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Psychophily and Anemochory in *Wendlandia tinctoria* (Roxb.) DC. (Rubiaceae): A Dry Season Blooming Tree Species in the Dry Deciduous Southern Eastern Ghats Forest, Andhra Pradesh, India

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Abstract. *Wendlandia tinctoria,* a semi-evergreen hermaphroditic tree species is a massive bloomer for about a month during March-April. The floral characteristics such as white colour of the flower, lack of odour, short-tubed corolla with deep seated hexose-rich nectar having 15-18% sugar concentration conform to psychophily. The nectar is also a source of two essential amino acids, arginine and histidine, and eight non-essential amino acids for butterflies. A variety of butterflies, especially nymphalids visit the flowers for nectar and in doing so, they pollinate them. Other foragers include bees, a fly and wasps. Wasps are occasional nectar foragers and effect pollination. Bees are consistent foragers of pollen and/or nectar. *Apis* bees collect pollen and nectar while *Trigona* and *Ceratina* bees collect only pollen due to the short length of the tongue. The fly is an occasional pollen feeder. The intense pollen collection activity of bees has been considered to be detrimental for the reproductive success of the plant. Therefore, *W. tinctoria* is primarily psychophilous and serves as a key forage source also for other insects at the study site where floral nectar sources are scarce during summer season.

Keywords: *Wendlandia tinctoria*, butterflies, psychophily, bees, wasps, nectar source

Introduction

The Rubiaceae is a large family with plants distributed over the temperate and tropical regions of the world. It shows a wide spectrum of floral mechanisms characterized by different types of gynoecium and androecium organization. Despite this diversity, Robbrecht (1988) pointed out the presence of three reproductive strategies common in Rubiaceae–distyly, morphologically characterized by the presence of two inter-compatible floral morphs, which is generally observed in species of Rubioideae (Barrett, 1992); stylar pollen presentation involving protandry and pollen presentation in the style which is generally recorded in Ixoroideae (De Block and Igerscheim, 2001; Nilsson *et al*., 1990) and the occurrence of unisexual flowers in certain species almost restricted to Theligoneae and Anthospermae (Robbrecht, 1988). In similar lines, Bir Bahadur (1968) documented three types of flower sex in the members of Rubiaceae - heterostyly, dioecy and hermaphroditic or gynodioecy. He mentioned that ninety-one genera with four hundred sixteen species are dimorphic and heterostylous. Consolaro *et al*. (2011) reported that Rubiaceae contains the largest number of distylous species in the angiosperms. The studies mentioned above do not give any details regarding pollination systems in the members of Rubiaceae. Raju *et al*. (1997) reported that *Xeromphis spinosa* is dioecious and adapted for pollination by hawkmoth pollination. In *Psychotria* genus, most of the species are bee-pollinated (Consolaro *et al*., 2011; Sakai and Wright, 2008; Bawa and Beach, 1983), while some are hummingbird-pollinated (Castro and Araujo, 2004).

Wendlandia is a genus of the subfamily Ixoroideae in Rubiaceae with over 90 species distributed in tropical and sub-tropical Asia; a single species of this genus is distributed in Africa (Misra and Bellwood, 1985). The literature does not provide any taxonomic and pollination biology details of this genus. Chetty *et al*. (2008) reported that *W. tinctoria* and *W. glabrata* occur in Seshachalam Hill range of Southern Eastern Ghats of India. Mishra *et al*. (2006) reported that *W. tinctoria* flowers during September-October in Similipal Biosphere Reserve in Eastern Ghats. In the present study, field investigations carried out in the entire stretch of Eastern Ghats showed that these two tree species do not grow together. *W. tinctoria* is confined to the Southern Eastern Ghats while *W. glabrata* has scattered distribution throughout the Eastern Ghats of Andhra Pradesh, India. Further, it

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was also found that both the species flower at the same time during March-April, show similar flower morphologies and play a crucial role in the nourishment of insects during dry season when floral resources are scarce. These two species have also not been investigated for their pollination biology despite their key role in the nourishment of local insect species. Therefore, the present study aims at providing complete taxonomic details and describing the aspects of pollination biology of hermaphroditic species, *W. tinctoria*. The findings of this study are discussed in the light of relevant information on the role of local insects in pollination and the attendant reproductive success.

