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On the reproductive ecology of *Premna latifolia* L. and *Premna tomentosa* Willd. (Lamiaceae)

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Abstract: *Premna latifolia* and *Premna tomentosa* shed their foliage during winter but produce foliage at different periods to avoid competition for nutrients, *P. latifolia* during the dry season while *P. tomentosa* during the wet season. Flowering also occurs at different seasons, *P. latifolia* during the wet season and *P. tomentosa* during the dry season. The flowering period of *P. latifolia* is comparatively longer than *P. tomentosa*; however, both of them display profuse flowering for 2–3 weeks only. The flowers are of gullet type, weakly bilabiate, and the stamens and stigma are situated near the upper corolla lip and are dichogamous, herkogamous and facultatively xenogamous in both species. In *P. latifolia, the* pollinators are wasps and butterflies while in *P. tomentosa*, in addition to wasps and butterflies, the bees and flies are also pollinators. *Camponotus* ants rob the nectar of *P. latifolia* by removing the entire corolla together with stamens and sometimes also with the style and stigma. Both the species display ornithochory, anthropochory and anemochory. The seeds germinate during wet season in both the species irrespective of their time of dispersal but their continued growth depends on the soil nutrient and moisture environment.

Keywords: Lamiaceae, pollination, *Premna latifolia, Premna tomentosa*, reproductive ecology.

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INTRODUCTION

The family Lamiaceae shows a wide spectrum of specialization in pollination (Huck 1992), with some species displaying classical examples of generalization (Herrera 1989), while other species appear to be fairly specialized (Nilsson et al. 1985; Wester & Claßen-Bockhoff 2006a,b). Lamiaceae members basically show flag and gullet blossoms; the former is adapted for sternotribic pollination whilst the latter for nototribic pollination (Faegri & van der Pijl 1979). Sternotribic pollination is most naturally performed by dipterans such as syrphids and primitive hymenopterans whereas nototribic pollination is performed by advanced hymenopterans, especially bees (van der Pijl 1972; Beker et al. 1989). In this family, dichogamy, herkogamy and self-compatibility are widespread (Owens & Ubera-Jimenez 1992). Different workers reported on seed dispersal aspects of certain Lamiaceae members but they have not drawn any generalizations regarding the predominant modes of seed dispersal (Ingrid 1987; Charles-Dominique 1993; Green 1999; Ahenda 1999; Zhi-Yong et al. 2007; Rogers et al. 2008; Santhoshkumar & Balasubramanian 2011). The species studied by them, however, display zoochory (especially endozoochory), myrmecochory, anemochory, barochory and hydrochory, and zoochory has been found to be the dominant mode of seed dispersal. This information suggests that a few species of Lamiaceae have been studied in detail for their reproductive biology despite it being one of the commercially important families. Therefore, this state of information warrants the study of the reproductive biology of individual species of Lamiaceae to understand their sexual reproduction and take measures for the protection of their habitats along with their pollinators and seed dispersers.

The genus *Premna* L. was traditionally included under the family Verbenaceae but in recent classifications it is treated under the family Lamiaceae (Harley et al. 2004; APG 2016). This genus consists of about 200 species distributed mainly in the tropical and subtropical regions of Asia, Africa, and Australia (Harley et al. 2004). Thirty one species and six varieties of *Premna* are reported from India (Rajendran & Daniel 2002). Leeratiwong (2008) reported that most *Premna* species are widespread but a series of morphologically closely related and generally geographically restricted species occur from India to New Guinea. deKok (2013) stated that most species of this genus are small trees or shrubs and rarely lianas and identified two basic calyx types based on the species distributed in Southeast Asia. The first type has four isomorphic lobes and its shape remains largely intact when the flower develops and fruits are formed. The second type has zero to five heteromorphic lobes and it increases in size during flower and fruit development; in most species, it obscures the number of lobes and their original shape. Further, de Kok recognized two fruit types: one is a globose drupe-like fruit consisting of four somewhat fleshy mericarps, each with one seed; the second type is a clavoid drupe-like fruit consisting of somewhat fleshy mericarp with one seed. In the second type initially four seeds develop but only one seed matures fully while the other three develop partially. Munir (1989) stated that *Premna* can readily be identified by its calyx and corolla being 4- or 5-lobed, corolla-tube mostly much dilated above the calyx, stamens and style scarcely or shortly exserted above the corolla lobes and drupe composed of one 4-locular pyrenes. Bodlah et al. (2016) reported that *P. foetida* is an important source of forage for bee-flies, *Thyreus himalayensis* and *T. ramosus* in Pakistan. Chopra et al. (1992) and Hymavathi et al. (2009) noted that different parts of *P. latifolia* are used in the traditional system of medicine for treatment of dropsy and boils. Various parts of the plant have been reported to have antioxidant and anticancer activity. Subbareddi (1990) reported that *P. latifolia* flowers are small, pollinated by the wasp, *Ropalidia spatulata* while collecting nectar. *Premna tomentosa* grows in primary to secondary forest, beaches or open types of vegetation. Flowering occurs during February-November while fruiting during May–September, rarely in February. The flowers are visited by insects while the fruits are eaten by birds.

Santhoshkumar & Balasubramanian (2011) reported that the Indian Grey Hornbill *Ocyceros birostris* is a legitimate seed disperser of *Premna tomentosa* in the Eastern Ghats, India. It consumes the fruits and excretes the seed undamaged; such seeds have enhanced germination potential. Since there is hardly any information on the reproductive ecology of *Premna* species, the present study is contemplated to provide information on phenology, floral biology, pollination, pollinators, sexual system, breeding system, fruiting behaviour and seed dispersal of *Premna latifolia* L. and *Premna tomentosa* Willd. The information will help to understand its sexual reproduction and the reasons for their scattered distribution.

MATERIALS AND METHODS

Twenty scattered individuals of *P. latifolia* growing at Sunkarimetta forest block of Araku Valley Reserve Forest in Visakhapatnam District ($17^0$15'–$18^0$32'N & $18^0$54'–

83^{0}30’E, 900m) and eighteen scattered individuals of *P. tomentosa* at Srungavarapukota (18^{0}07’N & 83^{0}10’E, 70m) in Vizianagaram District in the state of Andhra Pradesh, India, were used for the study.

Prior to collecting the data, visits were made to the study areas to have an idea on the timings of leaf fall, leaf flushing and flowering events. Later, field visits were made once in two weeks during 2015 and 2016 to record the timings of these three phenological events. The phenological observations were made from 07:00–18:00hr. The inflorescence type and the number of flowers per inflorescence were noted. Ten inflorescences prior to commencement of their flowering were tagged and followed daily to record the flowering duration. Twenty-five fresh flowers were used to record the flower type, sex, shape, colour, odour, symmetry, calyx, corolla, stamens and style. The floral configuration and rewards presentation aspects were examined in relation to the forage collection activity of insects. Anthesis was initially recorded by observing the marked inflorescences in the field. Later, the observations were made 3 to 4 times on different days in order to record the accurate anthesis schedule. Similarly, the mature buds were followed to record the time of anther dehiscence. The pollen presentation pattern was also investigated by recording how anthers dehisced and the same was confirmed by observing the anthers under a 10x hand lens. The presence of nectar was determined by gently pulling a flower from its calyx and firmly pressing its base against a hard surface. The protocols provided by Dafni et al. (2005) were used for measuring the nectar volume, sugar concentration, sugar types and amino acid types, and stigma receptivity. The method described by Cruden (1977) was used for calculating pollen ouput/anther/flower and pollen-ovule ratio.

After making preliminary observations on the foraging activities of insects, a thorough knowledge of the local insect species was obtained by observing the representative species available with the Department of Environmental Sciences, Andhra University, Visakhapatnam. All butterflies were identified to species level by consulting the books of Kunte (2007) and Gunathilagaraj et al. (1998) while other insects, some to species level while a few others to genus level only. The efforts to get the specimens identified to species level for the species, which were identified up to genus level by the Zoological Survey of India, Government of India were not successful during the study period. The insect species were observed visually and by using binoculars; the insect species that could not be identified on the spot were captured and later identified with the help of the identified specimens available in the Department. The foraging activities of insects were recorded for 10 min at each hour for the entire day on 3 or 4 occasions depending on the possibility and the data was tabulated to use the same for further analysis, especially to understand the foraging activity rate at different times of the day. For each species, 20–30 inflorescences were selected to record the foraging visits of insects. The data thus obtained were used to calculate the percentage of foraging visits made by each category of insects per day to evaluate their association and pollination role. The insects feeding on nectar and/or pollen were carefully observed to assess their role in effecting pollination. They were observed on a number of occasions for their foraging behaviour such as mode of approach, landing, probing behaviour, contact with essential organs to result in pollination, inter-plant foraging activity in terms of cross-pollination, etc. Based on this data, the association between floral rewards and insects was assessed. Further, nectar-robbing activity exhibited by an ant was carefully observed to record its role in the sexual life of the plants. Ten individuals of each insect species were captured while collecting pollen and/or nectar on the flowers; the collection was done during their peak foraging activity period. The captured specimens of insects were brought to the laboratory, washed in ethyl alcohol, stained with aniline-blue on a glass slide and observed under microscope to count the number of pollen grains present and evaluate their relative pollen carryover efficiency and pollination role.

Mature flower buds of some inflorescences on different individuals were tagged and enclosed in paper bags. They were tested in the following way for each mode of pollination.

1. The stigmas of flowers were pollinated with the pollen of the same flower manually by using a brush and bagged to observe manipulated autogamy.
2. The flowers were fine-mesh bagged as such without hand pollination to detect spontaneous autogamy.
3. The emasculated flowers were hand-pollinated with the pollen of a different flower on the same plant and bagged to test geitonogamy.
4. The emasculated flowers were pollinated with the pollen of a different individual and bagged to detect xenogamy.

