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PHENOLOGY AND REPRODUCTIVE ECOLOGY OF *VATERIA MACROCARPA* B.L.GUPTA. (DIPTEROCARPACEAE)- A CRITICALLY ENDANGERED TREE SPECIES OF WESTERN GHATS, KERALA, INDIA

Keshavanarayan, P., Rajkumar, K. & *Sivaram, V.
Laboratory of Biodiversity and Apiculture, Department of Botany, Bangalore University, Bangalore - 560056, India.
*Corresponding Author: E mail: sivaram900@gmail.com

ABSTRACT
Phenology has great significance because it is not only provides the knowledge about the plant growth patterns but also provides the inferences on the effect of environment and selective pressure on flowering and fruiting behavior. *Vateria macrocarpa* is a critically endangered tree species of Palakkad reserve forest, Western Ghats of Kerala, India. The study of phenology and reproductive ecology has been conducted during 2013-14. Phenological events showed leaf fall period during December and leaf flushing peak period in the months of January -February, whereas peak flowering and fruiting activity was found during the month of March - April and May - July respectively. Anthesis occurred at early evening and stigma receptivity was observed up to 12 hrs after anthesis. The pollen output per flower was 4, 40, 250 and pollen viability was about 97.21%. The maximum 86.19% pollen germination was observed in 15% sucrose concentration of Brewbaker and Kwack media. Controlled pollination experiments showed that *Vateria macrocarpa* is a self-compatible species. The fruit set in natural pollination and autogamy showed 22% and 18% respectively. Manual geitonogamous pollination showed 26% and manual xenogamous pollination revealed 24% of fruit set. Butter flies and wild bees are the regular flower visitors. It was noticed that, many of the flowers were found infested by Beetle larvae. The factors responsible for endangered status of this species were discussed.

KEY WORDS: *Vateria macrocarpa*, Western Ghats, phenology, floral biology, stigma receptivity, pollen viability, flower visitors.

INTRODUCTION
Phenology determines the different phases in the life cycle of the plant and plant productivity with regard to biotic and abiotic factor (Maiti and Rodriguez, 2015). The plant communities display conspicuous seasonal patterns in vegetative and reproductive phenology such as leafing, flowering and fruiting. Such studies on endangered, rare and threatened species may be useful for understanding the consequences of this endangerness. The study of phenology and reproductive ecology are important to conserve the tree genetic resources, forestry management and to understand the ecological adaptivity and growth of plant in the community (Desai and Patel, 2010). *Vateria macrocarpa* is considered as a critically endangered species according to IUCN Red data book and endemic to evergreen forests of Western Ghats, Kerala, India (Fig. 3B), belonging to the family Dipterocarpaceae. The members of this family comprises of 19 genera and around 580 or more species. *Vateria macrocarpa* commonly called as Vellakundrigum in Malayalam. Bark is dark grayish in color (Fig. 3B[1]), leaves are elliptic, oblong, lanceolate and rounded, coriaceous, glabrous on both surfaces rarely with few stellate hairs at the base on midrib beneath lateral nerves. Flowers are axillar and hairy, calyx brown and corolla white in color and five in number, stamens numerous, ovary 3-10 loculed, densely tomentose and fruit contains only one seeded. Several studies were carried out on Phenology (Borah and Devi, 2014; Bajpai *et al*., 2012; Kaur *et al*., 2013) and reproductive biology (Chan *et al*., 2011; Murali and Sukumar, 1994) of endangered and endemic plant species from different forest types of India. However the information on these factors with reference to *Vateria macrocarpa* is not reported. Thus the objective of the present investigation was to study the phenology and reproductive ecology of *Vateria macrocarpa* to determine the possible factors for limited distribution in natural conditions and also to formulate the conservation strategies.

MATERIALS & METHODS
Study area and species
The field study was conducted in Palakkad, Kerala at Singampara forest (Fig. 3A). Twenty individual trees were selected by using GPS. The location of study site lies between N10˚ 59.406 Latitude; E 76˚37.450 Longitude, (Fig.1A & B).

