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RESEARCH ARTICLE

Phenological studies of *Calophyllum apetalum* Willd., an economically important medicinal tree of Western Ghats

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Abstract

Calophyllum apetalum Willd., commonly called Poonspar of Travancore, is a multipurpose evergreen tree endemic to the Western Ghats of India. For phenology studies, individual trees were tagged and their vegetative and reproductive phases have been monitored from 2021 to 2023. The leafing phenophase - simultaneous flushing and leaf fall before the onset of rain - makes *C. apetalum* trees appear to be always leafy. Leafing, flowering and fruiting phenophases were significantly seasonal. Leaf flushing to flowering takes time and also from flowering to fruiting. Peak flowering is visible from September to October, fruiting maturity occurs in January. Phenological events may vary according to region as well. As with flowering the phenology of fruiting is governed by its own set of constraints. Phenological studies are essential for developing appropriate conservation strategies and to assist adequate germplasm collections since it is necessary to know the baseline data on their flowering and fruiting behavior.

Keywords: *Calophyllum, flowering, fruiting, leaf flushing, phenology*

Introduction

Phenology, from the Greek '*Phainein*' (meaning "to show or appear") is the timing of life history events (Fenner 1998) and it has been principally concerned with the dates of first occurrence of biological events in their annual cycle. In plants, this can include flowering, leaf unfolding (or budburst) seed set and dispersal and leaf fall in relation to climatic conditions (Davi *et al*., 2011). However, phenology data sets spanning many years are rare in the tropics, making it difficult to evaluate possible responses of tropical communities to climate change. *Calophyllum apetalum* Willd., is a species under the genus Calophyllum belonging to the Clusiaceae family. According to the IUCN Red List, *C. apetalum* is classified as vulnerable. Among the species of genus Calophyllum, only three species have been evaluated, while other species remain unevaluated and data-deficient (Chinthu *et al.,* 2022). Over the past thirty years there has been an increase in scientific research concerning the conservation and management of threatened or endangered species (Brigam 2003). *C. apetalum* yields edible fruits; its stem bark exudes a translucent resinous fluid used as a vulnerary, anti - inflammatory and anodyne (Chopra *et al*., 1956). According to IUCN, *C. apetalum* has no conservation actions yet and research is needed in the areas of taxonomy, population size, distribution & trends, life history and ecology, threats and conservation planning. Understanding *C. apetalum* phenology is crucial for the conservation of this species and its propagation.

Materials and methods

Calophyllum apetalum Willd., is a woody tree species endemic to southern Western Ghats in riparian habitats. The best representations of this species are found in the coastal areas of Alappuzha district in Kerala (Lat: 9.41279º N; Long: 76.34781º E / 9.54; 76.40). The area experiences a

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mean annual rainfall of 2763 mm and a mean maximum temperature of 32.75˚C (Government of India Meteorological Department, 2021-2023). A selected group of trees in Alappuzha was studied. The phenological events were studied by randomly selecting 10 trees from undisturbed areas of Alappuzha district, Kerala. The observations were made on different phenophases including (a) leaf emergence and drop (b) flowering and (c) fruit setting during the period from January to December 2021 to 2023. The floral phenology of the species was studied by tagging five inflorescences each on 10 randomly selected individual trees and the flowering stages were observed from bud initiation until senescence. The unopened flower buds were selected and tested for pollen viability (Acetocarmine test - Stanley R. G. and Linskens H. F 1974). Different phenophases were found to be cyclic and were assessed for the strength of cyclicity in a given event. Fruiting individuals and girth classes were marked and observed to record the production, maturation, ripening of fruit and to assess the DBH. The state of Kerala experiences distinct seasons and weather. Through recurrent trips to the areas where the populations were dispersed, phenological events like leaf flushing, flowering, fruit setting etc. were meticulously documented.

Results

Population

Calophyllum apetalum is a moderately sized evergreen tree with a height of 9 to 10 metre. The tree requires moist soil that is rich in organic matter and can grow well in light shade with sufficient rainfall. The common stature of the tree is straight, with a cylindrical stem and a girth ranging between 4.5 and 5 metre.

