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Article

Reproductive biology of *Cinnamomum sulphuratum* Nees. from wet evergreen forest of Western Ghats in Karnataka

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Abstract
In *Cinnamomum sulphuratum* the initiation of the buds occurred after the leaf initiation during October and initiation of buds started during November last week. Inflorescence is an axillary panicle with 62.48±7.01 floral buds that took 13±1.41 days to bloom. Flower offer both pollen and nectar as a floral reward to the pollinators. Foragers include honeybees, butterflies, wasps, flies and ants. The flowers are self-compatible, pollinate both by self and cross pollination. In Allogamy (Hand cross pollination), highest mean percentage of fruit set was observed as 71 and 75% respectively for the period 2012-13 and 2013-14.

Keywords *Cinnamomum sulphuratum*; phenology; pollination; breeding system.

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1 Introduction
The genus *Cinnamomum* belongs to the family Lauraceae, comprising of many commercial spices. Leaves of different species of *Cinnamomum* are used as a substitute to tamalapatra (Baruah et al., 2000; Sunil Kumar, 2006; Sunil Kumar et al., 2012a,b) on account of its easy availability and similarity in flavor, different parts of *Cinnamomum sulphuratum* are used as a substitute for commercial *Cinnamomum* derived spices.

Cinnamomum sulphuratum is a medium size tree, distributed in the southern Western Ghats of India. It is one of the 12 endemic south Indian species of *Cinnamomum* (Kostermans, 1983). It is also reported from North Cachar Hills of Assam and Northeast India (Nath and Barua, 1994; Ravindran et al., 2004). In South India it is distributed in Western Ghats regions of Tamilnadu *viz.*, including Nilgiris and Annamalai and Thiruvananthapuram and Wynad in Kerala. In Karnataka it is found in Coorg, Dakashina Kannada, Hassan, Mysore, Shimoga and Uttara Kannada districts (FRLHT, 2006; Sunil Kumar et al., 2013). The leaves and bark of the tree are aromatic (Baruah et al., 1999a, b) leaves are used as a spice and has vernacular name *tejpat* by the North-East Indian people (Baruah and Nath, 1998; Baruah et al., 2000). Medicinal uses of *C. sulphuratum*

is similar to *C. zeylanicum* which includes use for treating wounds, fever, intestinal worms, headache and menstrual problems.

Although many important investigations on reproductive ecology of tropical tree species have been undertaken (Whitmore, 1990; Richards, 1996), yet many species remain uninvestigated. Contrary to the situation in other countries, interest in the field of reproductive biology is rather dwindling in India. This trend is disheartening to the research community across India, harboring two biodiversity hotspots of the world. Many of these species are threatened and require developing of an effective conservation strategy. The study of phenology of tree species gives information on the time of appearance of floral buds, anthesis, fruit development and fruit fall during the reproductive phase of a tree (Morales et al., 2005). Besides these, it also provides essential input for many relevant ecological, most importantly concerned with the global carbon and water cycles (Menzel, 2002; Sparks and Menzel, 2002). In generally the information on phenological patterns of endemic tree species in tropical forests of the Western Ghats is limited. Bhat (1992), Murali and Sukumar (1994), Joseph (1981), Kubitzki and Kurz (1984) and Mohanakumar et al. (1985) studied the floral biology of Cinnamomum species, but information is still incomplete (Ravindran et al., 2004). Current threat status of *C. sulphuratum* is vulnerable at the global scale (FRLHT, 2013). Keeping this in view, the present study was conducted to study the vegetative and reproductive phenology, pollination biology and breeding systems of this important species.

2 Study Area

The study was carried out in the Agumbe region of Someshwara Wildlife Sanctuary, situated in Udupi-Shimoga districts within the central Western Ghats of Karnataka. Agumbe region falls within 13^{o}30’9.64”N and 75^{o}5’25.15”E with an elevation ranges 400-600 meters above mean sea level (MSL).These forests are composed of rich endemic flora (Pascal et al., 1988). Agumbe is one of the wettest regions in Karnataka, with a mean annual rainfall between 5000 to 8000 mm.

3 Material and Methods

3.1 Vegetative and reproductive phenology

The phenological events were studied by selecting 25 mother trees, marked randomly from the study location. The observations were made on phenophases such as, (1) leaf sprouting and maturation (2) flowering and anthesis, (3) Fruiting and (4) leaf and fruit drop, for a period of three consecutive years from January 2011 to January 2014. The phenological records were made every week during the high activity period of flowering season from October to March, till fruit maturation. The observations were continued on other phenophases with three week intervals during the rest of the year (Prasannakumar et al., 2013).