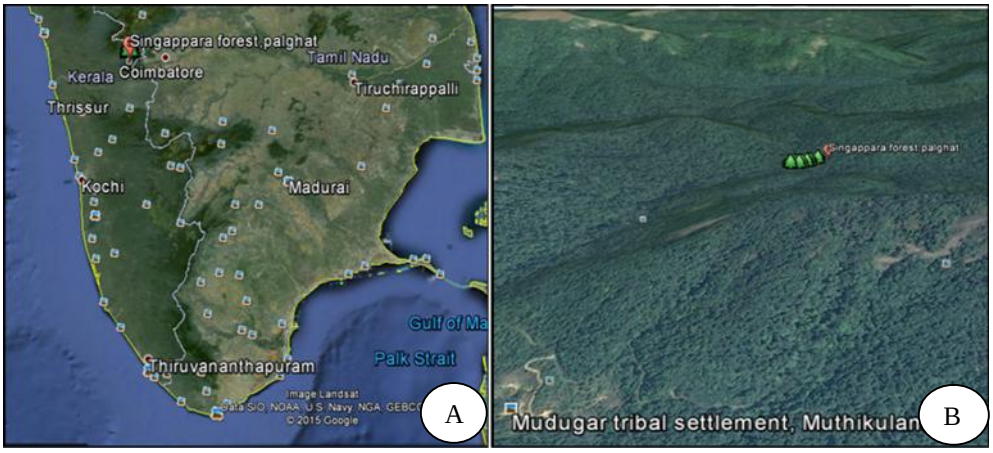

FIGURE 1. A. Study area**. B.** Enlarged view of study site (Singampara)

Phenology, floral biology and morphology

Phenological events were carried out by randomly selecting 20 individual trees in the study area and similar procedure was adapted to study phenological events in *Garcinia imbertii* (Rajkumar *et al*., 2015) and *Pterospermum reticulatum* (Keshavanarayan *et al*., 2015). Five flowers from each of ten trees were selected to study morphological details such as size of the flowers, sepals, petals, anthers, style and ovary (Solomon Raju *et al*., 2013; Antonysamy *et al*., 2014). Stigma receptivity was observed during anthesis by using hydrogen peroxide on mature buds according to Dafni *et al*. (2005). Stigma doesn't stain when hydrogen peroxide is applied on it but bubbles on stigma surface appear as a result of catalase enzyme activity. The duration of bubble production was taken as the duration of stigma receptivity (Solomon Raju *et al*., 2014).

Pollen output/anther/flower and pollen ovule ratio

Pollen counts were made by selecting five individual trees and 20 randomly selected flower buds. Average number of pollen grains per flower was calculated and was measured by Haemocytometer under the light microscope (Prasanna *et al*., 2013). Pollen and ovule ratio was calculated by Cruden's (1977) formula.

P/O ratio = Pollen count per anther× No. of anthers per flower/No. of ovules per flower.

Pollen viability, pollen germination and pollen tube growth

Pollen viability were assessed through 2,3,5 Triphenyl tetrazolium chloride (TTC) under different sucrose concentrations ranged between 0.01% -0.05%. Germination of pollen grains was carried out in Brew baker and Kwacks medium under various concentrations of sucrose ranging from 5% - 20%. Pollen grains from mature flower buds were dusted evenly on germination media in the cavity slides following the method of "Hanging Drop Technique". Percentage of pollen germination and pollen tube growth was calculated by observing under the light microscope (Shivanna and Rangaswamy, 1992).

Breeding system

Breeding experiments of *Vateria macrocarpa* was carried out manually by selecting five trees. Flower buds (n=50) from twenty five inflorescences were observed before anthesis and tagged to observe the fruit set through natural pollination.

a) Open/ natural pollination- Flower buds were tagged and fruit set at maturity was recorded.

b) Autogamy (isolated)- Mature flower buds were tagged and bagged with mesh cloth and isolated from pollinators, fruit set upon maturity was recorded.

c) Manual self-pollination (Geitonogamy)- Mature flower buds were tagged and bagged. The buds upon opening were hand self-pollinated with pollen collected from the same plant and re-bagged.

d) Manual cross– pollination (Xenogamy) - Mature flower buds were tagged and bagged. The buds after opening were hand cross-pollinated with pollen collected from two or three other plants and then re-bagged.

