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RESEARCH ARTICLE

FLORAL BIOLOGY AND BREEDING SYSTEM OF *GARCINIA IMBERTI* BOURD. - A CRITICALLY
ENDANGERED TREE SPECIES OF WESTERN GHATS, KERALA, INDIA

Rajkumar Kandasamy, Keshavanarayan Puttaramaiah, **Shubharani Ramnath and
*Sivaram Venkataramgowda

*Department of Botany, Bangalore University, Bangalore – 560056, India

**V Sivaram Research Foundation, # 132, 2nd main Road, Rajarajeswari Layout. Jnanabharathi, Bangalore –
560056, India

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ABSTRACT

Plant flowering and breeding characteristics are important to understand the reproduction of plant populations. *Garcinia imberti* belongs to the family Clusiaceae is a critically endangered tree species of Agasthyamalai hills and endemic to Western Ghats, Kerala, India. Present study is to investigate the floral biology and breeding system during 2012-13. The tree species is dioecious. The peak of male and female flowering was observed in Feb-May, but the male flowers were observed one week before the female flower. Female flowers are significantly larger than the male flower. Both sexual morphs have four petals and four sepals in imbricated arrangement. Controlled pollination experiment showed that *Garcinia imberti* was a self-incompatible species that produced fruits through cross-pollination. According to the field observations after pollination treatments fruit setting was observed as 53.33%, 30.00%, and 36.67% in cross pollination, apomixis and open pollination respectively. This indicates a functionally dioecious mating system and pollination limited fruit set in *Garcinia imberti*. Female flower bear fruits with 1-2 seeds. Floral visitors observed in only in male flowers. These are not pollinators and they never observed on female flowers.

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INTRODUCTION

Angiosperms are the most advanced and structurally intricate in the plant kingdom and their multiple components each have one or more specialised functions, most importantly the female and male generative organs, the pistil (gynoecium) and the anthers (androecium) respectively (Sedgley and Griffin, 1989). In flowering plants approximately 10% of species are dioecious, producing male and female flowers on different plants (Dellaporta and Calderon-Urrea, 1993). Male and females of many dioecious species are different from one another in terms of their life histories, including their phenology (Bullock and Bawa, 1981). In dioecious plants, sexual dimorphism may be apparent both in vegetative characters (Wilson, 1991) and in reproductive characters (Lloyd and Webb, 1977; Bawa, 1980). Although many variations in the sexual expression of dimorphic species may be affected by a variety of environmental factors and interpreted as intermediate stages in the evolutionary process leading to

dioecy (Delph and Wolf, 2005; Barrett, 2002, 2010). Many tropical tree species with low natural fecundity are predominantly out breeders and exhibit self-incompatibility to various extents (Bawa, 1974; Kress and Beach, 1994). Since dioecious plant species are largely pollinated by insects or by wind, the breeding system of dioecious populations will then largely depends on the spatial distribution of the sexes within the population (Bawa, 1990; Mucahy, 1967; Waser, and Price, 1982). Understanding the reproductive biology is essential for the successful conservation efforts, particularly of endangered species where there are very few populations to supply propagules for future generations (Rajkumar et al., 2015; Holsinger, 1991; Anderson, 1995). The genus *Garcinia* consists of over 400 species and is one of the largest genus in Clusiaceae (Guttiferae) (Cox, 1976). This species is distributed across the tropical regions of Asia, Africa and Polynesia (Ridley, 1922; Whitmore, 1973). They are evergreen polygamous trees, shrubs, and herbs. About 30 species of *Garcinia* are distributed in India, which includes *Garcinia combogia*, *Garcinia xanthochymus*, *Garcinia cowa*, *Garcinia indica* etc. However, many species of *Garcinia* are important for commercial use as timber, medicine, resin oil, pigment, fodder and edible fruits (Cox, 1976).

*Corresponding author: Sivaram Venkataramgowda,
Department of Botany, Bangalore University, Bangalore – 560056,
India.

