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POLLINATION BIOLOGY AND BREEDING SYSTEM OF *EUGENIA DISCIFERA* GAMBLE- AN ENDANGERED SPECIES OF WESTERN GHATS, INDIA

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ABSTRACT
Knowledge of the pollination biology and breeding systems is very important for understanding the life history of long-lived tree species. In case of endangered species, the information may have implication for conservation practices. In the present investigation on *Eugenia discifera* Gamble, an endangered tree species, belongs to the family Myrtaceae, the reproductive phenology, floral biology, pollination biology and breeding system has been studied during 2012-2013. Based on the observation, the lifespan of a single flower was about 1-2 days and to complete the whole process of fruit formation was 102-112 days. Inflorescence is terminal raceme or axillary, flowers are hermaphrodite (bisexual), zygomorphic, epigynous and complete. Sepals and petals are four. Stamens are conspicuous and numerous, stigma simple, fruits are globoid with crowed by calyx lobes. Breeding experiments showed there is no apomixis. The lowest mean fruit set were observed with autogamy pollination (34.00±16.73%) and highest mean fruit set were observed with geitonogamy pollination (48.00±21.68 %) respectively. However, there is no significant difference in the fruit set by geitonogamy and xenogamy. The flowers are often visited by various pollinating insects. The most frequent visitors are butterfly, *Apis* sp, *Anomala* sp, *Altica cyanea* (Weber) and they visit to collect food sources such as pollen and nectar for their survival in turn helps in pollination. The factors responsible for declining the population of *Eugenia discifera* were recorded.

KEY WORDS: *Eugenia discifera*, reproductive phenology, floral biology, breeding systems and floral visitors.

INTRODUCTION

Pollination mechanisms are greatly diverse in angiosperms. Understanding the ecology of critically endangered species is crucial both in comprehending the causes of their conservation status as well as formulating appropriate management measures. Nevertheless, insufficient appreciation of the ecology of a particular species is repeatedly cited as a shortcoming in the management of threatened and endangered plants (Schemske *et al*., 1994, Tear *et al*., 1995). Critically endangered species are at the sharp end of today's global extinction crisis. These are species judged most likely to become extinct in the immediate future unless conservation efforts are made (Rossetto *et al.,* 1997). Causes of rarity and critical endangerment are diverse, ranging from direct human destruction of wild ecosystems to processes such as recent evolutionary origin or reproductive failure of relict species under changed environmental circumstances (Fiedler & A house, 1992, Pate & Hopper, 1993). For example, several comparisons of rare common species pairs have demonstrated that reproduction and recruitment are often particularly low in rare species (Munzbergova, 2005, Young *et al.,* 2007). The need to gather information on the basic biology of rare species is vital to both species- and community-level conservation efforts (Saunders & Sedonia, 2006). Pollination is a fundamental aspect of plant reproduction, and pollination by animals is largely considered a co-adaptive process in which plants evolve traits to attract certain pollinators, where by pollinators then evolve traits to better exploit floral resources of particular plants, with the occurring natural selection mediated by that pollinator (Faegri & Van der Pijl, 1980, Heinrich, 1983). Conservation of biodiversity refers to different levels of ecological organization (species, biocenoses, and ecosystem). In conservation efforts, special interest is devoted to the protection of rare and endangered species, many of which may impact the stability of pollination webs, although mutualistic relations are highly asymmetrical (Bascompte *et al*., 2007 and Potts *et al*., 2010). The Western Ghats, extending along the West Coast of India, covers an area of 180,000 square kilometres. The Western Ghats comprises the major portion of the Western Ghats of India and Sri Lanka which is one of 34 global biodiversity hotspots for conservation and one of the two on the Indian subcontinent. This contains a lot of endemic and endangered species. Some of the forests of Western Ghats have been declared either as National parks or as Reserve forests. There are about 4500 species of flowering plants of the total estimated 17000 species (Ahmedullah and Nayar, 1987). Nayar (1996) recognized eight micro-endemic centres in the Western Ghats among which the Agasthyamalai region has a greater concentration of endemic species. Gopalan & Henry (2000) have reported 150 taxa as strict endemics to the Agasthyamalai region of which 24 are tree species. Species belonging to the genera as *Elaeocarpus, Garcinia, Myristica,* and *Syzygium* occur in swamps. The objective

of the present study of *Eugenia discifera* was to investigate pollination biology and breeding system occurring in Chemunji Hills of Western Ghats.