All the pollination treatments were studied after three days and re-bagged. Pollinated flowers were observed to record fruit initiation and the percentage of maturity was recorded. Self –incompatibility index for hermaphroditic plants was calculated as the ratio between percentage of the fruit set resulting from hand self-pollination over hand cross pollination. The obtained ratio< 0.25 are considered self – incompatible and those with ratio > 0.25 as self- compatible (Mohandass & Priya Davidar, 2014).

Floral visitors

Foraging activity of flower visitors with reference to pollination was observed during flowering season. Pollinator observation was recorded in different hours of day between 7:00 to 17:00 hrs. Frequencies of visits, kind of resource collected from the pollinators were recorded. Insect/ pollinators were collected and stored in 70% alcohol for identification of species (Krishna Kumar *et al*., 2014; Seema Chauhanand & Archana Shakya, 2014).

Floral predators and threats

Flowers, buds and fruits were observed carefully during flowering and fruiting season for pests and insects. They

were captured and stored in 70% alcohol and identified in Department of Entomology, University of Agricultural Science, GKVK, Bangalore.

Statistical analysis

The data obtained were analyzed statistically by using Megastat (Orris, 2003).

RESULTS & DISCUSSION

Phenological events

Vateria macrocarpa is an evergreen tree species showing vegetative phase containing green leaves throughout the year. Leaf fall starts in the month of December and leaf renewal starts in the first week of January and continues till the end of February. Figure 2 shows month wise phenological observations with phenogram.

Floral biology

Flowering in *Vateria macrocarpa* started during first week of March and continued up to last week of April. The average life span of each flower is 1-2 days as bud initiation started in March. The total period of flowering up to fruiting was found to be approximately 61-92 days and fruit initiation to fruit maturity took approximately 3 months. The young fruits were hard, light brown in colour during fruit initiation and turns to dark brown in maturity (Fig.3F)

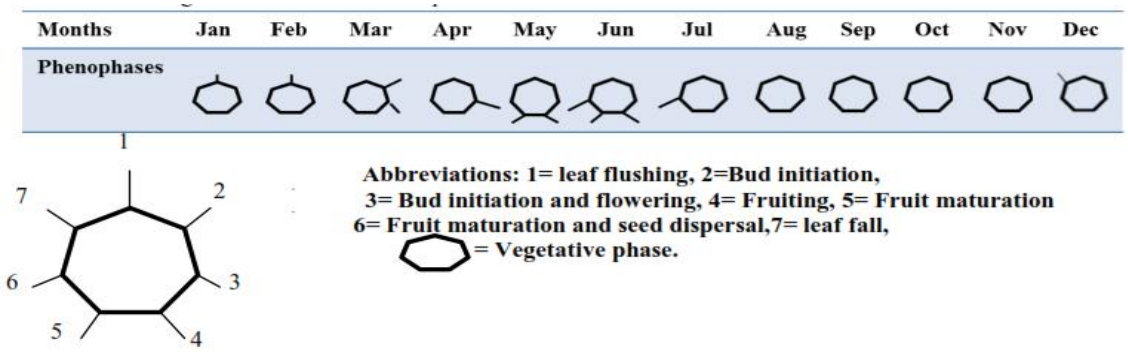

FIGURE 2. Phenogramof *Vateria macrocarpa*

Flowers are axillary in position (Fig.3C) measuring 3.22cm with hairy stellate pubescent panicles. Pedicels measuring 1.92cm long jointed a little below the middle. Calyx consists of 5 sepals measuring 2.40cm. Coralla measuring 1.88cm consisting 5 petals. Anthers measuring 0.70cm linear, glabarous and approximately 120 in number. Styles measuring 1.92 cm slightly longer than stamens. Ovary is 3 loculed, cross section showed 6 ovules. Floral characters, floral morphology and statistical data were shown in Table 1 and 2.

TABLE1. Floral characters of *Vateria macrocarpa*.