Garcinia imberti Bourd. is a critically endangered tree species in the IUCN Red list and World Conservation Monitoring Centre (1998), belongs to the family Clusiaceae of order Theales and sub class Dilleniidae. It is evergreen forest trees species restricted to two regions viz. Chemunji and Attayar of the Southern Western Ghats (Nair and Ahmedullah, 1988; Gopalan and Henry, 2000; Mohanan and Sivadasan, 2000). Recently Rameshkumar *et al.*, 2005) have investigated chemical constituents of stem bark oil of *Garcinia imberti*. The present investigation has been undertaken to study the floral biology and breeding system of *Garcinia imberti* as there is no sufficient information on reproductive biology. Hence the comprehensive investigations were carried out in their natural habitat.

MATERIALS AND METHODS

Study site and study period

The present research work was undertaken during the two consecutive flowering seasons, during 2012 and 2013 in the natural populations of *Garcinia imberti* at Agasthyamalai hills of Western Ghats, Kerala, India. The natural distribution zone of the species is located lies between N 8°41.204 Latitude; E 77°11.275 Longitude. The geographical co-ordinates and the distances among the study sites were obtained using a Global positioning system (GPS). The local mean average of annual temperature is approximately 18-35°C and the average annual rain fall varies from place to place ranging from 90 to 650 cm (Chandrababu *et al.*, 2009).

Study species

Garcinia imberti Bourd. is an evergreen tree reaches up to 15m tall (Fig. 3a, 4a.) and endemic to Western Ghats of Kerala, India. The morphological characters of the male and female plants are almost similar. The wood is yellowish grey and hard. The bark is brownish white, smooth, mild fragrant (Fig.4d). Leaves opposite, simple, lamina elliptic or lanceolate, greyish green on drying, cuneate-attenuate at apex, penninerved, midrib prominent on both surfaces and raised beneath, nerves 15- 25, parallel, close, obscure. The plants were identified with the help of Flora of Agasthyamalai and Endemic plants of India. Also the specimens were authenticated in consultation with Madras Herbarium and Botanical Survey of India (BSI), Southern circle, Coimbatore (Herbarium specimen numbers: 11402, 11412 and 162084).

Vegetative and reproductive phenology

Vegetative and reproductive phenological events of *Garcinia imberti* was carried out by randomly selecting each sexual plant (10 male and 10 female trees). Twenty individual trees ≥ 30 cm of diameter at breast height (DBH) were observed monthly intervals in two flowering seasons during 2012 and 2013. The observations of phenological events including leaf fall, bud break, leaf flushing, bud initiation, flowering, fruiting and vegetative phase were recorded during the study period. The identification of the phenological pattern was made according to the classifications proposed by (Gentry, 1974; Newstrom, *et al.*, 1994).

Floral biology and morphology

Flower biology and morphology was recorded in both sexual plants. Twenty mature male and female trees (each ten trees) were selected in the study site. Observations included the date of initiation of flowering, peak of flowering, end of flowering, and fruiting period were recorded during the entire flowering period from selected plants for two years (Luis, 2001; Margrit, 2002; Marco and Fernando, 2004). The floral morphology was studied in the field and also in the laboratory with the help of a stereo zoom microscope (LABEN, STZ-800BT model). Morphological observations included the flower length, width, and length of sepals, petals, width of sepals, petals, pistil length and stamens length of both sexual morphs of the species were measured. The floral morphometric characters of male and female flower measurements were made by using a digital calliper.

Breeding system

On the basis of stigma receptivity, the reproductive system was studied through hand pollination experiments in the field according to (Dafni *et al.*, 2005; Dafni, 1992; Radford *et al.*, 1974; Kearns and Inouye, 1993). The following experiments were conducted during the flowering season in five female plants from thirty inflorescences in each experiment and also fruit set percentage was recorded;

a) Natural conditions/open/control: Thirty female flowers from thirty inflorescences were tagged and observed to check the pollination under the natural conditions with (n=30 flowers, 5 plants, 30 inflorescences), the flowers were left without any intervention (no bag, no artificial pollination). The initiations of fruit set were observed after pollination and to estimate the number of seeds per fruit was dissected from twenty fruits.

b) Apomixis (Agamospermy): The possibility of asexual reproduction by apomixis was examined by covering thirty inflorescence on thirty female flowers by polythene bags (no=30 flowers; 5 plants; 30 inflorescences) and regularly observed in order to determine the initial fruit set and also pistil were observed until fruit initiation (Richards, 1986), and

c) Xenogamy (Manual cross-pollination): Thirty matured female flowers from thirty inflorescences were covered completely with polythene bags and the number of female flowers on each inflorescence was counted before bagging (n=30 flowers; 5 plants; 30 inflorescences). During the blooming period, the pollen of each inflorescence was artificially applied by rubbing the anthers of male flowers on the stigma of female flowers, after which the pollinated flowers were re-bagged and initial fruit set was observed.

