Annexure -VIII

UNIVERSITY GRANTS COMMISSION

BAHADUR SHAH ZAFAR MARG

NEW DELHI – 110 002.

Final Report of the work done on the Major Research Project.

1. Project report : **Final**

2. UGC Reference No.F.: **42-924/2013 (SR) dated 22.03.2013**

3. Period of report: from **01.04.2013** to **31.03.2017**

4. Title of research project **"Reproductive Ecology and conservation of *Garcinia imberti* Bourd. and *G.travancorica* Bedd.: Endemic and Endangered tree species from the Agasthyamalai Biosphere Reserve"**

5. (a) Name of the Principal Investigator ; **Dr. R. Ramasubbu**

(b) Department :**Biology**

(c) University/College where work has progressed: **The Gandhigram Rural Institute-Deemed University, Gandhigram, Tamil nadu.**

6. Effective date of starting of the project: **01.04.2013**

7. Grant approved and expenditure incurred during the period of the report:

a. Total amount approved :**Rs. 8,65,800/-**

b. Total expenditure : **Rs. 7,99, 206**

c. Report of the work done: (Please attach a separate sheet)

i. Brief objective of the project :

- Survey, inventory and identification of viable populations of candidate species.
- To study the population structure, size, distribution and dynamics.
- To study the phenology with special reference to flowering and fruiting.
- To study the pollinators and their foraging behaviors.
- To assess the reproductive capacity of the species in the natural environment.
- To develop suitable micropropagation protocol for multiplication and re-introduction.
- To regenerate the candidate species through Embryo culture, callus for the sustainable plantlet production.

ii. Work done so far and results achieved and publications, if any, resulting from the work (Give details of the papers and names of the journals in which it has been published or accepted for publication: **Refer Part I**

iii. Has the progress been according to original plan of work and towards achieving the objective. if not, state reasons : **Yes**, **the work has been done according to original plan of work**

iv. Please indicate the difficulties, if any, experienced in implementing the project:

The second installment of the grant has not been sanctioned on time and the amount has received at the end of the project period. Therefore, we felt difficult to travel to the study site and to execute the project work.

v. If project has not been completed, please indicate the approximate time by which it is likely to be completed. A summary of the work done for the period (Annual basis) may please be sent to the Commission on a separate sheet.

The project has been completed within the stipulated time period

vi. If the project has been completed, please enclose a summary of the findings of the study. One bound copy of the final report of work done may also be sent to University Grants Commission.

The detailed project report has attached separately as Annexure

vii. Any other information which would help in evaluation of work done on the project. At the completion of the project, the first report should indicate the output, such as

(a) Manpower trained : **01 Number (Project Fellow)**

(b) **Ph. D. awarded** : **01 Number (01 awarded)**

(c) Publication of results : 6 papers published : 04 papers Communicated

S.No.	Author(s)	Title	Name of Journal	Vol.	Page	Year
1.	R.Ramasubbu, G.Manikandan, P.Mehalingam and A.G. Pandurangan	*Impatiens courtallensis* (Balsaminaceae), a new species of *Impatiens* from Western Ghats, Tamil Nadu, India	Phytotaxa	203 (2)	199-204	2015
2.	Ramasubbu Raju, Manikandan Gurusamy and Sasi kala Nambi	*In vitro* propagation of *Garcinia travancorica* Bedd. – an endemic and endangered tree species of Western Ghats, India	Indian Journal of Tropical Biodiversity	24(1)	64-69	2016
3.	R. Ramasubbu, C. Divya, N. Sasi Kala, Anjana Surendran	*Impatiens megamalayana*, a new species of *Impatiens* from the Western Ghats, Tamil Nadu, India	Phytotaxa	302 (2)	193-197	2017
	Manikandan, G. and Ramasubbu, R.	Field notes on Distribution, Population status and threats of Garcinia imberti Bourd. and G. travancorica Bedd. critically endangered tree species from	Journal of Threatened Taxa.	10	1094	1001

		Agasthyamalai.				

Book Chapters

S. No.	Title	Author's Name	Publisher	Year of Publication
4.	Population degradation, seed predation and limited distribution of *Garcinia travancorica* bedd.: an endemic, endangered and medicinally important tree species	Manikandan, G. and Ramasubbu, R	Virudhunagar Hindu Nadar's Senthikumara Nadar College, Virudhunagar. ISBN-978-93-81723-18-0	2013
5.	Seed biology of *Garcinia imberti* Bourd. and *G. travancorica* Bedd.: Endemic endangered trees of Agasthyamalai	Ramasubbu, R. and Manikandan, G	Institute of Forest Genetics and Tree Breeding (Indian Council of Forestry Research and Education) Coimbatore ISBN- 978-93-82387-10-7	2015
6.	Antimicrobial activity of the tree bark extracts of *Garcinia travancorica* Bedd. (Clusiaceae). In proceedings Discources on past, present and future scenario on medicinal plant conservation in India	Manikandan Gurusamy, Stephan Anburaj Micheal and Ramasubbu R.	SN College, Madurai Conference Proceeding	2015

Manuscript communicated

- Manikandan, G. and Ramasubbu, R. Reproductive biology of *Garcinia travancorica* Bedd. (Clusiaceae): An endangered tree of Southern Western Ghats. *Current Science.*
- Manikandan, G. and Ramasubbu, R. Reproductive inability and unsuccessful pollination leads to the population reduction in *Garcinia imberti* Bourd: an endemic and endangered tree species from Southern Western Ghats. *Current Science.*
- Manikandan, G. and Ramasubbu, R. Phytochemical and antimicrobial analysis of leaf oil extracts of *Garcinia imberti* Bourd. and *G. travancorica* Bedd. critically endangered tree species from Agasthyamalai. *American Journal of essential oil Research*.

Annexure – IX

UNIVERSITY GRANTS COMMISSION

BAHADUR SHAH ZAFAR MARG

NEW DELHI – 110 002

PROFORMA FOR SUBMISSION OF INFORMATION AT THE TIME OF SENDING THE FINAL REPORT OF THE WORK DONE ON THE PROJECT

1. TITLE OF THE PROJECT

"Reproductive Ecology and conservation of *Garcinia imberti* Bourd. and *G.travancorica* Bedd.: Endemic and Endangered tree species from the Agasthyamalai Biosphere Reserve"

2. NAME AND ADDRESS OF THE PRINCIPAL INVESTIGATOR

Dr. R.Ramasubbu

Department of Biology,

The Gandhigram Rural Institute- Deemed University, Gandhigram – 624 302, Dindigul, Tamil Nadu, India.

3. NAME AND ADDRESS OF THE INSTITUTION

Department of Biology,

The Gandhigram Rural Institute- Deemed University,Gandhigram – 624 302, Dindigul, Tamil Nadu, India.

4. UGC APPROVAL LETTER NO. AND DATE: **42-924/2013 (SR) dated 22.03.20133**

5. DATE OF IMPLEMENTATION :**01.04.2013**

6. TENURE OF THE PROJECT : **3 years**

7. TOTAL GRANT ALLOCATED : **Rs. 8,65,800/-**

8. TOTAL GRANT RECEIVED : **Rs.7,95,500**

9. FINAL EXPENDITURE :**Rs.7,99, 206**

10. TITLE OF THE PROJECT **"Reproductive Ecology and conservation of *Garcinia imberti* Bourd. and *G.travancorica* Bedd.: Endemic and Endangered tree species from the Agasthyamalai Biosphere Reserve"**

11. OBJECTIVES OF THE PROJECT : Refer Part-I

12. WHETHER OBJECTIVES WERE ACHIEVED : Yes, achieved

13. ACHIEVEMENTS FROM THE PROJECT : Refer Part II

14. SUMMARY OF THE FINDINGS :

Garcinia imberti and *G. travancorica* are strict endemic tree species of Agasthyamalai Biosphere Reserve of Southern Western Ghats. After a few decades from its discovery, *G. imberti* was declared as extinct and recently rediscovered from its type locality. Both species were included in IUCN threatened category as Endangered and Vulnerable. *Garcinia imberti* and *G. travancorica* are closely associated tree species growing with restricted forest areas. The extent of occurrence of both the species was reported as less than 50 km^2 and the area of occupancy was restricted to less than 10 km^2. Both of the species were reported as dioecious trees, the tree has male and female flowers at two different individuals, however, the female tree produced a few bisexual flowers.