Fig. 1: leaf flushing stages of C. *apetalum*

Morphology

Calophyllum apetalum is an evergreen, moderately sized tree with a straight and cylindrical stem, distributed in the forests of the Western Ghats, often found along the banks of rivers and streams. The leaves exhibit uniqueness irrespective of regions and leaf flushing imparts a very impressive appearance. The young leaves are very thin, glossy and light green or light yellow in colour. Later, they become dark green. Leaves turn light yellow to dark red before flower initiation. Leaf flushing is also visible when new leaves arise. Newly developed leaves are seen along with mature leaves and their colour changes during maturity. The synchronization between leaf senescence and flushing keeps the trees evergreen (Fig: 1). Leaves are susceptible to a range of pathogenic agents. Young leaves are eaten by common caterpillars (Fig: 2). Seasonal studies of Peak leaf flushing (PLF), Leaf fall initiation (LFI), Peak flowering (PFL), Peak fruiting (PFR) was conducted during the period of 2021 to 2023 (Table: 1). The bark and roots of *C. apetalum* are yellowish; hence, it is called Manjapunna. The bark looks similar throughout the life of *C. apetalum*, but the diameter of the trunk varies dramatically (Trees

Fig. 2: Insect attack (caterpillar) on the young leaves of *C. apetalum*

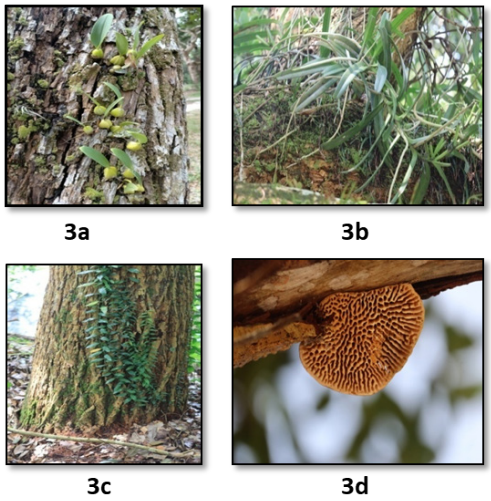

Fig. 3: a- Bulbophyllum Sp., b- *Vanda tessellata.*, c- *Pothos scsndans.*, d- Polyporus Sp.

Table 1: Seasonal study- During the period of 2021 - 2023

		2021				2022				2023			
District	*Populations*	*PLF*	*LF1*	*PFL*	*PFR*	*PLF*	*LF1*	*PFL*	*PFR*	*PLF*	*LF1*	*PFL*	*PFR*
Alappuzha	Vandanam	Sep-Oct	April-May	Aug- Sep	Feb-Mar	Aug-Sep	April-May	Nov- Dec	Jan-Mar	July- Aug	April-May	Sep-Oct	Nov- feb
	Kalavoor	July-Aug	April-may	Sep-Dec	Jan-Feb	Aug-sep	March-apri	Sep-oct	Jan-Feb	March-april	May-june	Sep-oct	Dec-Mar
	Kayippuram	Ovt-Nov	April- may	-	-	July-Aug	April-may	-	-	June – july	March - april	-	-
	Muhamma	July-Aug	April-May	Sep-Oct	Dec-Jan	Aug-Sep	April-March	Sep-Oct	Dec-Jan	Aug-Sep	April-March	Sep-Oct	Dec-Jan
	Vanaswargam	July-Aug	April-may	Sep-Dec	Jan-Feb	Aug-sep	March-apri	Sep-oct	Jan-Feb	March-April	May-june	Sep-oct	Dec-Mar

Peak leaf flushing (PLF); Leaf fall initiation (LFI); Peak flowering (PFL); Peak fruiting (PFR).

Table 2: Trees with their DBH (Diameter at Breast Height) - Alappuzha

Sl. Nos	*Locations*	*Populations*	*DBH (cm)*
1	Aikkarakkavu	P1	200
		P2	140
		P3	300
		P4	460
		P5	340
2	Illathukaavu	P1	180
		P2	280
		P3	110
		P4	420
		P5	370
3	Vandanam	P1	220
		P2	290
		P3	490
		P4	510
		P5	280

Fig. 4: Extensive root system of *C. apetalum*

Fig. 5: Common treehopper associated with *C. apetalum*

with their DBH (Diameter at Breast Height) - Alappuzha) (Table: 2). The bark serves as a storage space for water due to this peculiar quality of the structure. Many plants are associated with the tree, such as *Bulbophyllum Sp*; (Fig: 3a), *Vanda tessellata* **(**Roxb) Hook. ex.G.Don (Fig:3b), *Pothos scandens* (L.) (Fig: 3c), Polyporus Sp. (Fig: 3d), *Cuscuta reflexa* Roxb. and *Pyrosia heterophylla* (L.) M. Price inhabits the tree. On the bark, lichens, bryophytes, pteridophytes and even common treehoppers (Fig. 5) can be seen. The roots are

Fig. 6: Inflorescence of *C.* apetalum

highly extended to absorb water, forming a large network around the tree (Fig. 4). Flowers are bisexual, creamy white, with petals absent. Stigma is peltate with entire margins, about 1 to 1.5 mm across (Inflorescence - Fig: 6).