Floral visitors and their behavior

The floral visitors have been recorded and observed at the selected site. The insect floral visitors were observed at different hours of the day from both sexual morphs (male and female) by direct observations between 7:00 am - 17:00 pm for more than 72 hrs throughout the two consecutive years during the flowering period. The observations on the insect floral

visitors, purpose of visiting, foraging activity, and the time spent on each flowers were observed during different hours of the day. Some of these floral visitors were captured and preserved for identification during the flowering period randomly from the selected plants in the study site.

Statistical analysis

For floral traits of male and female flowers the Mean and Standard deviation was analyzed by using mega stat model (Programmed by J.B. Orris, Version 9.1).

RESULTS AND DISCUSSION

Vegetative phenology

Phenological patterns of vegetative phases were observed in both sexual plants. Vegetative shoots are produced annually and the leaf initiation occurred continuously throughout the year. Emergence of new leaves commenced during the January-March and leaf fall was observed fourth week of September and continued until December. Table 1 shows month wise phenological observations of *Garcinia imberti* with phenogram.

Table 1. Comparison of floral traits (Mean± SD) between male and female flowers of *Garcinia imberti*

S.No	Parameters	N	Male flower	Female flower
1	Flower length	10	0.54±0.05	0.60±0.08
2	Flower width	10	0.42±0.06	0.45±0.05
3	Pistil length	10	-	0.28±0.04
4	Stamen length	10	0.10±0.00	-
5	Petal length	10	0.28±0.06	0.32±0.06
6	Petal width	10	0.26±0.05	0.30±0.06
7	Sepal length	10	0.23±0.04	0.26±0.05
8	Sepal width	10	0.22±0.04	0.24±0.05

Reproductive phenology

The reproductive phenological data of *Garcinia imberti* was recorded in regular monthly periodicity. According to the field observation *Garcinia imberti* was confirmed as dioecious plant. The both sexual plants was covered fully with green leaf (vegetative phase) throughout the year because of evergreen forest tree species. Initiation of male floral bud primordia appeared on inflorescence begins in February followed by flowering, occurs during late February-March and terminated at the end of April. The female floral bud primordial start late in February followed by the flowering occurs during March-April and terminated at the end of May. Bud primordia eventually developed into green buds and then developed into yellow colour mature buds. Fruiting was initiated at the end of the dry season and beginning of the rainy season during the two years of observation (Fig. 1). The average days taken from floral bud primordia stage to flowering approximately was 18-26 days of both male and female flowers. The average days taken from flowering to fruiting was 26-36, and fruit initiation to maturity was approximately 6-8 months in female flowers, which is comparable with the result of (Rao *et al.*,2008). The fruit development was initiated during the peak of flowering in March-April and continued until end of December. The fruits

are smooth, leathery rind and green in the beginning, later turn yellow and finally to brown or black. Seed coat is brown with branched lines.

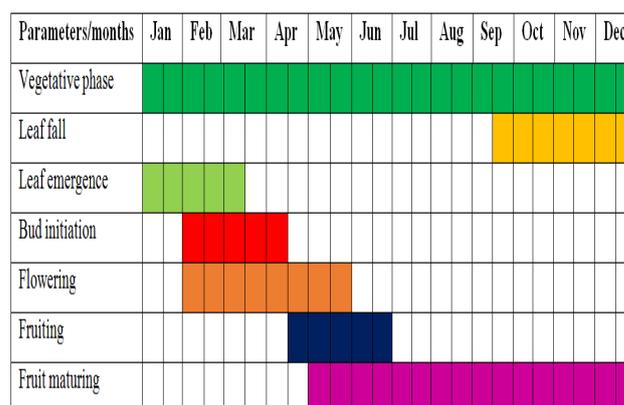


Fig. 1. Phenogram of *Garcinia imberti*

Floral biology and morphology

Garcinia imberti flowering once in a year and two types of flowers were observed. *i.e.*, Male (staminate) and female (pistillate) flowers (Fig. 3b and Fig. 4b).