In both species, male and bisexual flowers produced more number of fertile pollens. In *G.travancorica*, male and bisexual flowers produced an average of 526278±509 and 394935±833 pollen grains respectively. The pollen-ovule ratio was calculated as 10592:1 in *G.*

imberti and 12875:1. Among the three viability tests conducted in male and bisexual flowers, a higher percentage of pollen viability was recorded in TTC test in both the species. *In vitro* pollen germination was achieved through modified Brewbaker's medium with a different concentration of sucrose (5%, 10%, 15%, 20% and 30%). However, *in vitro* pollen germination through modified Brewbaker's medium with 15% of sucrose concentrations recorded the higher percentage (73-81%) in *G. imberti* and 63-71% of pollen germination in *G.travancorica*.

In both the species, the stigma is larger and discoidal shape and covered with a thin continuous non-permeable membrane. The female flowers hold the largest stigma when compared to bisexual flowers. In flowers of *G. travancorica*, small fluid bags (25-35 nos.) were developed on the stigmatic surface before anthesis. The stigmatic fluid bags enlarged rapidly, dehisce completely and enclosed the stigmatic surface, which helps to hydrate the pollen grains and proceed for germination. The receptivity of stigma observed through hydrogen peroxide test showed the higher percentage (90%) of receptivity in *G. imberti* in female and 76% in bisexual flowers of 8 days after anthesis. About 92% of female and 84% of bisexual flowers were reported as receptive on the 8^{th} day of anthesis in *G. travancorica.*

In both the species, the nectar-secreting glands were observed at the base of the ovary and secreting nectar with poor quantity. Flies, mites, moths, grasshopper, beetles, butterflies, ant and field cricketer were reported as floral visitors which attracted by the mild fragrance and nectar of male, female and bisexual flowers. The pollination took through flies, mites, moths, beetles, butterflies, unidentified ant and field cricketer in *G. imberti*. Whereas in *G. travancorica*, flies, mites, beetles, grasshopper, and butterflies were observed. The manual pollination experiments confirmed that lowest fruit set observed through autogamous pollination experiments and highest fruit set was observed through geitonogamous pollination in both the species. The maximum

percentage of seed germination was recorded as 49% in *G. imberti* and 59% in *G. travancorica* at 10×10 meter plot created in the natural habitat at 7-9 months. During the seedlings development, most of the seedlings were severely damaged by grasshopper and newly emerged seedlings in forests were trampled down by grazing cattle and other forest animals for its tastiest and nutritive leaves.

Shoot tip and nodal segment of *G. travancorica* were collected from natural habitat and used as explants. Shoot regeneration from *in vitro* cultured explants were tested on MS medium fortified with BAP (1.0 -10.0 mg/l) and Kn (1.0 - 10.0 mg/l) alone and in combination with lower concentrations (0.5 mg/l – 2.5 mg/l) of auxins (NAA and IAA). Nodal and shoot tip explants were cultured on MS medium supplemented with various concentration of BAP (1.0-10.0 mg/l) and Kn (1.0-10.0 mg/l) induced shoot bud initiation, with BAP being more efficient than kinetin. The low concentration of auxin facilitated better root formation. The maximum frequency of root formation and higher number (6.84±0.98) of roots were achieved in half strength MS medium containing 2.0 mg/l of IBA.

Immature flower buds and leaves were used for somatic embryos induction. Explants were inoculated on the MS medium supplemented with different concentration of 2,4 -D or NAA (1.0 - 5.0 mg/l) along with BAP (1.0 mg/l). The smooth and round embryos observed in direct somatic embryogenesis culture. The highest no. of somatic embryos (15.67) was observed in leaf explants followed by flower bud (10.54) in the combination of BAP and 2, 4 - D (2.0 mg/l) which was highly significant at 5% level. The result of present study indicated that 2,4-D and NAA at higher concentration reduced the number of somatic embryos per culture. After embryo germination and maturation, somatic embryos were transferred to half strength MS medium free of plant growth regulators for further plantlet development. The plantlets thus obtained through

somatic embryogenesis were transferred to a soil mixture (sand: peat moss, 2 : 1) and the rate of survival was 72%.

15. CONTRIBUTION TO THE SOCIETY

Management and conservation of wild species are impossible without a clear understanding of the reproductive biology of the species. Nonetheless, insufficient understanding of the biology of plant species is repeatedly cited as a shortcoming of endangered species recovery plans. By studying the reproductive biology of rare, endangered and threatened (RET) species, one can understand the exact causal factors inducing rarity and can overcome these factors through scientific intervention, so as to protect the plants from endangerment. The information obtained from reproductive biology and conservation studies of both species may be useful for evaluating alternative *in-situ* and *ex-situ* management strategies. Through the knowledge on reproductive biology of *Garcinia imbertii* and *G.travancorica*, the conservation, management strategies and recovery of endemic and endangered species can be achieved. This study will help to understand the viability of other rare plant population and their successful establishment of seedlings in the near future.

16. WHETHER ANY PH.D. ENROLLED/PRODUCED OUT OF THE PROJECT

Yes, one person has enrolled and awarded the Ph.D. degree

17. NO. OF PUBLICATIONS OUT OF THE PROJECT : **06** (**Refer Part III**)

PART-II

Field visit and Survey

Several field exploration trips were conducted to various forest areas of Agasthyamalai Biosphere Reserve in both Tamil Nadu and Kerala in the past 26 months to locate the candidate species. During the field exploration trips, *Garcinia imberti* Bourd. was located in Muthukuzivayal, Netterikal, Poonkulam of Tamil Nadu and Ponmudi, Chemugi of Kerala. The populations of *Garcinia travancorica* Bedd. were located at Muthukuzivayal, Netterikal of Tamil Nadu and Chemungi and Ponmudi of Kerala. *Garcinia imberti* and *G. travancorica* were reported only from the above mentioned forest areas of Tirunelveli and Travancore Hills. The distribution of these two trees was confirmed by various filed exploration trips. The trees are strict Endemic to this region and survival of the species is also questionable. The voucher specimens of both species were submitted in the Herbarium, Department of Biology, The Gandhigram Rural Institute – DU, Gandhigram, Dindigul.

REPRODUCTIVE BIOLOGY OF *GARCINIA IMBERTI* BOURD.

Garcinia imberti Bourd. in J. Bomb. Nat. Hist. Soc. 12: 349. T. 1.1899 & For. Tr. Trav. 24, 1908; Gamble. Man. Ind. Timb. 57. 1902; Rama Rao, Fl. Pl. Trav. 31, 1914; Dunn in Gamble, Fl. Pres. Madras 74. 1915 (1:53.1957, repr. Ed.); Maheswari, Bull. Bot. Surv. India 6(2-4): 117-118. 1964; Mohanan *et al.,* Ind. J. Forestry 20(4): 383-385, 1997; N.P. Singh in B. Sharma & Sanjappa, Fl. India 3:128.1993; Gopalan & Henry, 2000 Endemic plants of India CAMP for the strict endemics of Agasthyamalai hills, SW Ghats Bot. Sury. India 206-209; Ramasubbu and Manikandan in. Buvaneswaran *et al.,*(Eds.) Advances in Tree Seed Science and Silviculture. pp. 113-121, 2015; Muhammed Shareef *et al.,* in Taiwania 60(3): 148-149, 2015.

Garcinia imberti is a medium-sized evergreen tree, 9-12 m high, trunk about 30 cm in diam. Bark brown and white, smooth, about 6 mm thick; cut sweet scented, wood yellowish-grey, very hard; pores medium to small, scanty, evenly distributed; medullary rays indistinct; annual rings not visible. Leaves opposite, 4-8 × 1.5-3 cm, elliptic or lanceolate, acuminate, base narrowed, entire, dark green; midrib prominent on both surfaces and raised beneath, nerves 15-25, parallel, close, obscure; petiole 3 mm long. **Male flowers**: terminal fascicles 3-9, at the ends of branchlets, yellow, about 5 mm in diam., succulent, sessile. Sepals and petals 4 each, much imbricated. Stamens in a central globose mass, 31±6. **Female flowers**: terminal, solitary or geminate, yellow, succulent, sessile. Sepals and petals as in male. Staminodes about 18±3.19 in a ring surrounding the ovary. Inserted on a hypogynous ring; Ovary bilocular; ovules solitary in each locule; stigma broad, sessile, convex. Berry about 2.5 × 2.5 cm. Seeds 1-2, brown, smooth, enclosed in a leathery covering. **Bisexual flowers:** terminal fascicles 1-5, at the ends of branchlets, yellow, about 5 mm in diam., succulent, sessile. Sepals and petals 4 each, stamens long about 7±2 in a ring surrounding the ovary, inserted on a hypogynous ring; Ovary bilocular; ovules solitary in each locule; stigma broad, sessile and cone shaped, wet and non-papillate convex. Berry about 2.5 × 2.5 cm. Seeds 1-2, brown, smooth, enclosed in a leathery covering.

