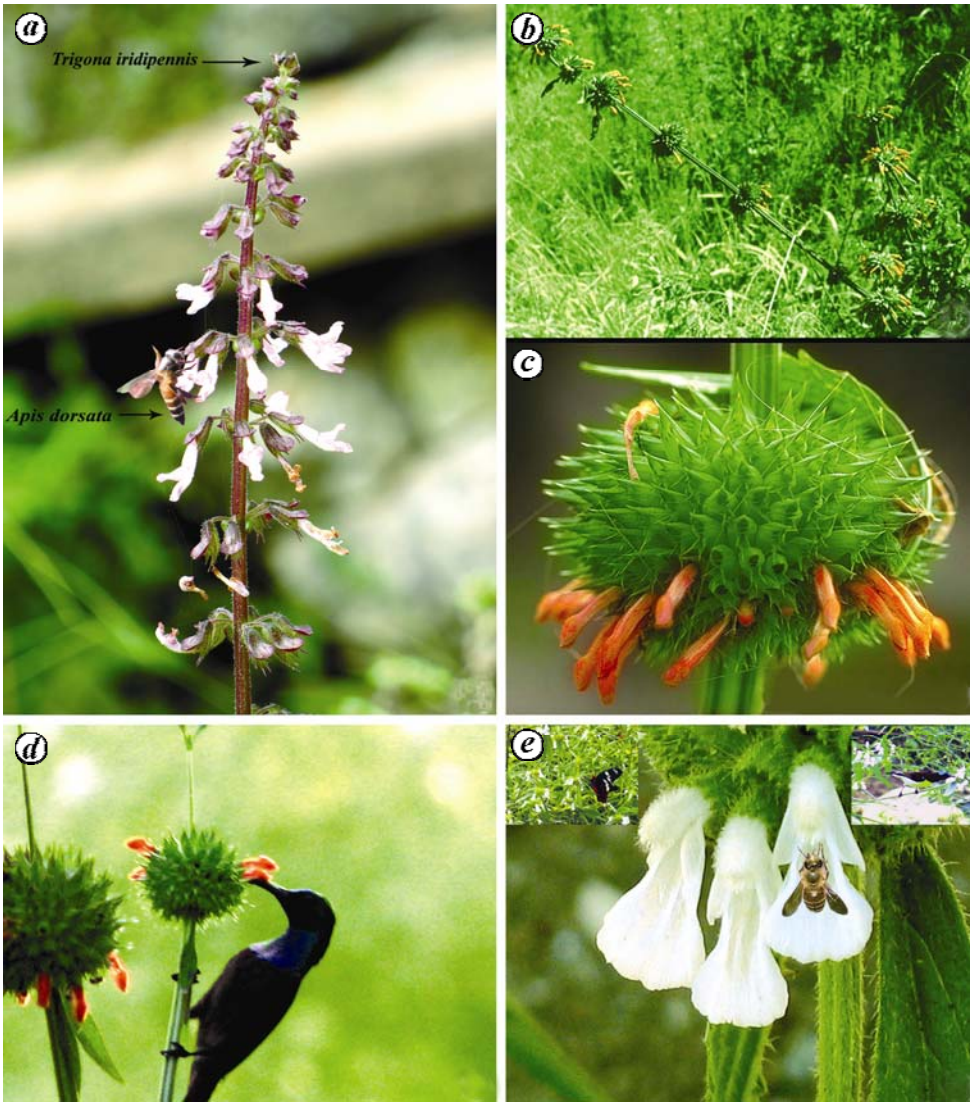

Figure 1. Pollinators of three Lamiaceae species. ***a***, *Orthosiphon thymiflorus* – *Apis dorsata* foraging nectar at inflorescence. ***b–d***, *Leonotis nepetifolia* Habit: (***b***), Inflorescence (***c***) and *Nectarina zeylanica* foraging nectar (***d***). ***e***, Pollinators of *Leucas aspera* – *Apis cerana* collecting flower rewards. (Inset) *Pachliopta aristolochaea* and *Nectarina asiatica* foraging flower resources.