All these categories of flower pollinations were kept under regular observation until fruit set. Then, the percentage of fruit set and seed set was calculated. The flowers/inflorescences were tagged on ten individuals prior to anthesis and followed for fruit and seed set in open-pollinations. The resulting fruit and seed output were pooled up for calculating fruit and seed set rates. Fruit maturation period, the fruit and seed characteristics were recorded. Field observations were made regularly to record fruit and seed dispersal modes. Casual observations were also made to record whether the seeds germinate immediately after they are dispersed or not.

RESULTS

Premna latifolia L.

It is a small or medium-sized semi-evergreen tree with a spreading crown (Image 1a). It grows to be a small tree in water-stress areas but it attains a robust medium-size near streams. The bark is greyish-white while the young branchlets are grey-pubescent. Branches and branchlets are sub-terete, or obtusely quadrangular, slightly lenticellate and nodes annulate. Leaves are petiolate (20mm long), simple, opposite, ovate to elliptic-ovate, base rounded, tip tapering, margin entire or saw-toothed, finely velvet-hairy above and hairless below, membranous. Leaf fall occurs during December–February. Leaf flushing occurs between late January and mid-April. Flowering begins in June, reaches the peak during July–August and ceases in November. Individual plants flower for about four months but show profuse flowering for about three weeks only. The flowers are borne in flat-topped 60–80 mm long compact corymbose inflorescences that are produced at branch-ends (Image 1b,c). Individual inflorescences produce 101 ± 56 flowers over a period of one week.

Flower Morphology

The flowers are pedicellate (10mm long), small (4.3±0.4 mm long and 4.2±0.3 mm wide), creamy white, mildly scented, bisexual and somewhat zygomorphic. The calyx is 2.7±0.4 mm long, cup-shaped, obscurely bi-lipped, upper lip 2-lobed, lower lip 3-lobed and pubescent outside. The corolla is white, tubular (2mm long) at base, obscurely bi-lipped; upper lip is 2-lobed, sub-orbicular, apex emarginate while lower lip is 3-lobed, obovate and blunt. The corolla tube is slightly narrow, glabrous, densely hairy at the throat and puberulous outside (Image 2d). The stamens are 4, inserted in the middle of the tube by slender creamy white filaments, didynamous, long stamens 5.4±0.4 mm long and short stamens 4.9±0.2 mm long, exserted; anthers are globose, 1mm long, 2-loculed, black and have versatile fixation. The ovary is superior, globose, 2.5mm long, bicarpellary syncarpous with four locules by a false septum; each locule is 1-ovuled, erect and anatropous on axile placentation. The style is 5mm long, linear and stigma shortly bifid (Image 2f–i). The stigma is situated either at the height of short or long stamens.

Floral Biology

The mature buds with stamens and pistil inside open during 07:00–10:00 hr with peak anthesis during 08:00–09:00 hr by diverging petal lobes. Petals slightly stretch out and reflex exposing the stamens, style and stigma beyond the rim of the corolla tube and height of the corolla lobes (Image 2a–c). During and immediately after anthesis, the stigma stands at the height of short stamens, grows further and then stands at the height of long stamens. But, the anthers and stigma never contacted each other during flower life although they are situated in close proximity to each other. The anthers dehisce during anthesis by longitudinal slits exposing the creamy white pollen. After the pollen is shed, the stamens slightly bend toward the stigma. After one hour of anther dehiscence, the stigma attains receptivity by stretching out its lobes gradually and ceases its receptivity by the evening of the 2nd day. While the stigma is in a receptive phase, the corolla and stamens fall off together by the evening of the day of anthesis. The style and stigma wither and dry up on the 3rd or 4th day. The calyx is persistent and remains so without any

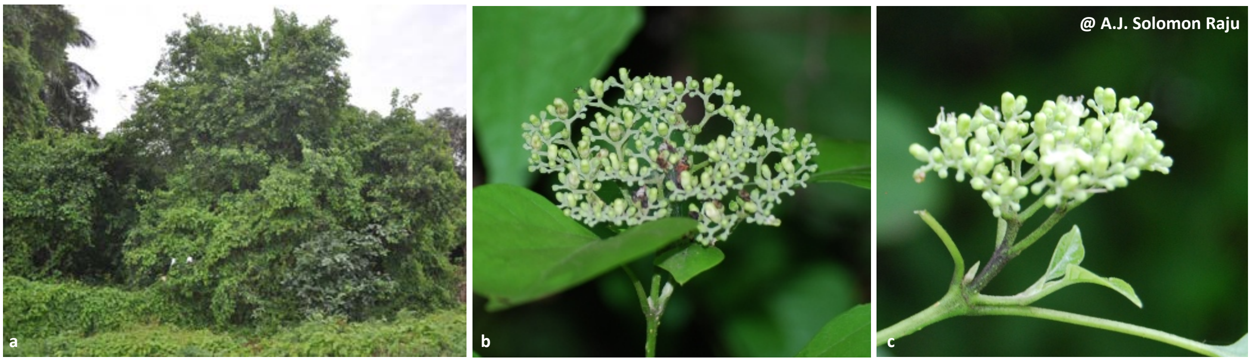

Image 1. *Premna latifolia:* a. Individual tree, b. Inflorescence with buds, c. Inflorescence with buds and flowers.

further development until fruit drop. The flowers are morphologically and functionally hermaphroditic. Weak protandry facilitates self-pollination at flower and plant level but self-pollination does not occur due to lack of physical contact between the stamens and stigma. The flowers, however, facilitate autogamy, geitonogamy and xenogamy mediated by insect vectors.

The pollen output per anther is 714±43.63. The total pollen productivity in individual flowers is 2,856±174.55. The pollen-ovule ratio is 714: 1. The pollen grains are spheroidal, tricolpate, prolate, 34.3µm long and 45µm wide (Image 2e). Nectar is secreted around the ovary inside the corolla tube during post-anthesis period on the day of anthesis only. Its secretion is gradual from anthesis onwards, reaches its peak after three hours and decreases gradually thereafter towards evening. A flower produces 3.2±0.42 µl of nectar. Half of the tube part of the corolla is filled with nectar. The nectar sugar concentration varied from 21–34 % and it averaged to 28±5.4 %. The sugars present in the nectar include sucrose, glucose and fructose of which sucrose is the dominant one. The total sugar content in the nectar of a flower is 1.01mg. The nectar also includes the essential amino acids such as lysine and tryptophan, and the non-essential amino acids such as cystine, hydroxyproline and tyrosine.

Breeding Systems

The result of breeding system indicates that the flowers are self-compatible and self-pollinating. The fruit set is absent in autonomous and un-manipulated autogamy, 20% in manipulated autogamy, 37% in geitonogamy, 59% in xenogamy and 42% in open pollinations. Seed set rate is constant and it is 25% in all modes of pollinations due to production of only one seed per fruit (Table 1). But, seeds produced from each mode of pollination were not examined for their viability and germination potential.

Foraging Activity and Pollination

The flowers expose the stamens and stigma beyond the rim and lobes of the corolla upon anthesis. The corolla lobes stretch out and reflex partially facilitating access to nectar by insects. The flowers were foraged by 13 wasp species, 16 butterfly species and one ant species during the day; all these insects foraged exclusively for nectar (Table 2). The wasps were *Scolia* sp. (Image 3e,f), *Campsomeris annulata* (Scoliidae, Image 3g), *Stilbum superbum*

Image 2. *Premna latifolia:* a. Bud, b. & c. Anthesed flowers showing different positions of stigma in relation to the stamens, d. Hairs inside the corolla tube, e. Pollen grain, f. Ovary, style and stigma, g. Ovary, h. Bifid stigma, i. Ovules.

Table 1. Breeding systems in *Premna latifolia*

Mode of breeding system	Number of flowers sampled	Number of flowers set fruit	Fruit set (%)	Seed set (%)
Un-manipulated Autogamy	25	0	0	0
Manipulated Autogamy	25	5	20	25
Geitonogamy	56	21	37	25
Xenogamy	78	46	59	25
Open-pollination	1408	586	42	25

(Chrysididae, Image 3d), *Eumenes conica* (Image 3h), *E. petiolata* (Image 4a), *Eumenes* sp. (Image 4b), *Phimenes flavopictus* (Eumenidae, Image 4c), *Rhynchium* sp. (Image 4d,e), *Polistes* sp. (Image 4f), *Parapolybiavaria* (Vespidae, Image 4g), *Ectemnius* sp. (Crabronidae Image 4h), *Sphex ichneumoneus* (Image 4i) and *Sphex* sp. (Sphecidae Image 4j,k). The ant was *Camponotus* sp. The butterflies were *Graphium doson* (Image 5a), *G. agamemnon* (Papilionidae, Image 5b), *Catopsilia pomona* (Image 5c), *Eurema hecabe* (Image 5d), *Pieris canidia* (Image 5e), *Anaphaeis aurota* (Pieridae Image 5f), *Junonia lemonias* (Image 5g), *J. hierta* (Image 5h), *Precis iphita* (Image 5i), *Danaus chrysippus* (Image 5j), *Euploea core* (Nymphalidae Image 5k), *Leptotes plinius* (Image 5l), *Jamides alecto* (Image 5m), *Spindasis vulcanus* (Lycaenidae Image 5n), *Hasora chromus* (Image 5o) and *Suastus gremius* (Hesperiidae Image 5p). All these insects foraged during the day from 08:00–17:00 hr with more activity during 10:00–11:00 hr. But, individual species showed variation in their activities by visiting the flowers either continuously or with one or two hours break or stopped after noon (Figs. 1, 2). Among wasps, scolid, chrysidid, eumenid, and the Vespid, *Rhynchium* visited the flowers more frequently. The ant made a fewer visits to the flowers. Among butterflies, the papilionid, *Graphium agamemnon*, all pierids, and Nymphalids, *Junonia lemonias* made more visits to the flowers. The wasps formed 49%, ants 4% and butterflies 47% of total foraging visits (Fig. 3). The body washings of these foragers collected from the flowers during peak foraging activity revealed that they carried pollen on their bodies. The mean number of pollen grains varied from 18.9 to 50.4 in the case of wasps (Table 3) and from 21.3 to 52.5 in the case of butterflies (Table 4).