Vernacular Name: Mania kanji (Tamil)

Population Studies

Garcinia imberti Bourd. is an endemic and critically endangered tree belongs to the family Clusiaceae (Guttiferae) and distributed in Western Ghats of Tamil Nadu and Kerala, India. It is generally home at evergreen, semi-evergreen forests of the tropical or in areas with relatively mild monsoon climate. In the forest, *Garcinia imberti* appears as medium-sized

straight stemmed tree with horizontal branches. The trees were distributed in the restricted patches of high altitude forest areas (700-1500 m asl) of Thiruvananthapuram and Tirunelveli hills of Agasthyamalai Biosphere Reserve of Southern Western Ghats. The large number of mature individuals of *Garcinia imberti* has been over exploited from Agasthyamalai and the very meager number of mature individuals and seedlings alone exist. *Garcinia imberti* is closely associated with many evergreen arboreals including *Calophyllum austro-indicum, Cullenia exarillata, Actephila cxcela, Euphorbia santapaui, Garcinia travancorica, Garcinia xanthochymus, Garcinia gummi-gutta, Schefflera bourdillonii, Syzygium mundagam, Elaeocarpous venustus, E. variabilis, E. tuberculatus, E. serratus, Litsea coriaceae,* etc., Based on field observation and standard literature (IUCN), the extent of occurrence was estimated to about less than 50 km^2 and the area of occupancy was restricted to less than 10 km^2. The populations were severely fragmented and exist in 15±3 locations. It was also observed that there were no sub- populations observed in the study area. The number of mature individuals (individuals which produce new recruits and individuals having reproducing units within the populations were counted as mature individuals) recorded was 127±14 in the entire distributional areas. The habitat of the tree species is being altered due to the extension of tea estates by private companies and also by raising commercial plantations by the forest department. There was an extreme fluctuation observed in every year in the case of populations and also in the number of individuals due to the disturbance in the forest ecosystem. Since, being an endangered tree species as categorized by IUCN (IUCN 2015.3), authenticated survey reports and other relevant information has to be communicated to IUCN and the species has to be included in the critically endangered category.

Phenology

The various field studies conducted at different forest areas of Western Ghats were confirmed that *Garcinia imberti* are a dioecious tree which produces male and female flowers at different individuals. However, the female tree produces a small proportion of bisexual flowers at each flowering period. The male flowers are normally developed at the branches of the lower part of the male trees whereas the female flowers developed mostly at the upper part of the female tree. Since it is an evergreen tree, the entire tree has fully covered with leaves throughout the year and there was no mass leaf shedding observed. However, a minimum percentage of leaf falling was observed from July-August in which the area receives high rainfall. The leaf flushing of the tree was started in the month of August at every year and continues until the end of September. The young leaves developed from the tender shoots were appeared as pale green colour and later it becomes deep green. Flower buds of male, female and bisexual flowers on the trees were initiated in the month of February-March and the number of flowers developed per branches was increased gradually till the month of April and the peak flowering period was observed in the month of May. The male flowers are terminal fascicles which grow as clusters of 3-9 flowers per inflorescence. The male flowers were started to bloom in the month of February in which only 6% of the flowers only attains maturity. The percentage of flowering was continuously increased and reached the peak in the month of May during which 94% of male flowers were bloomed. Further, the percentage of flowering was decreased suddenly at June and no more flowering observed after June. The female flowers were solitary and terminal. The female flowers were started to bloom in the month of February, continuous for subsequent months and reached the peak (91%) during the month of May. The percentage of flowering was decreased suddenly in the subsequent month and at June, only 17% of flowers were bloomed.

As like male and female flowers, the bisexual was also started to initiate the floral buds in the month of February, but the percentage of flowering was very low when compared to male and female flowers. Further, the bisexual flowering of individual was continued up to a month of July. The peak flowering period was also recorded on May in which 90% bisexual flowers were observed as bloomed. Further, the percentage of flowering was decreased up to 16% at July, further, no more flowers were observed in all flowering season. A strong correlation was observed among the flowering individuals of male, female and bisexual flowers. The floral buds of male, female and bisexual flowers took about 15-25 days to attains maturity and also for full bloom from the day of bud initiation. The average lifespan of male, female and bisexual flowers was recorded as 5±2 days. Fruit initiation and development were started from the month of July and matured fruits were observed in the month of August and extend up to September.

Floral Biology

The plant has yellow coloured male, female and bisexual flowers with four sepals and petals. The floral parts especially, the sepals were persistent up to fruit setting or withering. In male, female and bisexual flowers, sepals were observed as pale green coloured whereas the petals were observed as deep yellow in colour. The male and bisexual flowers were started to bloom at early morning (0300 – 0500 hrs) in which they produced mild fragrance. The female flowers were started to bloom one hour after the opening of male and bisexual flowers. But severe oscillation was observed on flowering of female flowers between 0400-0700 hrs. Both male and bisexual flowers produce fertile pollen, but the number of fertile pollen grains counted differed significantly between male and bisexual flowers. An average of 31±6 stamens was calculated from male flowers and no pistillode was observed. The female flowers are solitary,

terminal or rarely axillary. The stigma was reported as broad, sessile and cone shaped, wet and non-papillate type. An average of 18±3.19 staminodes has surrounded the tip of the pistil which contains sterile pollen grains.

In bisexual flower, 7±2 stamens have surrounded the pistil which contains both fertile and sterile pollen grains. Both female and bisexual flowers have receptive stigma which supports for pollen adhesion, hydration, germination and pollen tube growth which results in fruit production. During the fruit development, the stigmas were turned to brown colour and persist up to the maturation. The size of male and female flowers was almost similar and about 5.6 mm length and 4.5 mm width, but the size of bisexual flowers were reported as smaller in size about 5.0 mm length and 3.9 mm width when compared with male and female flowers. In terms of the size of stigma, the female flowers hold the largest stigma (3.5×2.9) mm when compare to bisexual flowers (2.5×1.4 mm).

Average numbers of pollen grains were calculated as 5141±72.50 per anther and 893716±831 per flower in male flowers. The pollen productivity of bisexual flowers was reported as 3842±65.92 pollen grains per anther and 43981±374 pollen grains per flower. The pollen grains were observed as spherical and tetra-colporate. The number of ovules developed per mature ovary was calculated as 2 in female flowers. But the number of mature ovules developed per ovary of bisexual flowers was 1-2. The pollen-ovule ratio of the species was calculated as 10592 pollens per ovule (10592:1). The average number of flowers observed for the fruit set in the female tree was 30 and number of fruits developed naturally from these flowers was only 9. Hence, the flower-fruit ratio in natural condition was calculated as 30:9. The average number of seeds developed per ovary was calculated as only one out of 14 ovules marked. Therefore, the ovule- seed ratio was calculated as 14:1.

Pollen Biology

The viability of pollen grains of male and bisexual flowers was assessed through randomly collected flowers of *G.imberti*. Various viability tests were adopted to assess the viability of pollen grains at different time intervals of both before and after anthesis. In male and bisexual flowers, the viability test conducted through 2, 3, 5-Triphenyl Tetrazolium Chloride (TTC) test confirmed that the pollen grains of 82% of male flowers and 72.51% of bisexual flowers were viable on the day of anthesis. Further, the viability test was continued for up to 84 hours after anthesis.

The pollen viability through Fluorochromatic Reaction (FCR) test revealed that 80.50% of pollen grains from male flowers and 73% of pollen grains of bisexual flowers were viable on the day of anthesis. The viability tests were continued for up to 84 hrs after anthesis.

Di-amino benzidine (DAB) test indicated that 80% of pollen grains of male and 71% pollen grains of bisexual flowers were viable at the time of anthesis. The Acetocarmine-glycerin staining technique revealed that 81% of pollen grains of male flowers and 74% of pollen grains of bisexual flowers were fertile on the day of anthesis. Further, the fertility test was continued for up to 84 hours after anthesis All the viability and fertility tests have confirmed the ability of pollen grains to survive and germinate up to 3 days after anthesis.