Male flowers

The male flower occurs in cluster 1-9 (Fig. 3b). The flower 0.54 cm length and 0.42 cm in width. The four sepals 0.23 cm length and 0.22 cm in width and green in colour. In contrast, the four free petals 0.28 cm length and 0.26 cm width yellowish in colour. The male trees have typical flowers with long pedicels, each flowers had stamens ranging from 16 to 20, all fertile, centrally crowded and hemispherical rudimentary, receptacle and the stamens form a round circle. The pistils were absent and pollen grains are present in this type. Flowering in male trees was observed more when compare to female trees. The wilted male flowers were observed from the tree branches within a few days (Fig. 3e)

Table.2. Floral visitors of male flowers in *Garcinia imberti*

Floral visitors	Order/family	Forage type	Time of visits (seconds)	Visit frequency
Cockroaches	Blattodea	Pollen	5-20	Very frequency
Black ants	Hymenoptera/ Formicidae	Pollen	5-10	Frequency
Snails	-	Resin	10-50	Very frequency
Unidentified	-	Pollen/ feeders	10-20	Frequency

Female flowers

The female flowers are solitary and occur single or occasionally in clusters (1-2). The female flowers are larger than male flowers (Fig. 4b). The flowers develop at both terminal and auxiliary buds. The four petals are yellow, 0.32 cm length and 0.30 cm width and four sepals 0.26 cm length and 0.24 cm in width green in colour. The female flowers were pedicellate having a length of 0.60 cm and 0.45 cm width and rudimentary structure, conical in shape present in place of pistil

and length 0.28 cm, pollen grains were absent. Ovary globose, pistils were fully developed and cross section showed two locules (Fig. 2a-d).

The developmental stages of both male and female flowers were similar and produced white colour liquid resin (Fig. 4b).

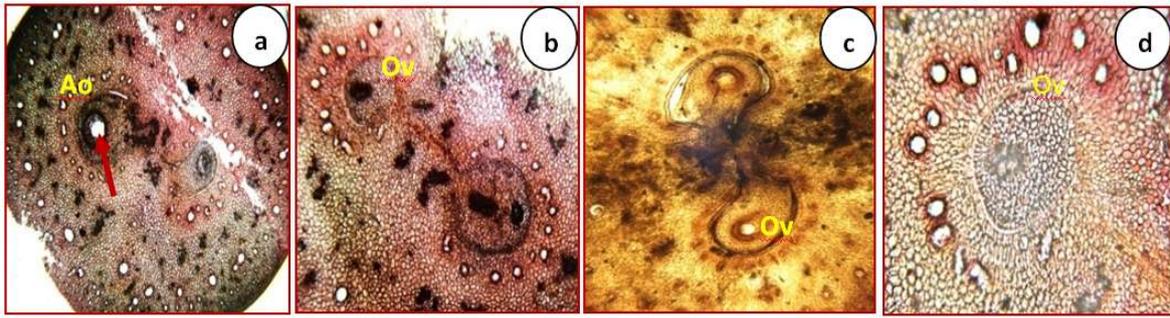


Fig.2. Breeding system in *Garcinia imberti*. Detail of the fully developed ovules in free-hand sections, showing, a. Open pollination aborted ovules, b. Manual cross-pollination, c. Apomixis, respectively, d. Enlarged view of ovule (Ao-aborted ovule, Ov-Ovule)