All wasps irrespective of their body size invariably approached the flowers in upright position, landed on the flat-topped corymbs and probed individual flowers for nectar with great ease due to the wide corolla throat. Further, the wasps with their sharp tongue (proboscis) penetrated easily through the mass of hair present at the corolla throat to access nectar. In this act, their forehead had contact with both the stamens and stigma facilitating the pollination. Further, their ventral side had contact

Image 3. *Premna latifolia:* a-c. *Camponotus* sp. collecting nectar from flower base by removing corolla together with stamens and sometimes also with style and stigma, d. *Stilbum superbum*, e. & f. *Scolia* sp., g. *Campsomeris annulata*, h. *Eumenes conica*

Table 2. List of insect foragers on *Premna latifolia*

Order	Family	Genus	Species	Common Name	Forage Sought
Hymenoptera	Scoliidae	*Scolia*	sp.	Blue Winged Wasp	Nectar
		Campsomeris	*annulata* F.	Flower Wasp	Nectar
	Chrysididae	*Stilbum*	*superbum* Spinola	Cuckoo Wasp	Nectar
	Eumenidae	*Eumenes*	*conica* F.	Potter Wasp	Nectar
		Eumenes	*petiolata* F.	Potter Wasp	Nectar
		Eumenes	sp.	Potter Wasp	Nectar
		Phimenes	*flavopictus* Zimmerman	Tiger wasp	Nectar
	Vespidae	*Rhynchium*	sp.	Black Potter Wasp	Nectar
		Polistes	sp.	Hunts Man Spider	Nectar
		Parapolybia	*varia*	Paper Wasp	Nectar
	Crabronidae	*Ectemnius*	sp.	Solitary Wasp	Nectar
	Sphecidae	*Sphex*	*ichneumoneus* L.	Great Golden Digger Wasp	Nectar
		Sphex	sp.	Great Black Wasp	Nectar
	Formicidae	*Camponotus*	sp.	Black Carpenter Ant	Nectar
Lepidoptera	Papilionidae	*Graphium*	*doson* C& R Felder	Common Jay	Nectar
		Graphium	*agamemnon* L.	Tailed Jay	Nectar
	Pieridae	*Catopsilia*	*pomona* F.	Common Emigrant	Nectar
		Eurema	*hecabe* L.	Common Grass Yellow	Nectar
		Pieris	*canidia* L.	Indian Cabbage White	Nectar
		Anaphaeis	*aurota* F.	Caper White	Nectar
	Nymphalidae	*Junonia*	*lemonias* L.	Lemon Pansy	Nectar
		Junonia	*hierta* F.	Yellow Pansy	Nectar
		Precis	*iphita* Cramer	Chocolate Pansy	Nectar
		Danaus	*chrysippus* L.	Plain Tiger	Nectar
		Euploea	*core* Cramer	Common Indian Crow	Nectar
	Lycaenidae	*Leptotes*	*plinius* F.	Zebra Blue	Nectar
		Jamides	*alecto* Felder.	Metallic Cerulean	Nectar
		Spindasis	*vulcanus* F.	Common Silverline	Nectar
	Hesperiidae	*Hasora*	*chromus* Cramer	Common Banded Awl	Nectar
		Suastus	*gremius* F.	Indian Palm Bob	Nectar

with the stamens and stigma causing pollination while moving from flower to flower on the corymbs. They never visited the flowers that did not have corolla, stamens and stigma intact. Their ventral side, however, had contact with the flowers that have only the ovary with style and stigma intact while approaching and searching for the fresh and rewarding flowers for nectar; such a contact was considered to facilitate pollination.

The ant, *Camponotus* was a resident forager crawling all over the plant during the day. At the time of nectar collection, it crawled to the corymbs and removed the corolla together with stamens and sometimes also the style and stigma to collect nectar situated around the ovary (Image 3a–c). Hence, the ant was treated to be a nectar robber and an agent negatively affecting the success rate of sexual reproduction to a certain extent.

All butterflies recorded were found to collect only nectar. They approached the flowers in upright position, landed and/or hovered at the flowers to collect nectar. They fluttered their wings in vertical position and inserted their proboscis through the hairy throat of the corolla tube to collect nectar. The length of proboscis of all butterflies enabled them to reach the flower base with great ease. During nectar collection, their proboscis and forehead came into contact with the stamens and stigma facilitating pollination. They never visited the flowers that did not have corolla with stamens and stigma intact. Their abdomen, however, came in contact with the flowers

Image 4. *Premna latifolia:* a. *Eumenes petiolata*, b. *Eumenes* sp., c. *Phimenes flavopictus*, d. & e. *Rhynchium* sp., f. *Polistis* sp., g. *Parapolybia varia*, h. *Ectemnius* sp., i. *Sphex ichneumoneus*, j. & k. *Sphex* sp.

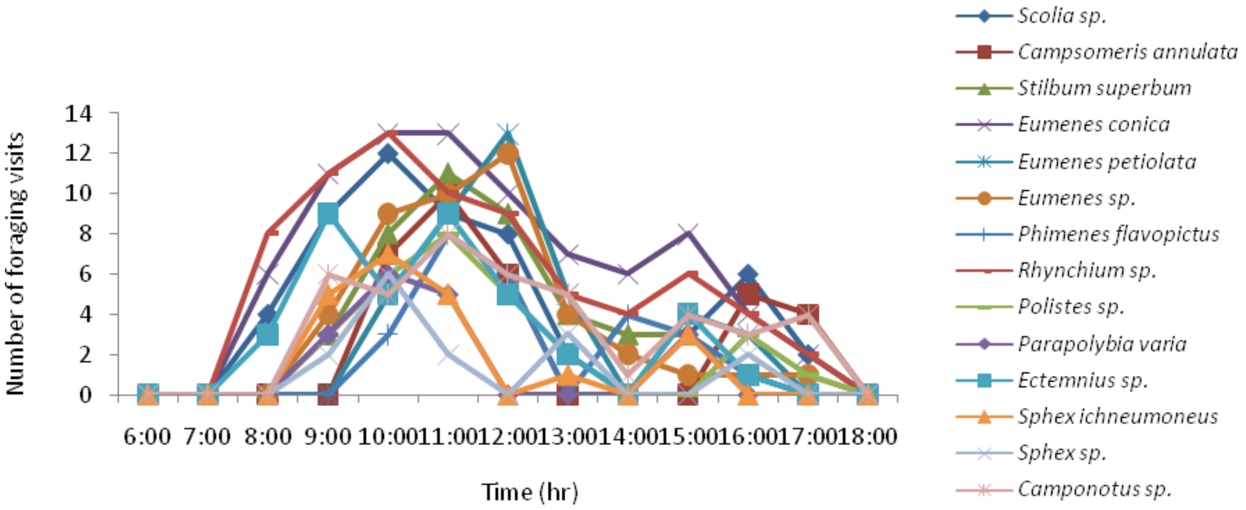

Figure 1. Hourly foraging activity of wasps and ant on *Premna latifolia*

that only had the ovary with style and stigma intact while approaching and searching the flowers for nectar; such a contact was considered to facilitate pollination.

The wasps and butterflies visited the flowers in quick succession and swiftly moved from one corymb to the other on the same and different plants for nectar and such a foraging activity was considered to be effecting both self as well as cross-pollinations. Several wasps and butterflies visited the flowers of the same plant at the same time harmoniously and such an activity was very pronounced during the peak flowering phase. Such intense foraging activity was found to be important to drive them off to different plants in search of more nectar and such an activity could be promoting out-crossing rate.

Fruiting Behavior

Pollinated and fertilized flowers initiate fruit development immediately and take approximately four weeks to produce mature fruits (Image 7a). The fruit is nearly a globose indehiscent drupe, 6x4 mm across, green initially, bluish-black or black upon ripening, and shiny (Image 6a–d). The drupe produces one fully developed seed and three partially or aborted seeds enclosed by the outer fleshy layer (Image 6f,g). The seed is oblong, cream-coloured and 3.6x3 mm across (Image 6e). Fruit dispersal occurs during July–October. The fleshy fruits against the green foliage are quite attractive to birds. Birds such as *Acridotheres tristis* Indian Myna (Image 7b–d), *Corvus splendens* House Crow, *Corvus macrorhynchos* Jungle Crow and *Turdoides caudatus* Common Babbler