Pollen grains of male and bisexual flowers were randomly collected for *in vitro* pollen germination studies at different time intervals. The *in vitro* pollen germination studies were carried out through modified Brewbaker's medium with different concentration (5%, 10%, 15%, 20% and 30%) of sucrose at different time intervals of before and after anthesis. In both male and bisexual flowers, *in vitro* pollen germination was not observed before anthesis. The *in vitro*

pollen germination through modified Brewbaker's medium with 15% of sucrose concentrations promoted 10-12% of pollen germination on the day of anthesis. The higher percentage (73-81%) of *in vitro* pollen germination was recorded through Brewbaker's medium with 15% of sucrose concentrations after 60 hrs of anthesis of pollens of both male and bisexual flowers. There was no pollen germination recorded after 72 hrs after anthesis at higher concentration (20-30%) of sucrose. Further, the *in vitro* germination was continued up to 84 hrs after anthesis and 16%-22% of pollen grains were observed as viable.

Stigma Receptivity

The stigma was confirmed as broad sessile, wet and non-papillate type. The stigma receptivity was analyzed with hydrogen peroxide test which showed that the receptivity was started from the day of anthesis and maintains its receptivity up to 14 days after anthesis. Of the 25 stigmas analyzed with hydrogen peroxide test, only 47% of female flowers and 35% of bisexual flowers showed receptivity on the day of anthesis. The percentage of stigma receptivity was gradually increased in the subsequent days and 77% (female) and 59% (bisexual) of receptivity were observed at 4^{th} day after anthesis. The highest percentage of receptivity was recorded as 90% in female and 76% in bisexual flowers of 8 days after anthesis in which the stigma produced the highest number of bubbles with hydrogen peroxide test. Further, the receptivity was continued for up to 14 days after anthesis.

To confirm the receptivity of stigmatic surface, about 20 flowers were marked for the studies on *in vivo* pollen germination at different time intervals. Stigmas of different day intervals were collected and allowed for *in vivo* pollen germination in laboratory condition. About twenty stigmas each of both female and bisexual flowers were observed for *in vivo* pollen germination,

40% of female and 30% of bisexual stigmas alone observed as receptive. There was no pollen adhesion and germination observed on the stigmas of flower buds before anthesis. The percentage of pollen germination on stigma was increased in the following days and reached the maximum at the 8th day of anthesis where 63% of female and 60% of bisexual stigmas showed pollen germination. On the 8th day of anthesis, about 1612 (female) and 3625 (bisexual) numbers of pollen grains were observed on the stigmatic surface of female and bisexual respectively. The number of pollen grains germinated on stigma on the 10th day of anthesis was comparatively very less in female (984) and bisexual (1567) flowers when compared to the pollen grains retained on stigma. Different receptivity test confirmed that stigmas of *G. imberti* was receptive from the day of anthesis and continued up to 14 days.

Volume and concentration of Nectar

The nectar secretion was measured through capillary tubes on randomly selected flowers at different time intervals. The nectar-secreting glands at the base of the ovary were started to secrete nectar from the day of anthesis. The nectar secretion was found to be high in the morning hours from 0700 to 1100 hrs and an average nectar secretion was measured from 0.3 to 0.9µl per flower per day. Before anthesis, the nectar glands were not active and no secretion was observed. On the day of anthesis, an average of 0.5±0.09µl of nectar was measured per flower per day. The volume of nectar was increased subsequently up to 0.9±0.05µl on the 3rd day after anthesis. Further, the nectar secretion was started to decrease and no secretion of nectar was observed after 6 days of anthesis.

The concentration of nectar was analyzed through hand refractometer (0-35%). After the measurement of nectar of each flower, about 1µl of nectar was poured at a panel of the refractometer and carefully observed the concentration of nectar and recorded. On the day of

anthesis, the concentration of nectar was observed as 20% and subsequently the concentration has increased up to 27%. The concentration of nectar observed on the 7th day of anthesis was only 15%. There was no correlation observed between volume and concentration of nectar observed from the flowers of *G. imberti*. Interestingly, the average volume and concentration of nectar were observed as high at 2-4 days after anthesis in which receptivity of stigma was also observed with receptivity.

Pollination Biology

The male, female and bisexual flowers have four yellow coloured petals with four pale green coloured sepals. At the time of anthesis, flowers of male, female and bisexual flowers emit a mild fragrance and withstand for up to 12 hours. Further, there was no characteristic fragrance observed in male, female and bisexual flowers. The flowers of *G.imberti* do not have any specific physical or chemical signals to attract pollinators. The present observation confirmed that the flowers offer both pollen and nectar to the floral visitors. Since being an evergreen tree, the tree always covered with deep green leaves and there was no clear visibility and distinct colour variation of flowers from the leaves to attract the pollinators or foragers. This preparation of sexual system did not properly attract the pollinators for the successful fruit production and ultimately affects the reproductive cycle.

Most of the flowers were bloomed in the early morning between 0400-0500 hrs and remain fresh until 5±2 days. The present observations confirmed that the flowers offer both pollen and nectar to attract the floral visitors. Pollination in *G.imberti* took place mostly through the wind (Anemophily) which was confirmed by various experiments. Transformation of natural pollen grains from male and bisexual flowers to female and bisexual flowers was trapped

through oil papers and stigmatic surfaces at different time intervals. Further, the stigmatic surface of bisexual flowers receives more number (4290) of pollen grains and female flowers received fewer numbers of pollen grains (2274). There were more than 60% of the pollen grains observed on the stigmatic surface were observed as sterile.

Flies, mites, moths, grasshopper, beetles, butterflies, unidentified ant and field cricketer were also observed as floral visitors in male, female and bisexual flowers. Flies, mites, moths, grasshopper, beetles, butterflies and ant were attracted by the mild fragrance and nectar of male, female and bisexual flowers which emits during anthesis. Flies, mites and beetles forage for pollen grains during the daytime between 0800-1600 h and regularly visited the flowers and spent 6-14 seconds in each visit. Pollen loads were found in the mouth, legs, and heads of most of the floral visitors and they pollinated the flowers by transferring the pollen grains to the stigma. However, the visits of all the insects are not sufficient to pollinate all the flowers in the populations.

Moths are nocturnal pollinators and mostly seen at branches and tree trunk of few trees. They occasionally visit the male and bisexual flowers during the time between 1500-2000 hrs. The number of individuals visited the male and bisexual flowers were also very less (1-9). In most of the case, moths visited the freshly anthesised flowers from older flowers. In terms of pollinators, moths could not be considered as effective pollinators.

Beetles are another visitor of male, female and bisexual flower during the day time between 0800-1600 hrs. The beetles were attracted by the mild fragrance emitted during anthesis and nectar secreted at the base of the floral parts. The number of individuals pollinators visited the flower was very less when compared to other floral visitors. But they effectively pollinate the bisexual flowers. During the floral visit, hindlegs, thorax and abdomen harvest the pollen grains

from the anthers and effectively transfer the pollen grains to the receptive stigmas. During the flying mechanisms, the wing speed created a small force to disperse the pollen grains from anthers to stigma at bisexual flowers. Beetles were effectively pollinating the bisexual flowers of *G.imberti*. Grasshoppers are frequent visitors of the flowers of male and bisexual flowers during the daytime between 1100-1700 hrs and completely fed the floral parts especially the sepals, petals and anthers. They are not real pollinators and forage the floral parts and affected the pollination mechanism.

Butterflies are another visitor of male, female and bisexual flower during the day time between 0700-1800 hrs. There was a strong relationship observed between the weather and butterflies activity. Butterflies were active during daytime and in fine weather, they actively visited the flowers and spending 4±1 seconds in freshly anthesised flower in an inflorescence. However, during the rainy season, butterflies were completely inactive. Butterflies land on the petals, slightly bend the body and insert the proboscis at the base of the ovary for collecting the nectar from the flower. While harvesting the nectar, the head portion of the butterflies having pollen grains from the previous visits was transferred to the stigma of another flower. Butterflies were effectively pollinating the female and bisexual flowers of *G.imberti*.

Few species of unidentified ants were observed as floral visitors and visit male, female and bisexual flower during the day time between 0700-1700 hrs. The ants were attracted by the mild fragrance emitted during anthesis and nectar secreted at the base of the floral parts. During the floral visit, hindlegs, thorax and abdomen parts harvest the pollen grains from the anthers and effectively transfer the pollen grains to the receptive stigmas.

Few insects, like field cricket (*Lepidogryllu* sp.) were also reported as frequent floral visitors. During the floral visit, legs and abdomen harvest the pollen grains from the anthers and effectively transfer the pollen grains to the receptive stigmas.