Table 1. Floral characteristics of the three species of Lamiaceae

Taxa	Habitat	Flowering period	Flower colour	Flower opening time
Leonotis nepetifolia (L.) R. Br.	Dry fields and roadside in forests	September–January	Scarlet–orange	Midnight
Leucas aspera (Willd.) Link	Fields, scrubs, jungles and wastelands	June–February	White	Early morning (0200–0330 h)
Orthosiphon thymiflorus (Roth) Sleesan	Roadside, wastelands and jungles	July–January	Pale, purple	Early morning (0230–0400 h)

solvent. The different components of sugar were separated on the chromatographic paper and eluted by dissolving the spots in 80% ethanol. The extract was analysed quantitatively by the colorimetric method of Yamm and Wills[39], using 0.2% anthrone dissolved in 70% sulphuric acid and recording absorbance at 620 nm for sucrose and fructose, and at 540 nm for glucose with ELICO SL171 Spectrophotometer (TBGRI).

Results and discussion

Floral biology and phenology

The floral characteristics of *Leonotis nepetifolia*, *Leucas aspera* and *O. thymiflorus* are shown in Table 1. The flowers of all selected species are zygomorphic, with faint scent. In all three species, the area of advertisement

Table 2. Nectar dynamics of the three species

Taxa	Nectar volume per flower (µl)	Nectar sugar concentration (%)	Nectar sugar type	Forage type
Leonotis nepetifolia (L.) R. Br.	6.90 ± 0.75	16.80 ± 1.75	SGF*	P + N†
Leucas aspera (Willd.) Link	0.66 ± 0.09	28.60 ± 2.68	GFS	P + N
Orthosiphon thymiflorus (Roth) Sleesan	0.70 ± 0.43	32.84 ± 0.98	FGS	P + N

*S, Sucrose; G, Glucose; F, Fructose, ±, Standard deviation; †P + N, Pollen and nectar.

is the lower lip of the corolla tube. The corolla has a well-developed upper lip and lower lip, an adaptation of the family Lamiaceae for insect pollination. The tubular portion of the corolla contains copious amount of nectar. The anthers are oriented towards the upper lip and located within the villous crown in *L. nepetifolia*, but in *L. aspera* and *O. thymiflorus*, the filaments and anthers are partly fused with the upper lip of the corolla tube. Floral details have already been reported for *L. nepetifolia*[31,32] and *L. aspera*[40].

Phenological data of the three species are given in Table 2. All the three species are annual, completing their vegetative growth following monsoon rains (Table 1). The vegetative and flowering phenology is already known for *L. nepetifolia*[32] and *L. aspera*[40]; but not for *O. thymiflorus*. The flowering period may vary with geographic phase. For example, the flowering period of these species in Mexico varies in their occurrence range. Such variation in the flowering period of the species in different regions is undoubtedly an adaptation to local weather conditions, which is imperative for these three species. A wide array of differences was noticed in flower opening time for the three species. In *L. nepetifolia*, the flowers open during midnight and remain so for about 40 h. In *L. aspera* (anthesis at 0200–0330 h) and *O. thymiflorus* (anthesis at 0230–0400 h), the flowers are available for pollinators till 20 h after anthesis.