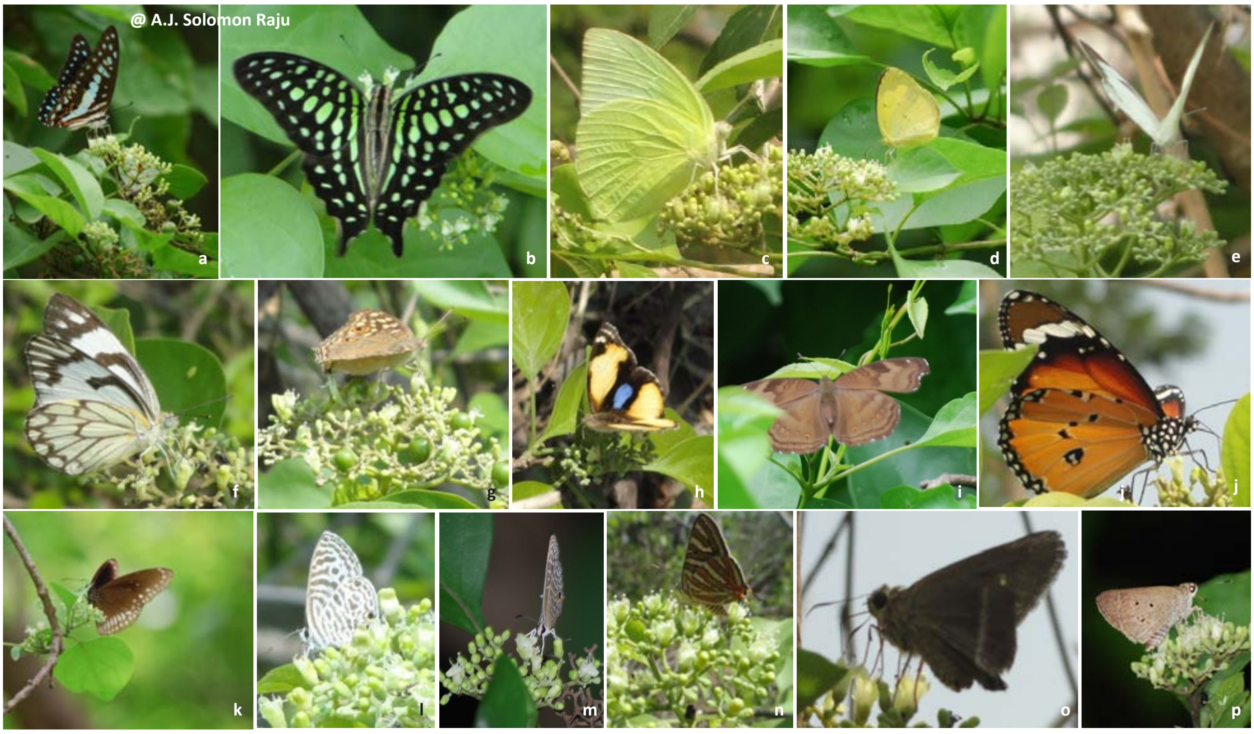

Image 5. *Premna latifolia*: a. & b. Papilionids – a. *Graphium doson*, b. *Graphium agamemnon*, c-f. Pierids – c. *Catopsilia pomona,* d. *Eurema hecabe*, e. *Pieris canidia,* f. *Anaphaeis aurota*, g-k. Nymphalids – g. *Junonia lemonias*, h. *Junonia hierta*, i. *Precis iphita*, j. *Danaus chrysippus*, k. *Euploea core*, l-n. Lycaenids – l. *Leptotes plinius*, m. *Jamides alecto*, n. *Spindasis vulcanus*, o. & p. Hesperiids – o. *Hasora chromus*, p. *Suastus gremius*

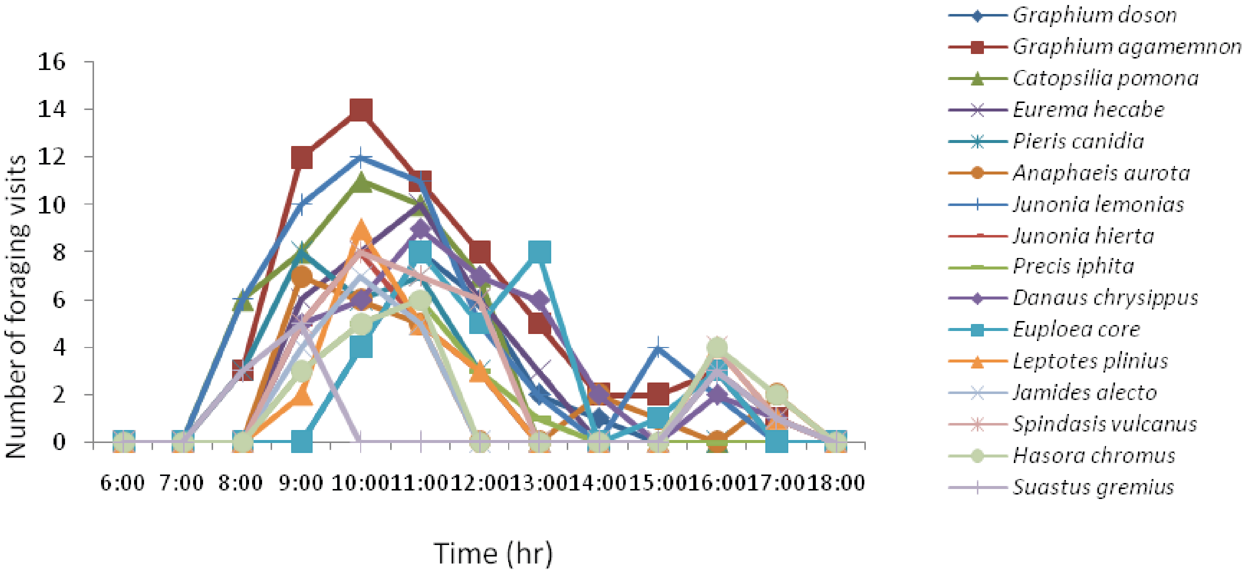

Figure 2. Hourly foraging activity of butterflies on *Premna latifolia*

were found to feed on the pulp of the drupe and in the process disperse them to different places. The dry drupe free from the fleshy part also disperses easily by wind due to the dry season. Local children eat the fleshy part of the fruit and throw the drupe and in this way they also take part in the dispersal of seeds. Hence, ornithochory (bird dispersal), anemochory (wind dispersal) and anthropochory (dispersal by humans) are functional in this species. Seeds germinate during rainy season soon after the occurrence of the first monsoon rains in June.

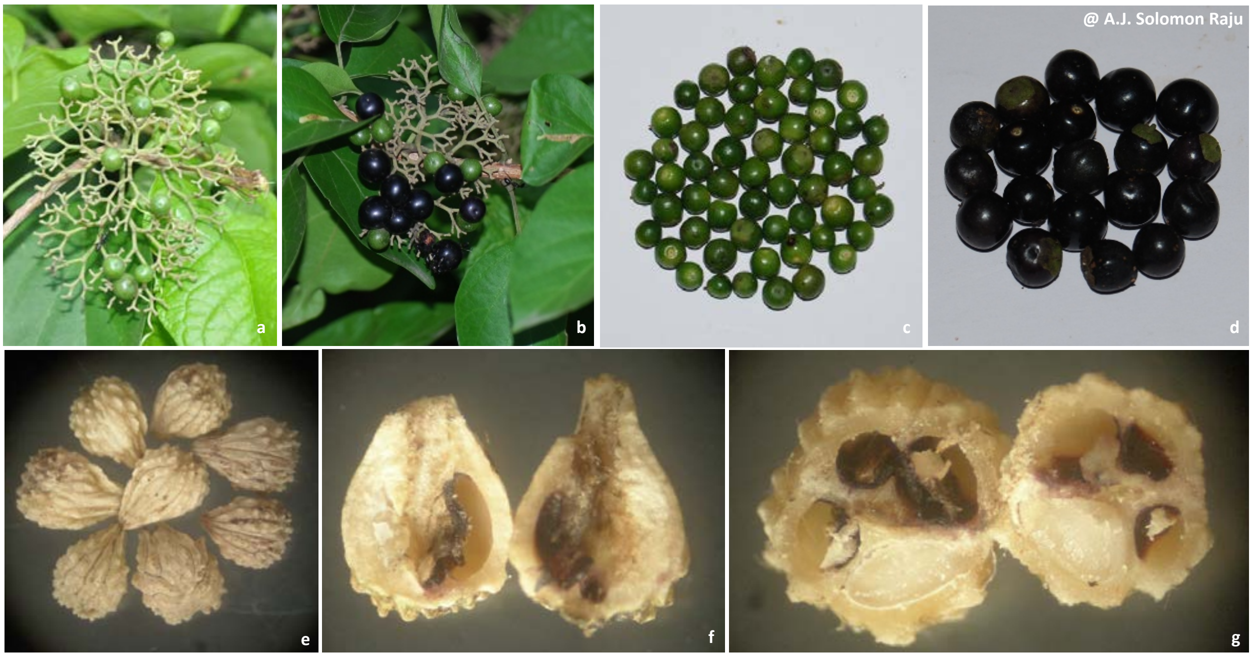

Image 6. *Premna latifolia*: a. Immature fruits, b. Ripe fruits, c. Green immature fruits, d. Ripe black pulpy fruits, e. Seeds, f. & g. Seed abortion during seed formation phase of the fruits.

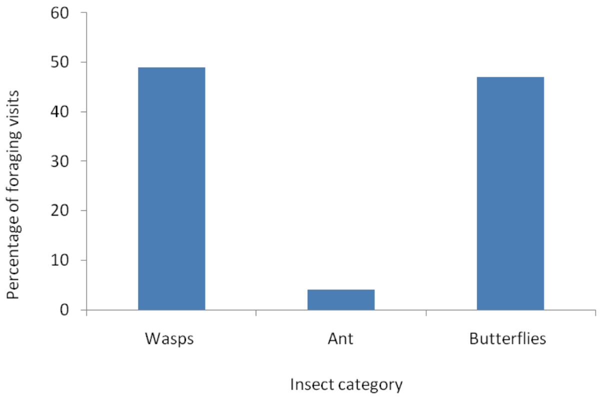

Figure 3. Percentage of foraging visits of different categories of insects on *Premna latifolia*

The seedlings grow and produce new plants if there is enough moisture in the soil, otherwise they soon perish.

Premna tomentosa Willd.