Breeding systems

Detailed breeding experiments were conducted through controlled field pollination experiments and subsequent observation on fruit and seed set were also made. Four types of manual field pollinations such as autogamous self, geitonogamous, xenogamous and apomixis were carried out. In *G. imberti*, the natural fruit set was low (17-20%) when compared to higher flower production and different factors could affect fruit set. Out of 100 flowers marked in female and bisexual flowers for fruit set through open pollination, the overall fruit set was recorded as 18.5%. The percentage of overall fruit set under the autogamous pollination experiments was recorded as 25.5%. The autogamous pollination experiments confirmed the similarity in the percentage of fruit set in both bisexual flowers and female flowers. The geitonogamous pollination experiments conducted at bisexual flowers set 43% of fruits. The percentage of fruit set observed through xenogamy (manual cross-pollination) was enhanced the fruit set rate up to 48% and 35% in female and bisexual flowers respectively and the overall fruit set was recorded as 41.5%. The lowest percentage of fruit set was observed in autogamous self-pollination and highest fruit set was observed in geitonogamous pollination. The experiments conducted through different breeding systems suggested that the tree species set more fruits with exclusive geitonogamous pollen grains. It was observed that the most part of buds and other floral parts were damaged by insects and other pests, which leads to the loss of fruits and seed productivity. The lower percentage of fruit set was observed in naturally pollinated flowers when

compared to manually cross-pollinated flowers which strongly suggested the requirement of external agents for the effective pollination. The closed pollination (apomixis) is possible to this tree and about 18.5% of fruit set was observed from the female tree through apomixis.

Fruit and Seed biology

Reproductive success of the wild species will be assessed on the basis of fruit and viable seed set in its natural habitat. The fruiting period was started from the month of July and matured fruits were observed in the month of August and extend up to September. Periodical observations on fruit set revealed that the fruit took 16-26 days from the initiation to attain maturity after anthesis. The morphological studies on fruits revealed that shape of the fruit was recorded as a globose or subglobose berry with persistent stigma. Since being a species growing at a higher elevation, the area receives a higher percentage of rainfall and heavy wind during monsoon in which most of the underdeveloped fruits were spoiled.

The colour of fruits was green in colour and the size of the fruit was recorded as 2.5-3.0×1.1-1.8 cm with one to two seeded. During the development of fruit, immature and mature fruits were foraged by Malabar Giant squirrel (*Ratufa indica*), an endemic tree mammals (Plate 11D&E). During fruiting period, 85% of mature fruits were foraged by Malabar Giant squirrel for its fatty rich seeds (Cocum butter), which is the most important diet supplement during fruiting period. The squirrel targeted to forage matured fruits with seeds and completely damage the entire seeds grown inside. During the fast interplant movement, they completely damaged the immature fruits along with anthesised flowers of the trees.

The colour of the mature seed was observed as brown and lined with thin seed coat. The moisture content, viability and germinability of seeds were assessed through randomly collected

seeds of *G. imberti* at different time intervals. The viability was assessed through 2, 3, 5-Triphenyl Tetrazolium Chloride (TTC) test and confirmed that about 84% of seeds were viable at the time of detached stage and the moisture content of the seeds was recorded as 87%. Further, the moisture content and viability tests were continued for up to 22 months. The moisture content and viability of the seeds were drastically reduced in the subsequent months and about 62% of seeds were observed as viable at 4-6 months. Further, the viability and moisture content of the seeds were drastically reduced up to 27% and 29% respectively at 19-22 months.

During the studies on seed germination, the seeds of *G.imberti* delayed germination to overcome the dry months after the fruiting seasons. The maximum percentage of seed germination was recorded as 49% in 10×10 meter plot created in the natural habitat. However, the seed germination trails at mist house have failed to germinate the seeds. The failure of germination of seeds may be due to a lower altitude, poor relative humidity and higher atmospheric temperature. Freshly harvested seeds have not shown immediate germination up to the first month. Further, about 16% of seed germinations were observed at 2-3 months. The highest percentage (49%) of seed germination was observed at 10-12 months. The germination ability of seeds was retained for more than 22 months and about 23% of the seeds showed germination.

Insect-pest association/associated with food habit of animals

The floral parts especially petals, sepals and anthers were fed by many insects due to its nutritive tissues. In addition, most of the seeds in the soil were affected by soil-dwelling insects, fungi and other pests. The seeds either fail to germinate or even if germinated, the seedlings showed poor establishment. Due to the above reasons, seedling recruitment in natural condition

was very poor. The seedling emerged in the month of October and continued up to January. Further, the seedlings were heavily browsed by herbivores which reduce the establishment of seedlings further. Most of the seedlings developed foraged through the rapid cut. It had also been noticed that newly emerged seedlings in forests were trampled down by grazing cattle and other forest animals for its tastiest and nutritive leaves.

REPRODUCTIVE BIOLOGY OF *GARCINIA TRAVANCORICA* BEDD.

Garcinia travancorica Bedd. Fl. Sylv. T. 173. 1872; T. And. In Fl. Brit. India 1: 268. 1874; Dunn in Gamble, Fl. Pres. Madras 74. 1915. (1.53.1957 repr.ed.); Maheswari, Bull. Bot. Surv. India 6(2-4): 120. 1964; Raman. in A.N. Henry *et al*., Fl. Tamil Nadu Ser. I Analy.1:281983; N.P.Singh in B. Sharma & Sanjappa, Fl.India 3:128.1993; Manikandan and Ramasubbu, in Mehalingam *et al.*, (Eds.). A trends on Medicinal plants and Herbal products. pp.339-345, 2013. Ramasubbu and Manikandan, G., Buvaneswaran *et al.*, (Eds.). Advances in Tree Seed Science and Silviculture. pp. 113-121, 2015.

Garcinia travancorica is a medium sized, slender evergreen tree grow up to 12-18 m tall. Branches obtusely 4-angled, shining. Wood yellowish-brown; sapwood pale yellow, hard, heavy. Latex yellow, sticky, Leaves 8-10×1.5-2.5 cm, linear-oblong to sub-spathulate, sometimes broader upwards, rotundate or obtuse, base acute, margin revolute, coriaceous, dark green above, pale beneath; midrib stout, prominent below, lateral nerves slender, numerous, horizontal. **Male Flowers:** few in terminal and subterminal short trichotomous cymes; about 1 cm in diam.; pedicels very short, thickened. Sepals 4, decussate, orbicular, concave, two outer ones much smaller than the inner pair. Petals 4, about twice as long as sepals, shortly clawed, rounded. Stamens numerous (84.5±15.44), in four multifid polyandrous masses; anthers bilocular, versatile, linear-oblong, longitudinally bi-valvular; filaments short. Pistillode columnar, with a circular peltate stigma. **Female Flowers:** solitary in the terminal axils; Slightly longer than male flowers; Sepals 4, decussate, orbicular, concave, two outer ones much smaller than the inner pair. Petals 4, about twice as long as sepals, shortly clawed, rounded. Staminodes many (28.5±2.01), free; inserted in a hypogynous ring; filaments complanate, linear, in bi or trichotomous branches; anther loculi often divaricate, oblong or kidney shaped. Ovary subglobose or pyriform, 4 locular,

half concealed by the large convex stigma. Berry oblong to subglobose, as a walnut, contracted into a short, thick style with a broad imbricate stigma. Style and stigma persistent. Seeds few, large 2.5 × 1.5 cm, shining, testa brown. **Bisexual flowers:** trichotomous, short, few-flowered, terminal and subterminal cymes; about 1 cm in diam.; pedicels very short, thickened. Sepals 4, decussate, orbicular, concave, two outer ones much smaller than the inner pair. Petals 4, about twice as long as sepals, shortly clawed, rounded. Stamens numerous (69.5±8.69), in four multifid polyandrous masses; anthers bilocular, versatile, linear-oblong, longitudinally bivalvular; filaments very short, sometimes absent. Ovary subglobose or pyriform, 4 locular, half concealed by the large convex stigma. Berry oblong to subglobose, as a walnut, contracted into a short, thick style with a broad imbricate stigma. Seeds few, large 2.5 × 1.5 cm, shining testa brown.