Materials and Methods

Wendlandia tinctoria trees occurring at Tirumala Hill region, a part of Seshachalam Hills of southern Eastern Ghats of Andhra Pradesh, India, were used for the study during the dry season of 2009. The study area is located at 13º 40’ N latitude and 79º 19’ E longitude, and at an elevation of 2,443 ft. Field surveys in the entire forest area covering an extent of 50 sq. km showed that the population of this species is limited to about 25 trees only. Of these, only ten trees were accessible due to certain restrictions stipulated by forest department. Twenty five tagged mature buds on 10 trees were followed for recording the time of anthesis and anther dehiscence; the mode of anther dehiscence was also noted using a 10x hand lens. The details of flower morphology such as flower sex, shape, size, colour, odour, sepals, petals, stamens and ovary were described. Ten mature but undehisced anthers of flowers on five trees were collected from five different plants and placed in a petri dish. Later, each time a single anther was taken out and placed on a clean microscope slide (75 × 25 mm) and dabbed with a needle in a drop of lactophenol-aniline-blue. The anther tissue was then observed under the microscope for pollen, if any, and if pollen grains were not there, the tissue was removed from the slide. The pollen mass was drawn into a band, and the total number of pollen grains was counted under a compound microscope (40x objective, 10x eye piece). This procedure was followed for counting the number of pollen grains in each anther collected. Based on these counts, the mean number of pollen produced per anther was determined. The mean pollen output per anther was multiplied by the number of anthers of a flower for obtaining the mean number of pollen grains per flower. The characteristics of pollen grains were also recorded. Ten fresh flowers collected from 5 trees were used to measure the total volume of nectar/flower. The nectar sugar concentration was measured using a Hand Sugar Refractometer (Erma, Japan) as per Dafni *et al*. (2005).

Nectar analysis for sugar types and amino acid were conducted as per the paper chromatography methods of Harborne (1973) and that of Baker and Baker (1973), respectively. The stigma receptivity was tested with hydrogen peroxide from mature bud stage to flower drop as per Dafni *et al*. (2005). Regular observations were made on the insect species visiting the flowers for forage. The insects were observed on ten trees for 8 h over a period of 3 weeks for their foraging behaviour such as mode of approach, landing, probing behaviour, the type of forage they collect, contact with essential organs to result in pollination, inter-plant foraging activity in terms of cross-pollination. Three to five specimens of each butterfly species collected at different times of the day were brought to the laboratory for examining their proboscis under microscope for the presence of pollen grains in order to assess their role in pollen transfer and pollination. Casual observations were also made on fruit, seed and seedling aspects. A sample set of ten tagged inflorescences consisting of 2987 flowers from 5 trees was used for assessing fruit set in open-pollination.

Results and Discussion

Wendlandia tinctoria is a semi-evergreen tree species with scattered distribution in the forest (Fig. 1a). The leaves are glabrous and elliptic-lanceolate borne opposite to each other. Leaf flushing occurs during rainy season adding bright green look to the foliage. Flowering occurs from the 3rd week of March to the 3rd week of April. The flowering is almost synchronous in all individuals. Flowers are borne in clusters of terminal paniculate inflorescences (Fig. 1b) and open at 0600 h. The petals unfold exposing the stigma and stamens beyond the length of the corolla tube (Fig. 1c-1e). The flowers are small, 6-7 mm long, tubular, white with yellow tinge, odourless, regular and bisexual. The calyx is very small with 5 light green fused sepals; it is persistent and transforms into fruiting calyx after fertilization. The corolla is tubate tipped with 5 lobes, 5 mm long, white and conceals nectar. The stamens are 5 each with dithecous anthers having versatile fixation, very small and situated below the stigma; the anthers appear star-like at mature bud stage but take different postures after anthesis. The anthers dehisce during mature bud stage by longitudinal slits. A flower produces an average number of 38,850 ± 25.6 pollen grains. The pollen grains are monads, tricolporate, reticulate, circular, powdery and 16.6 ± 0.76 µm in size (Fig. 1f).