Pollinator behaviour

Details of floral visitors are given in Table 3. In *L. aspera*, two night visitors are documented, *Macroglossum variegatum* and *Macroglossum heliophila*. But they are mere pollinators. Honey bees are the major pollinators in all three species (Figure 1 *a* and *e*). Their foraging is more than any other visitor, as listed in Table 3. Two ants (*Oecophylla smaragdina* and *Camponotes compressus*) were also recorded during the study. They intermittently visited the flowers of *L. nepetifolia* and *L. aspera*, but no ants were observed in *O. thymiflorus*. A variety of butterflies visited the flowers of all the species. The two pollen robbers, *Trigona iridipennis* and *Pithis binanira* (Figure 1 *a*) collected primarily pollen during their visits to the flowers. Sunbirds (*Nectarina zeylanica* and *Nectarina asiatica*) are also pollinators of *L. nepetifolia* and *L. aspera*, but are not observed in *O. thymiflorus* (Figure 1 *d*, *e*). Earlier, Solomon Raju[32] reported sunbirds as effective pollinators of *L. nepetifolia* and *L. aspera*. Vos *et al.*[33] reported that bees such as *Apis mellifera* and *Lassoglossum* sp. forage on the pollen of *L. nepetifolia*.

Sunbirds act as pollinators as well as robbers on *L. nepetifolia* and *L. aspera* flowers depending upon the quantum of available nectar. When they probe the flowers from the front, their bill contacts the stigma and anthers dorsally as it penetrates into the floral tube for nectar; in effect pollination occurs. Iwarsson[41] and Blundell[42] have reported that all *Leonotis* species are sunbird-pollinated, but the present study has reported a wide array of pollinators. Bees store pollen grains in the scopae of the hind leg and metasomal segments, whereas pollen is collected on the crown feathers of sunbirds[33]. Pollen grains were also found on the heads and long proboscis of captured butterflies and hawk months. It seems that while foraging for nectar, butterflies and hawk moths pollinated the flowers by transferring pollen grains from one flower to another. The peak period of activity of bees and butterflies was 0700–1200 h each day (Table 4). They fly rapidly and visit many flowers, spending on an average 2–4 s and 3–5 s at each flower respectively. Sunbirds spent on an average of 3–5 s at each flower. They visited fewers flowers than honey bees, as confirmed by visual observations in the field. In *L. aspera* and *O. thymiflorus*, one Dipteral member, i.e. *Pangania* sp. was an occasional visitor during the daytime.

In *O. thymiflorus*, an unidentified wasp was also noticed during close observations in the field. There was a strong relationship between the weather and butterfly activity. When it was sunny, butterflies actively visited the flowers of all the three species. However, when the weather was cloudy, butterflies were less active. On rainy days, they were completely inactive[43]. Cruden[44] suggested that flowers open for more than 12 h are exposed to visitations by a number of different diurnal and nocturnal pollinators. Ants are great lovers of nectar and regularly collect it from flowers of two species (Table 3). They have to reach the flowers by crawling up the stems and are unlikely to cause cross-pollination between different plants. However, Procter and Yeo[45] have reported that ants are legitimate pollinators of Rupturewort (*Herniaria ciliolata*).

Table 3. Floral visitors of the three species in Kalakkad Mundanthurai Tiger Reserve