Vernacular Name: Halambungu (Tamil); Malampongu (Malayalam); Travancore Gamboge (English)

Population Studies

Garcinia travancorica Bedd. is an endemic and Vulnerable tree belongs to the family Clusiaceae (Guttiferae) which is growing in high altitude (2500-4619ft. asl) forest areas of Chemungi, Bonnacud, Muthukuzhivayal, Upper Kodayar, Sengaltheri, Naterikal, Kannikatty, Yanai Elumbuodai and Poonkulam of Agasthiyarvanam in Western Ghats of Tamil Nadu and Kerala, India. Like other evergreen trees of Western Ghats, *Garcinia travancorica* is a medium sized, slender evergreen tree grow up to 12-18m tall. It is a dioecious slow growing tree, the seed germination and seedling establishment in the natural habitat were very poor. *Garcinia travancorica* is closely associated with many evergreen arboreals including *Calophyllum austro-indicum, Cullenia exarillata, Actephila cxcelsa, Euphorbia santapaui, Garcinia imberti,*

Garcinia xanthochymus, Garcinia gummi-gutta, Scheflera bourdillonii, Syzygium zeylanicum, S. mundagam, Elaeocarpous venustus, E. recurvatus, E. variabilis, E.tuberculatus, E. serratus and *Litsea coriaceae,* etc. Based on several reports and field study, the extent of occurrence was estimated to about less than 50 km^2 and the area of occupancy was restricted to less than 10 km^2. The populations were severely fragmented and exist in less than 13±2 locations. It was also observed that there were no subpopulations in the study area. The number of mature individuals counted (individuals which produce new recruits and individuals having reproducing units within the populations were counted as mature individuals) was 112±14 in the entire distributional areas. There was an extreme fluctuation observed in every year in the case of populations and also in the number of individuals due to the disturbance in the forest ecosystem. The habitat of the tree species is being altered due to the extension of tea estates by private companies and also by raising commercial plantations by the forest department. The adult tree secretes yellowish gamboges as an important key indicator to identify the tree. According to IUCN, the tree has been included under Vulnerable (B1+2c ver 2.3) category. However, only eight populations are alone reported from the protected areas. This species can be considered as Critically Endangered on the basis of its high altitude habitat specificity, reduced geographic range and lesser population size.

Phenology

The field studies conducted at different forest areas of Western Ghats were confirmed that *Garcinia travancorica* is a typical evergreen and dioecious trees which produce male and female flowers at different individuals. Approximately, 25-45 days took for the primordial initiation to the full bloom of all type of flowers. The female trees also produce a small

proportion of bisexual flowers at each flowering period. The male flowers are normally developed at the branches of the lower part of the male trees whereas the female flowers developed mostly at the upper part of the female tree. As it was an evergreen tree, it has fully covered with leaf throughout the year and there was no mass leaf shedding observed in a particular season. However, a minimum percentage of leaf falling was observed from June and July in which the area receives heavy rainfall. The leaf flushing of the tree was started in the month of May and extended up to June. The younger leaves developed from the tender shoots appeared as crimson red colour and later it becomes deep green colour. Male flowers are shortly pedicellate and formed at the terminal and subterminal trichotomous cymes which were grown as 7-9 flowers per inflorescence. The male flowers were started to bloom in the month of May in which only 15% of the flowers only attains maturity. The percentage of flowering was continuously increased and reached the peak in the month of August during which 91% of male flowers were bloomed. Further, the percentage of flowering was decreased suddenly at October and no more flowering observed after October.

The female flowers are solitary or twin in the terminal axis. The flowering period of female flowers was started in the month of June and continuously increased for the subsequent month of August and reached the peak in the month of September during which 98% of flowers were bloomed. The percentage of flowering was suddenly decreased in the month of October and about 56% of flowers were bloomed. Further, the flowering was continued up to the month of December. Bisexual flowers are shortly pedicellate and formed at the terminal and subterminal trichotomous cymes which grown as 5-7 flowers per inflorescence. As like male flowers, the bisexual was also started to bloom in the month of May, but the percentage of flowering was very low when compared to male and female flowers. Further, the flowering period of bisexual

flowers was continued up to the month of October. The peak flowering period was also recorded on August in which 93% of bisexual flowers were observed as bloomed. Further, the percentage of the flowering of bisexual was decreased suddenly up to 7% at October, further, no more flowers were observed in all flowering season. In general, the peak flowering period of male and bisexual flowers was recorded in the month of August. The rate of flowering was decreased from the month of September whereas the female tree started to bloom in the middle of July and attains maximum blooming in the month of September. This has lead to wastage of many fertile pollen grains during the peak flowering period of male and bisexual flowers. The average lifespan of male, female and bisexual flowers was recorded as 4-7 days and the total period needed to complete the whole process of fruit formation was 40-120 days. Fruit initiation and development were started from the month of November and matured fruits were observed in the month of December and extend up to April.

Floral Biology

Systematic research on several species of *Garcinia* has recorded the occurrence of variations in the morphology of sexual systems in which few authors considered *G. travancorica* as dioecious due to the non-availability of all three types of flowers in a tree on time. The tree produced male and female flowers at two different individuals also the female tree produced bisexual flowers at peak flowering time. The flower of all sexes produced four green colour sepals and white coloured petals and the floral parts were persistent up to the fruit development or detachment. The maximum number of flowers was opened at 0430-0530 hrs. Also, a minimal percentage of flowers were started to bloom up to 0600 hrs. The male flowers were started to bloom between 0300-0630 h. In male flowers, four multifid polyandrous mass hold numerous

short, filamentous, bilocular and versatile anthers. In bisexual flowers, anther dehisced and released the pollen grains one day before anthesis .The female flowers were started to open one hour after the bloom of male and bisexual flowers. But, severe oscillation was observed on flowering of female flowers between 0400-0700 hrs. Both male and bisexual flowers were produced fertile pollen, but the numbers of fertile pollen grains differed significantly between male and bisexual flowers. An average of 84.5±15.44 anthers was calculated from the male flowers whereas bisexual flowers hold 69.5±8.69 anthers in a concentric ring below the ovary. An average of 28.5±2.01 staminodes was calculated from the female flowers. The staminode of female flowers also consists of pollen grains which were underdeveloped with a crystalline structure. Both female and bisexual flowers have yellow coloured receptive stigmas which support for pollen adhesion, hydration, germination, pollen tube growth and fruit production. The size of male and bisexual flowers was almost similar (7-7.5 × 4-4.8 mm) but the size of female flowers was reported as larger (8-8.6 × 5-5.4 mm) when compared with male and bisexual flowers.

Pollen biology

In male flowers, the average numbers of pollen grains were calculated as 3791±87.07 per anther and 526278±509 per flower. The pollen productivity of bisexual flowers was reported as 2184.2±70.46 pollen grains per anther and 394935±833 pollen grains per flower. Also, few numbers of pollen grains were observed in female flowers, with a mean value of 3±2.01 pollen grains per anther and 17±6.72 pollen grains per flower. In male and bisexual flowers, pollen grains were observed as spherical and tetra-colporate.

In *G.travancorica,* the ovary is pyriform or globose and 3-3.9 mm size with circular and peltate stigma in both female and bisexual flowers. In male flowers, the ovary was reduced as pistillode with pinhead shape. The stigma is well developed and convex shaped in female and bisexual flowers and an average of 7-7.2 mm and 4-4.1mm size. The globose ovary was slightly elongated to ovate-lanceolate. The style and stigma were enlarged and persistent up to the development and detachment of fruits. Before anthesis, small fluid bags (25-35 nos.) were developed randomly on the surface of the stigma. The number and position of fluid bags developed were varied from female flowers to bisexual flowers. Further, the fluid bags enlarged at different parts rapidly on the stigmatic surface up to 50 µm size, dehisce and discharge the fluid content in the entire convex stigmatic surface. This stigmatic fluid helps to adhere the pollen grains and effectively harvests the pollen grains during pollination. The exact chemical constituents of fluid dispersed on the stigmatic bag were not known.

The viability of pollen grains of male, female and bisexual flowers was assessed through randomly collected flowers of *G.travancorica.* Various viability tests were conducted to assess the viability of pollen grains at different time intervals of both before and after anthesis. In male, female and bisexual flowers, the viability test calculated through 2, 3, 5-Triphenyl Tetrazolium Chloride (TTC) test confirmed that about 78% of pollen grains of male flower and 70% of bisexual flowers were viable at one day before anthesis. Further, the viability test was continued for up to 84 hours after anthesis. The viability of pollen grains was retained for more than 84 hrs. Interestingly, very few numbers (4-6) of viable pollen grains were recorded in some female flowers at 24 hrs after anthesis.