The ovary is well seated in the calyx, bicarpellary, syncarpous with a total of 97 ± 3 ovules (Fig. 1g); the style is erect and terminated with spathulate bifid stigma. The pollen : ovule ratio is 400.5:1. Stigma attains receptivity about two hours after anthesis by gradual unfolding of the stigmatic lobes and the receptivity remains so until the evening of the 2nd day. Stigmatic lobes gradually turn brown indicating the loss of receptivity. Same duration was evidenced in the hydrogen peroxide test also. Nectar is produced in minute amounts of approx. 0.6 ± 0.12 µL per flower and is collected at the base of the corolla tube. Nectar sugar concentration ranged from 15% to 18%; the sugar types includes sucrose, fructose and glucose but the last one is more dominant. Nectar contains essential amino acids, arginine and histidine, and non-essential amino acids alanine, aspartic acid, cysteine, glysine, hydroxy-proline, tyrosine, glutamic acid and serine. The flowers remain in place for four days and fall off, subsequently.

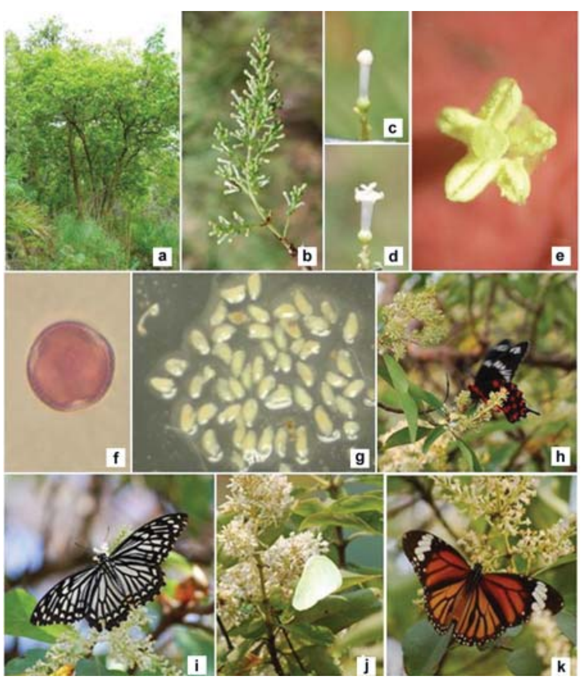

Fig. 1. *Wendlandia tinctoria*: a. Habit, b. Inflorescence, c. Bud, d - e. Flower, f. Pollen grain, g. Ovules, h. *Pachliopta hector*, i. *Papilio clytia*, j. *Catopsilia pyranthe*, k. *Danaus genutia*.

Mature buds contained thrips. Each mature bud/flower showed a minimum of 3-4 thrips. The thrips partly fed on nectar before anthesis and hence the flowers offered only minute amount of nectar after opening to nectar foragers. Insects began foraging at the flowers soon after anthesis; they included butterflies, bees, wasps and a fly species. Butterflies foraged throughout the day while all the other insects during forenoon only. Butterflies included 25 species representing Papilionidae, Pieridae, Nymphalidae, Lycaenidae and Hesperiidae (Table 1). Papilionidae and Pieridae each was represented by 2 species (Fig. 1h-1j), Nymphalidae by 15 species (Fig. 1k, Fig. 2a-2j), Lycaenidae by 5 species (Fig. 2k-2n), and Hesperidae by a single species (Fig. 3a). The foraging activity of these butterflies gradually increased from morning to noon and gradually decreased towards the evening (Fig. 4-9). Of these, the individuals of Nymphalid butterflies were more than those of other

Fig. 2. *Wendlandia tinctoria*: a. *Euploea core*, b. *Tirumala septentrionis*, c. *T. limniace*, d. *Hypolimnas bolina*, e. *H. misippus*, f. *Precis iphita*, g. *Phalanta phalantha*, h. *Neptis hylas*, i. *Junonia lemonias*, j. *Parantica aglea*, k. *Jamides celeno*, l. *Arhopala amantes*, m. *Lampides boeticus*, n. *Spindasis vulcanus*.