Taxa	Order	Visitor	Common name	Foraging type	Foraging time
Leonotis nepetifolia (L.) R. Br.	Hymenoptera	*Apis cerana*	Honey bee	P + N	Day
		Apis florea	Honey bee	P + N	Day
		Apis dorsata	Honey bee	P + N	Day
		Trigona iridipennis	Bee	P	Day
		Pithitis binghami	Pollen bee	P	Day
		Xylocopa pubescens	Carpenter bee	P + N	Day
		Xylocopa latipes	Carpenter bee	P + N	Day
		Oecophylla smaragdina	Ant	N	Day + night
	Lepidoptera	*Jamides bochus*	Small butterfly	N	Day
		Junonia lemonias	Brown butterfly	N	Day
		Spindasis vulcanus	Spotted butterfly	N	Day
		Pachliopta aristolochaea	Common rose	N	Day
		Pachliopta hector	Common marmon	N	Day
		Delias eucharis	Yellow pancy	N	Day
		Azanus ubaldus	Blue tiger	N	Day
	Aves	*Nectarina zeylanica*	Purple sunbird	N	Day
		Nectarina asiatica	Yellow spot sunbird	N	Day
Leucas aspera (Willd.) Link	Hymenoptera	*Apis florea*	Honey bee	P + N	Day
		Apis dorsata	Honey bee	P + N	Day
		Apis indica	Honey bee	P + N	Day
		Xylocopa latipes	Carpenter bee	P + N	Day
		Trigona iridipennis	Bee	P	Day
		Camponotus compressus	Black ant	N	Day + night
		Anthophora Zonata	Pollen bee	N	Day
	Lepidoptera	*Azanus ubaldus*	Glossy blue tiger	N	Day
		Precis orithya	Pioneer butterfly	N	Day
		Delias eucharis	Yellow pancy	N	Day
		Pachliopta hector	Common mormon	N	Day
		Pachliopta aristolochaea	Common rose	N	Day
		Anaphaes aurota	Common rose	N	Day
		Eurema blanda	Gross yellow	N	Day
		Papilio demoleus	Lime butterfly	N	Day
		hosora chromus	Bonded awl	N	Day
		Macroglossum heliophila	Hawk moths	N	Night
		Macroglossum variegatum	Hawk moths	N	Night
	Aves	*Nectarina zeylanica*	Purple sunbird	N	Day
		Nectarina asiatica	Yellow spot sunbird	N	Day
	Diptera	*Pangania* sp.	Fly	N	Day
Orthosiphon thymiflorus (Roth) Sleesan	Hymenoptera	*Apis florea*	Honey bee	P + N	Day
		Apis dorsata	Honey bee	P + N	Day
		Apis indica	Honey bee	P + N	Day
		Trigona iridipennis	Bee	P	Day
		Xylocopa latipes	Carpenter bee	P + N	Day
		Megachile sp.	Cuttila bee	P + N	Day
	Lepidoptera	*Pachliopta clytia*	Common rose	N	Day
		Pachliopta hector	Common mormon	N	Day
		Pachliopta aristolochaea	Common rose	N	Day
		Parantica aglea	Dark blue tiger	N	Day
		Tirumala limniacea	Blue tiger	N	Day
		Precis hierta	Blue pancy	N	Day
		Azanus ubladus	Glossy tiger	N	Day
		Danus chrysipes	Plain tiger	N	Day
		Unidentified wasp	Wasp	P + N	Day
	Diptera	*Panganis* sp.	Fly	P + N	Day

Table 4. Nectar volume and concentration in relation to tube length at different time intervals (2 h) in three species (unbagged)

Taxa	Time interval (h)	Mean flower length (mm)	Mean petal tube length (mm)	Nectar/ flower (μl)	Nectar concentration (%)
Leonotis nepetifolia (L.) R. Br.	0700	24.37 ± 1.69	11.50 ± 1.48	07.400 ± 0.58	17.37 ± 0.68
	0900	23.00 ± 2.32	11.25 ± 1.20	07.735 ± 0.69	17.62 ± 1.17
	1100	23.32 ± 1.69	11.25 ± 1.18	07.560 ± 1.05	19.37 ± 0.74
	1300	22.12 ± 1.80	10.50 ± 2.05	06.100 ± 0.42	14.25 ± 0.54
	1500	22.50 ± 1.96	10.80 ± 0.98	06.000 ± 0.36	15.68 ± 0.96
	1700	23.50 ± 1.76	11.85 ± 1.46	06.800 ± 1.29	16.90 ± 0.25
Leucas aspera (Willd.) Link	0700	16.62 ± 0.51	08.15 ± 0.21	0.6550 ± 0.05	28.37 ± 4.45
	0900	14.25 ± 0.65	07.00 ± 0.13	0.7025 ± 0.07	28.50 ± 0.21
	1100	15.25 ± 0.32	07.87 ± 0.32	0.5900 ± 0.04	30.87 ± 1.12
	1300	15.50 ± 0.76	07.87 ± 0.42	0.5500 ± 0.12	32.37 ± 3.54
	1500	15.20 ± 0.67	07.50 ± 0.37	0.8020 ± 0.06	25.00 ± 1.05
	1700	15.58 ± 0.60	07.25 ± 0.25	0.7125 ± 0.04	25.68 ± 1.25
Orthosiphon thymiflorus (Roth) Sleesan	0700	14.37 ± 0.91	07.87 ± 0.64	0.7375 ± 0.47	34.25 ± 3.41
	0900	13.25 ± 0.46	07.27 ± 0.47	0.6625 ± 0.06	32.25 ± 1.48
	1100	13.00 ± 0.53	06.50 ± 0.53	0.7800 ± 0.03	31.50 ± 3.54
	1300	13.37 ± 0.51	06.87 ± 0.35	0.6870 ± 0.05	32.75 ± 2.18
	1500	13.50 ± 0.49	07.50 ± 0.29	0.6890 ± 0.09	33.65 ± 2.58
	1700	13.80 ± 0.43	07.80 ± 0.32	0.6900 ± 0.10	32.68 ± 3.18