The pollen viability through Fluorochromatic Reaction (FCR) test revealed that 78% of pollen grains from male flowers and 71% of pollen grains of bisexual flowers were viable on one

day before anthesis. The viability of the pollen grains was drastically reduced in the subsequent hours and 59-66% of pollen grains were observed as viable at 24 hrs of after anthesis. The viability tests were continued for up to 84 hours after anthesis and 32-36% of pollen grains were observed as viable. But, the viability of pollen grains was retained for more than 84 hrs after anthesis. 3.3-Di-Amino Benzidine (DAB) test indicated that 76% of pollen grains of male and 72% pollen grains of bisexual flowers were viable at one day before anthesis. About 39-48% of pollen grains were observed as viable on 72 hours of anthesis.

The Acetocarmine-glycerin staining technique revealed that 78% of pollen grains of male flowers and 73% of pollen grains of bisexual flowers were fertile on one day before anthesis. The fertility of the pollen grains was drastically reduced up to 40-47% at 72 hrs after anthesis. Further, the fertility test was continued for up to 84 hours after anthesis in which 33-36% of pollen grains were alone observed as fertile. All the viability and fertility tests have confirmed the ability of pollen grains up to 84 hrs after anthesis.

Pollen grains of male and bisexual flowers were randomly collected for *in vitro* pollen germination at different time intervals. The *in vitro* pollen germination studies were observed through modified Brewbaker's medium with a different concentration of sucrose (5%, 10%, 15%, 20% and 30%) at different time intervals of before and after anthesis. In both male and bisexual flowers, *in vitro* pollen germination was observed as very low at before anthesis. However, *in vitro* pollen germination through modified Brewbaker's medium with 15% of sucrose concentrations, recorded 10-13% of pollen germination on one day before anthesis. The higher percentage (63-71%) of *in vitro* pollen germination was recorded in Brewbaker's medium with 15% of sucrose concentrations after 48 hrs of anthesis.

Stigma receptivity

The stigma receptivity was analyzed with hydrogen peroxide test which showed that the receptivity started from the day of anthesis and maintains up to 14 days after anthesis. Of the 20 stigmas analyzed with hydrogen peroxide test on the day of anthesis, only 58% of female flowers and 53% of bisexual flowers were observed with receptivity. The highest percentage of receptivity was recorded as 92% in female and 84% in bisexual flowers on the 8^{th} day of anthesis in which the stigma produced the highest number of bubbles. Further, the receptivity was continued for up to 14 days after anthesis and 35% (female) and 23% (bisexual) of stigma were observed as receptive. The receptivity of stigma was retained for more than 14 days.

Volume and Concentration of Nectar

The nectar secretion was measured by using capillary tubes on randomly selected flowers at different time intervals. The nectar-secreting glands positioned at the base of the ovary was started to secrete nectar from the day of anthesis. The yellow coloured stigma of the base indicated the secretion of nectar. The nectar secretion was found to be high in the morning hours from 0600 to 0900 hrs and nectar secretion was measured from 0.4 to 1.2µl per flower per day. Before anthesis, the nectar glands were not active and no secretion was observed. On the day of anthesis, an average of 0.5±0.1µl of nectar was measured per flower per day. The volume of nectar was increased subsequently up to 1.2±0.3µl on the 4^{th} day after anthesis. Further, the nectar secretion was started to decrease and no secretion of nectar was observed after the 6^{th} day of anthesis. The amount of nectar secreted per flower was very low when compared to other tree species of evergreen forests.

The concentration of nectar was analyzed through hand refractometer (0-35%). After the measurement of nectar of each flower, about 1µl of nectar was poured at a panel of the refractometer and carefully observed the concentration of nectar and recorded. On the day of anthesis, the concentration of nectar was observed as 20% and subsequently the concentration has increased up to 30%. The concentration of nectar observed on the 7^{th} day of anthesis was only 17%. There was no correlation observed between volume and concentration of nectar in the flowers of *G. travancorica*. Interestingly, the average volume and concentration of nectar were observed as high at 2-4 days after anthesis in which stigma started its receptivity.

Pollination Biology

The flower of all sexes produces four green coloured sepals and white coloured petals which emit mild fragrance at anthesis and withstand for about 24 hours. Further, there was no characteristic fragrance observed in all the three types of flowers. The flowers of *G.travancorica* did not have any characteristic physical or chemical signals to attract pollinators. Since being an evergreen tree, the tree always covered with deep green leaves and the flowers were dull coloured and do not have distinct colour variation from the leaves to attract the pollinators or foragers. The present observations confirmed that the flowers offer both pollen and nectar to the floral visitors, but there was no significant visitors/pollinators observed in the natural habitat. Flies, mites, beetles, grasshopper and butterflies were also observed as floral visitors in male, female and bisexual flowers.

Pollination in *G.travancorica* takes place mostly through the wind (Anemophily) which was confirmed by various experiments. Transformation of natural pollen grains both male and bisexual flowers to female and bisexual flowers were trapped through oil papers and stigmatic

surfaces at different time intervals up to 12 hours of anthesis and significant numbers (about 50,000) of pollen grains were observed. Due to the availability of fertile anthers, the stigmatic surface of bisexual flowers receives more number (8698±321.27) of pollen grains when compared to female flowers (4715±162.80). There were more than 60% of the pollen grains observed on the stigmatic surface as sterile.

Flies, mites, grasshopper, beetles and butterflies were also observed as floral visitors in male, female and bisexual flowers. The pollination was carried out through flies, mites, grasshopper, beetles and butterflies. Flies and butterflies forage for nectar and pollen grains during the daytime between 0700-1800 h and regularly visited the flowers and spent 3-5 seconds in each visit. The pollen loads were also found in the mouth, legs and head of most of the floral visitors and transferred the pollen grains to the stigma effectively. All the insects were found as pollen carriers and their frequent interplant movement facilitates cross-pollination. However, the visits of all the insects were not sufficient to pollinate all the flowers in the populations.

The flies were the active floral visitors during the daytime between 0700-1600 hrs and spent 3±1 seconds per visit. They were active during the day time; however, the number of individual pollinators visited per flower was comparatively higher between 1400-1500 hrs. Mites played a major role as pollinator which was found inside the flowers and contributed for efficient transfer of pollen grains within the flower. The mites have visited the flower during the day time between 0800-1700 hrs and spent 6±2 seconds per visit. Hence, it has a greater role when the absence of activity of other pollinators during cloudy and rainy days and also during the late flowering season.

Beetles were another floral visitor of male, female and bisexual flower during the day time between 0800-1500 hrs. The beetles were attracted by the mild fragrance emitted during

anthesis and nectar secreted at the base of the floral parts. The number of individuals pollinator visited the flower was very less when compared to other floral visitors, but they effectively pollinate the bisexual flowers. During the floral visit, hindlegs, thorax and abdomen of different floral visitors harvested the pollen grains from the anthers and effectively transfer to the receptive stigmas. During the flying mechanisms, the wing speed created a small force to disperse the pollen grains from anthers to stigma at bisexual flowers. Beetles were effectively pollinating the bisexual flowers of *G.travancorica.*

Grasshoppers were frequent visitors of male, female and bisexual flowers during the daytime between 0800-1400 hrs. They were visited all the trees bearing male, female and bisexual flowers and completely fed the floral parts especially the sepals, petals and anthers. They were not real pollinators and frequently visited the flowers to forage the floral parts and damage the pollination mechanism.

Butterflies were another visitor of male, female and bisexual flower during the day time between 0700-1800 hrs. Butterflies were active during daytime and in fine weather, they actively visited the flowers and spending 4±1 seconds in each visit on freshly anthesised flower in an inflorescence. Butterflies land on petals, slightly bend the body and insert the proboscis at the base of the ovary for collecting the nectar from the flower. While harvesting nectar, the head portion of the butterflies having pollen grains from the previous visits was transferred to the stigma of another flower. Butterflies were effectively pollinating the male, female and bisexual flowers of *G.travancorica.*

Flies, mites, beetles, grasshopper and butterflies were attracted by the mild fragrance and nectar of male, female and bisexual flowers which emits during anthesis. The stigma of female and bisexual flowers secretes strong stigma fluid during peak receptivity period to adhere the

pollen grains. This strong sticky fluid act as a fly trap which mostly appended the legs of insects, floral visitors and mostly kills. Therefore, the insects avoid visiting female and bisexual flowers. In most cases, female flowers suffered from pollen scarcity which leads to poor fruit and seed set. This preparation of sexual system strongly affects the reproductive cycle.