Table 1. List of insect foragers on *Wendlandia tinctoria*

Family	Scientific name	Common name
Papilionidae	*Pachliopta hector*	Crimson rose
	Papilio clytia	Common mime
Pieridae	*Catopsilia pyranthe*	Mottled emigrant
	C. pomona	Common emigrant
Nymphalidae	*Danaus genutia*	Striped tiger
	D. chrysippus	Plain tiger
	Junonia lemonias	Lemon pansy
	J. hierta	Yellow pansy
	Precis iphita	Chocolate pansy
	Euploea core	Common Indian crow
	Ariadne merione	Common castor
	Acraea violae	Tawny coster
	Tirumala septentrionis	Dark blue Tiger
	T. limniace	Blue Tiger
	Hypolimnas bolina	Great Eggfly
	H. misippus	Danaid Eggfly
	Phalanta phalantha	Common Leopard
	Neptis hylas	Common Sailer
	Parantica aglea	Glassy Tiger
Lycaenidae	*Jamides celeno*	Common Cerulean
	Arhopala amantes	Large Oak blue
	Lampides boeticus	Pea blue
	Spindasis vulcanus	Common silver line
	Everes lacturnus	Indian Cupid
Hesperiidae	*Borbo cinnara*	Rice swift
Sphingidae	*Cephonodes hylas*	Coffee Hawk moth
Apidae	*Apis dorsata*	Rock honey bee
	A. cerana	Indian honey bee
	A. florea	Dwarf honey bee
	Trigona iridipennis	Stingless honey bee
	Ceratina sp.	Small carpenter bee
Crabronidae	*Stizus* sp.	Digger wasp
Eumenidae	*Eumenes petiolata*	Potter wasp
	E. conica	Mason wasp
Scoliidae	*Scolia* sp.	Blue-winged wasp
Scoliidae	*Campsomeris annulata*	Flower wasp
	Vespa cincta	Yellow wasp
Syrphidae	*Helophilus* sp.	Hover fly

families at the flowers throughout the flowering season (Fig. 10). This foraging trend was observed almost throughout the flowering season for butterflies of all families recorded in this study. The data collected on the foraging visits of butterflies of each family showed that Nymphalids made 63%, Lycaenids 18%, Papilionids 9%, Pierids 7%, and Hesperiids 3% of total visits (Fig. 11). The aggregated arrangement of flowers provided comfortable landing place for butterflies and also this arrangement enabled them to probe several flowers in each visit in succession for nectar before their departure. The clusters of paniculate inflorescences borne terminally stand out prominently and the butterflies were found to be attracted to them even from a long distance. A sample of 3-5 specimens of all the butterfly species was used to examine the pollen carrying capacity of their proboscides. The results indicated that the proboscides invariably contained pollen grains ranging from 23-156 in Papilionids, 15-89 in Pierids, 35-213 in Nymphalids, 22-69 in Lycaenids and 31-93 in Hesperiids. All these butterflies probed the flowers with their proboscis to reach the nectar located at the flower base; while doing so, the proboscis invariably contacted the stigmatic lobes and the exposed dehisced anthers affecting pollination. The butterflies frequently moved between individual plants of *W. tinctoria* which were scattered in the forest; this inter-tree foraging activity was considered to be promoting cross-pollination. Further, the diurnal hawkmoth, *Cephonodes hylas*