Nectar analysis

Nectar is an aqueous solution consisting almost exclusively of sugars and only three of these occur in quantity, viz. sucrose, fructose and glucose[45]. Nectar secretion started 2 h before flower opening in all the three species, and increased soon after flower opening and continued till early morning. Nectar is secreted by nectar glands inside the corolla tube. The length of the tube varies in all three species. In *O. thymiflorus*, the corolla tube varies from 5 to 8 mm in length and in *L. aspera* it ranges from 6 to 9 mm, whereas the tube is long in *L. nepetifolia* (9–12 mm; Table 4). Nectar lies within 3, 5 and 7 mm of the tube length respectively, where it is accessible to all the foragers. Measurable volumes of nectar are produced in all the three species and volume correlates with the length of the corolla tube (Table 4). The nectaries lined in the long tube of *L. nepetifolia* produced 6–8 μl nectar in flowers undisturbed by pollinators. In *O. thymiflorus* as well as in *L. aspera*, the amount of nectar is lower than in *L. nepetifolia*, i.e. 0.5–0.8 μl/flower (Tables 2 and 4).

Nectar sugar concentration is lower in *L. nepetifolia* and higher in *O. thymiflorus* (Table 2). The changes in the concentration and volume of nectar in each species are well marked. The amount and composition of total sugars of nectar varied from hour to hour (Table 4). There are low and high peaks in nectar which are highly earmarked in all the three species. Most plants show daily peaks of nectar secretion and often of sugar concentration as well. The stress on plants caused by water shortage is greatest during the middle of the day, so that maximum nectar secretion easier at other times often; however it is in the middle of the day when maximum secretion takes place, which provides good evidence that the peak is an adaptation to the time of pollinator activity[40]. In addition to these variations, the concentration of nectar may be increased by evaporation or decreased by dilution with rain or removal by insects[46]. Goldblatt *et al.*[47] found that moth-pollinated species produce nectar of 26–31.7% mean sucrose equivalents. The present results of *L. aspera* correlate with these findings. Sunbird-pollinated species produce the largest volume of nectar, usually followed by large proboscis fly pollinators and moth-pollinated plant species. Nectar concentration shows the same pattern as observed in some genera of Iridaceae[48]. Bee- and moth-pollinated flowers produce the most concentrated nectar. Bird-pollinated flowers often produce relatively dilute nectar[47] and *L. nepetifolia* also shows this trend.

Sugar analysis has revealed that sucrose, glucose and fructose were the main components in the three species (Table 5). The ratio of glucose/fructose was more than one in all nectar samples. The glucose/fructose ratio was maximum in *L. aspera* and minimum in *L. nepetifolia*, whereas the fructose/glucose ratio was maximum in *L. nepetifolia* and minimum in *L. aspera*. The percentage of sucrose was higher in *L. aspera* than in *O. thymiflorus*. *O. thymiflorus* had greater concentration of glucose than the remaining two species (Table 5). Similar results have been reported earlier in *Medicago sativa* L. and *Parkinsonia aculeata*[49]. Floral nectar comparison has been related to pollinator type[2] and may interference with pollinator foraging and therefore, the plant reproductive success. Frost and Frost[50], and Gill and Conway[28] studied the nectar of *L. nepetifolia* and *Leonotis leonursus* and suggested the presence of sucrose, glucose and fructose,