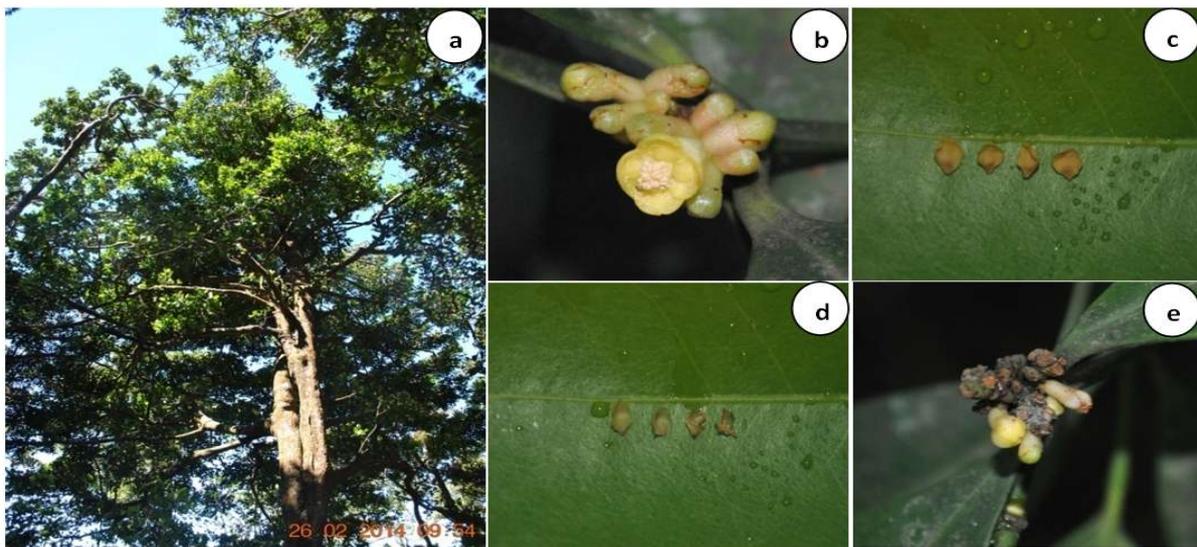


Fig.3.a. *Garcinia imberti* male tree, b. Male flower, c. Petals, d. Sepals, e. Male Flower dried and abscised

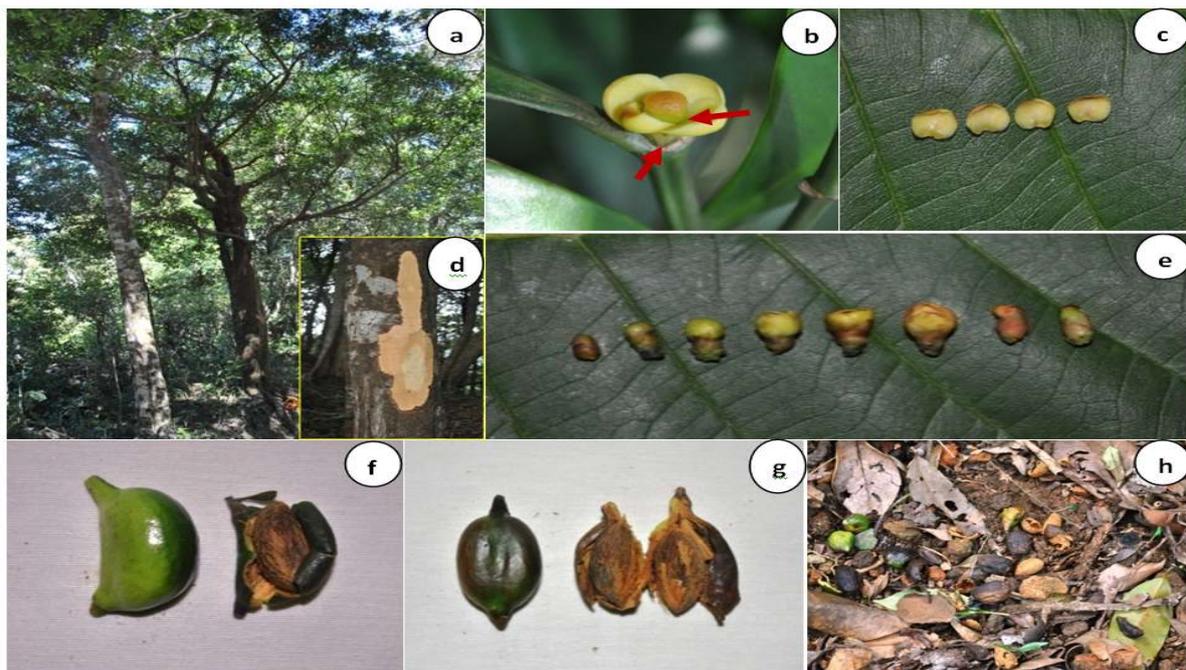


Fig. 4. a. *Garcinia imberti* female tree, b. Female flower (arrow-conical shape pistil and white colour resin) c. petals, d. Stem bark, e. Reproductive structures at different stages of female floral development, f. Single seed fruit, g. Double seed fruit, h. Seed dispersal by wild animals