3.2 Floral biology

The studies pertaining to floral biology started from the very beginning of floral bud initiation. The inflorescences were selected and marked on matured mother trees to observe the flowering period and different stages of floral development. The observations continued until fruit formation. The time of anthesis was noted. To observe the dehiscence of anther and stigma receptivity hand lens (*10x*) was used before and after the opening of flowers (Tidke and Thorat, 2011).

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

Pollen production was determined from randomly selected matured anthers taken from flower buds (Nair and Rastogi, 1963; Nanda et al., 2006), for the three consequent flowering seasons between November 2011 and December 2013. The number of ovules were counted by taking a cross section of ovary (Cruden, 1977).

In-vitro pollen germination studies were carried out using Breawbaker media. Freshly dehisced pollen grains were placed in requisite concentration of sugar following "Hanging Drop Technique" (Brewbaker and Kwack, 1963). Also, various concentrations of sucrose solution such as 5, 10, 15, 20, 30, 40 and 50% and, distilled water was used as control media maintained at room temperature. A pinch of boric acid was added to each concentration to facilitate initiation of germination. The pollen viability was assessed by aniline blue fluorescence microscopy and by 0.5% acetocarmine solution; the stainability was taken as an index of viability as described by Shivanna and Rangaswamy (1992).

3.4 Flower visitor's dynamics and behavior

Pollinators were observed over 24 hours during the flowering period for three consecutive years and particularly between 0600-1800 hrs. and the duration of time spent by each pollinator and floral visitors was noted (Fenster et al., 2004).

The behavior of insect visitors during flowering period was observed at different hours of the day, at each study site. The observations were also made on their mode of approach, the type of forage they collect, contact established with the essential organs of the flowers and the activities of the forager during the visits. Number of flowers visited per bout by floral visitors and the time spent on each flower were noted (Tidke and Thorat, 2011).

3.5 Breeding studies

Breeding experiments were carried out manually by hand pollination of the flowers as briefed here under

I. Apomixis: mature flowers were selected from the inflorescence before anthesis and emasculation was carried out followed by bagging.

II. Autogamy: matured flowers were selected from the tagged inflorescences and bagged.

III. Allogamy: matured flowers were selected from the tagged inflorescences emasculated before anthesis and pollen grains from a mature flower of another plant as deposited on the receptive stigma.

IV. Natural pollination: Mature flowers from the inflorescence were marked and observed for the pollination.

V. One time insect pollination: matured flowers were selected from the inflorescences before anthesis and observed for insect visit. In all the cases visited flowers were bagged and observed.

4 Results and Discussion

4.1 Vegetative and reproductive phenology

The leaf initiation in *C. sulphuratum* started during the first week of October in 2011, second and third week of October during 2012 and 2013. Flowering started by first week of December in 2011 and 2012 and during 2013, the flowering started in the last week of November. Fruit initiation started during the third week of March during 2012 and 2013. The three year phenological observations showed insignificant difference between the occurrence of vegetative and floral phenological events. Phenological events were on par within a week difference during the three consequent years from 2011 to 2013. Similar phenological observation has been recorded by Chauhan et al. (2008) in *Terminalia arjuna*; Dhillon et al. (2009) in *Pongamia pinnata*; Prasannakumar et al. (2013) in *Madhuca neriifolia*.