Table 5. Sugar composition of nectar in the three species

Taxa	Flowering period	Percentage of total sugars				Ratio*	
		Glucose	Fructose	Sucrose	Maltose	G/F	F/G
Leonotis nepetifolia (L.) R. Br.	September–January	45.35 ± 1.95	36.05 ± 1.60	18.60 ± 1.83	0	1.25	0.77
Leucas aspera (Willd.) Link	June–February	30.48 ± 1.65	15.62 ± 1.81	53.90 ± 1.55	0	1.95	0.51
Orthosiphon thymiflorus (Roth) Sleesan	July–January	58.20 ± 3.55	31.50 ± 1.79	18.10 ± 1.09	0	1.84	0.54

*G/F, Glucose–fructose ratio; F/G, Fructose–glucose ratio.

which was also confirmed by Vos *et al.*[33] and Dafni *et al.*[34] for eight species of *Leonotis* and nine species of other Lamiaceae members, contrary to the findings of Percival[9]. Nectar also reflects the lineage with related plants sharing similar genetic constituents, which govern nectar production. Within Lamiaceae, hymenophily and sucrose-dominant nectar are thought to be ancestral. There is great dynamism in the nectar of all the three species due to the interaction of a number of factors that influence the amount and concentration of nectar present in the flowers. The volume of nectar also determines its concentration. The three species showed temporal variation in nectar production and concentration of total sugars. A variety of foragers comprising honey bees, butterflies, flies, ants, birds and hawk moths have been recorded. Honey bees (~ 66% of the total visits made by the insects) have been the major pollinators; birds and the butterflies are implicated as opportunists, rather than effective pollinators.