Detailed floral morphometric data are given in Table 1. The gynoecium is superior and it has only two ovules and each in one locule. Stigma is sessile, convex and capitates. During the development stage of fruits, the stigmas turn into brown in colour. Stigmatic surface has four circle distinct lobes.

Breeding system

The observations on the breeding system of *Garcinia imberti* indicates that, the species is self-incompatible and fruit formation takes place only after manually transfer of pollen from male flowers to the stigma of female flowers.

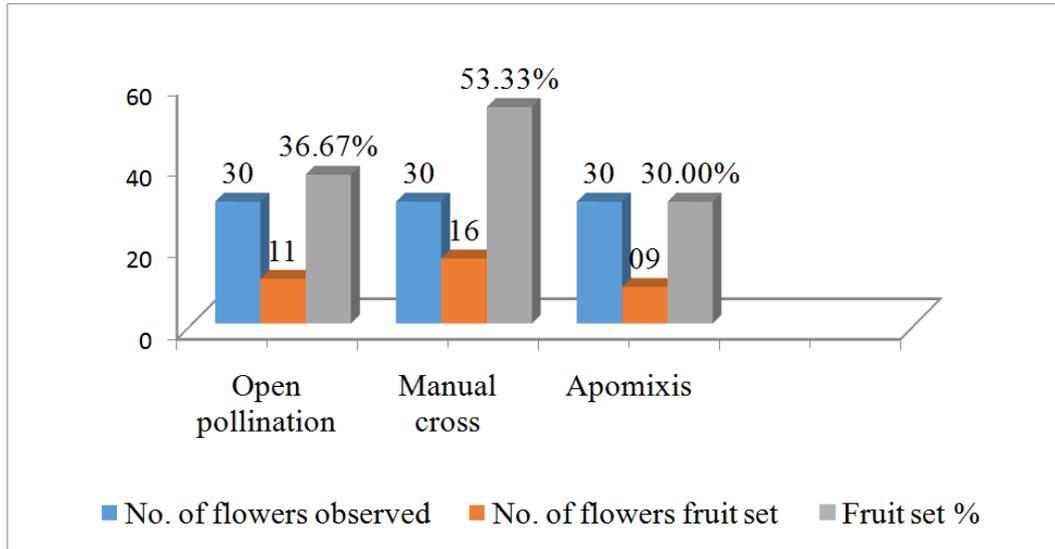


Fig. 5. Breeding system in *Garcinia imberti*



Fig. 6. Breeding system in *Garcinia imberti*. a & b. Female flower tagged for open pollination, c. Young fruit with persistent sepals and petals, d. initiation of fruit, e-g. Manual cross-pollination shows fruit initiation, h-j. Apomixis shows initiation of fruit