It is a small or medium-sized semi-evergreen tree with a spreading crown. It grows both in water-stressed areas and by the side of streams. The bark is greyish-brown, smooth, and young parts with velvety hairs. Branches and branchlets are stellately yellow tomentose. Leaves are petiolate, simple, opposite, slender, velvet-hairy, broadly ovate, base cordate, tip long-pointed, margin entire, densely tawny yellow, stellately tomentose on both sides, yellowish and papery. Leaf fall occurs during January–February (Image 8a), leaf flushing during June-September (Image b–e, Image 9a) and flowering during March–May (Image 9b,c). Individual plants flower for five weeks but show profuse flowering for about two weeks only. The flowers are borne in flat-topped 70–75 mm long compact corymbose inflorescences which are produced in axillary and terminal positions. Individual inflorescences produce 144.25± 53.1 flowers over a period of one week.

Table 3. Pollen recorded in the body washings of wasps on *Premna latifolia*

Insect species	Sample size (N)	Number of pollen grains		
		Range	Mean	S.D
Scolia sp.	10	23–67	50.4	11.52
Compsomeris annulata	10	12–41	32.1	8.62
Stilbum superbum	10	7–32	22.1	7.50
Eumenes conica	10	18–55	42.9	9.90
Eumenes petiolata	10	11–34	28.7	6.53
Eumenes sp.	10	10–30	23.9	5.64
Phimenes flavopictus	10	8–29	19.8	6.51
Rhynchium sp.	10	14–63	46.3	12.93
Polistes sp.	10	21–48	33.3	8.13
Parapolybia varia	10	11–43	32.5	9.50
Ectemnius sp.	10	20–51	41.8	9.00
Sphex ichneumoneus	10	11–29	18.9	5.27
Sphex sp.	10	8–34	24.8	8.08

Table 4. Pollen recorded in the body washings of butterflies on *Premna latifolia*

Butterfly species	Sample size (N)	Number of pollen grains		
		Range	Mean	S.D
Graphium doson	10	21–51	36.8	9.73
Graphium agamemnon	10	26–76	51.6	12.73
Catopsilia pomona	10	23–55	43.5	8.98
Eurema hecabe	10	15–53	42.3	10.71
Pieris canidia	10	19–40	29.2	5.65
Anaphaeis aurota	10	20–54	38.9	9.24
Junonia lemonias	10	22–60	42.3	10.47
Junonia hierta	10	17–42	33.2	7.23
Precis iphita	10	16–53	40.7	9.91
Danaus chrysippus	10	31–68	52.5	9.84
Euploea core	10	15–56	40.4	10.47
Leptotes plinius	10	13–37	25.3	6.63
Jamides alecto	10	11–28	21.3	4.57
Spindasis vulcanus	10	19–40	29.3	6.27
Hasora chromus	10	10–34	26.5	6.43
Suastus gremius	10	6–35	22.6	7.15

Flower Morphology

The flowers are pedicellate, small (5.1±0.3 mm long and 3.8±0.4 mm wide), creamy white, mildly scented, bisexual and somewhat zygomorphic. The calyx is green, 3.9±0.3 mm long, 2.8±0.4 mm across, campanulate, obscurely bi-lipped, upper lip 2-lobed, lower lip 3-lobed and stellate pubescent. The corolla is white, 5±0.52 mm long, 7.5±0.3 mm across, tubular at base (c. 3mm long), obscurely bi-lipped; upper lip is 2-lobed, sub-orbicular, apex emarginate while lower lip is 3-lobed, obovate and blunt. The corolla tube is slightly narrow, glabrous, densely hairy at the pale yellow throat and puberulous outside. The stamens are 4, inserted at the throat of the corolla by slender white filaments, didynamous, long stamens c. 4mm long and short stamens c. 3mm long, slightly exserted; anthers are globose, c. 1mm long, 2-loculed, black and have versatile fixation. The ovary is superior, globose, puberulous, 2mm long, bicarpellary syncarpous with four locules by a false septum; each locule is 1-ovuled, erect and anatropous on axile placentation. The style is c. 3.5mm long, linear and stigma shortly and unequally bifid. The stigma is situated either at the height of short or long stamens.

Floral Biology

The mature buds with stamens and pistil inside open during 07:00–09:00 hr with peak anthesis at 08:00hr by diverging petal lobes. The floral biology is similar to that in *P. latifolia*.

Table 5. Breeding systems in *Premna tomentosa*

Mode of breeding system	Number of flowers sampled	Number of flowers set fruit	Fruit set (%)	Seed set (%)
Un-manipulated Autogamy	25	0	0	0
Manipulated Autogamy	25	7	28	25
Geitonogamy	25	13	52	25
Xenogamy	86	71	82	25
Open-pollination	875	473	54	25

Image 7. *Premna latifolia*: a. Fruiting phase, b-d. *Acridotheres tristis* (Indian Myna) approaching the inflorescences for collecting fruits.

The pollen output per anther is 1,225±180.54. The total pollen productivity in individual flowers is 4,900±722.17. The pollen-ovule ratio is 1,225: 1. The pollen grains are spheroidal, tricolpate, prolate, 33.86µm long and 42µm wide. Nectar is secreted around the ovary inside the corolla tube during post-anthesis period on the day of anthesis. Its secretion is gradual from anthesis onwards, reaches its peak after three hours and decreases gradually thereafter towards evening. A flower produces 2.8±0.37 µl of nectar. Half of the tube part of the corolla is filled with nectar. The nectar sugar concentration varied from 23–36 % and it averaged to 30.3±4.21 %. The sugars present in the nectar include sucrose, glucose and fructose with a dominance of sucrose. The total sugar content in the nectar of a flower is 0.95mg. The nectar also includes the essential amino acids such as arginine, lysine, threonine and tryptophan, and the non-essential amino acids such as alanine, aspartic acid, aminobutyric acid, cysteine, glutamic acid, glycine, hydroxyproline, proline, serine and tyrosine.

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Image 8. *Premna tomentosa*: a. Leaf shedding phase, b-d. Leaf flushing phase, e. Leaf flushing from cut base stock.

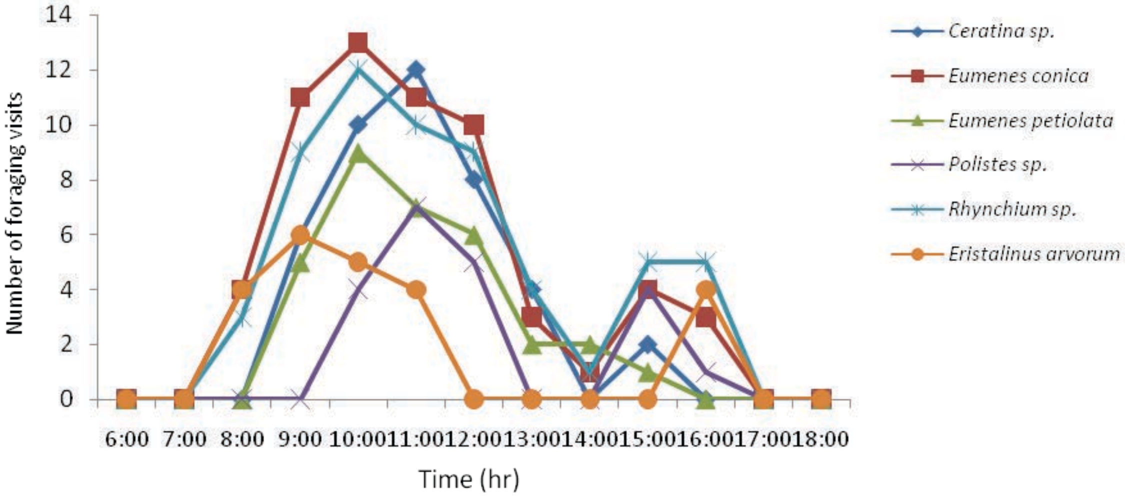

Figure 4. Hourly foraging activity of bees, wasps and fly on *Premna tomentosa*

Breeding Systems

The results of breeding systems indicate that the flowers are self-compatible and self-pollinating. The fruit set is absent in autonomous and un-manipulated autogamy, 28% in manipulated autogamy, 52% in geitonogamy, 82% in xenogamy and 54% in open pollinations. Seed set rate is constant and it is 25% in all modes of pollination due to the production of only one seed per fruit (Table 5). But, seeds produced from each mode of pollination were not examined for their viability and germination potential.

Foraging Activity and Pollination

The flowers expose the stamens and stigma slightly beyond the rim and lobes of the corolla upon anthesis. The corolla lobes stretch out and reflex partially facilitating access to nectar by insects. The flowers were foraged by one bee species, four wasp species, one fly species and