Study area

The present research was conducted during the two consecutive flowering seasons, from 2012 to 2013 in natural populations of *Eugenia discifera* in the Agasthyamalai at Chemunji Hills of Western Ghats, Thiruvananthapuram district, Kerala, India. The natural distribution zone of the species is located between N 8°41. 274 Latitude; E 77° 11.205 Longitude. Figure 1 and 2 shows the general distribution locality and approximately covers an area of 1500 sq km. The co-ordinates and the distances among the study sites were obtained using a GPS. The local mean average of annual temperature is approximately 16-35°C and the average annual rainfall is approximately 2,800 mm/yr. The relative humidity about 85%, and the soil is latric acidic and red loamy.

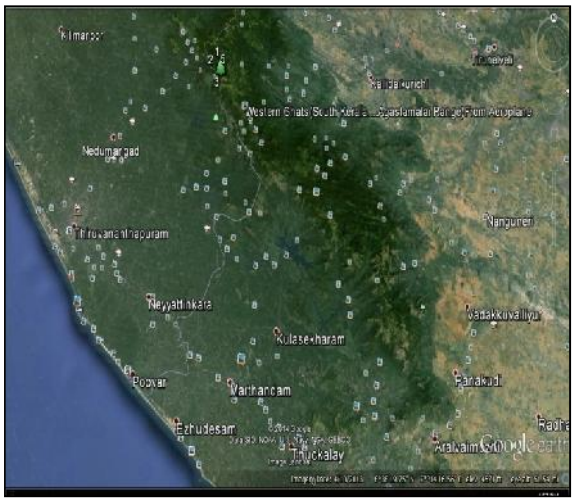

FIGURE 1. Location of study area

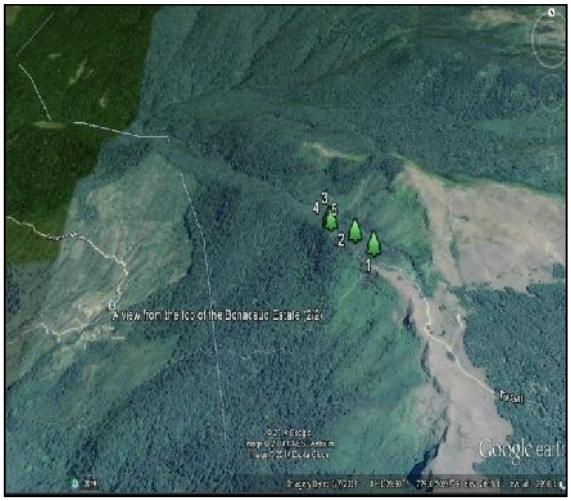

FIGURE 2. Enlarged view in the study area

MATERIAL & METHODS

Plant species

Eugenia discifera is an endangered tree species, belongs to the family Myrtaceae. It is evergreen medium size tree growing up to 5-10 m tall (Fig. 6A) and endemic to Western Ghats. Bark is grey thin (Fig. 6 A1), branchlets slender, subterete at the tip, with simple leaves, opposite, 3-6×1.5-3.3 cm, elliptic-obovate, acute-narrowly attenuate at base, shortly acuminate at apex; petioles 3-5 mm long. Inflorescences are short terminal raceme or pair below the leaves. A detailed study on their reproductive phenology, floral biology, pollination biology and breeding system was conducted two consequent flowering periods.

Reproductive phenology

In order to determine the sequence of reproductive phenological events of *Eugenia discifera* was carried out by randomly selecting five individual plants during the period 2012 to 2013. Observations were made on vegetative phase and reproductive phenology of selected individuals, with respect to time of leaf fall, leaf flushing, bud initiation, flowering, and fruiting were recorded monthly on the selected plants. The intensities of these phenological events were estimated using the semi-quantitative scale of Fournier (Fournier, 1974) and identification of the morphological patterns was made according to the classification proposed by Newstrom (Newstrom *et al*., 1994).