Breeding systems

In *G. travancorica*, fruit set was observed in all the breeding experiments. The open fruit set (natural/control) was low when compared to the higher flower production and different factors could affect fruit set. Out of 100 flowers marked for fruit set through open pollination, overall fruit set was recorded as 20.5%. Further, the percentage of fruit set recorded under the autogamous pollination experiments was 31%. The autogamous pollination experiments confirmed the similarity in the percentage of fruit set in both bisexual and female flowers. Further, geitonogamous pollination experiments conducted at bisexual flowers set 42% of fruits. The percentage of fruit set observed through xenogamy (manual cross-pollination) enhanced the fruit set rate up to 46% and 31% in female and bisexual flowers respectively and the overall fruit set was recorded as 38.5%. The lowest percentage (20.5%) of fruit set (42%) was observed in open pollination and highest fruit set was observed through geitonogamous pollination. The experiments conducted through different breeding systems suggested that the tree species set more fruits with exclusive geitonogamous pollen grains. Since, being a dioecious tree with polygamous flowers, unisexual flowers were functionally more fertile and set more percentage of fruits. However, bisexual flowers also set considerable percentage of fruits, but the fertility of the seeds was reported as very less. Natural pollen grains transformation efficiency from male and bisexual flowers to female and bisexual flowers was analyzed by various field experiments. It

was observed that the most part of buds and other floral parts were damaged by insects and other microbes, which leads to the loss of fruits and seed productivity. The lower percentage of fruit set was observed in naturally pollinated flowers when compared to manually cross-pollinated flowers which strongly suggest the requirement of external agents for the effective pollination. The closed pollination (apomixis) was also possible to this tree with 8% of fruit set

Fruit and Seed biology

The fruiting period was started from the month of November to April. The shape of the fruit was recorded as globose or subglobose and berry and the size of the fruit reported to about 2.0-3.5×1.8-3.5 cm with one or two seeded. Since being a species growing at a higher elevation, the area receives a higher percentage of rainfall and heavy wind during monsoon in which most of the underdeveloped fruits were spoiled. During the fruiting period, a considerable amount (4-7%) of fruits was infested with the larvae of flies and the seeds were also infested with various fungi. The higher percentage (70%) of the fruits was infected by some seed borer insects, unidentified larvae and Malabar Giant squirrel. This squirrel forages mature and immature fruits and completely damaging the entire seeds grown inside.

The number of ovules developed per mature ovary was calculated as 2 in female flowers. But, the number of mature ovules developed per ovary of bisexual flowers was only one. The pollen-ovule ratio of the species was calculated as 12875 pollens per ovule (12875:1). Hence, the flower-fruit ratio in natural condition was calculated as 34:1. The average number of ovules required to set single seed was 16 in female flowers. Therefore, the ovule- seed ratio had been calculated as 16:1.

In a natural habitat, the seeds were viable for about a year only and susceptible to fungus infection. The selected candidate species showed varying results with respect to the study of seed

biology. The marked seeds of the selected plants showed the varied percentage of seed moisture content, viability and germination. The colour of the mature seed was reported as leathery brown and the shape of the seed was ovate-lanceolate the size of the seed was reported as 1.7-2.5x1.6-2.5 cm. The seeds showed the highest percentage of seed moisture content of about 78.04% and 67.5% of seed viability at detached stage. During the seed germination trials, the seeds of *G.travancorica* took one month time to germinate and there may be to overcome the dry months after the fruiting seasons. The germination percentage of the seeds was recorded as 59.75% in 10×10 meter plot created in the natural habitat. However, the seed germination trails at mist house showed the poor result (1.3%). The failure of germination of seeds may be due to a lower altitude, poor relative humidity and higher atmospheric temperature. Freshly harvested seeds have not shown immediate germination up to the first month. The highest percentage (59%) of seed germination was observed at 7-9 months. The germination ability of seeds was retained for more than 22 months.

In addition, most of the fruits and seeds in the soil were affected by soil-dwelling insects, fungi and other pests. The seeds were either fail to germinate or even if germinated, the seedlings showed poor establishment. Due to the above reasons, seedling recruitment in natural condition was very poor. The seedling emerged rarely in the month of May and regular seedlings development started to emerge in the month of June and July. The germinated seedlings also had slow growth rate and were highly prone to infection, which accounts for the absence of young seedlings in the natural habitat. Further, the seedlings were heavily browsed by herbivores which reduce the establishment further.

Insect-pest association/associated with food habit of animals

The beetles and grasshoppers have also damaged the stigma, anthers, petals and sepals of male, female and bisexual flowers. So, the flower was unable to attract the pollinators to fertilize and ultimately failed to set fruits. During the seedlings development, most of the seedlings were severely damaged by grasshopper and foraging the growing seedlings. It had also been noticed that newly emerged seedlings in forests were trampled down by grazing cattle and other forest animals for its tastiest and nutritive leaves. The fungi were born as surface contaminants and mostly present on the seed coat. The unknown fungi were also penetrated deep into the seeds and attack the embryo, thus damaged the seeds. Since the mother plant was found near the stream, most of the seeds were washed away to the plains during monsoon.

Tissue culture studies /Restoration

***In vitro* propagation**

Shoot tip and nodal segment of *G. travancorica* were collected from natural habitat used as explants. Shoot regeneration from *in vitro* cultured explants were tested on MS medium fortified with BAP (1.0 -10.0 mg/l) and Kn (1.0 - 10.0 mg/l) alone and in combination with lower concentrations (0.5 mg/l – 2.5 mg/l) of auxins (NAA and IAA). Nodal and shoot tip explants were cultured on MS medium supplemented with various concentration of BAP (1.0-10.0 mg/l) and Kn (1.0-10.0 mg/l) induced shoot bud initiation, with BAP being more efficient than kinetin. With response to the initiation and subsequent proliferation of shoots, medium supplemented with 4.0 mg/l BAP induced a mean value of 8.17±0.84 shoots per nodal explants, while medium supplemented with kinetin resulted 5.64±0.98 of shoots. Albeit, frequency of regeneration and number of shoots per explants were found maximum at 4.0 mg/l and minimum

at 10.0 mg/l. For *G. travancorica,* nodal segment was the best in terms of percentage of shoot induction and also the number of shoots per explants was better at 4.0 mg/l of BAP. The effect of auxin and cytokinin combination was also evaluated on multiple shoot induction from nodal segments and shoot tip. The highest shoot regeneration frequency and number of shoots per explants were recorded in the combination of MS medium supplemented with BAP along with NAA. BAP with NAA was found more effective combination for multiple shoot regeneration in which 13.04±1.27 mean multiple shoots per explants were induced. Although, a low induction and multiplication of shoots were recorded when NAA was replaced with IAA.

The regenerated shoots from explants were excised and transferred to half strength MS medium supplemented with different concentration of IBA and IAA (0.5-2.5 mg/l). The low concentration of auxin facilitated better root formation. The maximum frequency of root formation and higher number (6.84±0.98) of roots were achieved in half strength MS medium containing 2.0 mg/l of IBA.

Somatic embryogenesis

Immature flower buds and leaves were used for somatic embryos induction. Explants were inoculated on the MS medium supplemented with different concentration of 2,4 -D or NAA (1.0 - 5.0 mg/l) along with BAP (1.0 mg/l). The smooth and round embryos observed in direct somatic embryogenesis culture. The highest no. of somatic embryos (15.67) was observed in leaf explants followed by flower bud (10.54) in the combination of BAP and 2, 4 - D (2.0 mg/l) which was highly significant at 5% level. Flower buds and leaves produced 09.73 and 11.21 somatic embryos respectively in the combination of BAP and NAA (2.0 mg/l). Among different portions of the flower bud explants, only flower head segments produced luxuriant embryogenic

callus. Our studies proved that, the production of embryogenic cultures and number of somatic embryos per explants depends upon the strength of the auxin in the induction medium. The lowest no. of somatic embryos were produced in the concentration of 5.0 mg/l of 2,4-D and NAA in both flower buds (06.62) and leaf (08.25). Similarly, leaf (08.11) and flower bud (07.62) induced somatic embryos at the same concentration (5.0 mg/l) of NAA. The result of present study indicated that 2,4-D and NAA at higher concentration reduced the number of somatic embryos per culture. After embryo germination and maturation, somatic embryos were transferred to half strength MS medium free of plant growth regulators for further plantlet development. The plantlets thus obtained through somatic embryogenesis were transferred to a soil mixture (sand: peat moss, 2 : 1) and the rate of survival was 72%.