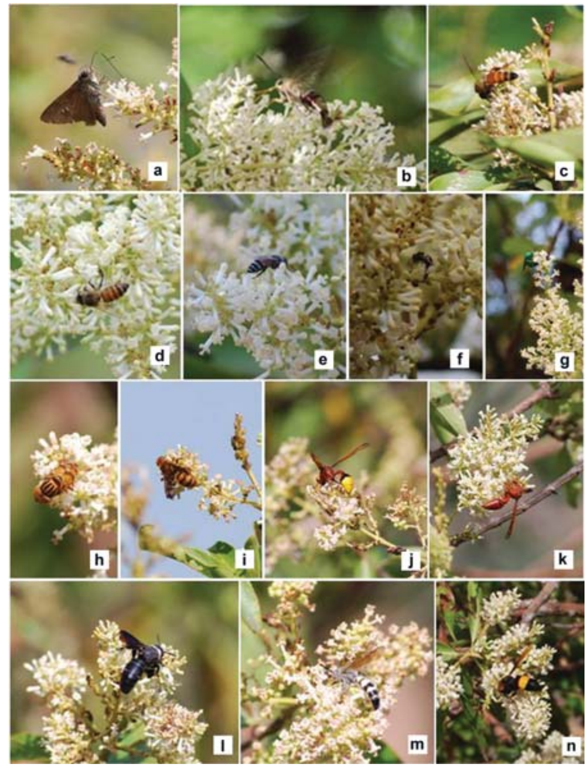

Fig. 3. *Wendlandia tinctoria*: a. *Borbo cinnara*, b. *Cephonodes hylas*, c. *Apis dorsata*, d. *Apis cerana*, e. *Apis florea*, f. *Trigona iridipennis*, g. *Ceratina* sp., h. *Helophilus* sp., i. *Stizus* sp., j. *Eumenes petiolata*, k. *Eumenes conica*, l. *Scolia* sp., m. *Campsomeris annulata*, n. *Vespa cincta*.

(Fig. 3b) also foraged for nectar but its foraging activity was confined to dawn hours when flowers just open and offer fresh nectar. The bees visiting the flowers were consistent foragers of pollen and/or nectar. They were *Apis dorsata* (Fig. 3c), *A. cerana* (Fig. 3d), *A. florea* (Fig. 3e), *Trigona iridipennis* (Fig. 3f) and *Ceratina* sp. (Fig. 3g). Of these, *Apis* bees collected pollen and nectar while the other bees collected only pollen due to the short length of the tongue. The wasps foraging at the flowers were *Stizus* sp. (Fig. 3i), *Eumenes petiolata* (Fig. 3j), *Eumenes conica* (Fig. 3k), *Scolia* sp. (Fig. 3l), *Campsomeris annulata* (Fig. 3m) and *Vespa cincta* (Fig. 3n) while the fly was *Helophilus* sp. (Fig. 3h). Both wasps and flies were occasional foragers

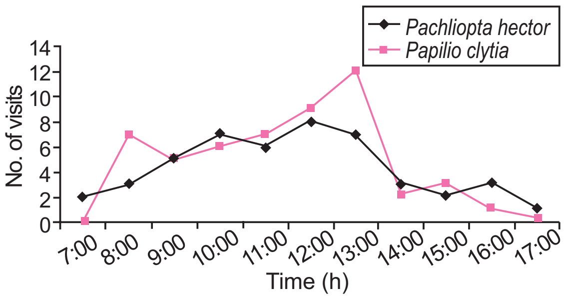

Fig. 4. Hourly nectar foraging activity of Papilionid butterflies on *W. tinctoria*.

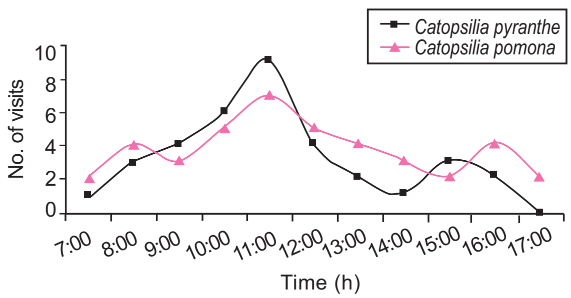

Fig. 5. Hourly nectar feeding activity of Pierid butterflies on *W. tinctoria*.