Floral biology

Observations were recorded during the entire flowering period from selected plants. The floral biology of *Eugenia discifera* was studied in twenty tagged mature flower buds from ten inflorescence (one inflorescence per tree) were followed for recording the time of anthesis and anther dehiscence was also recorded. The presence of pollen powder on anther surface was considered to be anther dehiscence. Buds and flowers were collected and analysed in the laboratory. Morphometric analyses were performed the following characters of individual flowers of *Eugenia discifera* with a digital caliper. (i) Flower length, (ii) diameter, (iii) length of sepals, (iv) petals, (v) pistil length, and stamens length were measured and other floral characters were visually observed through extensive field exploration.

Breeding system

To determine the breeding system, pollination experiments were performed on randomly chosen from five trees in the population. Various types of breeding experiments including open pollination, autogamy, geitonogamy, xenogamy and apomixis were carried out during the two subsequent years with two flowering seasons (Radford *et al*., 1974; Dafni, 1992 and Kearns and Inouye, 1993). Open pollination (control): flower buds were tagged and observed the fruit set. Autogamy: mature flower buds were tagged and bagged with a cloth mesh bag, and fruit set at maturity was recorded, geitonogamy (manual self-pollination): mature flower buds were tagged and bagged, the buds upon opening were hand self-pollinated with pollen collected from the same plant, re-bagged and fruit set observed. Xenogamy (manual cross-pollination): mature flower buds were tagged and bagged, the buds upon opening were hand cross-pollinated with pollen collected from two or three other plants and then re-

bagged and fruit set observed. Apomixis: mature flower buds were emasculated and bagged without pollination (Richards, 1986). The Index of self-incompatibility (ISI) was calculated using the method of Zapata & Arroyo (1978). The ratio of fruit set through manual self-pollination to those formed through manual cross-pollination was taken as the Index of self-incompatibility. The species with ratios <0.25 are considered self-incompatible and those with ratios >0.25 as self-compatible (Subasi and Guvensen, 2011; Mohandass, 2013; Bawa, 1974; Nayak and Davidar, 2010).

Pollinator observation

The observations of flower visitors were made for a total of approximately 72 hours during three consecutive days of high diurnal pollinator activity (8:00hrs to 15:00hrs). The types of insect floral visitors, purpose of visiting, time interaction with flowers and the foraging activity of insect floral visitors were observed during different periods of a day. They were observed with reference to the type of forage they collected, contact with essential organs to result in pollination and inter-plant foraging activity in terms of cross-pollination. They were thereafter classified either as pollinators or robbers (Dafni, 1992, Inouye, 1980). Some of these insect floral visitors were captured fixed in 70% alcohol for identification.

Flower, fruit and seed predation

The insect parasites were observed from flower, fruit and seed on the plants as well as from the litter. Further many fallen fruits were collected to record fruit infestations and stored in vials for further identification.

Statistical analysis

The statistical analyses were calculated for floral traits, and breeding behaviour. Mean and standard deviation was analyzed using mega stat model (Programmed by J.B. Orris, Version 9.1.).

RESULTS & DISCUSSION

Reproductive phenology

The reproductive cycle of *Eugenia discifera* had a regular monthly periodicity (Fig.3). Leaf fall, leaf emergence, leaf flushing, flowering and fruit sets are annual events in *Eugenia discifera*. The plant was covered fully with green leaf (vegetative phase) was observed throughout the year. The leaf fall was observed during the last week of September and continued till second week of December. The leaf flushing occurs during the season from December to January. About two week later, the flower bud primordial eventually developed into green buds and then developed into mature buds initiate along with new leaves sprouting from the tip of the whole inflorescences. The new leaves appeared light green as compared to mature leaves which remained dark green. Although the flowering started in last week of February and the Peak flowering were observed the first week of March. The flowering ceases by end of April at the most of population level. A various phase of floral development were observed in the flowering season. The initiation of buds takes to the emergence within 6-8 day. During this stage fully emerged flower begin to unfold, the average life span of each flower is 1-2 days. It was shown that the total period needed to complete the whole process of fruit formation was 102-112 days (Fig.4). As comparisons, Schmidt-Adam *et al*. (1999) recognized six stages of development on *Metrosideros excel* (Myrtaceae).