Floral parameter	Observations
Inflorescence	Axillary, hairy, stellate pubescent panicles
Flowering period	March-April
Flower type	Bisexual
Flower colour	White
Number of anthers per flower	120
Pollen per anther	3668.75
Pollen per flower	4,40,250
Pollen ovule ratio	73375:1

TABLE 2. Floral morphology of *Vateria macrocarpa*

Sl. no	Parameters	Measurements Mean ± SD (cm)
1	Flower	3.22±0.19
2	Flower pedicel	1.92±0.08
3	Petals	1.88±0.00
4	Sepals	2.40±0.50
5	Anthers	0.70±0.10
6	Style	1.92±0.08

Blooming of flowers starts only after sunset (Fig.3D). Anthesis and anther dehiscence occurs at night while tetrasporangiate anthers of all the stamens dehisced simultaneously. Stigma becomes receptive after flower opening. The peak activity of stigma receptivity is noticed by tiny droplets of stigmatic exudates and bubbles appeared

on the surface of stigma indicating receptivity (Fig.4A). Stigma remains receptive up to 12hrs. Pollinated and fertile flowers indicate fruit development and sometimes flower falls if they are not pollinated. It is observed that pollination is affected by beetle larvae (Fig.3 J&K) and some of flowers were not pollinated because of these larvae damaged the flowers.

Pollen production, pollen ovule ratio, pollen viability and pollen germination

The maximum number of pollen grains per anther is 3668.75 and pollen per flower is 4, 40,250. Pollen ovule ratio is 1:73375 (Table 3 & 4). Pollen viability test (TTC) indicated that pollen grains showed maximum viability showing pale pink and red coloration of pollen grains (Fig. 4B). The maximum pollen viability was observed in 0.05% concentration of TTC and percentage of viability is 97.21% (Table.5). The average pollen germination was observed as 86.19% in 15% of sucrose concentration (Fig. 4C & D; Table 6).

Breeding system

Observations on the breeding system of *Vateria macrocarpa* shows open/ natural pollination produced fruit set of 22% and autogamy produced 18% of fruit set. Manual pollination experiments such as Geitonogamy and Xenogamy produced fruit set of 26% and 24% respectively. Based on self-incompatibility ratio between self and cross pollination showed 1.08 which was above exceeding value >0.25 ratio indicating that the species is self- compatible (Fig. 3E& F and Table.7).

Floral visitors

During flowering only two types of floral visitors such as honeybees and butterflies were observed as major pollinators (Fig. 3G & H). The most frequent visitors are honeybee *i.e. Apis dorsata* which collect both nectar and pollen from morning to afternoon (Fig. 5). Butterflies visited flowers for pollen and nectar are seen foraging at less frequency (Table 8).

Pests and predators

Bruchid beetle larvae were observed damaging flower buds, fruits and seeds. Larvae are whitish in colour and found feeding on flower buds and flowers, they continue to affect the fruit set. In many of the flowers 3-10 beetle larvae were observed (Fig. 3J & K).