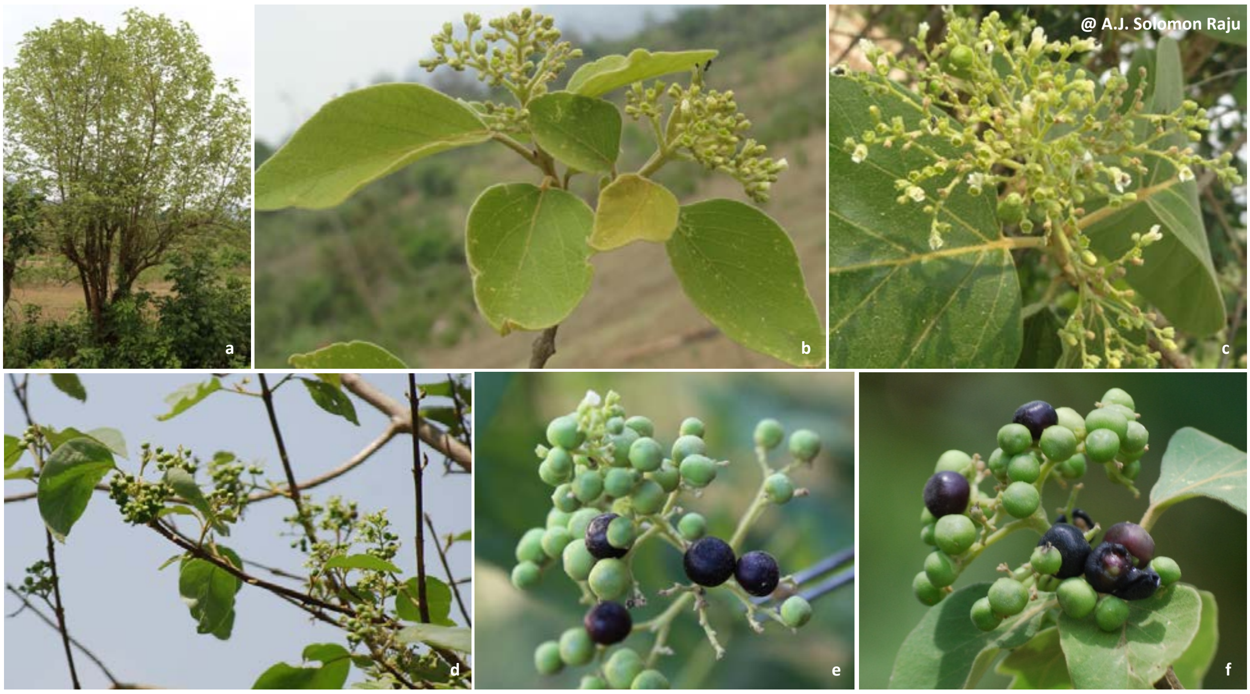

Image 9. *Premna tomentosa:* a. Tree with complete leaf flushing, b. Floral bud initiation, c. Flowering initiation, d-f. Different stages of fruit maturation.

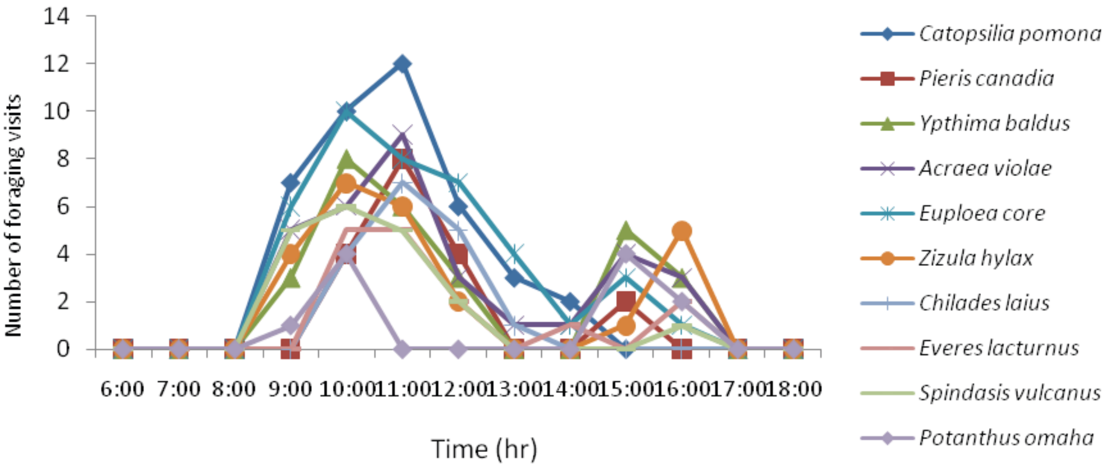

Figure 5. Hourly foraging activity of butterflies on *Premna tomentosa*

10 butterfly species during the day-time; the bee species for both nectar and pollen while all other insects foraged exclusively for nectar (Table 6). The bee was *Ceratina* sp. (Image 10a). The wasps were *Eumenes conica* (Image 10e), *E. petiolata* (Image 10f), *Rhynchium* sp. (Image 10d) and *Polistes* sp. (Image 10b,c). The fly was *Eristalinus arvorum* (Image 10g,h). The butterflies were *Catopsilia pomona* (Image 11a,b), *Pieris canidia* (Pieridae, Image 11c,d), *Ypthima baldus* (Image 11g,h), *Acraea violae* (Image 11f), *Euploea core* (Nymphalidae, Image 11e), *Zizula hylax* (Image 11l), *Chilades laius* (Image 11i), *Everes lacturnus* (Image 11j), *Spindasis vulcanus* (Lycaenidae, Image 11k), and *Potanthus omaha* (Hesperiidae, Image 11m). The bee, wasps and the fly foraged from 08:00–16:00 hr with slightly more activity during 10:00–11:00 hr (Fig. 4) while the butterflies foraged from 09:00–16:00 hr with slightly more activity during 10:00–11:00 hr (Fig. 5). But, individual species showed variation in their activity by visiting the flowers either continuously or with one or two hours' break. The fly was not a consistent and regular

Table 6. List of insect foragers on *Premna tomentosa*

Order	Family	Genus	Species	Common name	Forage Sought
Hymenoptera	Apidae	*Ceratina*	sp.	Small Carpenter Bee	Pollen+Nectar
	Eumenidae	*Eumenes*	*conica* F.	Potter Wasp	Nectar
		Eumenes	*petiolata* F.	Potter Wasp	Nectar
	Vespidae	*Rhynchium*	sp.	Black Potter Wasp	Nectar
		Polistes	sp.	Paper Wasp	Nectar
Diptera	Syrphidae	*Eristalinus*	*arvorum* F.	Spotted-eye Syrphid Fly	Nectar
Lepidoptera	Pieridae	*Catopsilia*	*pomona* F.	Common Emigrant	Nectar
		Pieris	*canidia* L.	Indian Cabbage White	Nectar
	Nymphalidae	*Ypthima*	*baldus* F.	Common Five Ring	Nectar
		Acraea	*violae* F.	Tawny Coster	Nectar
		Euploea	*core* Cramer	Common Indian Crow	Nectar
	Lycaenidae	*Zizula*	*hylax* F.	Tiny Grass Blue	Nectar
		Chilades	*laius* Stoll	Lime Blue	Nectar
		Everes	*lacturnus* Godart	Indian Cupid	Nectar
		Spindasis	*vulcanus* F.	Common Silver line	Nectar
	Hesperiidae	*Potanthus*	*omaha* Edwards	Skipper Butterfly	Nectar

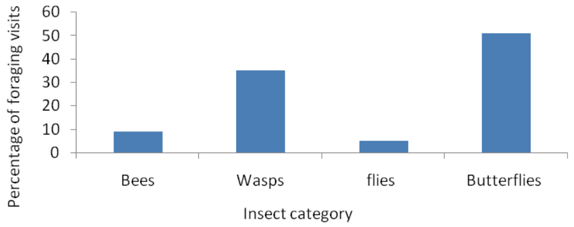

Figure 6. Percentage of foraging visits of different categories of insects on *Premna tomentosa*

Table 7. Pollen recorded in the body washings of bees, wasps and fly on *Premna tomentosa*

Insect species	Sample size (N)	Number of pollen grains		
		Range	Mean	S.D
Ceratina sp.	10	65–167	109.8	27.65
Eumenes conica	10	24–58	42.5	8.65
Eumenes petiolata	10	18–51	36.8	8.49
Polistes sp.	10	21–59	41.5	9.38
Rhynchium sp.	10	34–63	48.3	7.51
Eristalinus arvorum	10	9–31	26.6	6.53

forager. All other insects were consistent and regular foragers during the entire period of the flowering period. The bee formed 9%, wasps 35%, the fly 5% and butterflies 51% of total foraging visits (Fig. 6). The body washings of these foragers collected from the flowers during peak foraging activity revealed that they carry pollen on their bodies. The mean number of pollen grains was 109.8 in case of the bee species, from 36.8 to 48.3 in case of wasps, 26.6 in case of fly species (Table 7), and from 21.8 to 48.5 in the case of butterflies (Table 8).

The bee, wasps and flies irrespective of their body size invariably approached the flowers in upright position, landed on the flat-topped corymbs and probed individual flowers for nectar. The bee mainly concentrated on pollen collection due to difficulty in accessing nectar because the latter is insulated by a hairy throat. It, however, attempted to collect nectar but the flowers that were visited by wasps appeared to be enabling it to access nectar. The wasps and the fly with their sharp tongue (proboscis) penetrated easily through the mass of hair present at the corolla throat to access nectar. In this act, their forehead came into contact with both the stamens and stigma facilitating the occurrence of pollination. Further, their ventral side came into contact with the stamens and stigma causing pollination while moving from flower to flower on the corymbs. All these insects visited the fresh flowers only. They never visited the flowers that did not have corolla, stamens and stigma intact. Their ventral side, however, came into contact with the flowers that have only the ovary with style and stigma intact while approaching and searching for the fresh and rewarding flowers for nectar; such a contact was considered to facilitate pollination.

All butterflies recorded were found to collect only nectar. They approached the flowers in an upright

Image 10. *Premna tomentosa* a. *Ceratina* sp., b. & c. *Polistes* sp., d. *Rhynchium* sp., e. *Eumenes conica*, f. *Eumenes petiolata*, g. & h. *Eristalinus arvorum*.