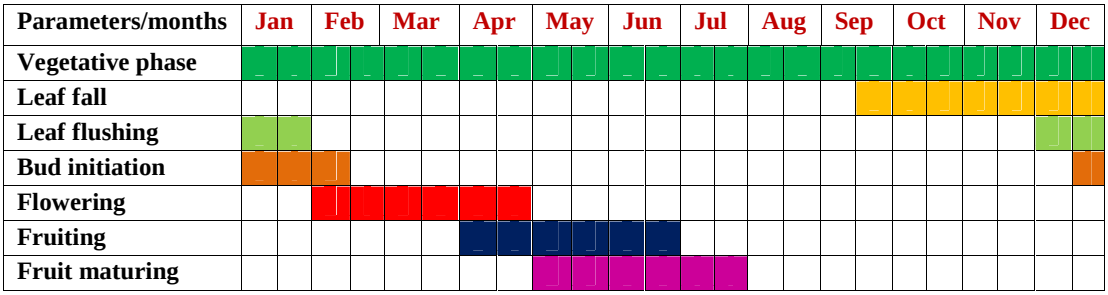

FIGURE 3. Phenograms in *Eugenia discifera*

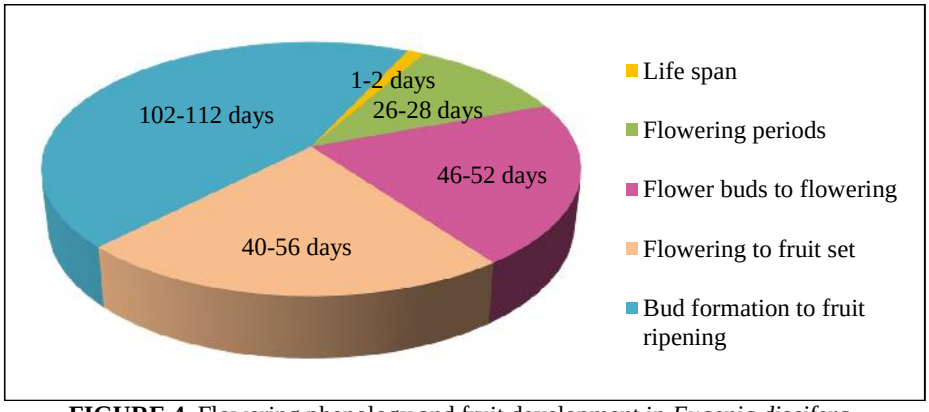

FIGURE 4. Flowering phenology and fruit development in *Eugenia discifera*

Floral biology

Flowering starts from February and continues till the last week of April, with maximum bloom in March. The inflorescence is terminal raceme or axillary or solitary pair, peduncles up to 1 cm long, rusty villous; pedicels 2-10 mm long, rusty villous; bracts small linear; caducous, rusty villous; bracteoles 2. Flowers are hermaphrodite (bisexual), zygomorphic, epigynous and complete. (Fig.7.C). Calyx tube (ovary), campanulate with four sepals, and corolla is orbicular, pale yellow with the same number of petals that are arranged alternate to sepals (Fig.7.D&E). Stamens are conspicuous and numerous and anthers orbicular with orange red glands at apex, basifixed. Disc hairy around style base; style (3.5-4 mm) long; slout, glabrous, ovule numerous; stigma simple, fruits are globoid with crowed by calyx lobes, glabrous (Fig.7). Anthesis started at 07:30 am and the flowers were completely opened by approximately 09:30 am and following the anther dehiscence at 08:30 am. The flower parameters measured are shown in table.1.

