A STUDY ON ECOLOGY, CONSERVATION AND UTILIZATION OF *DIOSPYROS CRUMENATA* THWAITES IN KERALA - AN ENDANGERED TREE OF THE WESTERN GHATS

Thesis submitted to the University of Calicut in partial fulfillment of the requirements for the Degree of

DOCTOR OF PHILOSOPHY IN BOTANY

By

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KSCSTE - Jawaharlal Nehru Tropical Botanic Garden and Research Institute
An institution of Kerala State Council for Science, Technology & Environment
National Centre of Excellence

15 /12/2025

CERTIFICATE

This is to certify that the work embodied in the thesis entitled **A STUDY ON ECOLOGY, CONSERVATION AND UTILIZATION OF *DIOSPYROS CRUMENATA* THWAITES IN KERALA - AN ENDANGERED TREE OF THE WESTERN GHATS** is a bona fide record of research work carried out by **Mr. Rahul Jose** under our supervision and guidance at KSCSTE - Jawaharlal Nehru Tropical Botanic Garden and Research Institute. This submission is in partial fulfilment of the requirements for the degree of Doctor of Philosophy of the University of Calicut. This work has not been submitted to anywhere else for any other degree, diploma, associateship, or other similar titles to any candidate of any other University.

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DECLARATION

I hereby declare that the work presented in the thesis entitled A STUDY ON ECOLOGY, CONSERVATION AND UTILIZATION OF *DIOSPYROS CRUMENATA* THWAITES IN KERALA - AN ENDANGERED TREE OF THE WESTERN GHATS is based on the original work done by me under the guidance of Dr. Anilkumar C. (Retd.) and Dr. Jothish P. S., Senior Principal Scientists of KSCSTE-Jawaharlal Nehru Tropical Botanic Garden and Research Institute Palode, Thiruvananthapuram and has not been included in any other thesis submitted previously for the award of any degree. The contents of the thesis are undergone plagiarism check using the iThenticate software at C.H.M.K. Library, University of Calicut, and the similarity index found within the permissible limit. I also declare that the thesis is free from AI generated contents.

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CONTENTS

LIST OF TABLES	**vii-viii**
LIST OF MAP & FIGURES	**ix-x**
LIST OF PLATES	**xi**
ABSTRACT (English)	**xii**
ABSTRACT (Malayalam)	**xiii**
Chapter 1 **INTRODUCTION**	**1 – 8**
Chapter 2 **REVIEW OF LITERATURE**	**9–26**
Chapter 3 **MATERIAL AND METHODS**	**27–42**
Chapter 4 **RESULTS**	**43 – 110**
Chapter 5 **DISCUSSION**	**111– 126**
SUMMARY AND CONCLUSIONS	**127–131**
RECOMMENDATIONS	**132**
REFERENCES	**133 – 171**
PUBLICATIONS AND CERTIFICATES	

LIST OF TABLES

SL. No.	Title	Page No.
1	Table 1. *Diospyros crumenata*: Location details across population sites	43
2.	Table 2. *Diospyros crumenata*: Population structure within the sampled and non-sampled area at Vazhachal/ Karanthode	47-49
3	Table 3. *Diospyros crumenata*: Population diversity at Vazhachal/ Karanthode	53-56
4.	Table 4. *Diospyros crumenata*: Population structure within the sampled and non-sampled area at Chakkapara	57
5.	Table 5. *Diospyros crumenata*: Population diversity at Chakkapara	61-62
6.	Table 6. *Diospyros crumenata*: Population structure within the sampled and non-sampled area at Vellanimala	63-64
7.	Table 7. *Diospyros crumenata*: Population diversity at Vellanimala	69-70
8.	Table 8. *Diospyros crumenata*: Vegetative propagation through stem rooting	87
9.	Table 9. *Diospyros crumenata*: Vegetative propagation through air-layering	88
10.	Table 10. *Diospyros crumenata*: Seed pre-treatments vs. germination	90
11.	Table 11. *Diospyros crumenata*: Comparison of seed germination parameters in various seed pre-treatment	91
12.	Table 12. *Diospyros crumenata:* Effect of storage conditions on the seed germination	93
13.	Table 13. *Diospyros crumenata:* Chemical composition of fruit - Comparative analysis with cultivated fruits	96
14.	Table 14. *Diospyros crumenata*: Mineral composition of seeds	99
15.	Table 15. *Diospyros crumenata*: Chemical composition of seeds (Proximate)	100
16.	Table 16. *Diospyros crumenata*: Phytochemical composition of seeds	100

17.	Table 17. *Diospyros crumenata:* Biological activities of the top five phytocomponents identified in the chloroform extract of seeds	102
18.	Table 18. *Diospyros crumenata*: Biological activities of whole phytocomponents identified in the chloroform extract of seeds.	104
19.	Table 19. *Diospyros crumenata*: Estimates of relative contributions and permutation importance of the predictor environmental variables to the MaxEnt model	106

LIST OF MAP & FIGURES

SL. No.	Title	Page No
1.	Figures 1 &2. Male tree (inset) Inflorescence; Figure 2. Female tree (inset) fruiting primodium	28
2.	Map 1. *Diospyros crumenata*: Distribution in Kerala showing population sites	29
3	Figure 3. *Diospyros crumenata*: Estimation of the extent of Occurrence and Area of Occupancy using the GeoCat app.	45
4.	Figure 4. *Diospyros crumenata:* Total individuals along with the Girth class from the four study sites	46
5.	Figure 5. *Diospyros crumenata:* Total individuals along with the Girth class at Vazhachal/ Karanthode	47
6	Figure 6. *Diospyros crumenata*: Vegetation profile (Vertical) at Vazhachal forest (Karanthode)	51
7.	Figure 7. *Diospyros crumenata*: Vegetation profile (Horizontal) at Vazhachal forest (Karanthode)	52
8.	Figure 8. *Diospyros crumenata*: Age-wise distribution profile at Vazhachal forest (Karanthode)	52
9.	Figures 9 &10. *Diospyros crumenata*: Crown projections at Vazhachal forest (Karanthode) **(**Fig.9. Vertical plane; Fig.10. Horizontal plane)	53
10.	Figure 11. Total individuals along with the Girth class at Chakkapara	57
11.	Figure 12. *Diospyros crumenata*: Vegetation profile (Vertical) at Chakkapara forest	59
12.	Figure 13. *Diospyros crumenata*: Vegetation profile (Horizontal) at Chakkapara forest	60
13.	Figure 14. *Diospyros crumenata*: Age-wise distribution profile at Chakkapara forest	60
14.	Figures15&16. *Diospyros crumenata*: Crown projections at Chakkapara forest (Fig.15. Vertical plane; Fig.16. Horizontal plane)	61