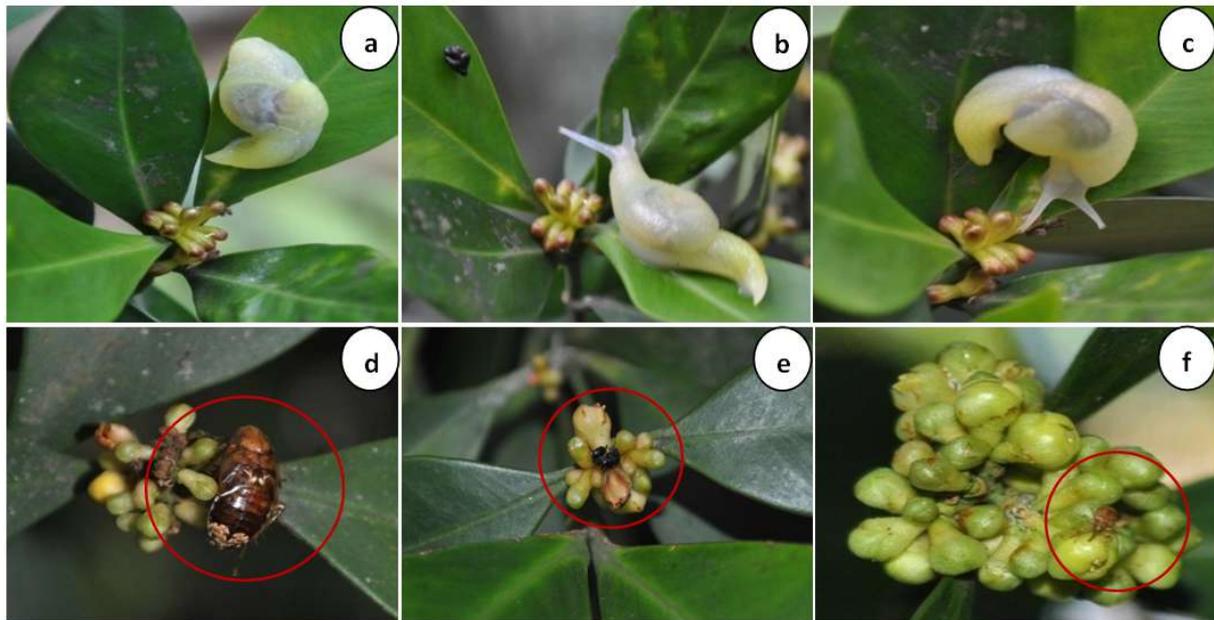


Fig. 7. Floral visitors of male flower in *Garcinia imberti*, a-c. Snail collecting resin in rainy day, d. Cockroach collecting pollen, e. A large black ant feeding on the pollen, f. Unidentified insect feeding on the pollen and damaging floral buds

The fruit formation in open pollination experiment was 36.67%, indicating the absence or lack of pollinators wherein the ovaries showed the presence of aborted ovules. In manual cross pollination the fruit set percentage was observed as 53.33% and the female flowers increased the fruit set considerably as compared to open pollination because of artificially applied pollen on the stigma of female flowers. Therefore, pollinators are necessary for pollen transfer and successful pollination. The results of apomixis experiment in female flowers of *Garcinia imberti* fruit sets was 30.00%, indicating that evidence of apomixis occurs in this species and we have verified the presence of seeds in the fruits (Fig. 6. h-j). Based on the observation of free-hand section in open pollination experiment there were 2 locules in the ovary and two ovules in each locule. It was observed that several of the ovules are aborted in open pollination experiment. Similarly, it was observed in case of apomixis and manual cross-pollination experiment (Fig. 2a-d). The significantly lower number of seeds per fruit in open pollination and apomixis may be due to ovule abortion at the time of fruit development (Raju and Ezradanum, 2002). However, Richards (1990a, 1990b) recorded facultative apomixis for two species of the genus *i.e.*, *Garcinia parvifolia* (Miq.) Miq. where fertile seeds produced from gaged female flowers.

Floral visitors and their behavior

Floral visitors and their behaviour were studied in both male and female flowers during the entire flowering season. In female flowers, any kinds of floral visitor were not observed during the flowering season because of lack floral rewards. Resins are present in both male and female flowers.

The male flowers are visited by various insects *e.g.* Cockroaches (Blattodea), Ants (Formicidae), unidentified insect and snails (Fig. 6 & Table 3). The visiting insects were observed on male flowers and were not observed on female flowers.

Seed dispersal

Garcinia imberti mature fruits usually fall to the ground of mother trees and also the seeds get dispersed through wild animals (Fig. 4.h).

DISCUSSION

Vegetative and reproductive phenology

Phonological and reproductive data are important in order to understand persistency and distribution of the plants (Rathcke and Lacey, 1985; Munguia-Rosas *et al.*, 2011). The present observations showed that the vegetative phase of both sexual plants had similar phonological characters throughout the season. Bud primordia started during spring season and flowering occurred during summer and this is in accordance with the general findings in the tropics, particularly where there is climatic seasonality (Schaik *et al.*, 1993). Although the vegetative and reproductive phenology of male and female trees of *Garcinia imberti* was similar, however, male trees start flowering earlier than the female flowers (Lloyd and Webb, 1977; Bawa, 1983). In dioecious species, usually the male plants bear more number of flowers than the female plants (Kay *et al.*, 1984, Armstrong and Irvine, 1989; Muenchow and Delesalle, 1994; Vaughton and Ramsey, 1998).