Phenology of flowering

Calophyllum apetalum sets out to bloom at the onset of leaves fall in the dry season. Trees experience concomitant leaf transition states-leaf initiation and leaf senescence. Flowering in *C. apetalum* occurs in axillary inflorescences (solitary flowers), extending for a short period of time with high intensity. Peak flowering is observed between September and November, with more than 80% blooming. The peak leaf fall is observed during March to April. When fresh leaves arise in July to August, followed by leaf flushing, the flowering period begins. Peak flowering is observed between September and October. The average duration taken by the flowers to mature is 13 to 18 after initiation. The peak flower anthesis occurs between 08:15 a.m. and 09:30 a.m. hours (Prasanna Kumar et al., 2013). Fruit set initiation was observed 2 to 3 days after pollination and fertilization. Characteristics of flowers and their production are listed (Table: 3)

Table 3 : Characteristics of *C. apetalum* flowers and flower production

Sl. Nos	*Flower characteristics*	*Observations*
1	Flower colour	Creamy White
2	Inflorescence	Axillary inflorescence
3	No of flowers per inflorescence (initial)	10 ± 2.04
4	Number of flowers per inflorescence (mature)	4 ± 1.02
5	Number of flowers / trees	Numerous
6	Mean length of flower (cm)	2.2 ± 0.05-3.5 ± 0.1 cm
7	Mean width of flower (cm)	2.0 ± 0.05-3.5 ± 0.1 cm
8	Mean pedicel length (cm)	0.5 ± 0.1-1.8 ± 0.2 cm
9	Anthesis	At night
10	Longevity (hrs).	24 – 48 hrs
11	Flowering months	September - October
12	Flowering duration (maximum)	1 month

Pollination and fruit set

Fruit set initiation was observed 2 to 3 days after pollination and fertilization. Pollination occurs primarily by cleistogamy (self-pollination) and other types of pollination such as entomophily (insects) and entomophily (wind). Allogamy (cross-pollination) is also observed. A single flower consists of 23 to 46 anthers; this number may vary from bud development to mature flower. Each anther contains numerous pollen grains. Viable pollen grains were detected using the acetocarmine test. 94.5 % of the pollen grains exhibited staining, indicating their viability. The major pollinators of the species include honeybees (*Apis dorsata*, *Apis mellifera*) and carpenter bees (*Xylocopa violacea* and *Xylocopa pubescens*) with high visitation frequency.

Fruiting phenology

The fruit is an indehiscent drupe, ovoid or globose, with a dry mesocarp and stony or spongy endocarp. It contains one seed, with large cotyledons, exalbuminous and with rich oil content. Following pollination and fruit set, fruit initiation occurs. The fruit matures within 2 to 3 months (November to January). In *C. apetalum*, peak fruiting is observed during January, when the maximum number of flowers becomes

Fig. 7: Mature fruits of *C. apetalum*

Table 4: Characteristics of fruit and fruit production

Sl. Nos	*Fruit characteristics*	*Observations*
1	Fruit	Drupe
2	Fruit colour -Young / Mature	Green / Yellow (Occasionally Red)
3	Fruiting period (maximum)	January - March
4	Shape of the fruit	Ovoid
5	Seed colour	White
6	Moisture content of mature fruit (10 Nos)	48.19 ± 0.59 %
7	Fruit fresh weight (single fruit)	3.88 ± 1.03 gm
8	Fruit dry weight (single fruit)	2.01 ± 2.10gm
9	Fruiting duration (months)	2 - 3 months
10	Number of fruits per inflorescence	Numerous
11	Fruit length (cm)	3.6 ± 0.10
12	Fruit width (cm)	2.5 ± 0.10

Values are the mean of three annual cycles (2021-2023)

Table 5: Reproductive biology of *C. apetalum*

Sl. Nos	*Reproductive characters*	*Observations*
1	Pollination	Cleistogamy/Anaemophily
2	Anthers per flower	24 – 48, free
3	Pollen grains per anther	Numerous
4	Inflorescence per branch	May-13
5	Sepals	4
6	petals	Absent
7	Carpel	single
8	Ovule	Single
9	Ovary	Superior
8	Fruits per inflorescence	15 - 20
9	Fruit	Drupe
10	Seeds per fruit	One
11	Albumin	Absent
12	Oil content	present
13	Fruit dispersal	Hydrochory, Zoochory