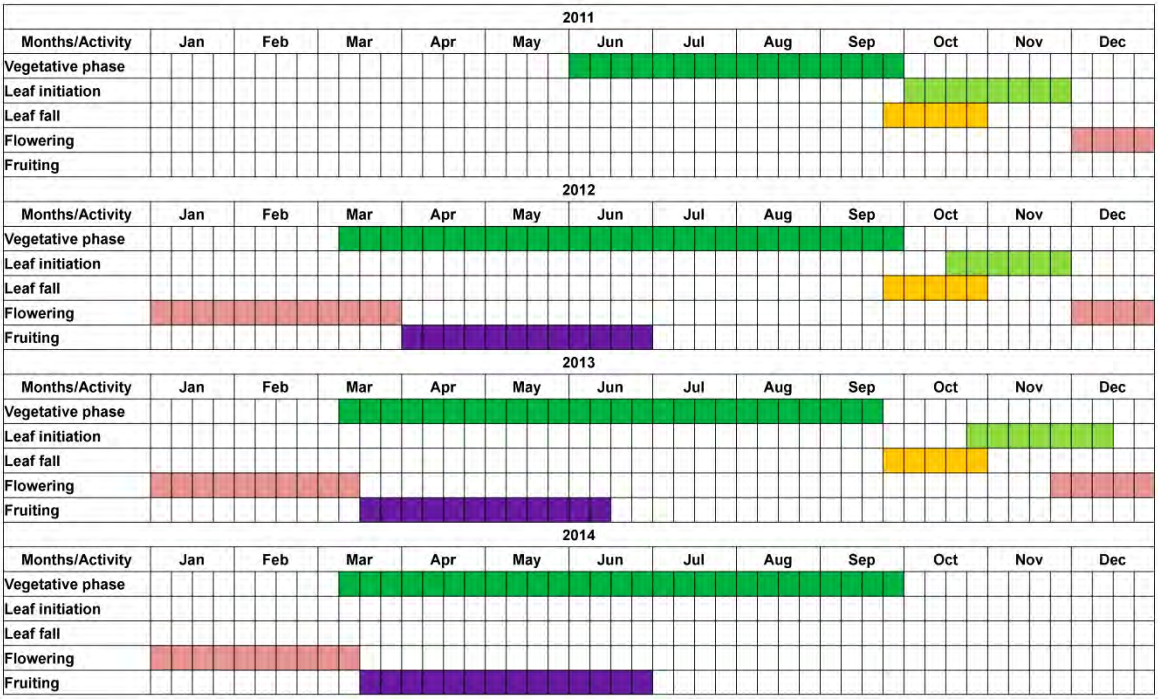

Fig. 1 Phenological events for the period of 2011 to 2013.

4.2 Floral biology

Flowers of *C. sulphuratum* is hermaphroditic, occur in axillary panicles, Average length of the inflorescence is 12.37±2.40 cm, average number of flowers per inflorescence is 62.48±7.01 and the average number of anthers per flower is 12 arranged in two whorls. Flowers of *C. sulphuratum* have greenish white colour peduncles, bracteate, actinomorphic, bisexual flowers, trimerous, perigynous, perianth six in two whorls of three each, free, stamens. Stamens and staminode filaments are provided with minute hairs. Ovary superior, unilocular with a single pendulous anatropous ovule.

Opening of the flowers occurred in two stages. On an average flower takes 13±1.41 days for development from the day of initiation. In the first stage after the flower opened stigma appeared to be receptive and there was no anther dehiscence. After five hours, the flowers closed and opened again the next day *i.e.*, second stage when anther dehisces before second time opening of the flower, after five hours flowers again got closed and opened again. Similar observations are recorded in *C. verum* and *C. camphora* (Kubitzki and Kurz, 1984; Mohanakumar et al., 1985).

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

Average number of anther per flower is 12. Pollen production was 7536, 7753 and 8470 during 2011, 2012 and 2013 respectively. Percentage pollen germination is observed to be 60.97±13.91 in Brew baker media. The average percentage of pollen viability is 82.60, 80.69 and 87.73% during 2011, 2012 and 2013 respectively.

4.3.1 Pollen-ovule ratio

The pollen: ovule ratio is 1256 during 2011; 1292 during 2012 and 1411 during 2013. The average nectar volume is 0.9±0.51, 1.02±0.56 and 1.09±0.68. Whereas, nectar concentration is 4.59±1.89, 5.79±1.68 and 6.16±1.70 during 2011, 2012 and 2013 (Fig. 3). Nectar production in *C. sulphuratum* is very meagre due to the

small size of the flower. Nectar glands are produced regularly at the base of the stamens in Lauraceae (Weberling, 1989). Nectar secretion is strongly influenced by floral type, plant age, position of inflorescence on the stem and light etc. as observed by Cawoy et al. (2008).

Table 1 Pollen – ovule ratio for the period 2011 to 2013.

Year	Total pollen production	No. of ovule	Pollen-Ovule ratio
2011	7536.00	6	1256.00
2012	7753.20	6	1292.20
2013	8470.80	6	1411.80

Table 2 Pollen production in *Cinnamomum sulphuratum* during 2011-2013.

Year	Sample size (Flower No.)	Mean no. of pollen per flower	S.D	S.E	Range	Total pollen production per flower
2011	10	7536.00	2208.00	698.23	3840-10800	7536±2208.00
2012	10	7753.20	2140.76	676.97	5664-11760	7753.2±2140.76
2013	10	8470.80	2346.51	742.03	4608-11772	8470.8±2346.51

Table 3 Floral visitors of *Cinnamomum sulphuratum*.