Flower biology and morphology

Garcinia imberti flowers have a typical set of characters with male and female flowers are separate in different tree. Male trees flowering in February and female in March, these variations may be related to climatic and edaphic factors of the area. In dioecious species, the timing of flowering generally differs between males and females, with males beginning to flower earlier than females (Lloyd and Webb, 1977; Wilson, 1979; Beach, 1981).

Breeding system

Breeding system is the mode of transmission of genes from one generation to the next generation through sexual reproduction. It largely reflects the extent of self and cross-pollination (Richards, 1986). In the present investigation the breeding system analysis through controlled pollination experiments revealed that female individuals of *Garcinia imberti* are self-incompatible which means that efficient, reliable pollinators are very essential for successful pollination process. The predominantly self-incompatibility nature of forest trees is directly related to selective pressure to maintain genetic variability (Bawa, 1974). Moreover, the study site had very less population of female trees which produced fruits and seeds under natural conditions which supports the view that the female flowers are complete and can set fruit in the absence of male trees. Bawa and Opler, (1975) described the mean fruit set in some tropical forest dioecious trees as about 26.3%, while Sutherland and Delph, (1984) reported an average of 73.8%. In the present observation, very less fruit set in open pollination. The low fruit set in natural pollinated flowers as compared to artificial cross pollinated flowers strongly suggest the requirement of some external agents necessary for pollination (Sreekala *et al.*, 2008). These fruit failures are because of pollinator limitation (Calvo, 1990) or may be due to self-incompatibility which is quite common in tropical trees (Bawa, 1974; Kaur *et al.*, 1977). In manual cross pollination of *Garcinia imberti* significantly produced higher number of fruit set when compared to open pollination and apomixis. Fruit set observed in the female flowers (bagged) indicate the evidence of apomixis and developed ovule (Fig. 2c). Pangusban, (2009) has reported more fertilized ovules in manually pollinated flowers in *Garcinia atroviridis*, a facultative apomictic tree, than in open pollinated and unpollinated flowers. Many apomictic plants belong to lineages of plants that have reproductive systems of self-incompatibility, dioecy (Asker and Jerling, 1992). Kaur *et al.* (1986) suggested that apomixis may have a significant role in the speciation of tropical trees and similar opinion expressed in case of *Garcinia scortechinii* (Thomas, 1997), *Garcinia hombroniana* (Richards, 1990a), and *Garcinia gummi-gutta* (Rai, 2003).

Floral visitors and their behavior

Observations on the insect floral visitors and their foraging behaviour were carefully observed during flowering period at regular intervals throughout the day accordingly the procedure suggested by Faegri and Vander Pijl, (1980) and Dafni *et al.* (2005). In the present observation the floral visitors were more often visited on male flower, however, the pollinators were not observed on female flowers. The preference of floral visitors for both male and female flower is more attractive and that offers food resources (Vaughton and Ramsey, (1998), Ashman *et al.* (2000), Miller and Venable, (2003). Farwing *et al.* (2004).

Conclusion

The present research clearly demonstrates that *Garcinia imberti* is functionally dioecious and the male and female flowers are separate in the different trees. Female flowers have well

developed ovary with two locules and produced two types of fruits single and double seeded fruits. Based on the breeding experiments there is evidence of apomixis and further investigations of genetic and cytological is needed. *Garcinia imberti* is self-incompatible species and dependent on pollinators for fruit set and insect vectors are essential for successful reproduction. The low fruit set was observed in open pollination as compared to that in manual cross pollination. The flowers were visited by cockroach, ants and snail on male flowers for food sources and there is scope to study the specific pollinators transferring pollen from male to female flower. The population of *Garcinia imberti* is a critically endangered due to the large areas have been exposed to fire, grazing, fragmentation, establishment of large scale commercial plantations and cutting fuel wood by local population. In order to increase the population density in the Agasthyamalai hills and adjoining areas in-situ and ex-situ conservation programmes are highly recommended.

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