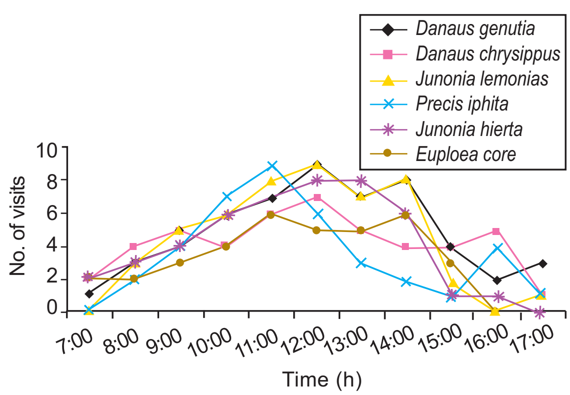

Fig. 6. Hourly nectar foraging activity of Nymphalid butterflies on *W. tinctoria*.

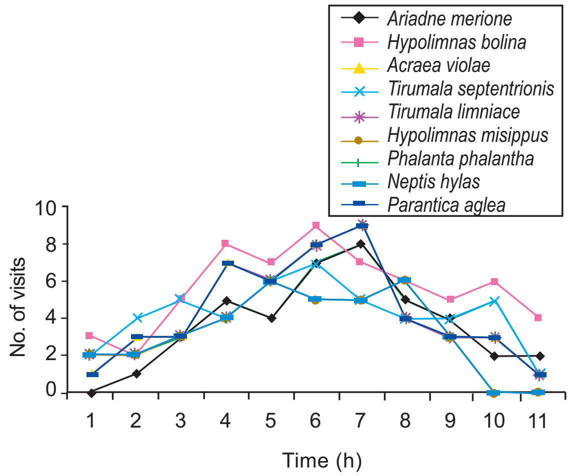

Fig. 7. Hourly nectar foraging activity of Nymphalid butterflies on *W. tinctoria*.

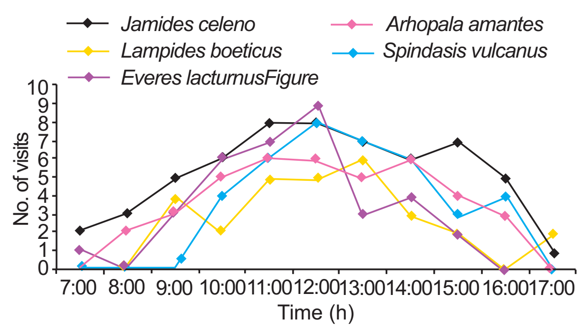

Fig. 8. Hourly nectar feeding activity of Lycaenid butterflies on *W. tinctoria*.

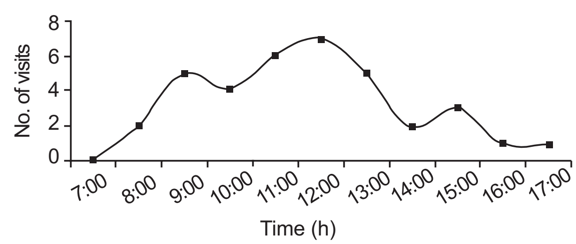

Fig. 9. Hourly nectar feeding activity of Hesperiid butterfly, *Borbo cinnara* on *W. tinctoria*.

only; the former collected only nectar while the latter, only pollen. The intense pollen feeding activity of bees was considered to be limiting the pollen availability for seed set while wasps during nectar collection were found to be contacting the stigma as well as the dehisced anthers thereby contributing to pollination.

The fruit growth and development began immediately after pollination and fertilization (Fig. 12a-12c). Fruit set in open-pollinations was 7%. The fruit was a small globose dry capsule, light green initially and brown when mature. Each fruit produced 45-53 seeds out of which viable seeds ranged from 34 to 46 (Fig. 12d-12f), while the remaining ones were either half-filled or ill-formed (Fig. 12g). Fruits matured in about 5 months and dehisced to release the tiny seeds into the air. This occured during late rainy season but it was unfavourable for seed dispersal, germination and establishment.