Table 8. Pollen recorded in the body washings of butterflies on *Premna tomentosa*

Butterfly species	Sample size (N)	Number of pollen grains		
		Range	Mean	S.D
Catopsilia pomana	10	27–64	48.5	9.13
Pieris canidia	10	20–48	41.3	8.20
Ypthima baldus	10	28–51	40.6	6.23
Acraea violae	10	24–57	46.6	8.73
Euploea core	10	35–60	43.7	6.83
Zizula hylax	10	6–29	21.8	6.26
Chilades laius	10	14–32	26.1	5.60
Everes lacturnus	10	10–36	27.2	7.20
Spindasis vulcanus	10	22–41	35.2	4.98
Potanthus omaha	10	12–30	22.4	5.14

position, landed and/or hovered at the flowers to collect nectar. They fluttered their wings in vertical position and inserted their proboscis through the hairy throat of the corolla tube to collect nectar. The length of proboscis of all butterflies enabled them to reach the flower base with great ease. During nectar collection, their proboscis and forehead came into contact with the stamens and stigma facilitating pollination. They never visited the flowers that did not have corolla with stamens and stigma intact. Their abdomen, however, came into contact with the flowers that have only the ovary with style and stigma intact while approaching and searching for the fresh and rewarding flowers for nectar; such a contact was considered to facilitate pollination.

All insects visited the flowers in quick succession and swiftly moved from one corymb to the other on the same and different plants for nectar and such foraging was considered to affect both self as well as cross-pollinations. Several individuals of these insects, especially wasps and butterflies visited the flowers of the same plant at the same time harmoniously and this was very pronounced during peak flowering phase. Such intense foraging was found to be important to drive them off to different plants in search of more nectar and this could promote out-crossing rate.

Fruiting Behavior

Pollinated and fertilized flowers initiate fruit development immediately and take approximately four weeks to produce mature fruits (Image 9d–f). Fruit is nearly a globose indehiscent drupe, c. 8x5 mm across, green initially, bluish-black or black upon ripening, hairy and shiny. The drupe produces one fully developed seed and three partially or aborted seeds enclosed by the outer fleshy layer. The seed is oblong, light brown and 7x5 mm across. Fruit dispersal occurs during April-June. The fleshy fruits against green foliage are quite attractive to birds. Birds such as *Acridotheres tristis* Indian Myna (Image 12g), *Pycnonotus cafer* Red-vented Bulbul (Image 12a), *Dicaeum erythrorhynchos* Tickell's Flowerpecker (Image 12b–d) and *Tephrodornis pondicerianus* Common Woodshrike (Image 12e–f) were found to feed on the pulp of the drupe and in the process disperse them. The dry drupe free from the fleshy part also dispersed easily by wind due to dry season. Local children eat the fleshy

Image 11. *Premna tomentosa:* **a-d. Pierids – a. & b. *Catopsilia pomona*, c. & d. *Pieris canidia*, e-h. Nymphalids - e. *Euploea core*, f. *Acraea violae*, g. & h. *Ypthima baldus*, i-l. Lycaenids - i. *Chilades laius*, j. *Everes lacturnus*, k. *Spindasis vulcanus*, l. *Zizula hylax*, m. Hesperiid, *Potanthus omaha.*omaha.**

part of the fruit and throw the drupe and in this way they also take part in the dispersal of seeds. Hence, the plant displays polychory involving ornithochory, anemochory and anthropochory. During rainy season, seeds germinate and produce seedlings. Their continued growth is subject to the availability of moisture in the soil.

DISCUSSION

Leeratiwong (2008) reported that most *Premna* species are widespread but a series of morphologically closely related and generally geographically restricted species occur from India to New Guinea. In India, 31 species and six varieties of *Premna* are reported by Rajendran & Daniel (2002). None of these species have been studied thus far for their reproductive ecology. In this context, the present study described floral morphology for two *Premna* species, *P. latifolia* and *P. tomentosa*. deKok (2013) reported two basic calyx types in the *Premna* species of South East Asia. The first type has four isomorphic lobes and its shape remaining largely intact during flower development and fruit formation. The second type has zero to five heteromorphic lobes and it increases in size during flower and fruit development. The present study indicated that *P. latifolia* and *P. tomentosa* have bi-lipped calyx consisting of 2-lobed upper lip and 3-lobed lower lip but they are not clearly bilabiate as in typical Lamiaceae flowers. The calyx does not increase in size and hence does not change its shape during flower development and fruit formation. During fruit development, the calyx provides protection only to the bottom part of the fruit suggesting that it has no role in protecting the fruit from frugivores.

Chopra et al. (1992) and Hymavathi et al. (2009) noted that *P. latifolia* is an evergreen tree, widely distributed in India and other tropical and sub-tropical and coastal areas. deKok (2013) reported that *P. tomentosa* is distributed in India, Sri Lanka to South China, south to Java and the Philippines, except Borneo. It grows in primary to secondary forest, beaches or open types of vegetation. The present study shows that both *Premna latifolia* and *P. tomentosa* are not widely distributed and a few individuals occur in the study areas which are parts of Eastern Ghats of Andhra Pradesh. Dash et al. (2009) also reported that these two tree species are not widely

Image 12. *Premna tomentosa:* a. *Pycnonotus cafer* (Red-vented Bulbul), b-d. *Dicaeum erythrorhynchos* (Tickell's Flowerpecker, e. & f. *Tephrodornis pondicerianus* (Common Woodshrike), g. *Acridotheres tristis* (Indian Myna).

distributed in the Eastern Ghats.

Both the plant species are small to medium-sized semi-evergreen trees. The plants growing near streams show healthy growth while those growing in water-stressed areas show relatively stunted growth with light yellowish foliage. The present study indicates that both the species shed their foliage during winter but produce foliage at different periods, *P. latifolia* during the dry season and *P. tomentosa* during the wet season. Flowering also occurs at different periods, *P. latifolia* during the wet season and *P. tomentosa* during the dry season. Their flowering in different seasons seems to have evolved over a period of time in order to avoid competition for the same pollinators in areas where these two tree species grow together or in close vicinity to each other. Further, the same types of insects pollinate both of them but the two species do not grow in the same habitat.

van der Pijl (1972) stated that Labiatae flower is typically bilabiate and zygomorphic. The flowers are basically flag or gullet type. The flag blossoms present the sex organs ventrally and are primarily adapted for sternotribic pollination while gullet blossoms present the sex organs dorsally and are primarily adapted for nototribic pollination. The sternotribic pollination is most naturally performed by dipters such as syrphids and primitive hymenopterans. During probing, the pollen gets dusted all over on the ventral side of the insects and there would be wastage or loss of pollen. The nototribic pollination is naturally performed by advanced hymenoterans, especially bees. In this pollination mechanism, pollen is deposited precisely on the back of the insect preventing the wastage or loss of pollen. Both flag and gullet blossoms in certain plant species also facilitate foraging by birds and even lepidopterans due to which pollination occurs. Kleber et al. (2009) categorized three different pollination systems based on the number of different taxa pollinating a plant species, namely polyphily, oligophily and monophily. Polyphily refers to plants pollinated by different taxonomic orders of visitors. Oligophily refers to plants pollinated by more than one family of the same taxonomic order. Monophily refers to plants pollinated by only one species, one genus or different genera of the same taxonomic family. In *Premna latifolia* and *P. tomentosa*, the flowers are of gullet type, weakly bilabiate, and the stamens and stigma are situated near upper corolla lip. But, the sex organs are curved towards the lower corolla lip with the stigma extending beyond the height of long stamens without any contact with each other. Such an arrangement of sex organs is the characteristic of nototribic pollination mechanism, *sensu* van der Pijl (1972). In both, the gullet blossoms with nototribic pollination mechanism are pollinated by wasps and butterflies and hence are polyphilous. Additionally, bees and flies also pollinate the flowers of *P. tomentosa*. This study does not agree with the generalization made by van der Pijl (1972) that the gullet blossoms are pollinated by bees and in certain cases by birds. This study, however, agrees partly with his note that gullet blossoms of certain plant species are pollinated even by lepidopterans. Further, this study also does not agree with his statement that gullet blossoms represent monophily as the gullet blossoms in both the species are pollinated by different species, genera and families of wasps and butterflies. Apart from these insects, the small carpenter bee, *Ceratina* and the syrphid fly, *Eristalinus arvorum* also pollinate the flowers of *P. tomentosa*. The functionality of polyphily characterizes generalist pollination system in these tree species. At community level, polyphily is the most appropriate situation that guarantees reproductive success throughout time where there are as many pollinator classes as possible acting in many plant species (Kleber et al. 2009). In both the tree species, the flowers are pollinated nototribically by all these insects by striking the stigma and then stamens in quick succession with their forehead. Since the flowers are too small, the insects either land on the lower corolla lip alone or on other nearby flowers for nectar and/or pollen collection. The insects also move from flower to flower on the same or other corymbs of the same or different plants for forage collection during which sternotriby occurs by striking the stamens and stigma with their abdomen. The functionality of both nototriby and sternotriby in the same plant species is quite unusual and hence this study for the first time reports such a dual pollination mechanism in *P. latifolia* and *P. tomentosa*. Further, this study also does not agree with Subbareddi (1990) who reported that the wasp, *Ropalidia spatulata* pollinates *P. latifolia* in mess and soil fashion. The present study indicates that the foraging activity pattern, especially of wasps and butterflies is in accordance with the pattern of standing crop of nectar in the both the tree species. The corymbose inflorescence presenting numerous flowers in close proximity to each other is advantageous for foragers to reduce flight time as well as search time for flowers and hence is energetically profitable for them. Additionally, the wasps and butterflies quickly move from plant to plant in search of more nectar to quench their thirst during the dry season. Such foraging promotes cross-pollination in addition to self-pollination.