1. Reddy, T. B., Rangaiah, K., Reddi, E. U. B. and Reddi, C. B., Consequences of nectar robbing in the pollination ecology of *Vitex negundo* (Verbinaceae). *Curr. Sci.*, 1992, **62**(10), 690–691.
2. Fenster, C. B., Cheely, G., Dudash, M. R. and Reynolds, R. J., Nectar reward and advertisement in hummingbird pollinated *Silene virginia* (Caryophyllaceae). *Am. J. Bot*., 2006, **93**(12), 1800–1807.
3. Simpson, B. B. and Neff, J. L., Evolution and diversity of floral rewards. In *Handbook of Experimental Pollination Biology* (eds Jones, C. E. and Little, R. J.), Van Nostrand Reinhold, New York, 1983, pp. 117–141.
4. Baker, H. G. and Baker, I., Chemical constituents of nectar in relation to pollination mechanisms and phylogeny. In *Biochemical Aspects of Evolutionary Biology* (ed. Nitecki, M. H.), University of Chicago Press, Chicago, USA, 1982, pp. 131–171.
5. Baker, H. G. and Baker, I., The occurrence and significance of amino acids in floral nectar. *Plant Syst. Evol*., 1986, **151**, 175–186.
6. Baker, H. G., Baker, I. and Hodges, S. A., Sugar composition of nectars and fruits consumed by birds and bats in tropics and subtropics. *Biotropica*, 1998, **30**, 559–586.
7. Galetto, L. and Bernardello, L. M., Nectar sugar composition in angiosperms from Chaco and Patagonia (Argentina): an animal visitors matter? *Plant Syst. Evol*., 2003, **238**, 69–80.
8. Jurgens, A., Nectar sugar composition and floral scent compounds of diurnal and nocturnal *Conophytum* species (Aizoaceae). *South African Journal of Botany*, 2004, **70**, 191–205.
9. Percival, M. S., Types of nectar in angiosperms. *New Phytol*., 1961, **60**, 235–281.
10. Freeman, C. E., Worthington, R. D. and Jackson, M. S., Floral nectar sugar composition of some South and Southeast Asian species. *Biotropica*, 1991, **23**, 568–574.
11. Herrera, C. M., Perez, R. and Alonso, C., Extreme interplant variation in nectar sugar composition in an insect pollinated perennial herb. *Am. J. Bot*., 2006, **93**(4), 575–581.
12. Van Wyk, B. E., Nectar sugar composition in Southern African papilionoideae (Fabaceae). *Biochem. Syst. Ecol*., 1993, **21**, 271–277.
13. Barnes, K., Nicolson, S. W. and Van Wyk, B. E., Nectar sugar composition of *Erica*. *Biochem. Syst. Ecol*., 1995, **23**, 419–423.
14. Galetto, L. and Bernardello, L. M., Floral nectaries, nectar production dynamics and chemical composition in six *Ipomoea* species (Convolvulaceae) in relation to pollinators. *Ann. Bot*., 2004, **94,** 269–280.
15. Southwick, E. E., Nectar biology and nectar feeders of common milkweed, *Asclepias syriaca* Linn. *Bull. Torrey Bot. Club*, 1983, **110**(3), 324–334.
16. Southwick, E. E., Photosynthate allocation to floral nectar: a neglected energy investment. *Ecology*, 1987, **65**, 1775–1779.
17. Pyke, G., What does it cost the plant to produce floral nectar? *Nature*, 1991, **350**, 58–59.
18. Anand, C., Umranikar, C., Shintre, P., Damle, A., Kare, J., Joshi, J. and Watve, M., Presence of two types of flowers with respect to nectar sugar in two gregariously flowering species. *J. Biosci*., 2007, **32**, 769–774.
19. Heinrich, B., The foraging specialization of individual bumble bee. *Ecol. Monogr*., 1976, **46**, 105–128.
20. Wolf, L. L. and Hainsworth, F. R., Foraging efficiencies and time budges in nectar-feeding birds. *Ecology*, 1975, **56**, 117–128.
21. Heinrich, B., Resources partitioning among some eusocial insects: bumble bees. *Ecology*, 1976, **57**, 874–889.
22. Nicolson, S. W. and Van Wyk, B. F., Nectar sugars in Proteaceae: patterns and process. *Aust. J. Bot*., 1998, **46**, 489–504.
23. Nicolson, S. W., Pollination by passerine birds: why are the nectars so dilute? *Comp. Biochem. Phys. B*, 2002, **131**, 645–652.
24. Lotz, C. N. and Schondube, J. E., Sugar preferences in nectar- and fruit-eating birds: behavioural patterns and physiological causes. *Biotropica*, 2006, **38**(1), 3–15
25. Solomon Raju, A. J., Pollination ecology of the genus *Leonotis* (Lamiaceae). *Indian J. Bot. Res*., 2005, **1**(2), 243–256.
26. Ahmedullah, M. and Nayar, M. P., *Endemic Plants of the Indian Region: Lamiaceae*, Botanical Society of India, Calcutta, 1987.
27. Clark, L. J., *Wild Flowers of British Columbia*, Grays Publishing Ltd, Canada, 1973.
28. Gill, F. B. and Conway, C. A., Floral biology of *Leonotis nepetifolia* (L.) R. Br. (Labiateae). *Proc. Acad. Nat. Sci. Philadelphia*, 1979, **131**, 244–250.