Pollinators	Forage type	Time of visit (hrs)	Length of visit (sec)	Flowers visited per bout	Visit frequency
Apis dorsata	P	09.00 - 13.00	8-25	3-8	VF
Apis indica	P	09.00 - 16.00	5-12	4-8	VF
Apis florae	P	09.00 - 16.00	3-10	4-6	VF
Butterfly	P	09.00 - 12.00	2-5	1-3	VR
Trigona sp.	P	13.00 - 16.00	10-40	5-9	VF
Vespa sp.	P	12.00 - 17.00	5-25	2-6	VF
Formicidae sp	N	07.00 - 18.00	Long period	-	VF

Note: VF – Very frequently and VR – Very rarely

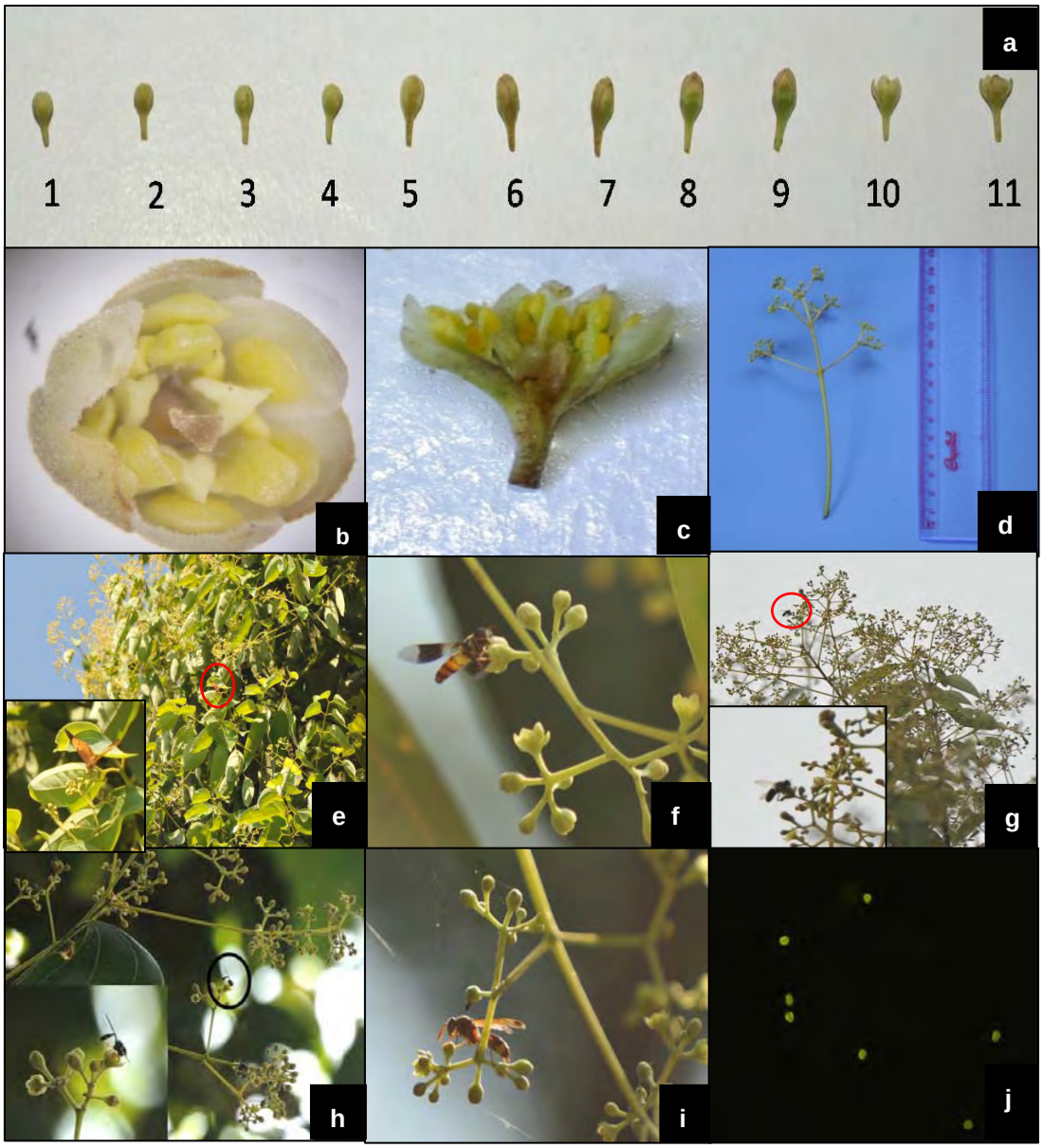

Fig. 2 a**)** Floral stages b) Matured flower c) Longitudinal section of flower d) Inflorescence e) Butterfly f) Hoverfly g) Bee h) Trigona sp. i) Vespa sp. j) Pollen viability test.