15	Figure 17. *Diospyros crumenata*: Total individuals along with the Girth class at Vellanimala	63
16.	Figure18. *Diospyros crumenata*: Vegetation profile (Vertical) at Vellanimala	67
17.	Figure19. *Diospyros crumenata*: Vegetation profile (Horizontal) at Vellanimala	67
18.	Figure 20 *Diospyros crumenata*: Age-wise distribution profile at Vellanimala	68
19.	Figures 21 & 22. *Diospyros crumenata*: Crown projections at Vellanimala (Figure21. Vertical plane; Figure 22. Horizontal plane)	68
20.	Fig. 23. Total individuals along with the Girth class at Chimmony WLS	71
21.	Figure 24. *Diospyros crumenata*: Phenology calendar	79
22.	Figure 25. *Diospyros crumenata*: Chemical composition of fruit: comparative analysis with cultivated fruits generated by ggplot	97
23.	Figure 26. *Diospyros crumenata*: GC-MS spectrum of the chloroform extract of seeds	103
24.	Figure 27. *Diospyros crumenata*: Habitat model	107
25.	Figure 28. *Diospyros crumenata*: Predicted distribution, emphasising favourable zones in both southern and northern parts of Kerala	108

LIST OF PLATES

SL. No.	Title	Page No.
1.	Plate 1. *Diospyros crumenata* : Population structure	72
2	Plate 2. *Diospyros crumenata*: Vegetative Dynamics	75
3.	Plate 3. *Diospyros crumenata*: Reproductive Dynamics (Male tree)	76
4	Plate 4. *Diospyros crumenata*: Reproductive Dynamics (Female tree)	80
5.	Plate 5. *Diospyros crumenata*: Fruit Abscission and Predation	81
6.	Plate 6. *Diospyros crumenata*: Lichen associations	82
7.	Plate 7. *Diospyros crumenata*: Reproductive constraints	84
8.	Plate 8. *Diospyros crumenata*: Reproductive constraints	85
9.	Plate 9. *Diospyros crumenata*: Vegetative propagation	89
10.	Plate 10. *Diospyros crumenata*: Seed propagation	94
11.	Plate 11. *Diospyros crumenata*: View of fruit and pulp	98
12.	Plate 12. *Diospyros crumenata*: Seed pathogenic fungi	110

Abstract

Keywords: *Diospyros crumenata***,** Threatened tree, Western Ghats, Kerala, Ecology, conservation, utilization.

An extensive field survey of *Diospyros crumenata*, a threatened tree distributed in the Western Ghats of Kerala, identified major populations in the Vazhachal, Peechi-Pattikkad forests and isolated individuals in the Chimmony WLS in Thrissur District. Based on population size, the species was assessed as Critically Endangered. The low percentage of pre-reproductive trees, as assessed by an inverted J-shaped curve, lower values of the Importance Value Index (IVI), and poor seedling counts indicate a declining trend in the population. The species primarily inhabits wet soils and watercourse areas, serving as microhabitats. The species' habitats are under threat due to power projects, road expansion, and the risk of forest fires. The dioecious trees showed irregularities in flowering, abnormal abscission of flowers and immature fruits, assumed to be ineffective pollination due to insufficient insect pollinators in a short period of blooming. The sweet, aromatic, fleshy fruit pulp was consumed along with the seeds by mammals, resulting in an estimated 70% seed loss, which negatively impacted the soil seed bank and seedling regeneration. The recalcitrant seeds on scarification followed by soaking in tap water for 24 hours improved germination to 100% within 15 days compared to 66% germination within 24 days of sowing in control sets. The seeds stored in closed polycarbonate containers in the controlled conditions of the seed bank, 20± 2°C; 40% RH, showed extended viability for up to 10 months. The application of auxin, IBA at 3000 ppm, resulted in stem rooting of 70% within 60-70 days under controlled nursery conditions, compared to 20% in the control set. Air -layering success of 100% was achieved with NAA at 5000 ppm, while the control set did not induce rooting. Acute oral toxicity tests of the ripe fruit pulp on adult Wistar rats revealed no toxicity. The nutritional analysis of the ripe fruits indicated that they are more nutrient-dense than commonly cultivated fruits. The evaluation of the seeds revealed significant mineral content, chemical content, antioxidant activity, and the presence of promising bioactive compounds. The habitat distribution model, developed using MaxEnt software, identified potential distribution areas in the Western Ghats of Kerala, pinpointing suitable niche models for species reintroduction. Seed and seedling infestations caused by fungal pathogens were identified at the molecular level, enabling effective disease management.

സംഗ്രഹം

കേരള പശ്ചിമഘട്ടത്തിൽ നടത്തിയ സർവ്വേ പ്രകാരം ഡയോസ്പൈറോസ് ക്രൂമനേറ്റ എന്ന അപൂർവ വൃക്ഷത്തിന്റെ പ്രധാന സമൂഹങ്ങൾ തൃശൂർ ജില്ലയിലെ വാഴച്ചാൽ, പീച്ചി - പട്ടിക്കാട് പ്രദേശത്തും ഒറ്റപ്പെട്ട വൃക്ഷങ്ങൾ ചിമ്മിണി വന്യജീവിസങ്കേതത്തിലും ഉണ്ടെന്നു കണ്ടെത്തി. വൃക്ഷങ്ങളുടെ എണ്ണത്തിന്റെ അടിസ്ഥാനത്തിൽ ക്രിട്ടിക്കലി എൻഡേൻജേഡ് സംരക്ഷണ വിഭാഗത്തിൽ ഉൾപെടുത്താവുന്നതാണെന്നു വിലയിരുത്തി. ഇൻവെർട്ടഡ് 'ജെ' ആകൃതിയിലുള്ള മാതൃക അവലംബിച്ചു അതാത് സമൂഹങ്ങളിലെ പ്രത്യുല്പാദനത്തിനു മുൻപുള്ള വൃക്ഷങ്ങളുടെ കുറഞ്ഞ ശതമാനവും, ഐവിഐ വിശകലനത്തിലുടെ സഹവൃക്ഷങ്ങളിൽ നിന്നും വൃക്ഷത്തിന്റെ ആനുപാതികമായ കുറഞ്ഞ സംഖ്യയും, വളരെ കുറഞ്ഞ തൈകളുടെ എണ്ണവും, വൃക്ഷ സമൂഹത്തിന്റെ വളർച്ച കുറയുന്ന പ്രവണതയാണെന്നു വിലയിരുത്തി. ഈർപ്പമുള്ള മണ്ണും ജലസാമിപ്യ സ്ഥലങ്ങളും വൃക്ഷത്തിന്റെ സൂക്ഷ്മ ആവാസമേഖല വഹിക്കുന്നതാണെന്ന് മനസ്സിലാക്കി. വൃക്ഷത്തിന്റെ ആവാസമേഖലകൾ ജലവൈദുതി പദ്ധതികളുടെ നിർമാണം, റോഡ് വികസനം, കാട്ടുതീ എന്നീ ഭീഷണികൾ നേരിടുന്നവയാണ്.