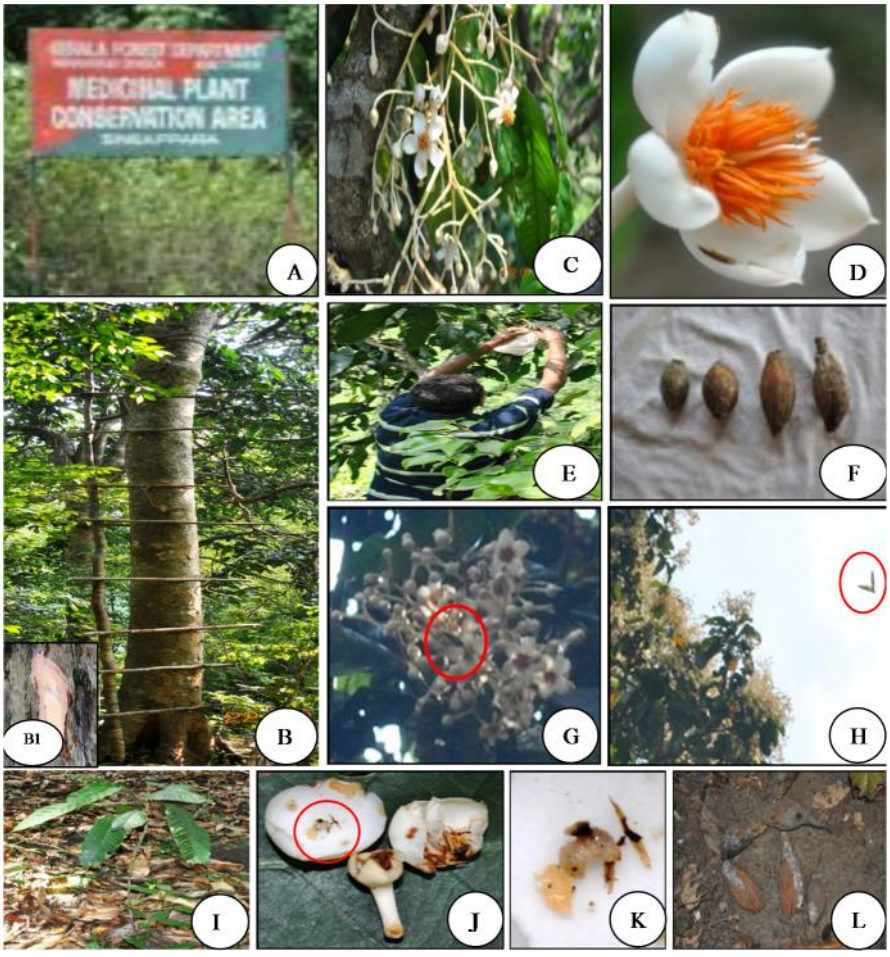

FIGURE 3A. Singamppara forest station **B. 40 m** tall tree, **B1.** Bark, **C.** Inflorescence and flowers buds, **D.** Flower, **E.** Breeding experiments (Bagging), **F.** Mature fruits, **G.** *Apis dorsata*, **H.** Butter fly**, I.** Natural regeneration, **J** and **K.** Bruchid beetle larvae**, L.** Predated fruits.

TABLE 3. Pollen production.

Year	No. of flowers sample	Range	Total pollen production per flower
			Mean ± std. dev
2013	10	269000-459000	3,58,850±60,932.59
2014	10	210000-473700	3,68,760±79,321.35

TABLE 4. Pollen ovule ratio

Year	no of samples	No of ovules	Pollen– ovule ratio	Mean ± std. dev
2013	10	6	59832:6	59832.50±10.139.24
2014	10	6	61460:6	61460.00±13220.22

TABLE 5. Pollen viability(TTC)

Sucrose concentrations (%)	No of samples observed	Percentage of germination	Mean ± st.dev
0.01%	5	92.00%	92.01±2.27
0.02%	5	88.81%	88.81±6.69
0.04%	5	90.54%	90.54±4.94
0.05%	5	97.21%	95.21±4.43

TABLE 6. Pollen germination

Sucrose concentrations (%)	No of samples observed	Percentage of germination	Mean ± st.dev
5%	5	78.14%	78.34±6.50
10%	5	84.86%	84.86±6.09
15%	5	86.19%	86.19±2.49
20%	5	85.58%	85.58±2.72