4.4 Pollinator observation and breeding systems

Flowers of *C. sulphuratum* are pollinated by insects such as honey bees (*Apis indica*, *A. dorsata* and *A. florae*), Hoverfly (*Episyrphus balteatus*), Wasps (*Vespa spp.*), bee (*Trigona iridipennis*), Butterfly (*Cupha erymanthis*) and Ants (*Formicidae sp*) (Fig. 2). They forage daily during day hours from 0600-1800h collecting pollen and nectar.

The average duration of visit made by wasps is 5-25 sec; Trigona with 10-40 sec between 1400 and 1800h, *Apis dorsata* visits for 8-25 sec between 09.00 to 13.00h. *Apis indica* (5-12 sec) and *A. florae* (3-10 sec) during 09.00 to 16:00h, Butterfly visit for 2 to 5 sec during 09.00 to 12.00h and the duration of Ants visit is highest between 07.00 to 18:00h. The bees and trigona are the dominant pollinators. Apart from their regular

pollinators, *C. sulphuratum* is also reported to be pollinated by Thrips (Devy and Davidar, 2003). The floral colour of *C. sulphuratum* attracts a few pollinators, as observed by Sharma et al. (1999) in *Boswellia serrate.*

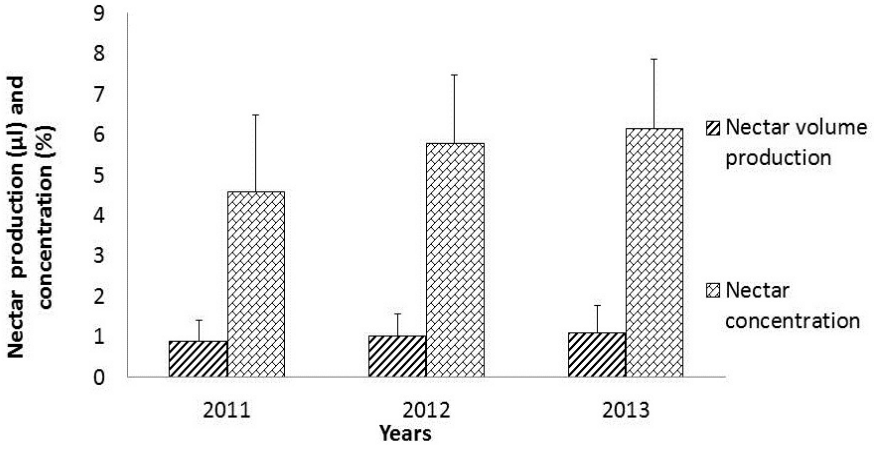

Fig. 3 Nectar production and concentration during 2011 to 2013.

Table 4 Breeding data of *C. sulphuratum* during 2012 to 2014.

Treatments	Sample size (Flowers)	2012-2013		2013-2014	
		No. of Fruits set	Fruit set (%)	No. of Fruits set	Fruit set (%)
Apomixis	100	00.00	00	00.00	00
Allogamy (HC)		71.00	71	75.00	75
Autogamy (HS)		63.00	63	70.00	70
Natural pollination		58.00	58	63.00	63
One time insect pollination		51.00	51	55.00	55

From the breeding studies it is observed that this species is both cross and self-compatible as fruit setting was observed are both controlled self and cross pollinated flowers. However, apomixis is absent and percentage of fruit set in apomixis is zero; allogamy (71.00%), autogamy (63.00%), natural pollination (58.00%) and one time insect pollination (51.00%) during 2012 and 2013, whereas during 2013. While in the year 2014, it was 75% in allogamy, 70% in autogamy, 63% due to natural pollination and one time insect pollination accounted for 55%. No significant difference between autogamy and allogamy is observed. Also the naturally pollinated and one time insect pollinated flowers showed significant fruit set. Joseph (1981) observed that the flowers of *Cinnamomum* are highly adapted for cross-pollination. A similar observation was made in *Madhuca neriifolia* an endangered species from Western Ghats of Karnataka (Prasannakumar et al., 2013). In insect pollinated species the pollen and nectar are the major rewards and a pollen vector having visited one flower is most likely to find the next attractive flower in the same or a neighboring tree contributing to both self and cross pollination as opined by (Bawa, 1974).

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