ഡയേഷിസ് വിഭാഗത്തിൽ ഉൾപ്പെടുന്ന വൃക്ഷത്തിന്റെ പെൺവൃക്ഷങ്ങളിൽ പൂവിടൽ ക്രമരഹിതമായ വർഷങ്ങളിലാണ്. അസാധാരണമായി പൂവുകളും ഇളം ഫലങ്ങളും പൊഴിഞ്ഞുകാണുന്നതു കുറഞ്ഞ ദൈർഘ്യമുള്ള പൂക്കാലവും പ്രാണികളുടെ കുറവും മൂലം ഉണ്ടാകുന്ന ഫലപ്രദമല്ലാത്ത പരാഗണത്താലെന്നു അനുമാനിക്കുന്നു. മധുരവും, മണവുമുള്ള മാംസളമായ പഴുത്ത പഴങ്ങൾ വിവിധങ്ങളായ സസ്തനികൾ കഴിക്കുന്നതിനാൽ 70% വിത്തുകളുടെ നഷ്ടം കണക്കാക്കുകയും തന്മൂലം മണ്ണിലെ ജീവനക്ഷമതയുള്ള വിത്തുകളുടേയും തൈകളുടെയും പുനരുൽഭവവും പ്രതികൂലമായി ബാധിക്കുന്നു.

റിക്കൽസിട്രന്റ് വിഭാഗത്തിൽപെടുന്ന വിത്തുകൾ സാധാരണയായി 24 ദിവസത്തിനുള്ളിൽ 66 ശതമാനവും, വിത്തുകൾ ഉരച്ചു ടാപ്പ് വെള്ളത്തിൽ 24 മണിക്കൂർ കുതിർത്തു പാകുമ്പോൾ 15 ദിവസത്തിനകം 100 ശതമാനവും മുളച്ചുകാണപെടുന്നു. വിത്തുകൾ പോളികാർബണേറ്റ് ബോട്ടിലിൽ 20± 2°C; 40% RH, നിയന്ത്രിത കാലാവസ്ഥയിൽ, സീഡ്ബാങ്കിൽ സൂക്ഷിക്കുമ്പോൾ 10 മാസം ജീവനക്ഷമത ദീർഘിപ്പിക്കാവുന്നതാണെന്ന് കണ്ടെത്തി. IBA 3000 ppm സഹായത്താൽ കമ്പുകൾ 60-70 ദിവസത്തിൽ നിയന്ത്രിത നഴ്സറി കാലാവസ്ഥയിൽ സാധരണയായുള്ള 20 ശതമാനത്തിൽ നിന്നും 70 ശതമാനവും, എയർ ലെയറിങ്ങിലൂടെ NAA 5000 ppm സഹായത്താൽ 100% സസ്യവർദ്ധനവ് നടത്താവുന്നതാണെന്നു കണ്ടെത്തി. ഹോർമോണിന്റെ അഭാവത്തിൽ വേരുകൾ ഉണ്ടാകാറില്ല.

വെള്ളെല്ലികളിൽ നടത്തിയ പരീക്ഷണങ്ങളിലൂടെ പാകമായ ഫലങ്ങൾ വിഷരഹിതമാണെന്നു കണ്ടെത്തി. ഫലങ്ങളുടെ പോഷകമൂല്യ നിർണയത്തിലുടെ സാധരണയായി കൃഷി ചെയ്യുന്ന ഫലവൃക്ഷങ്ങളേക്കാൾ ഉയർന്ന മൂല്യം കണ്ടെത്തി. വിത്തുകൾ മൂല്യനിർണ്ണയം നടത്തിയതിലൂടെ വിവിധ മിനറലുകൾ, രാസപദാർത്ഥങ്ങൾ, ആന്റിഒക്സിഡന്റുകൾ, ഭാവിസാധ്യതയുള്ള രാസസംയുക്തങ്ങൾ അടങ്ങിയിട്ടുള്ളതാണെന്ന് കണ്ടെത്തി.

മാക്സ്ന്റെ സോഫ്റ്റ്വെയർ ഉപയോഗിച്ച് കേരള വനപ്രദേശത്തു വൃക്ഷത്തിന്റെ അനുയോജ്യമായ ആവാസ പ്രദേശങ്ങൾ കണ്ടെത്തി. ഭാവിയിൽ വൃക്ഷസമൂഹങ്ങൾ വെച്ച് പിടിപ്പിക്കുന്നതിനു ഇത് ഏറെ സഹായകരമാണ്. വിത്തുകൾ മുളക്കുമ്പോൾ തൈകളെ സാരമായി ബാധിച്ചു വരുന്ന രോഗഹേതുവായ ഫങ്കസുകളെ മോളിക്യൂലർ പഠനത്തിന്റെ അടിസ്ഥാനത്തിൽ തിരിച്ചറിയുവാനും അതുവഴി രോഗപരിപാലനവും സാധ്യമാക്കി.

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CHAPTER 1

INTRODUCTION

Globally, the Western Ghats of the Peninsular Indian region is one among the 36 biodiversity hotspots (Subashree et al., 2021; Balasubramanian & Sangha, 2023). The Ghats traverse Tamil Nadu, Kerala, Karnataka, Goa, Maharashtra and Gujarat. These mountain forests cover an area between 1,40,000 and 1,60,000 km² in a long stretch of 1,600 km. The Ghat region is broken by a 30 km length and known as Palghat Gap at around 10° 45' North latitude. A significant feature of the Western Ghats is the immensely high level of species diversity and distribution of endemic flora. It is home to a large number of unique and endangered plant species. But at the same time, it is prone to various developmental activities and considered one of the threatened landscapes (Hrdina & Romport, 2017). Encompassing just 5% of India's land area, this region is home to 30% of all flora and fauna (Deepu et al., 2023). Among the 7,402 flowering species documented, an impressive 5,558 are indigenous, of which 2,253 species are found only in India, and 1,273 are unique to the Western Ghats (Nayar et al., 2008; 2014). However, this ecological treasure trove is under siege, largely from anthropogenic sources. The extinction rate is alarmingly high and estimated to be up to 10,000 times higher than that which naturally occurs. If this trend continues, 30–50% of the species will be lost by the year 2050 (Myers, 1980).

The man-made perturbations on natural ecosystems caused fragmentation of the original habitat into isolated patches, subsequently accelerating species extinction (Rands et al., 2010; Haddad et al., 2015; Edwards et al., 2019). Most parts of the Western Ghats come under this category. In such a fragmented, smaller ecosystem, there are more species than it can hold at equilibrium. The rate of species depletion is

faster in such smaller groups than in a bigger habitat, as ecological niches available for survival will be proportionally reduced (Liu et al., 2019). Most of the endemic species with small distribution ranges will move to a rare species and later become threatened species if adequate protection is not given to their habitats. In response to this, the Western Ghats Ecology Expert Panel (2011) assessed the Western Ghats region as an Ecologically Sensitive Area (ESA) and categorised it into three levels of ecological sensitivity regions.

The southern part of the Western Ghats is exceptionally rich in terms of floral diversity and the distribution of endemic taxa. The endemism in this area extends Kali Nadi of Karnataka to the Agasthyamalai in Kerala and Tamil Nadu. This region hosts approximately 1,286 endemic species (Nayar, 1996). This richness is directly influenced by unique climatic, edaphic, and other geographic conditions that support abundant plant diversity and endemism. Considering the rare plants' distribution, the southern states of the Ghats are home to 760 flowering species, including broadly under the threatened categories (Jose et al., 2014).