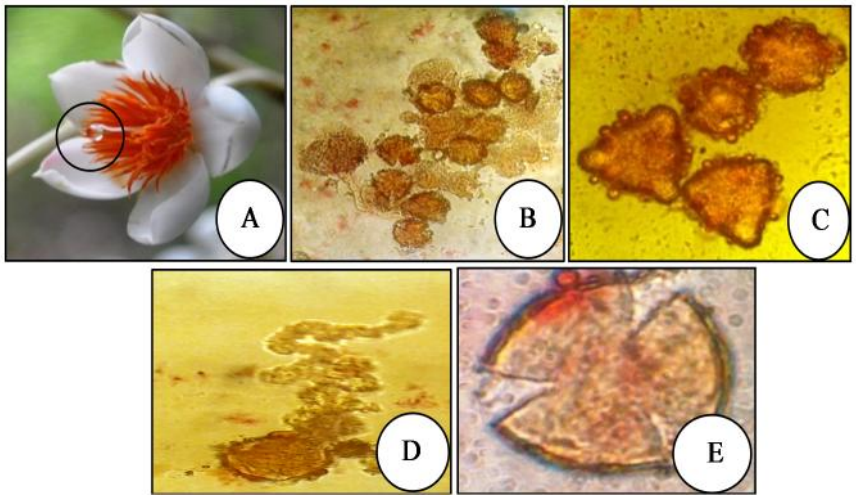

FIGURE 4. A. Stigma receptivity in flowers indicating emission of bubbles. **B .**Viability of pollen grain under TTC. **C.** Pollen germination**. D.** Pollen tube growth in Brew Bakers media**. E.** Pollen grain.

TABLE 7. Results of breeding systems**.**

Breeding system	No of pollinated flowers	No of flowers set fruit	Fruit set %
Open/Natural pollination	50	11	22%
Autogamy(Isolated)	50	9	18%
Geitonogamy (Manual self-pollination)	50	13	26%
Xenogamy(Manual cross pollination)	50	12	24%

TABLE 8. Pollinatorforagerson *Vateria macrocarpa*

Family	Scientific name	Nature	Time of visits (Sec)	Frequency
Apidae (Hymenoptera)	*Apis dorsata* (Rock bee)	Pollen/nectar	5-16	Very frequent
Pieridae (Lepidoptera)	Butterfly	Pollen/nectar	4-9	Very frequent

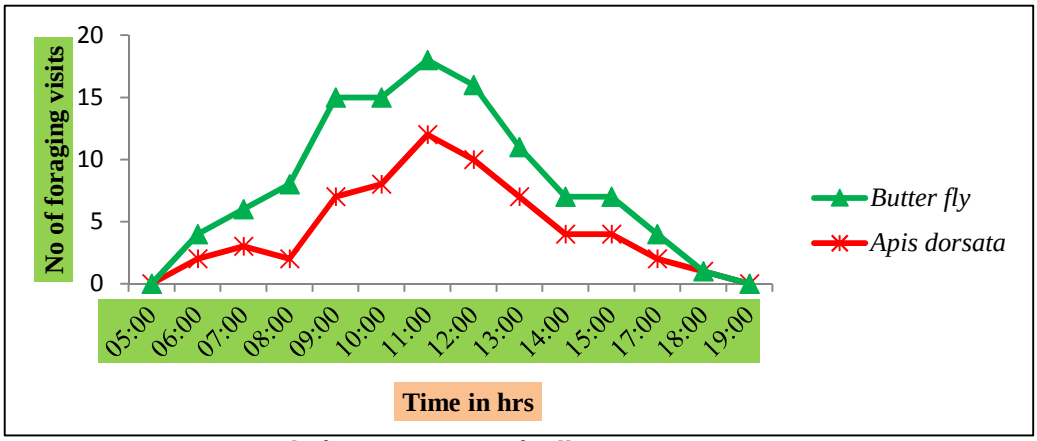

FIGURE 5. Hourly foraging activity of pollinators in *Vateria macrocarpa*

DISCUSSION

Phenology and floral biology

Phenological studies are important to understand ecosystem process such as plant growth pattern, plant responses to various biotic and abiotic factors as competition of light and pollinators (Reich, 1995). In some of the Dipterocarpaceae members like *Shorea roxburghii*, *S. robusta* and, *S. tumbaggaia* leaf flushing and flowering occurs sequentially one after the other (Solomon Raju *et al*., 2011). In the present investigation similar phenological events were observed and all the annual events, secondary events and growth pattern of leaf initiation, leaf flushing, and flowering to fruiting occurs sequentially depending on the biotic and abiotic factors. The phenological observations were made in *Vateria macrocarpa* where the leaf flushing occurs in the month of January to February, flowering begins in March – April. Fruit initiation and fruit maturation occurs from May to July. In the present study, the flower characters of *Vateria macrocarpa* is closely related to *Vateria indica*, since it belongs to same family. Roby *et al.* (2014) reported in the member of Dipterocarpaceae, phenological events such as leaf flushing of *Vateria indica* occurs in the month of January–February. Flowering begins in January and continues till May. Fruit maturation was observed in June and August. These are two types of temporal matching of phenological events in *Vateria indica* and *Vateria macrocarpa* (Nagamitsu *et al*., 1999). Compared to phenological events of *Vateria indica* the present phenological events of *Vateria macrocarpa* shows similarities in leaf flushing and fruit maturation is different which occurs in May-July.