TABLE 1. Flower Characteristics in *Eugenia discifera*

Sl.no	Parameters	Measurements (Mean ± Std.dev.)
1	Flower length	4.850±0.341
2	Flower diameter	2.670±0.359
3	Pistil length	3.170±0.157
4	Stamen length	2.080±0.210
5	Petals length	0.420±0.123
6	Sepals length	0.200±0.094

Breeding system

Fruit set were properly formed in all the treatment except that of the apomixis. The results of the breeding system experiments in autogamy and geitonogamy (manual self-pollinated) produced different level of mean fruit set (34.00 ± 16.73%) and (48.00 ± 21.68%) respectively. Xenogamy (manual cross-pollinated) produced the mean fruit set (42.00 ± 23.87%) and open pollination (control) produced the mean fruit set (38.00 ± 24.90%) respectively. Open pollination was significantly higher fruit set than autogamy. However, no fruit set was observed in the emasculated and bagged flowers (apomixis), which fell soon after the treatment, indicating the absence of agamospermy of this species. The lowest mean fruit set were observed with autogamy pollination (34.00 ± 16.73%) and highest mean fruit set were observed with geitonogamy (manual cross) pollination (48.00 ± 21.68 %) respectively. Moreover, the ratio of percentage of fruit set between self and cross pollination showed 1.14 (more than >0.25 ratio). Thus, fruits were produced after the treatments of geitonogamy and xenogamy, indicating that *Eugenia discifera* was completely out crossing fertile and self-compatible (Table. 2 & fig.5, 9).

TABLE 2. Breeding system results in *Eugenia discifera*

Treatments	No. of flowers observed	No. of flowers fruit set	Fruit set (%)	Mean ± Std.dev.
Open pollination (control)	50	19	38.00	38.00 ± 24.90
Autogamy	50	17	34.00	34.00 ± 16.73
Geitonogamy	50	24	48.00	48.00 ± 21.68
Xenogamy	50	21	42.00	42.00 ± 23.87
Apomixis	50	00	00.00	00.00 ± 00.00

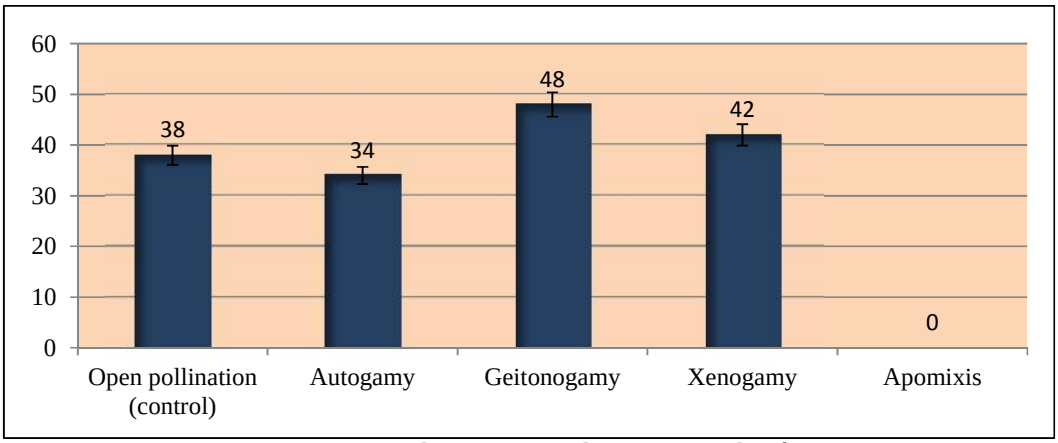

FIGURE 5. Breeding system results in *Eugenia discifera*

Pollinator observation

The flowers were observed at different times and days. Floral insects visited the flower and were attracted due to the presence of nectar, pollen grains, fragrance or colour of the flower. Floral visitors were observed in during the day time by seven insect species belonging to *Apis sp* (Honey bee); Butterfly, *Anomala sp*, *Altica cyanea* (Weber) and also some rare unidentified insect floral

visitors were observed. *Apis sp*. (Honey bee) and butterfly were collecting the pollen and nectar; they were regular foragers activity throughout the flowering season time (8:00-9:00hrs & 9:00-10:00hrs) (Fig.10 A & B). *Anomala sp* were collecting nectar from calyx part *and Altica cyanea* (Weber) were found to be consistent flower-feeder on the flower parts and those insects were contacting the anthers and stigma invariably and such contact with the floral reproductive parts was considered to be resulting in pollination at the same time (10:00-11:00hrs & 12:00-13:00hrs) (Fig.10 C & D). Further, unidentified1 insect collecting nectar and other two rare unidentified insect floral visitors were damaged the floral parts (14:00-15:00hrs) (Table 3 & Fig.10 E-I).