In Kerala, the Western Ghats cover approximately 11,522 km², and about 30% of the state's total land area is occupied by forests (India's State of Forest Report, 2023). Kerala represents a major species-rich portion of the Southern Western Ghats, with a total of 5,699 angiosperms, which contains 1,905 endemic species, of which 706 species are listed as threatened (Sasidharan, 2025). Tree species play a significant role in Kerala's flora.

Ecology

The ecology of threatened plants involves studying their distribution range, number and size of populations, relative abundance among associates, and natural regeneration, as well as the factors that affect their survival and the risk of extinction.

Conservation measures aim to counter these threats by evaluating species status, mapping their distribution, safeguarding habitats through *in-situ* approaches, and employing *ex-situ* methods such as clonal repositories and seed banks to ensure their long-term preservation.

The decline in biodiversity primarily results from human activities, with pressure to exploit medicinal and economically important endemic species. Endemics are, in a way, rare groups, and once lost, they cannot be replaced. Most endemic species with small distribution ranges eventually become rare species, and later, if adequate protective measures are not provided, they may become threatened species. Consequently, conserving rare and endemic species is crucial for ecosystem health. Species with small populations are more susceptible to extinction, experiencing genetic drift and inbreeding depression.

The main causes of plant rarity are coupled with ecological and biological factors, which ultimately lead to the untimely endangerment of the species (Reveal, 1981). This includes habitat loss or modifications, unscientific harvesting of genetic resources, spread of exotic plants, pests and diseases, ineffective pollination, poor fruit set, natural regeneration, etc. (Daniel & Jayanthi,1996; Jose, 2001; Pandurangan, 2003; Swarupanandan et al., 2013). According to Jose et al. (2014), among the 760 threatened flowering plants recorded from the southern Ghats, around 500 species are have not attended any kind of conservation programmes. Many other study reports across the country are suggesting the imminent need for the conservation of threatened plants before their untimely endangerment (Ramesh et al., 2003; Nayar et al., 2008; Sasidharan, 2003, 2011, 2017; Yasodharan & Jose, 2011). Recent studies on *Hopea erosa* (Praveena et al., 2024), *Litsea deccanensis* (Sreeraj et al., 2025), *Atuna travancorica* (Praveena et al., 2025), and *Coscinium fenestratum* (Sumod et al., 2025)

demonstrate the successful recovery of endemic and threatened plants in the Western Ghats of Kerala. Therefore, the sustainability of natural resources for their consistent utilisation is essential for the well-being and sustenance of man. Understanding the causes of rarity requires in-depth information on the species' natural dynamics and ecological interactions. Hence, the conservation of threatened plant species becomes an unavoidable process in maintaining the forest ecosystems and their functions.

Since the process of untimely endangerment causes concern about the ecological and biological aspects of species, it has diverse ecological consequences. The data on age classes of the populations, including seedling/ saplings, natural regeneration performance, associated species, spatial and stratic distribution, abundance in terms of the relative dominance of each species, etc., are integral ecological elements affecting population growth and the rarity of species *in situ* (Misra, 1968; Pandurangan, 2003). The monitoring of dynamic changes or phenophases helps in understanding the breeding behaviour and recruitment of the species. Pollination, fertility, fruit/seed dispersal, and natural regeneration performance are important aspects regarding the functional dynamics of species. The biotic associations of species are influencing factors in the behaviour of species. The negative influence of biotic interactions includes leaf, fruit and seed infestations, cause threats to species distribution and survival (Bawa, 1983; Jose, 2001). Therefore, knowledge of the population structure, population dynamics, associated species in the respective ecosystems, their dominance, spatial and vertical distribution, and size of the population is inevitable to understand the factors responsible for the rarity of a species (Pascal, 1988; Parthasarathi & Sethi, 1997). Similarly, knowledge about the biology of a threatened species is crucial for understanding the factors leading to the endangerment of the species. The time of anthesis, insect associations, pollen-ovule ratio, and identifying biotic interaction in the

natural dynamics of the species, such as insect-pest, birds and mammals, is of great significance for assessing the population trends in the ecological studies of the threatened species (Bawa, 1983; Pandurangan, 2003; Ravikanth et al., 2018). Hence, adopting the species recovery studies of each threatened species is the need of the hour to conserve, manage and utilise the fast-depleting plant resources of the Western Ghats.

Conservation

The conservation status assessment, as per IUCN norms, is a primary task before initiating conservation studies of threatened species. The population survey, identification of the number of populations, area of occurrence and occupancy of the species, number of mature trees and habitat threats are prerequisites for species assessments (IUCN, 2020). The data is essential for the downlisting of the conservation status of a threatened species.

Developing appropriate conservation strategies through vegetative and seed propagation methods and seed storage practices after identifying causes of rarity enables the large-scale multiplication and extended shelf life for the species. Macro propagation through rooting of stem cuttings and air-layering by the application of auxins at different concentrations has led to the multiplication of many endemic and threatened trees of the Western Ghats (Jose et al., 1995). Most wild edible fruit trees lack a definite protocol for their propagation and subsequent multiplication (Shareef, 2014; Anita, 2016; Rasmi & Jose, 2023a).

Standard methods for the optimum period for fruit collection, seed processing, moisture content analysis, viability period, seed germination and dormancy, etc., were described for many fruit trees (Anilkumar et al., 1996; ISTA,1976,1999; Pandurangan, 2003; Anandalakshmi et al., 2005; Jaryal et al., 2025). These studies enabled the

development of a comprehensive conservation protocol for the species both *in- situ* and *ex-situ.*

Utilization

Evaluation regarding the properties of the threatened plants is not much explored, whereas many of the species are underutilised. Among these, the nutritional value of edible fruits of threatened species can be a key step towards conservation. These fruits often possess superior nutritional content compared to cultivated varieties, making them a valuable resource (Valvi & Rathod, 2011). Studies on underutilised wild fruits are urgently needed, not only for their potential to enrich the local diet but also for their potential in conservation efforts (Nazarudeen, 2010; Biswas et al., 2022; Dharmappa, 2023). Since the plant resources are limited and shrinking globally due to the increase in human population, studies on underutilised fruits from the wild are an urgent necessity, which will enrich the fruit diversity of the State and also help in the popularization of these fruits for the benefit of society.

Therefore, a comprehensive study approach is necessary for the conservation, management and sustainable use of the threatened plants. Thus, the present study aims to understand ecology and biology, leading to the identification of the factors responsible for the rarity of the species. Further, developing suitable conservation strategies could be utilised for the successful multiplication and availability of species as and when required. Evaluation of fruit nutritional qualities of target species initiates intensive conservation measures and novel utilisation strategies for the species. The whole study, including identifying suitable areas for population replenishment through niche modeling, ensures a comprehensive conservation protocol of the species *in situ* and *ex-situ*.