29. Solomon Raju, A. J., Reproductive ecology of *Ocimum americanum* L. and *O. basilicum* L. (Lamiaceae) in India. *Plant Species Biol*., 1989, **4**, 107–116.
30. Solomon Raju, A. J., The explosive pollination mechanism and mating system of the weedy *Hyptis suavolens* (Lamiaceae). *Plant Species Biol*., 1990, **5**, 235–241.
31. Solomon Raju, A. J., The mint genus *Anisomeles* (Lamiaceae). *Proc. Indian Natl. Sci. Acad. Part B*, 1992, **58**, 209–216.
32. Solomon Raju, A. J., Reproductive biology of *Leonotis nepetifolia* R. Br. (Lamiaceae). *J. Natcon*., 1994, **6**, 1–10.
33. Vos, W. T., Edwards, T. J. and Van, S. J., Pollination biology of annual and perennial *Leonotis* species (Lamiaceae). *Plant Syst. Evol*., 1994, **192**, 1–9.
34. Dafni, A., Lenski, Y. and Fahn, A., Flower and nectar characteristics of nine species of Labiateae and their influence on honey bee visits. *J. Apic. Res*., 1988, **27**, 103–114.
35. Udayan, P. S., Tushan, K. V., George, S. and Balachandran, I., Notes on a new rare, endemic and red listed plants from the Western Ghats of Mundandurai forest Thirunelveli districts, Tamil Nadu, India. *Indian For*., 2008, **134**(1), 88–96.
36. Dafni, A., Kevan, P. G. and Husband, B. C., *Practical Pollination Biology*, Enviroquest Ltd, Cambridge, Ontario, Canada, 2005.
37. Dafni, A., *Pollination Ecology: A Practical Approach*, Oxford University Press, Oxford, London, 1992.
38. Patridge, S. M., Filler paper partition chromatography of sugars. *Biochem. J*., 1948, **42**, 238.
39. Yamm and Willis, A. J., The estimation of carbohydrates in plant extracts by anthrone. *Biochem. J*., 1954, **57**, 508.
40. Jagadish, K. S., Gowda, T. N. V., Lakshmikantha, B. P. and Gowda, A. N. S., Foraging behaviour of honey bee and other insect visitors on the bloom of two medicinal herbs, *Hygrophila auriculata* (Schum) Heine and *Leucas aspera* (Willd.) Link. *Pest Manage. Econ. Zool*., 2002, **10**(1), 67–70.
41. Iwarsson, M., Leonotis. *Flora S. Afr*., 1985, **28**, 31–37.
42. Blundell, M., *Collins Guide to the Wild Flowers of East Africa*, Collins, London, 1987.
43. Tian, J., Keming, L. and Guangwan, H., Pollination ecology and pollination system of *Impatiens reptans* (Balsaminaceae) endemic to China. *Ann. Bot*., 2004, **93**, 167–175.
44. Cruden, R. W., Reproductive ecology of weedy and cultivated *Mirabilis* (Nyctaginaceae). *Am. J. Bot*., 1973, **60**, 802–809.
45. Proctor, M. and Yeo, P., The pollination of flowers. In *The New Naturalist: A Survey of British Natural History* (eds Huxley, J. *et al*.), Collins, London, 1973.
46. Jaeger, P., Les aspects actuels du probleme de lentomogamie. *Bull. Soc. Bot. Fr*., 1957, **104**, 179–222, 352–413.
47. Goldblatt, P., Bernhardt, P. and Manning, J. C., Evidence of bird pollination in the Iridaceae of Southern Africa. *Adansonia*, Ser. 3, 1999, **21**, 25–40.
48. Bernhardt, P. and Goldblatt, P., The role of phylogenetic constraints in the evolution of pollination mechanism in the Iridaceae of Sub-Saharean Africa. In *Monocots: Comparative Biology and Evolution* (eds Columbus, J. J. *et al*.), Rancho Santa Ana Botanic Garden, Claremont, California, 2006, pp. 434–444, vol. 1.
49. Jain, K. L. and Kapil, R. P., Foraging rhythm of megachilid bees in relation to the flowering of *Medicago sativa* L. and *Parkinsonia aculeata* L. *Indian Bee J*., 1980, **42**, 35–38.
50. Frost, S. K. and Frost, P. G., Territoriality and changes in resources use by sunbirds at *Leonotis leonursus* (Lamiaceae). *Oecologia* (*Berlin*), 1980, **45**, 109–116.

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