Fateryga (2010) stated that Lamiaceae members possess concealed nectaries and their flowers are visited by a large number of wasp species. Johnson et al. (2007) reported that wasps are short-tongued and cannot exploit nectar in deep tubular flowers. They are recorded most frequently as components of the visitor fauna of generalist flowers with a shallow perianth and exposed nectar. There are, however, many reports of nectar-producing flowers that are specialized for pollination by wasps. Faegri & van der Pijl (1979) did not regard the definition of a wasp pollination syndrome (sphecophily) as a possibility because of the lack of uniformity in characteristics of flowers visited by this group. Wasps are generally restricted to foraging on allophilic flowers, which are typically dull coloured, actinomorphic, and have exposed floral rewards. While butterflies, birds and bees can also forage from allophilic flowers, they have exclusive access to euphilic flowers exhibiting their particular syndromes. Possession of a long proboscis in butterflies allows them to access nectar from narrow tubular corollas, in addition to those with more exposed nectar. These butterfly-pollinated (psychophilous) flowers are typically brightly coloured with little scent. Brodmann (2010) stated that wasp-flowers exhibit physiological and morphological adaptations for the visitation and pollination of social wasps. Amongst others, the flowers are often described as reddish-brown (Muller 1881), dirty purple (Werth 1956) and dirty brown (Schremmer 1962) and, according to Wiefelsputz (1970), these colours attract wasps. Furthermore, Schremmer (1962) noticed relatively small, mostly bulbous flowers with a broad entrance and sucrose-rich nectar (Baker & Baker 1983). In the present study, the flowers of *P. latifolia* and *P. tomentosa* with short corolla tube extended into a broad weakly bilabiate throat and nectar concealed by the hairy growth at the throat display generalist pollination system. This pollination system is further substantiated by pollination effected by different species, genera and families of social and solitary wasps (sphecophily), and butterflies (psychophily). In these tree species, the flowers are not dull-coloured but they are bright white and fragrant. Cruden et al. (1983) stated that lepidopteran-pollinated flowers are rich in nectar volume and sucrose-rich with the sugar concentration ranging from 15–25 %. In *P. latifolia* and *P. tomentosa*, the corolla tube is half-filled with sucrose-rich nectar. But, the nectar sugar concentration recorded in both the species exceeds the range for the same noted for butterflies by Cruden et al. (1983). The sugar concentration evidenced in the nectar of these flowers appears to be evolved in tune with the requirement by both wasps and butterflies. Further, the nectar of both the tree species is a source of certain essential and non-essential amino acids. *P. latifolia* and *P. tomentosa* floral nectars are sources of the essential amino acids, lysine and tryptophan; *P. tomentosa* floral nectar is also a source of arginine and threonine. The non-essential amino acids, cystine, hydroxyproline and tyrosine are produced by both the tree species but alanine, aspartic acid, aminobutyric acid, glutamic acid, glycine, proline and serine are produced by *P. tomentosa* only. The study suggests that amino acid types can be used as a phytochemical criterion to differentiate *P. latifolia* and *P. tomentosa*.

Bodlah et al. (2016) reported that *P. foetida* is an important source of forage for bee-flies, *Thyreus himalayensis* and *T. ramosus* in Pakistan. The present study reports that *Premna latifolia* and *P. tomentosa* are potential nectar sources for both wasps and butterflies. Further, the small carpenter bee, *Ceratina* and the syrphid fly, *Eristalinus arvorum* as forage sources; the former as pollen and nectar source while the latter as nectar source only.

Inouye (1980) stated that nectar robbing pertains to the behavior of consuming nectar from a perforation in the floral tissue rather than from the floral opening. Nectar robbers are subdivided into primary nectar robbers which make the holes and then extract the nectar and secondary nectar robbers which obtain nectar by using holes made by primary robbers. Irwin et al. (2004) used the term “floral larceny” to include the entire suite of foraging behaviors for floral rewards that can potentially disrupt pollination. They include “nectar theft” (floral visits that remove nectar from the floral opening without pollinating the flower), and “base working” (removing nectar from in between petals, which generally bypasses floral reproductive structures). Nectar robbing is a common phenomenon in entomophilous plants. It is a behavior exhibited by some species of bees in which nectar is obtained through holes bitten near the bases of the corolla tubes (Inouye 1980). Ants may also act as nectar robbers or thieves (Willmer & Corbet 1981; Schaffer et al. 1983), thus decreasing floral rewards without contributing to pollination. Nectar robbing ants may reduce the reproductive success of the plants they visit (Inouye 1980). The present study shows that *Camponotus* ant serves as a nectar robber in a different way on *Premna latifolia*. It removes the entire corolla together with stamens and sometimes also the style and stigma to collect nectar situated around the ovary. The foragers avoid visiting the flowers with only calyx and ovary. Therefore, the nectar robbing activity by the ant may either indirectly compel the pollinating insects to pay multiple visits to the same or different plants in quest of more nectar promoting pollination rate or reduce the reproductive success of the plant by reducing the visits of pollinators depending on the state of standing crop of nectar in the flowers. Nectar robbing by this ant or other ants is not evidenced on *P. tomentosa*.

Subtle variations in the morphology of male and female sex organs can cause significant differences in the probability of spontaneous selfing or autonomous autogamy (Goodwillie et al. 2005; Eckert et al. 2010). Autogamy can generally be achieved by changes in the development of key floral traits that separate the sexual organs (anthers and stigma) in relation to space (herkogamy), time (dichogamy) or both (Schoen 1982; Wyatt 1986). The reduction in the distance between anthers and stigmas has been considered a major adaptive change towards a greater capacity for autonomous seed production, especially when plants are affected by limitations caused by cross-pollination (Herlihy & Eckert 2002; Moeller & Geber 2005; Brys & Jacquemyn 2012). Dichogamy is a reproductive strategy to avoid intra-floral interference between pollen donation and reception (Bertin & Newman 1993). In case of complete dichogamy, self-pollination is totally impaired (Webb & Lloyd 1986), but an overlapping in the expression of the two sexual functions (partial dichogamy) can generate opportunities for self-pollination (Schoen & Lloyd 1992). Small changes in the level of dichogamy can result in major changes in the ability of self-pollination (Webb & Lloyd 1986; Barrett 2003; Kalisz et al. 2012). In the present study, *P. latifolia* and *P. tomentosa* are both dichogamous and herkogamous. Dichogamy represented by weak protandry facilitates fruit set through selfing but herkogamy precludes fruit set through autonomous autogamy. Fruit set, however, occurs through facilitated autogamy, geitonogamy and xenogamy which together constitute a facultative xenogamous breeding system. The pollen-ovule ratio also substantiates the operation of this breeding system and the ratio falls in the range of pollen-ovule ratio (244.7–2,588) for facultative xenogamy provided by Cruden (1977). Further, the fruit set rate recorded in open-pollinations also substantiate the same. Holsinger (1991) stated that the mixed mating system forms in sparse than in dense populations and seems evolutionarily stable. This proposition is true in case of the two *Premna* species studied as they are sparsely distributed. The function of this breeding system is totally dependent on pollinator activity and ensures fruit set in isolated trees through vector-mediated selfing.

deKok (2013) recognized two fruit types in Lamiaceae. The first type is globose drupe-like consisting of four somewhat fleshy mericarps, each with one seed. The second type is clavoid drupe-like consisting of one somewhat fleshy mericarp with one seed; in this type initially four seeds develop but only one seed develops fully while the other three develop partially. *Premna latifolia* and *P. tomentosa* follow the second type fruits with one well developed seed and three partially developed or aborted seeds. Rogers et al. (2008) noted that in Guam, *Premna obtusifolia* is a bird-dispersed tree species and the absence or loss of birds here reduced its long-distance dispersal. Sengupta et al. (2014) reported that *Premna benghalensis*is is a tree species that produces fleshy fruits. Rhesus Macaque *Macaca mulatta* consumes the fruits of this plant, excretes the seeds undamaged and hence acts as an important long distance seed disperser. Most of the seeds excreted by this monkey germinate. deKok (2013) reported that *P. tomentosa* fruits are eaten by birds. Santhoshkumar & Balasubramanian (2011) reported that Indian Grey Hornbill *Ocyceros birostris* is a legitimate seed disperser of *P. tomentosa* in the Eastern Ghats, India. It consumes the fruits, defecates the seed undamaged and enhances its germination potential. In the present study, both the tree species produce ripe bluish-black shiny fleshy fruits within a month after pollination and fertilization. The fruits disperse during July–October in *P. latifolia* and during April-June in *P. tomentosa*. But, de Kok (2013) noted that *P. tomentosa* fruits disperse during May-September. In both the tree species, birds (ornithochory), humans (anthropochory) and wind (anemochory) act as fruit or seed dispersal agents. Initial observations indicated that the seeds of both the species germinate during wet season irrespective of their time of dispersal. Detailed studies on seed germination and establishment rates were not carried out and hence these aspects could not be explained with reference to their regeneration through seeds.

Premna latifolia is widely used in the traditional system of medicine for the treatment of dropsy and boils (Chopra et al. 1992; Hymavathi et al. 2009). *P. tomentosa* is widely used as a medicinal plant and its wood is used for making paddles and house posts in India and peninsular Malaysia (Rajendran & Daniel 2002). Its bark mixed with the sap of *Vitex* is used for diarrhoea. The ground leaves are used to clean wounds and in the preservation of dried fish and meat in South Sumatra (Heyne 1950). These reports suggest that both the tree species are medicinally important and traditionally used for treating different health problems. Therefore, these tree species should be rationally utilized for traditional medicine without affecting the surviving plants.

CONCLUSION

Premna latifolia sheds foliage during the winter season, produces foliage during the dry season and flowers during the wet season. *P. tomentosa* sheds foliage during the winter season, produces foliage during the wet season and flowers during the dry season. The flowers in both the species are dichogamous, herkogamous and facultatively xenogamous. Both the species are pollinated by wasps and butterflies; *P. tomentosa* is also pollinated by bees and flies. *Camponotus* ant robs the nectar of *P. latifolia* by removing the entire corolla together with stamens and sometimes also with the style and stigma. Both the species display ornithochory, anthropochory and anemochory. In both, the seeds germinate during wet season but their continued growth depends on the soil nutrient and moisture environment.

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Aritlces

Communications

Short Communications

Notes

Miscellaneous