TABLE 3. Floral visitors in *Eugenia discifera*

Sl.No	Family	Order	Scientific name	Common name	Foraging nature
1	Apidae	Hymenoptera	*Apis sp*	Honey bee	Pollen/nectar
2	Nymphalidae	Lepidoptera	-	Butterfly	Pollen
3	Scarabaeidae	Coleoptera	*Anomala sp*	-	Nectar
4	Chrysomelidae	Coleoptera	*Altica cyanea*	Weber	Pollen
5	Unidentified1	-	-	-	Nectar
6	Unidentified2	-	-	-	Pollen/feeder
7	Unidentified3	-	-	-	Pollen/feeder

FIGURE 6. *Eugenia discifera.* **A.** Habitat. **B.** Flowering tree. **C**. Dried flowers. **D**. Flower petal and sepals partially destroyed by the insects. **E.** Fallen fruit. **F**. Infected mature fruit & seed. **G.** *Curculio c-album Fabricius* feeding fruit. **H&I.** Fruit infested by insects. **J.** Creamy white larva. **A1.** Bark.

FIGURE 7. A. Flower bud initiation. B. An inflorescence. **C.** Flowering panicle showing expanded flower. **D.** A single flower 1 hour after anthesis. The Anthers have dehisced. **E.** Disc hairy around style base. **F.** Four petals (pale yellow). **G.** Four sepals (green colour)**. H**. Flower buds at various stages of development.