Faegri and van der Pijl (1979) and Burkhardt (1964) characterized butterfly-flowers. Flowers conforming to this type usually possess large, white, pink, red, yellow or blue, narrow, tubular flowers with deep nectaries with often yellow rings or other markings on the petals which function as nectar guides. Baker and Baker (1983) reported that the short-tubed flowers tended to be hexose-rich and if grouped in conspicuous inflorescences provided excellent standing platforms for foragers, and attracted both butterflies and short-tongued bees. Cruden *et al.* (1983) also stated that hexose-richness appeared to prevail in the nectar of short-tubed flowers. These authors also documented that nectars of the most butterfly-pollinated flowers fell within the range of 15 to 25% sugar concentration. Kingsolver and Daniel (1979) suggested that the nectar sugar concentrations of 20-25% optimized the net energy gain by the butterflies. In *W. tinctoria*, the floral characteristics such as white colour of the flower, lack of odour, short-tubed corolla with deep seated hexose-rich nectar accounting for 15-18% sugar concentration characterized psychophily.

Fig. 12. *Wendlandia tinctoria*: a-c. Fruiting, d-f. Healthy seeds, g. Ill-formed seeds.

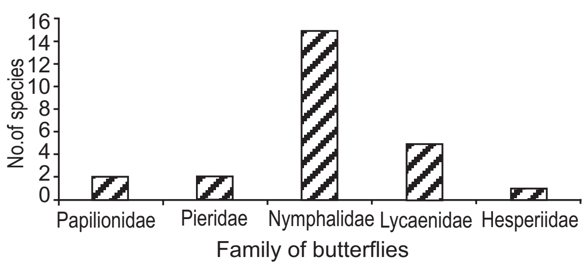

Fig. 10. Family-wise number of butterfly species foraging for nectar on *W. tinctoria*.

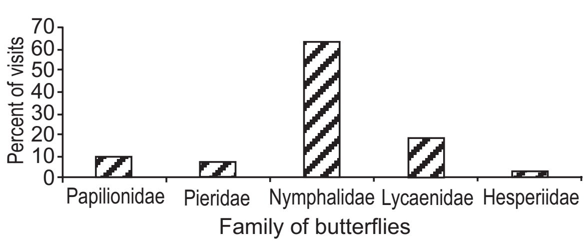

Fig. 11. Family-wise percentage of foraging visits of butterlies on *W. tinctoria*.

Nectar is a potential source of amino acids for the nutrition of butterflies. Naturally, butterfly nectars are rich in amino acids (Baker and Baker, 1983). Butterflies require ten essential amino acids but all of them are not normally found in all nectars. Usually, three to four essential amino acids and several non-essential amino acids are found in floral nectars (Baker and Baker, 1983;

1982). Baker and Baker (1986) reported that the amino acids added taste to the floral nectar and it depended on the concentration. Their presence serves as an important cue for butterflies to make flower visits and in the process affect pollination. In *W. tinctoria*, the nectar contained two essential (arginine and histidine) and eight non-essential amino acids. Its nectar is an important source of only two essential amino acids required by butterflies during adult life for their growth and development; other essential amino acids included isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine (DeGroot, 1953). Non-essential amino acids are metabolized by butterflies from the food they take; however, floral nectar provides some of these amino acids instantaneously. The nectar of *W. tinctoria* provides alanine, aspartic acid, cysteine, glysine, hydroxyproline, tyrosine, glutamic acid and serine. Gardener and Gillman (2002) mentioned that if local soil conditions favour higher amino acids in the nectar then local populations of butterflies may derive certain benefits. Adult feeding on amino acid rich food has been shown to increase the longevity and the reproductive ability in certain heliconine butterflies (Dunlap-Pianka *et al*., 1977; Gilbert, 1972). A later study on a temperate species *Euphydryas editha* showed that amino acids in the adult diet led to heavier eggs (Murphy *et al*., 1983). Jervis and Boggs (2005) reported that the butterflies are agents of selection for higher nectar amino acid production. The requirement of amino acids during adult stage of the butterfly is related to the larval nutritional condition. The larval food plant has a key role in the evolution of the flower-butterfly mutualism, and demonstrates that the importance to butterfly reproduction, and of different nutrient sources vary with butterfly nutritional state. Gardener and Gillman (2001) reported that soil conditions can affect the amino acid complement of nectar. This may have implications for plant-butterfly interactions, as local populations of butterflies may benefit from the increased amino acid content of the nectar and preferentially visit plants growing in high nutrient conditions. The fidelity of butterflies to *W. tinctoria* during flowering phase attests the fact that butterflies are in need of amino acids present in the nectar and in the process contribute to pollination.