***Diospyros crumenata* Thwaites (Ebenaceae)**

The members of the plant family Ebenaceae are a significant source of economically important products, with *Diospyros* as its major genus. These dioecious and polymorphic trees exhibit high morphological diversity and yield edible fruits (persimmons), timber (ebony) and medicinal resources (Singh, 2005). Leaves of the Coromandel ebony (*D. melanoxylon*) are used to roll South Asian beedi cigarettes. Many species are cultivated as ornamentals, such as *Diospyros buxifolia* (Blume) Hiern; *Diospyros discolor* Willd., and endemics such as *Diospyros foliosa* Wall. ex A. DC. *Diospyros ghatensis* Ramesh & Franceschi, *Diospyros paniculata* Dalz. etc., holds ecological value, while traditional medicine attributes them with uses against diarrhoea, hypercholesterolaemia, cognitive decline, and inflammatory disorders. Further demonstrate anti-inflammatory, antioxidant, antidiabetic, anti-atherosclerotic, hypocholesterolemic, and anticancer properties (Park et al., 2006). Phytochemically, *Diospyros* species are rich in naphthoquinones and naphthol, which serve as reliable taxonomic markers.

Globally, the genus comprises 607 species, distributed across Asia and the Pacific (300 spp.), Madagascar and the Comoro Islands (98 spp.), mainland Africa (94 spp.), the Americas (100 spp.), and Australia (15 spp.) In India, 66 taxa are reported, of which nearly 50% originated locally, and ~45% are endemic (Singh, 2005; Shashwathi & Krishnamurthy, 2024). The Western Ghats of Kerala alone support about 30 species (Sasidharan, 2011).

Diospyros crumenata Thwaites, an endangered tree species of the Ebenaceae family, is restricted to a few populations in the Western Ghats of Kerala. Traditionally, indigenous communities have consumed the fruit pulp during the fruiting season. Ecologically, the species plays an important role in semi-evergreen to evergreen forest

ecosystems at low to medium elevations, where its fruits serve as a food source for a wide range of mammals. However, a comprehensive strategy for its conservation, management, and sustainable use is still lacking. Thus, the present study aims to understand the population ecology and biology with reference to the rarity of the species in the Kerala part of the Western Ghats and to develop conservation protocols of *D. crumenata* with the following objectives. The findings of this study can provide valuable insights to support the long-term recovery and resource-based sustainable utilization of this threatened species.

With this backdrop, the objectives set for the study are:

1. Population survey and mapping of target species as per IUCN guidelines in the Kerala part of Western Ghats.
2. Analysis of population structure, diversity and dynamics with reference to the rarity of species *in situ.*
3. Development of vegetative and seed propagation protocol for multiplication and germplasm storage of species.
4. Evaluation of the nutritional composition of ripened fruits as an additional fruit tree resource for its popularization.
5. To develop a comprehensive conservation protocol, both *in situ* and *ex-situ,* of the candidate species.

CHAPTER 2

REVIEW OF LITERATURE

The Western Ghats, a hill range running parallel to India's western coast, are renowned for its exceptional biodiversity and high levels of endemism. Recognized globally, it is one of the world's eight "hottest hotspots" of biological diversity and one of the 36 biodiversity hotspots (Myers et al., 2000; Aravind et al., 2007; Merritt et al., 2019). This ecologically significant region has earned international acclaim, including designation as a UNESCO World Heritage Site and recognition as a Global Biodiversity Hotspot. Overall, the flora of this region accounts for approximately 25% of India's total plant species, highlighting its remarkable endemism and ecological value (Revathy& Sundarapandian, 2025; Chitale et al., 2014).

However, like many biodiversity hotspots, the Western Ghats face substantial ecological challenges, resulting in the rarity and endangerment of many plant species (Nayar, 1997; Nair et al., 2014; Jose, 2001; BSI, 2008-2020). Threats such as habitat loss, fragmentation, deforestation, the encroachment of invasive species, agricultural expansion, mining, and resource overexploitation are predominantly induced by human activities (Myers, 1980, 1988, 1996; Ehrich & Ehrich, 1992). The widespread destruction of tropical forests is considered a global ecological crisis, with far-reaching consequences for climate change, leading to floods, droughts, the loss of valuable species, and the degradation of their natural habitats (Raven, 1977; Soule, 1991; Groombridge, 1992; Primack, 1994).

The southern states of the Western Ghats, which cover Karnataka, Kerala, and the southern part of Tamil Nadu, are rich in species abundance and endemism. Most

endemic plants fall under various threat categories (Ramesh, 1997; Jose et al., 2014). The Southern part of the Western Ghats has large heterogeneity in the environmental conditions, resulting from geographical and demographic differences, which favoured the species diversity and endemism largely (Sasidharan, 2003; Pechimuthu & Arumugam, 2020; Gopal et al., 2023). The most striking characteristic of the southern Western Ghats is the presence of tropical rainforests along the windward slopes, notable for their exceptional biodiversity. Across the entire Western Ghats, the variation in endemism is primarily influenced by two factors: the rising number of dry months from south to north and the decline in temperature with increasing altitude (Pascal, 1988).

Kerala, located along India's western coastline and bordered by the Lakshadweep Sea, host a rich array of ecological habitats that support a wide diversity of species, including many that are endemic and threatened. Forests cover roughly 30% of the state's total area (Kerala Forest Statistics 2022-23). Kerala is home to over 5,699 species of flowering plants, accounting for nearly one-fourth of India's floral diversity. Of these, around 4,509 species are native to the region, including 1,905 species that are endemic to the Western Ghats (Sasidharan, 2025). Notably, about 16% of these native plant species are listed in the IUCN Red List, indicating their threatened status (Sasidharan, 2017, 2025). Three major centers of plant endemism in Kerala have been identified: Agasthyamala, the Anamalai-High Ranges, and the Silent Valley–Wayanad regions (Sasidharan, 2003). The state's flowering plant species belong to 1,315 genera across 228 families (Sasidharan, 2011, 2025). Among the 812 trees recorded in Kerala, 327 species are endemic to peninsular India (Sasidharan, 2003). According to a recent study by Jose et al. (2020), 53 woody species were found exclusively within the state. An updated enumeration enlists 571 taxa exclusively endemic to Kerala state (Mohanan, 2025).

The threatened plant species of South India have been documented by several researchers, including Joseph (1977), Henry et al. (1979), Jain & Sastry (1984), and Ahmedullah & Nayar (1987), with further extensive work by Nayar and Sastry (1987, 1988, 1990). Nayar (1997, 1997a) listed 1,272 plant species endemic to Kerala, of which 483 were categorised as threatened. Recent botanical studies have led to the identification of many new species (Mohanan, 1995; Sasidharan, 1997, 1998, 2002; Robi et al., 2014; Maya et al., 2021; Page, 2023).

According to the IUCN (2025) assessment of 551 *Diospyros* species, 7.4% are classified as Critically Endangered, 19.2% as Endangered, 17.6% as Vulnerable, 7.3% as Near Threatened, 44.5% as Least Concern/Lower Risk, and 3.8% as Data Deficient. Although several Red-listed tree species have been rediscovered in recent times, many of them are represented by only a few surviving individuals and show very poor regeneration (Sasidharan, 1998). This situation highlights the urgent need for conservation efforts, either through assisted natural regeneration or artificial propagation, to enhance the survival and population of these species.