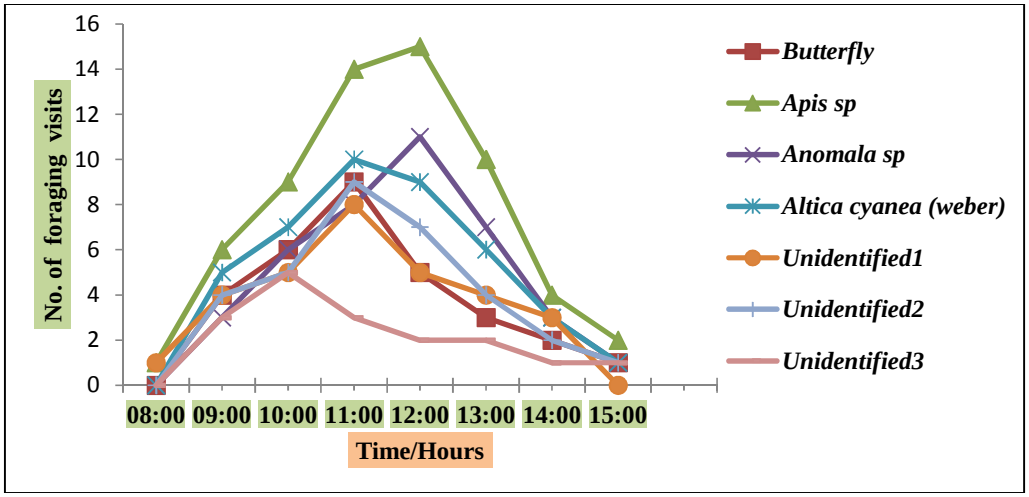

FIGURE 8. Hourly foraging activity of insects on *Eugenia discifera*

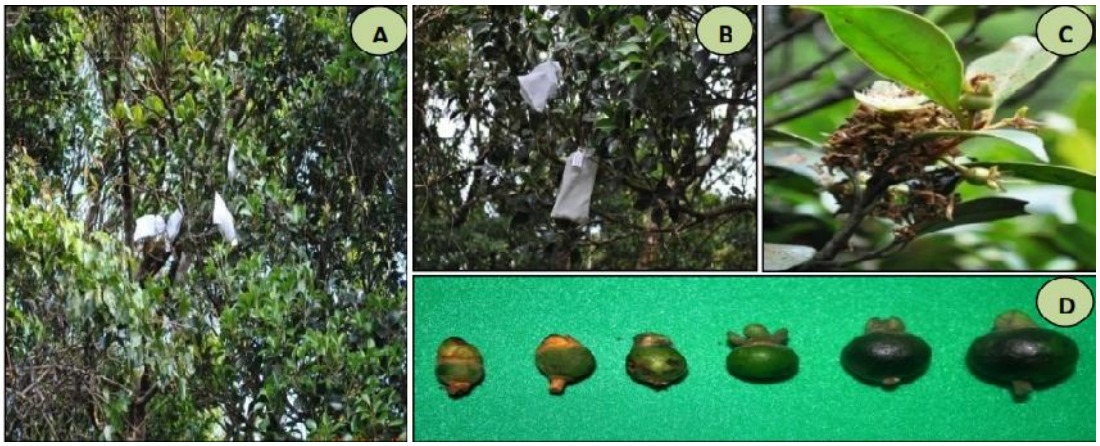

FIGURE 9 A. & B. Breeding systems (Bagged with mesh cloth). **C.** Open pollination fruit set. **D.** Stage wise fruits after the breeding treatments.

FIGURE 10 A. *Apis sp*. collecting pollen. **B.** Butterfly collecting pollen/nectar. **C.** *Anomala sp*. collecting nectar after making a hole in the calyx. **D.** *Altica cyanea* (Weber) collecting pollen. **E.** Unidentified 1 collecting pollen/nectar. **F.** Unidentified 2 collecting pollen. **G, H & I.** Unidentified 3 collecting pollen.

Flower, fruit and seed predation

Flower parts of petals, sepals and anthers were feeder by *Anomala sp., Altica cyanea* (Weber), resulting the flower were unable to get fertilize and failed to develop in to fruits and some rare unidentified species, as pollinators at the same time (Fig.10C & D). The fruits are berries and globoid shape. Young fruits were green in color (Fig. 9), which appeared during April-July and fruits matured in the month of May- July. The immature and unripe fruits contained the insects. The fruits were feeder by *Curculio c-album Fabricus (*Fig. 6G*).* Some of insects puts hole on the immature and unripe developing fruit of seeds. It has produce single larva which was creamy white in colour, when the fruit affected from this insects, fruit falls to the ground (Fig. 6H-J).

DISCUSSION

Reproductive phenology and floral biology

The present investigation provides the first detailed study about the pollination biology and breeding systems of

Eugenia discifera. The basic knowledge on reproductive biology is not only essential for evolutionary and systematic studies (Anderson, 1995) but also important for effective conservation strategies (Holsinger, 1991; Bernardello *et al.,* 1999) for endangered species like *Eugenia discifera*. In the present study we found the reproductive phenological observations and the floral transition is a major development event in the life cycle of flowering plants where by plants switch from a phase of vegetative growth to one of reproductive growth. The timing of this event is covered by many factors like season and climatic (Mark Doyle *et al.,* 2002) like season and climatic factors. From our study results suggest that *Eugenia discifera* has a regular flowering season from February to the end of April and peak of flowering in March. Based on our observation of leaf falls under some climatic variation occurs because of the plant is an evergreen tree species. The vegetative phase was observed throughout all the two years. The flower buds initiate along with new leaves spouting in January to continue till the end of April. Approximately, 102-116 days was taken for completion of whole process from the bud initiation to fruit ripening (Fig. 4). The flowering pattern of *Eugenia discifera* is defined as continuous by Newstrom *et al.* (1994). Onset and duration of flowering, relative maturation of male and female sex organs and the number and arrangement of flowers in a plant profoundly influence the pollinator visitation pattern in the taxa which has a direct bearing on the success of their life cycle (Siddique, 1991).