Values are the mean of three annual cycles (2021-2023)

fruits. Sometimes fruiting may be extended until March or April. Seeds are dispersed by hydrochory and zoochory. The mature fruits on the tree, as well as fallen fruits (both young and mature) are severely attacked by small weevils, ants etc. Fruits are chlorophyllous, with green color changing to dark yellow during maturity. Between development and maturity, some fruits occasionally attain a dark reddish color, later changing to dark yellow. Premature abscission of flower buds, flowers and fruit can be observed. Insect attacks are evident during all stages of development to maturity. Fruits may occasionally dry out instantly in hot weather before falling to the ground. Characteristics of fruit and fruit production are listed (Table: 4) (Mature fruit Fig: 7)

Reproductive biology of Calophyllum apetalum

Self-pollination is brought about by cleistogamy; therefore, the species does not depend on pollinators for pollination. This process helps produce an assured seed set even in adverse conditions. *C. apetalum* is devoid of petals, which supports the observation that the species is predominantly pollinated by self-pollination. One of the most notable observations is that phenological events such as leaf flushing, flowering, fruiting and leaf fall can all be observed simultaneously on the same tree. Reproductive biology of *C. apetalum* (Table:5)

Major threats facing Calophyllum apetalum

Unsustainable collection of seeds, resin and flowers for medicine, along with extraction of timber, has led to habitat loss. The species faces a high crisis status, primarily due to unscientific harvesting of high-quality timber. Chopping branches of even delicate trees to make organic compost has exacerbated the situation. Plastic pollution has worsened the region as it prevents fruits from flourishing either on the ground or in water. Plastics diminish the chance of seed germination. Construction of roads and buildings adversely affects the natural regeneration of seeds and may cause habitat destruction. Plants typically have residents and associates; however, many associates can eventually bring down the entire tree. All these factors are deeply interconnected and provide useful knowledge to identify possible factors that can reduce the reproductive ability of the species. Thus, conservation proposals are best supported when based on phenological studies.

Discussion

Phenological research is also crucial for understanding species interrelationships and interactions with the environment. Pollinator preferences are among the biotic elements influencing phenological trends (Lobo *et al*., 2003). The existence of species that can grow to a great size and the presence of disturbance regimes that allow for the formation of large diameter individuals are both necessary for the dynamics of large diameter trees. A forest may be more resilient to disturbances if the large diameter assemblage has high species richness (Musavi *et al*., 2017), which keeps its ecological function and structure intact. Endophytic fungi are visible on the tree; this agrees with the data of Chandrappa *et al*., (2016), which revealed that silver nanoparticles from Alternaria Sp. (an endophytic fungus) inhabit the bark of *C. apetalum*. Chandrappa *et al.,* (2016) also reported the endophytic synthesis of silver chloride nanoparticles by the aqueous extract of Penicillium *Sp.* from *C. apetalum* stem bark. In *C. apetalum*, flower initiation was observed following leaf flushing from September to October. Nanda *et al.,* (2011, 2013) observed synchronization of leaf flushing and flowering, which appears to be connected to moisture, temperature and day length in *Schleichera oleosa* and *Lagerstroemia lanceolata***.** One of the most notable observations in *C. apetalum* is that phenological events such as leaf flushing, flowering, fruiting and leaf fall can all be observed on the same tree. This is in line with the data of Sharma and Khanduri (2007), who noted that a phenophase was regarded as having begun when 20% of individuals were seen in that phase and it was judged to have ended when less than 20% of individuals remained in the same phase without any change. Flowering is infrequent and there is disparity in the flowering season among *C. apetalum* populations, which varies by region. However, timing of leaf flushing varies depending upon the region as well. Leaf flushing observed one to twice in an year. But the flowering observed only once. Some trees never flower at all only leaf flushing. Leaf senescence peaks in dry season such as March-May. Similar observations could be seen in most canopy trees. The rate of leaf fall during the dry season was correlated with the decline in soil moisture