Ecological studies

Bramwell (1991) pointed out that in ecological studies of plant communities, higher plants, especially trees, are prioritised as it is considered one of the most threatened members of a complex ecosystem. With the enforced limitations on population size, caused by habitat destruction, the chances of gene exchanges in woody endemics are also very much limited, which may ultimately lead to the collapse of the breeding system, paving the way for extinction. Unfortunately, once endemics are lost, the loss is forever, and genetic resources associated with the species will also be wiped out (Nayar, 1996).

Population survey and mapping

The IUCN criteria (2021) are used to assess the conservation status of species by estimating two key measures: the extent of occurrence, which refers to the minimal continuous imaginary boundary encompassing the overall distribution of species, and the area occupied, which indicates specific areas within that boundary where the species exists and can sustain its survival.

Evaluating the conservation status according to IUCN standards is a crucial and initial step in undertaking conservation efforts for endangered species. To conduct a comprehensive species conservation assessment, it is imperative to conduct a population survey, determine the number of populations, assess the area of occurrence, evaluate the area occupied, estimate the number of mature trees, and identify potential habitat threats, among other factors. Various individuals and organisations have published assessments on the conservation status of threatened plants. These assessments are based on the study of herbarium specimens, regional camp workshops, symposia, and seminars. Notable publications include those by Nayar and Sastry (1987, 1988, 1990), Ravikumar and Ved (2000), Molur and Walker (1996, 1997), Nair et al. (2008), and Sasidharan (2017, 2025). Conducting or reevaluating assessments of threatened plants in line with IUCN guidelines is essential. Many studies reported on the distribution and population studies of threatened trees of Western Ghats (Chandran et al., 2008; Ramachandran & Swarupanandan, 2013; Balan et al., 2019; Sreekumar et al., 2020; Rahul et al., 2020).

Population structure and Diversity

The population structural analysis helps to understand the population behaviour of the species and to predict endangerment or the various events of the extinction process. The population structure encompasses various lifeforms of the species, which

include the number of adults (GBH ≥ 30 cm), saplings (GBH 10–30 cm) and seedlings (GBH < 10 cm) (Richards, 1952; Halle et al., 1978; Pascal, 1988; Jose, 2001). Determining the effective or viable population size is pivotal for species conservation and management, as populations that diminish in size become more susceptible to endangerment. The vertical and spatial arrangement of individuals influences detection patterns and the distribution of the species in terms of height strata and ground-level distribution. Classification based on height distinguishes species into distinct layers, such as the first layer (26 to 35 m), the second layer (16 to 25 m), the third layer (5 to 15 m), and everything below includes shrubs and herbs. Tree height, girth measurements, and age structure serve as indicators for assessing the reproductive potential of the species (Prasad et al., 2008; Irwine et al., 2013; Somanathan & Borges, 2000; Jayakumar & Nair, 2013).

The Importance Value Index (IVI) denotes a species' relative significance within its ecosystem. All species with a GBH above 30 cm will be quantified and measured. The IVI is computed by the sum of relative frequency (rf), relative density (rd), and relative dominance (rD) of species using the INVENT-NTFP software (Sivaram et al., 2006). The age structure was categorised based on the flowering phase of the trees, with trees displaying flowers classified as 'reproductive' (set of present); yet to flower as 'pre-reproductive' (set of future) and old aged, non- productive trees as set of past (Richards, 1940, 1952; Odum, 1959; Ramakrishnan, 1960; Misra, 1968; Harper, 1977; Pascal, 1988; Parthasarathy & Sethi,1997).

Population Dynamics

Population dynamics of a species cover both vegetative and reproductive dynamics, which include the phenological phases of leaf, flower, fruit development, dispersal, natural regeneration and biotic association of the species. The knowledge of

the phenology and lifecycle of a species is critical for identifying the constraints responsible for the rarity of species. Information on fruit senescence, dispersal and natural regeneration would be facilitated for resource-based utilisation of the species and to develop the management of the populations *in situ* (Janzen,1969; Daubenmire,1972; Harper,1977; Pijl, 1978; Howe,1984; Parthasarathy et al., 1988; Armstrong & Irvine, 1989; Swarupanandan & Sasidharan, 1992; Murali & Sukumar,1994; Daniel & Jayanthi, 1996; Jose, 2001, Pandurangan, 2003; Ramasubbu & Irudayaraj, 2016; Sivaprasad, 2017; Anto et al., 2018; Nadarajan & Pujari, 2019; Reghunath & Raju, 2020; Jose et al., 2020; Kasi & Ramasubbu, 2021; Subin, 2023).

Causes of plant rarity, endangerment and extinction are due to several anthropogenic and intrinsic factors. Perturbations induced by humans, directly or indirectly, enhance the rate of rarity. Habitat loss, destruction, fragmentation of populations, over collection of species, introduction of exotic species, etc., are usually recognised as the major threats to the viability of endangered species (Lucas & Synge, 1978; IUCN, 1978; Rabinovitz et al., 1986; Raven, 1988; Tilman et al., 1994; Pandurangan, 1995).

In population dynamics, biotic interactions play a crucial role in the functional dynamics of the species. Their influence directly or indirectly affects the population behaviour and ultimately the existence of the species in the ecosystem. Ecologically and economically, insects are very important to plants as they facilitate the cross-pollination of flowers and thus assist in seed setting and growth of valuable fruits. On the other hand, insects injure many plant parts in the form of leaf feeders, seed consumers, sap suckers, etc. Most of the insect families, such as Pyraustidae, Bostrychidae, Chrysomelidae, Curculionidae, Jassidae, etc., are causing damage to tree

opulations in the Western Ghats (Janzen,1971; Mani,1973; Mathew,1982, 1994, 1995; Mathew & Rahmathulla,1995).

Conservation studies

Species conservation attempts to identify species that are on the verge of endangerment or are moving towards that. Conventionally, therefore, the species that are considered for conservation are the ones that belong to the various scheduled categories, such as Rare, Endangered and Threatened (RET). Species-based conservation programmes are acquiring importance in a variety of contexts. The long-standing practice of exploitative forestry is gradually shifting towards environmental forestry. As a result, Working Plans, which once prescribed strategies for resource extraction from forests, are being supplemented by Management Plans that encompass environmental values, including the preservation of rare flora and fauna. Programmes and strategies are to be instituted for the conservation of such species that are on their way to disappearance. Designing effective conservation measures demands precise information on distribution, population size, causes of rarity, reproductive traits, threat factors, methods of propagation and re-introduction possibilities. Jose (2001) carried out recovery studies on threatened tree species such as *Gluta travancorica* and *Ochreinauclea missionis*. Pandurangan (2003) conducted the species recovery of ten endemic and RET medicinal plants of Agasthyamalai, Kulamavu and Wayanad MPCAs, Kerala. Swarupanandan et al. (2013) studied two critically endangered trees, viz. *Humboldtia bourdillonii* and *Dipterocarpus bourdillonii.* Kunhikannan et al. (2004) conducted ecological and recovery studies in a few threatened plants of Silent Valley and Kolli Hills.