Breeding system

Based on the breeding experiments, it was observed that the flowers are morphologically and functionally hermaphrodite. Pollination is one of the prerequisites for fertilization and seed set in angiosperms (Faegri and Pijl, 1979). Open pollination experiments showed that it is self-compatible and out crossing fertile. According to Sunnichan *et al.* (2004) the fruit set under open pollination is poor and is highly variable from tree to tree. In *Eugenia discifera*, the open fruit set (natural/control) is low when compared to the high flower production and different factors could affect fruit set. It was observed that the bud and floral parts were damaged by insects, which leads to the loss of productivity. Secondly, the intensive pollen collecting behaviour of attending bees and their tendency to confine to the same plant that they first forage may result in more wasteful self-pollen transfer. Several factors may be responsible for the low fruit set under open-pollination (Tandon *et al.,* 2003). This finding was also reported in *Eugenia dysenterica* (Proenca & Gibbs, 1994), and in some Myrtaceae species (Butcher *et al*., 1992); Beardsell *et al*., 1993 a; Torezan-Silingardi & Del-Claro 1998, Schmidt-Adam *et al*., 2000; Gressler *et al*., 2006). In addition there is no apomixis occurred. Furthermore, because exclusion of pollinators resulted in the absence of fruit set, pollinators would seem to be necessary for the sexual reproduction of these species. As the flowers are at the canopy level, the wind force can easily make flowers release pollen into the air and then carry the same to the receptive stigmas of different flowers and trees. The fruit set geitonogamy is higher than that from open pollination (control) and xenogamy. 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 effective pollination (Sreekala *et al.,* 2008). According to the study report of breeding experiment in *Eugenia neonitida* and *Eugenia rotundifolia* did not produce fruit in hand-self pollination experiments, indicating self-incompatibility. In the similar type of experiments, *Eugenia uniflora* and *Eugenia punicifolia* produced fruits, showing self-compatible (Proenca and Gibbs, 1994; Gressler *et al*., 2006; Sobrevila & Arroyo, 1982; Wyk & Lowrey, 1988; Gressler *et al.,* 2006) and these results agree with studies on diversity in *Eugenia dysenterica* (Telles *et al*., 2001; Zucchi *et al*., 2003) and in *Eugenia uniflora* Margis *et al*., 2002; Salgueiro *et al*., 2004).

Pollinator observation

Plant species of floral traits that facilitate pollination efficiency in most aspects of reproduction (Ashman and Majestic, 2006; Sharma *et al*., 2008). Bees are the most common visitors of Myrtaceae in general (Beardsell *et al*.,1993; O'Brien & Calder, 1993; Nic Lughadha & Proenca, 1996; Gressler *et al.,* 2006). However, some authors described flies as floral visitors of Myrtaceae species (Beardsell *et al.*,1993; O'Brien & Calder, 1993; Silva & Pinheiro, 2007). In the present observations, bees (Hymenoptera) and butterflies (Lepidoptera) are found to visit the flowers of *Eugenia discifera*. It was observed that, honeybees were responsible for pollination either by bringing the stigmas near to the anthers or by transferring pollen grains. It was noticed that, *Anomala sp*, *Altica cyanea* and some other insects cause damage by feeding floral parts like petals, sepals, and anthers. Pollinator availability has been considered as probable reason for differential flowering time in tropical communities (Stiles, 1978; Bawa *et al.,* 1985).

Flower, fruit and seed predation

It was observed that the flowers and fruits of *Eugenia discifera* were damaged in large scale due to flower feeders and larval development inside the fruits. Hence, there is a drastic decline in fruit and quality seed production and it bears negative impact on natural regeneration. Ganesh and Davidar (2001) observed that biotic agents involved in seed dispersal and seed predation were six species of birds and five species of mammals. *E. uniflora* is also parasitized by Tephritidae (Diptera) and Eurytidae (Hymenoptera) larvae, common in Myrtaceae species (Lima 1916; Lughadha & Proenca, 1996; Menezes *et al.,* 2001).

CONCLUSIONS

The results suggest that *Eugenia discifera* flowers are hermaphrodite (bisexual), zygomorphic, epigynous and complete. Open pollination experiments showed that it is self-compatible and out crossing fertile. The decline of this tree species is mainly because of habitat loss, over exploitation and fragmentation. In addition, the floral, fruit and seed damage caused by the insects could be the reason for regeneration success and limited distribution of *Eugenia discifera* in the wild. In the present investigation on the reproductive phenology, floral biology, breeding systems and pollinator visitation are important because the evolutionary success and survival of the population. There

is an urgent need to formulate strategies for the conservation of the *Eugenia discifera* populations in the Western Ghat forests of South India.

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