In *W. tinctoria*, the inflorescences with clusters of flowers provide excellent platform for foraging by butterflies. The retention of flowers for two more days after the cessation of stigma receptivity appears to be an adaptive trait for the plant to enhance the attractiveness to butterflies and other foragers. With these floral structural and functional characteristics, *W. tinctoria* has been found to be foraged by butterflies of all five families of Lepidoptera and by other insects. The short-tubed flowers facilitate butterflies with any length of proboscis to collect nectar easily. The flowers being small in size with minute amount of nectar compel the butterflies to do a more laborious search for nectar from a greater number of flowers. The presence of thrips in flowers prior to anthesis showed that the thrips use the plant species for breeding. The reduced standing nectar crop due to nectar feeding by thrips prior to and after anthesis further drive butterflies to visit as many flower as possible either from the same or different individual plants to quench their nectar thirst. But, the clustered state of flowers is energetically profitable for butterflies to reduce search time and also flight time to collect good amount of nectar. Overall, the search for nectar by butterflies due to the production of minute amount of nectar at flower level contributes to both self- and cross-pollination. The presence of pollen on the proboscides of butterflies substantiates this conclusion. *W. tinctoria* attracts more number of individuals and species of Nymphalid butterflies when compared to those of other families of butterflies suggesting that Nymphalid butterflies play a key role in the pollination. The psychophily is advantageous for the plant because butterflies do not collect pollen for themselves but only carry pollen on their proboscis and affect pollination while collecting nectar.

W. tinctoria also attracts bees, wasps and a fly species. The bees being consistent pollen feeders and the fly being an occasional pollen feeder collect huge amounts of pollen; *Trigona* and *Ceratina* bees being exclusive pollen feeders due to their short tongue length further empty the pollen of dehisced anthers quickly. Such an intense pollen feeding activity of bees drastically reduces the pollen availability for pollination though they affect pollination while collecting pollen and nectar. This pollen feeding activity is highly detrimental for the plant as it reduces the availability of pollen for pollination which is to be affected by butterflies. Therefore, bees act as effective pollen thieves of *W. tinctoria*. Wasps being nectar feeders also affect pollination but they are not consistent visitors. The study suggests that butterflies are the prime pollinators while other insects, especially wasps contribute to additional pollination enabling the plant to slightly enhance seed set rate. *W. tinctoria* with massive flowering is an important nectar and/or pollen source for local insects and hence, it is a keystone tree species for them since it provides them with nectar for a period of about one month during summer season in the dry deciduous forest ecosystem of Seshachalam hills. There are no other plant species in flowering which attract a diversity of butterflies to this extent during the flowering period of *W. tinctoria* and hence this tree species plays a crucial role for the local butterflies for their nutrition for one month during summer season.

In *W. tinctoria*, the low fruit set rate despite pollination by butterflies and other insects could be the result of inadequate pollination due to pollen thievery by bees and to low resource availability. Each fruit produces a small percentage of half-filled seeds and these seeds do not germinate to produce new plants. The occurrence of such seeds could be attributable to fertilization of certain ovules with inferior pollen and to poor nutritional environment. The fruits split apart to disperse seeds into the air but they do not have characteristic adaptations usually found in anemochorous species (Howe and Westley (1997). Further study is required to understand the seed dispersal mode in this species. Seeds germinate as soon as they fall to the ground but most of the seedlings soon dry up due to non-penetration of sunlight, over-growth of herbaceous vegetation and space limitation. Therefore, inadequate pollination, low resource availability and poor competitive ability of seedlings in *W. tinctoria* seem to be contributing to the small population size and hence it attains the status of "rare" plant in the Southern Eastern Ghats.

The outcome of this work is that *W. tinctoria* being a dry season bloomer is psychophilous and butterflies are available during this period despite the dry season assuring the reproductive success for the plant while using it as an important adult nectar host.

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