In any plant biodiversity conservation programme, endemics, especially woody plants, get more attention when compared to other lifeforms of plants (Bramwell, 1991;

Bachman et al., 2024). This is often because endemic woody plants face a higher risk of extinction due to their limited geographic ranges and specialised habitats. According to Nayar (1980), the woody habit among endemics is considered a relic character or a derived condition, resulting from insular isolation.

Propagation protocols

Conventional propagation methods, such as vegetative and seeds, are regarded as one of the most effective methodologies for the sustainable maintenance and utilisation of plant resources. These methods enable the mass production of plants through seeds and the maintenance of desired traits through vegetative methods. Traditional propagation methods are often more cost-effective compared to advanced biotechnological methods.

Vegetative propagation

Vegetative propagation is regarded as a key technique in modern conservation-oriented programmes, as it allows for the selection of superior "plus trees" to develop true-to-type clones for establishing viable populations or orchards (Jose et al., 1995; Barthwal et al., 2025). This method is particularly valuable because it offers a rapid and cost-effective way to produce genetically uniform, high-quality progenies. Moreover, clonal propagation can help address the limitations associated with seed-based regeneration in many species (Aslam et al., 2007; Singh &Tomar, 2023). Auxins have been found to play a significant role in promoting adventitious root formation in a wide range of tree species (Nanda et al., 1970; Nanda & Kochhar, 1984). The use of rooting hormones (auxins) has also played a crucial role in the initiation, as well as the increased percentage of rooting (Mesen et al., 1997; Palanisamy et al., 1998). Vegetative propagation with the aid of auxin allows the cloning of plants with a higher success rate, ensuring that the exact genetic traits of the parent plant are maintained in the

offspring compared to sexual reproduction for the production/establishment of viable populations or orchards (Shekhawat & Manokari, 2016; Saini & Anmol, 2024).

Auxins are recognised as key regulators of organ development, playing a vital role in promoting cell division, elongation, and differentiation. The type and concentration of auxin are critical in regulating the differentiation of phloem ray parenchyma cells into root primordia (Thimann 1956; Hartmann & Kester, 1983). Exogenous application of auxins also played a significant role in the propagation of conservation-prioritised plants (Waheed et al., 2015; Shahzad et al., 2019; Aghdaei et al., 2019). Auxin is essential for effective plant propagation through stem cuttings, as supported by several studies (Wahab et al., 2001; Sevik & Guney, 2013). Stem rooting and air layering methods are usually employed in vegetative multiplication, particularly of trees (Nanda & Kochhar, 1984; Palanisamy et al., 1998; Swamy et al., 1999).

Vegetative propagation methods also benefit by reducing the time it takes for a tree to reach the flowering and fruiting stage since the ramets carry over the age of the parent trees. This is especially important in fruit orchards where early production is economically advantageous (Leakey, 2017; Saroj & Singh, 2020; Haokip et al., 2023). The auxins, such as Indole Acetic Acid (IAA), Indole Butyric Acid (IBA), and Naphthalene Acetic Acid (NAA) in low to high concentrations, viz.,1000, 3000, 5000 ppm, were used for rooting in both stem cuttings and air-layering methods. Thimann (1956) explained that different plant parts respond differently to auxins. Each organ has its own level of auxin that helps it grow or stops it from growing. A concentration of auxin that promotes shoots to grow might actually inhibit root growth, and the opposite can also happen. Young plants under 3-4 years old are usually subjected to stem rooting and layering experiments. Apical stem cuttings were found to be more responsive to rooting than other types of cuttings (Dick & Leakey, 2006; Kalyoncu et al., 2008;

Edizer & Demirel, 2012; Erol & Ercan, 2023). The rooting ability of *Persea macrantha* stem cuttings was significantly influenced by auxin treatment (Praveena et al., 2023).

Cuttings taken from juvenile plants responded better to rooting compared to those from adult trees. It has been reported that auxin application stimulates cambial activity in juvenile plants more effectively than in older trees, leading to the accumulation of reserve food materials at the nodal region and promoting rooting of vegetative cuttings and layering (Kamlesh et al., 1995; Singh et al., 2004; Praveena et al., 2023). The success of air-layering was also significantly influenced by various auxin treatments. Girdling during air-layering increases the reserve of carbohydrates and auxins, which accumulate in the basal portion of the girdled branches. These accumulated food reserves cause the formation of callus, the subsequent development of adventitious root primordia, and the formation of roots (Hartmann et al., 2011). Seasonal variations in the effect of auxins have been reported by many studies (Nanda, 1970; Roberts & Fuchigami, 1973). South-West monsoon months (June- September) were ideal for vegetative propagation studies, where atmospheric humidity plays a critical role in the success of rooting (Soumya et al., 2012; Soumya et al., 2014; Hrideek et al., 2019). So, in a same plant species, an auxin can encourage root formation during one season, but have no effect in another season, and even arrest rooting in a different season. Coarse river sand was found to be an ideal planting medium for early and maximum rooting (Borpuzari et al., 2023; Oak, 2009). The Mist house conditions, maintained with high humidity and moderate temperature, were promising nursery conditions for the success of vegetative propagation (Philip, 1979; Chauhan & Dua,1982; Nagpal et al., 1982; Surendran & Seethalakshmi, 1987; Gurumurti et al., 1994; Kamila & Panda, 2019; Swamy et al., 2002).

Seed propagation

The seeds represent a basic life form in the plants. They transfer genetic material from one generation to the next. Seed germination and early growth of seedlings are the most important stages in the plant life cycle (Pathak et al., 1980), and this will help to understand ecosystem functions, natural regeneration, succession and strategies for the conservation of tropical forests (Khurana & Singh, 2001). The small population of mature individuals and low seed production are two constraints found in threatened species (Fischer & Matthies, 1998; Luitjen et al., 2000). Information on seed germination, storage, behavioural and *in- situ* requirements of many of the threatened tree species remains unexplored (Iralu et al., 2019). Seed propagation is a common method for plant multiplication, and it involves the collection, processing, germination, storage, etc. This method is widely used in forestry due to its efficiency, cost-effectiveness, and ability to maintain genetic diversity (Pathak et al., 1980; Akeroyd & Jackson,1995; Anilkumar et al., 2000). Seed pre-treatments are usually adopted for many hard-coated seeds for their early germination. The method of scarification has become an efficient way of pre-treatment in the germination process (Leonar,1992; Bockarie, 1993; Sinu & Shivanna, 2016).