Floral biology and Morphology

The present investigation of floral biology shows that *Vateria macrocarpa* reveals mass flowering by producing more flowers. Similar results and the phenomena of mass flowering were observed in *Shorea roxburghii* and *S. tumbuggia*. The mass flowering is considered as a property of the individual of plant species and this flowering pattern has made effective movement of pollen between trees (Bawa, 1983). The present observation during the field study flowering of *Vateria macrocarpa* has been observed once in every year with fruit maturity, but *Vateria indica*a member of Dipterocarpaceae did not produce flowers for two years. Similarly, *H. parviflora* and *Dipterocarpus indicus*, members of Dipterocarpaceae produced flowers and fruits once in three years (Sundarapandian *et al*., 2005). However, Sundarapandian *et al*. (2005) stated that occasional flowering with an interval of about 3–8 years is more common in Dipterocarps.

Stigma receptivity, pollen production (pollen output per anther/flower), pollen viability and pollen germination

Stigma receptivity is a very important factor influencing effective pollination. Stigma is reported to be receptive at the time of anthesis in many trees (Sanzol and Herrero, 2001). In the present observation stigma receptivity test in *Vateria macrocarpa* showed bubbling activity on the surface of stigma and receptivity remained up to 12hrs. Ghazoul *et al*. (1997) reported that in flowers of *Dipterocarpus obtusifolius* the stigma remains receptive for 24 hrs. Solomon Raju *et al*. (2011) also reported the stigma of *Shorea roxburghii* remains receptive for two days. In some plant species stigmatic receptivity decreases as the flower ages (Stpiczynska, 2003).

In-vitro pollen germination can be useful to detect alterations in germination or tube growth (Cole *et al*., 2005). In the present observation pollen viability of *Vateria macrocarpa* was seen up to two days in germination media. Similar observation was seen in *Dipterocarpus obtusifolius showing* pollen viability decreases in next successive days. However, Shivanna and Harrison (1981) concluded that pollen germination percentages in many species can be increased considerably if pollen grains are equilibrated in moisture environment keeping in incubation in a suitable in-vitro germination media (Boavida and Mecormick , 2007).

Pollen production gradually increases due to approximately 120 stamens in *V. macrocarpa* with ratio of 1:73375 when compared to pollen ovule ratio of *D. obtusifolius*, existing ratio of 45,000: 6 with maximum thirty stamens (Ghazoul, 1997; Nagamitsu *et al.*, 1999).

Breeding system and pollination

Self-incompatibility is an important aspect which determines the percentage of fruit set from self and cross pollination in flowering plants (Krishna Kumar *et al*., 2014). In the present investigation controlled pollination of *Vateria macrocarpa* showed that the species is self- compatible. Similar studies of self-compatibility *Dipterocarpus tuberculatus* was reported by Chan (1981) and Ashton (1988). Some members of Dipterocarpaceae such as *Shorea tumbuggaia* and *Shorea robusta* are self- compatible (Raju *et al.*, 2009; Atluri *et al*., 2004). Ghazoul (1997) noticed that self- incompatibility in *Dipterocarpus obtusifolius* due to early abortion of ovules in self-pollinated flowers. Solomon Raju *et al*. (2011) also reported that in *Shorea roxburghii* absence of fruit set in autogamy and geitonogamy suggest that plant is self - incompatible and also suggested that the species exhibits weak protogyny. However Bertin and Newman (1993) stated that protogyny is a characteristic associated with self-compatible anemophilous flowers to reduce selfing rate. In the present investigation in breeding system of *Vateria macrocarpa* the similar results was observed due to numerous stamens and the species is self- compatible with strong protogyny. Since, *Vateria macrocarpa* grows up to certain canopy heights, wind force can easily vibrate the flowersto release pollen in air and same is carried to receptive stigmas of different flowers and trees. Natural fruit set was limited and percentage of fruit set in artificial cross pollination was higher in the present study. Compared to the results of natural pollination, it strongly indicates that some external agents are required for effective pollination (Solomon Raju *et al*., 2011; Sreekala *et al*., 2008; Raj Kumar *et al*., 2015).