content and increased water stress of the tree (Reich and Borchert 1982). Long term phenological records in trees, including spring events such as leaf unfolding and autumnal events of leaf colouring, have shown that a rise in global temperature general leads to earlier timing of spring events (Chen and Xu 2012, Doi and Katano 2008, Menzel 2006). The bark of *C. apetalum* is highly unique in appearance. In *C. apetalum*, the diameter of the trunk varies dramatically from 125 to 510 cm. The notion of large diameter is intrinsically dependent on species and habitat type. In frigid, continental woodlands, a large diameter tree might only be 20 cm DBH (Baltzer *et al*., 2014). In fertile temperate or tropical woods, a large diameter tree can be more than 100 cm DBH (Lutz *et al.,* 2012, 2013). In *C. apetalum*, many plant associates such as *Bulbophyllum Sp*; *Cuscutta reflexa* Roxb., *Pyrosia heterophylla* (L.) M. Price., *Vanda tessellata* (Roxb.) Hook. ex G.Don., *Pothos scandans* (L) and *Polyporus Sp*; inhabit the tree. Common treehoppers are visible on the tree. On the bark, lichens, bryophytes and pteridophytes can be found. Phenological variance in populations may also be influenced by variations in environmental conditions, but genetic modulation may also play a role in these phenotypic plastic responses (Quinn and Wetherington 2002). Sometimes flower buds can also be visible in March and April. Variations in flowering time relative to vegetative phenology caused by a range of variables, result in a multitude of flowering patterns in tropical trees (Borchert *et al*., 2004). In that situation, the bud will flower but fall due to high temperatures and will not develop into a fruit. The timing and duration of bud burst, leaf expansion, maturity, senescence and fall of individual leaves are all critical to plant fitness (Fenner 1998). The peak flowering is distinguished by late September to October. It has a short flowering period once blooming start. Approximately 35-40 days or sometimes 10-14 days are required for a flower to fully bloom from a bud. Endogenous genetic variables and environmental signals like photoperiod, temperature and stress all influence blooming timing (Andres F & Coupland G, 2012). Pollination is by cleistogamy (self-pollination) or allogamy (cross-pollination). It is also encouraged by a large number of pollen grains. The flowers are homogamous with a reduced outcrossing index, suggesting that they prefer fruit set with self-pollination (Cruden 1977). *C. apetalum* has white flowers with little to no fragrance, which supports self-pollination. The production of pollen grains per flower is numerous. The flowers solely generate pollen as a pollination reward and do not produce nectar (Prasannakumar *et al*., 2013). Premature fruit abscission and other phenological anomalies contribute to the scarcity of the species in its environment (Murali and Sukumar 1994, Lokesha and Vasudeva 1997). The duration of solar radiation, temperature and humidity all significantly impact flower production and fruit set (Fewless 2006). Following fruit initiation, they attain maturity within 2-3 months. There is a clear distinction between temperate and tropical fruiting patterns (Jordano 1992). *C. apetalum* yields edible fruits (Chinthu *et al.,* 2022). Fallen fruits (both young and mature) are severely attacked by small weevils, ants, etc. Individual reproductive performance is determined by seed predation, which can impact plant abundance, distribution and community structure (Kolb *et al*., 2007). The fruits are chlorophyllous and their green color changes to dark yellow during maturity. Between development and maturity, the fruit turns dark reddish before finally changing to dark yellow. Fully grown fruits are distinguished by their well-developed abscission zone, pleasant fruity odor and fleshy character, which serve as visual indicators. Seeds develop germinability gradually (Luo *et al*., 2005, Shao *et al*., 2006). Premature abscission of flower buds, flowers and fruits can be observed. Insect attacks are also seen during all stages of development to maturity. Certain plants located in isolated habitats show unusual flowering behavior during the off-season. One of the most notable findings from field research is a group of trees primarily located beneath sacred groves with ponds. This result aligns with the data of Nair M S (2014), who conducted comprehensive studies on sacred groves in Kerala, particularly six taluks of Alappuzha district. Nair assessed a total of 32 sacred groves, of which fifteen contained *C. apetalum*. Our studies identified many populations in the Aikkarakkavu region, which agrees with the findings of in their book Keralathile Kavukal (2021).

Conclusion

The present study revealed that leaf flushing occurs before flowering and when new leaves arise, based on the plant's developmental phase. If the phase is vegetative, new leaves emerge; if the phase is reproductive, flower formation occurs. After leaf flushing (reproductive phase), flower bud set occurs, with peak flowering visible during September. Fruit initiation begins about a month after flowering, with peak fruiting observed from December to January. In January, fruits ripen and begin to fall. Flowering period lasts for about 2–3 weeks, while fruit ripening takes about 80-90 days. In coastal regions such as Alappuzha, moist sandy soil allows fruits to fall naturally, promoting natural germination. However, natural regeneration is problematic because the species are scattered in riparian environments and most seeds are drowned, leaving only a few on the riverbanks to form saplings. These saplings are also at risk during the rainy season due to the high chance of being swept away. Additionally, seeds of *C. apetalum* attain maturity in January and the subsequent dry season (March-April) pose a risk increases the risk of desiccation if water is unavailable. The potential for seed germination increases if the seed lands in a damp area or a location with a water supply. Thus, water/moisture content is a pivotal factor for the natural seed germination of *C. apetalum*.

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