Floral visitors

The flowers of *Vateria macrocarpa* shows white colour petals and red stamens grouped in middle are found to be important character which attracts Pieridbutter flies. However, Dronamraju (1960) demonstrated that flowers with white – pink colored flowers of Dipterocarpaceae are favored largely and foraged by butterfly species such as *Catopsilia pyranthe pyranthe*. Similar results were reported by Ghajoul *et al*. (1997) in *D. obtusifolius where* colour is the important character which attracts butter fly species such as *Deliasdes combesi, D. pasithoethyra fruhstorfer and Catopsilia pomana fabricus* (Pieridae). In the present investigation similar observations were recorded, butterflies were seen foraging flowers for pollen and nectar and considered that colour is the important character to attract butterfly species in visiting *V. macrocarpa* flowers for pollination.

Honeybees like *Apis dorsata* and *Apis cerana* were the important pollinators with a maximum foraging range over 10 km in Dipterocarpaceae members (Rhett *et al*., 2005). In our present investigation of pollinator observation of *Vateria macrocarpa* included honeybee such as *Apis dorsata* the main pollinator. Similar observations were recorded in *Dipterocarpus crinatus, Dipterocarpus geniculatus* flowers were visited by *Apis dorsata (*Rhett *et al*., 2005). Dayanandan *et al.* (1990) reported that effective pollinators of *Vateria copallifera* in Sri Lanka were honey bees. In the present observations it is confirmed that *Apis dorsata* is the main pollinator among Dipterocarpaceae members pollinating flowers of *V. macrocarpa* with high frequency.

Pests/predators and threats

In the present study the flower buds of *Vateria macrocarpa* were usually predated in large quantities due to Bruchid beetle larva (Fig .3 J & K)destroying the reproductive cycle of flower and fruit maturation (Fig.3L) are one of the factors contributing the endangered status and increased declining of populations of *Vateria macrocarpa*. Fruits are seen below the mother tree with one or two saplings with poor natural regeneration due to the larval infestation of fruits (Fig.3I) and damage made by Bruchid beetle larvae. The local tribal population extract timber, and resin from *V.macrocarpa,* and more or less same in the closely related species of *Vateria indica,* these substances are sold by the tribal as they are use full in making candles and soaps and are also used as varnishes with coconut oil. Loss of habitat and other human activities has contributed critically to the endangered status of *Vateria macrocarpa* according to IUCN red list category (Venkatesh *et al*., 2010; Solomon Raju *et al*., 2011).

CONCLUSIONS

Vateria macrocarpa is a critically endangered species of Western Ghats and highly restricted to evergreen forests of Palakkad, Kerala, and some parts of Western Ghats, Karnataka. Results concludes that flowers of *Vateria macrocarpa* are axillary and hairy, calyx brown and corolla white in colour and 5 in number, stamens numerous, ovary 3loculed, densely tomentose and fruit one seeded. Anthesis starts in late evening and the floral visitors such as *Apis dorsata* and butterflies are important pollinators. The open pollination experiments revealed that the species is self - compatible. Fruits and seeds are largely predated by Bruchid beetle larvae and seeds are recalcitrant. It was observed that bark of *Vateria macrocarpa* was seen damaged by local tribes for obtaining resin as it has commercial value and sold by locals in nearby markets. Deforestation is continued by dam constructions and other developmental activities and also over exploitation of human activity. The study suggests that ex-situ and in-situ conservation strategies are to be developed for restoration. To protect these valuable species, the forest department has to create awareness among local population about the importance of *V. macrocarpa,* the endangered plant species in the forest ecosystem.

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