Seed moisture content and storage temperature are the major environmental factors for seed viability and longevity (Roberts,1973; King & Roberts, 1979; Tompsett, 1982, 1992; Hong & Ellis,1998; Anilkumar et al., 2000). Recalcitrant seed behaviour is commonly observed among species distributed in evergreen and riparian forests (Lah et al., 2023; Pratibha, 2024). These seeds are suited for rapid germination, as the soil moisture increases when the fruits senesce. Key characteristics of recalcitrant seeds include large size and high moisture content. However, these seeds are facing difficulties in a longer storage period, as they are highly intolerant to desiccation and

low temperatures. Critical moisture content (CMC) for recalcitrant seeds usually ranges from 21 to 31%, depending on the nature of the species (Roberts, 1972; Chin & Roberts, 1980; Pammenter et al., 1991; Baskin & Baskin, 1998). These seeds lose significant moisture during the shedding process and cannot tolerate the desiccation levels needed for extended storage. The CMC is the lowest safe moisture level of recalcitrant seeds and varies from species to species. Such types of CMC have been worked out in *Nephelium lappaceum* (Chin, 1975), in wild nutmegs (Jose & Pillai, 2016), in NTFP plants (Jose et al., 2022), in threatened medicinal plants (Pandurangan, 2003) and in Meliaceae and Araucariaceae (Tompsett, 1994). Optimising seed storage methods is inevitable for raising plants per their availability (Pandurangan, 2003). Seed longevity is influenced by climatic factors, such as storage temperature, relative humidity, seed moisture content, etc. Better storage conditions are essential for raising healthy seedlings (Roberts, 1973; Agarwal, 1980). According to Tiwari and Kuntal (2014), suitable storage materials with optimum environmental conditions can sustain the viability of seeds, resulting in improved germination even after a prolonged period. The successful cultivation of any crop depends on the seed's viability during storage and its ability to germinate. Mozumder et al. (2005) revealed that in bushbean seeds, germination and seedling emergence depend on the temperature and humidity maintained during storage.

Utilization studies

Fruit nutritional analysis

Wild edible fruits have historically been important for meeting human dietary needs. The lesser-known and underutilized varieties of these fruits are especially valuable and should be cultivated and promoted to benefit wider communities. These fruits often have higher concentrations of nutrients and bioactive compounds (Getahun,

1974; Martins et al., 2011). Wild fruits are a rich source of antioxidants, vitamins, and minerals, offering essential nutrients to rural populations and serving as a crucial source of micronutrients. Wild fruit and vegetables also play a positive role in providing a balanced diet (Callistus & Dharini, 2017; Rahul et al., 2024). In many parts of Ethiopia, hundreds of wild edible plants are known to be consumed by rural communities (Getachew et al., 2013). Biswas et al. (2022) investigated the nutrient composition and antioxidant properties of six wild edible fruits from Tripura, a Northeastern state in India. Therefore, understanding the composition of fruits and their nutritional potential is essential for a healthy human diet. Widening the base of agriculture by the promotion of underutilized and wild fruit crops aligns with global goals related to sustainable agriculture, food security, and nutrition, as outlined in initiatives like the Sustainable Development Goals (United Nations- Sustainable Development Goals, No.2, 2015). The effective management of the lesser-known and underutilised wild edible fruit trees is still at a bottleneck due to a lack of nutritional analysis, knowledge of propagation methods, and long periods of seed storage practices. Many studies have been carried out on the documentation and evaluation of wild edible fruits of the Western Ghats. The important species include *Flacourtia montana* Graham (Flacourtiaceae) *Baccaurea courtallensis*, (Wight) Muell. -Arg. (Euphorbiaceae), *Garcinia gummi-gutta* (L.) Robs. var. *gummi-gutta* (Clusiaceae), *Artocarpus hirsutus* Lam. (Moraceae), *Elaeocarpus tuberculatus* Roxb. (Elaeocarpaceae) (Sasi & Rajendran, 2012; Tripathi et al., 2019; Rasmi & Jose, 2023). Nazarudeen (2010) reported 218 wild edible fruits from the forests of Kerala. According to Valvi and Rathod (2011), wild edible fruits are considered more nutritious than cultivated fruits. In developing countries, a variety of wild edible plants are used to enhance people's nutritional intake. These plants, which are not widely cultivated for commercial purposes or traded on a large scale, are often

underutilised. They may either be cultivated or naturally occur, and are typically consumed locally. Recently, there has been an increasing interest in evaluating the nutritional properties of different wild edible plants (Aberoumand & Deokule, 2009; Nazarudheen, 2010; Vunchi et al., 2011). Many tribal communities in India live near forests and depend on wild products and biomass to meet their food and energy needs (Mahapatra & Mitchell, 1997; Bahuguna, 2000; Mohapatra & Sahoo, 2010). Tribal people have traditionally identified and collected plants from forests for both food and medicinal purposes, developing local processing methods suited to their needs. However, with the rise of modernisation and settled agriculture, this traditional knowledge is slowly fading, which threatens the diversity of local diets and overall nutrition. It is crucial to validate, quantify, and share this valuable knowledge to preserve it for future generations (Edison et al., 2006; Dwebe & Mearns, 2011; Deepadarshan et al., 2023).

Evaluation of seed potentials

Many lesser-known tree species remain largely unstudied and underutilised for their potential benefits. While research has primarily focused on well-known crops and tree species, several underexplored trees thrive in forests or specific ecosystems, producing seeds that may offer valuable solutions to global health, nutrition, and sustainability challenges. Investigating the mineral and chemical composition, phytoconstituents, antioxidant properties, and bioactive compounds present in the seeds could unlock their potential. However, only a few studies have been conducted to assess the value of these plant seeds. Anuforo et al. (2017) evaluated seed potentials of *Terminalia catappa*; Nkoli et al. (2019) analysed seeds and leaves of *Irvingia* spp. Omale et al. (2021) evaluated some wild edible seeds and stems in Makurdi, Nigeria; Arockianathan et al. (2019) investigated the chemicals, minerals and antioxidant

activities of *Vigna mungo,* and Nwaehujor et al. (2019) studied the seed potential of *Annona muricata.*

Conservation protocol for *in- situ* and *ex-situ*

Preserving threatened plant species in the Western Ghats is imperative, necessitating both *in-situ* and *ex-situ* conservation efforts, as many face extirpation before a comprehensive study (Jose, 2001; Swarupanandan et al., 2013). Predominantly human-induced physical factors pose a significant threat to these plants, exacerbated by the impact of climate change in recent decades (Jose et al., 2024). Biological constraints in flowering, fruiting, seed regeneration, and dispersal further impede these species. To assess population status and threats, conducting surveys, estimating population sizes, determining the extent of occurrence, and assessing the area of occupancy are essential (Deepu et al., 2021, 2021a; 2023; Subin et al., 2022). Analysing population structure and diversity, identifying associates, understanding microhabitat preferences, assessing habitat threats, and examining age classes, relative abundance, natural regeneration, and dispersal mechanisms provide crucial ecological insights to the species. Phenological data, including vegetative and reproductive aspects, including flushing, flowering, pollinator interactions, immature fruit abscission, mature fruit predation, and insect-pest incidences, aid in identifying causes for species rarity *in situ*. A few of the species studied in this line include *Gluta travancorica* and *Ochreinauclea missionis* (Jose, 2001), *Canarium strictum, Persea macrantha , Myristica malabarica, Myristica beddomei, Knema attenuata, Dysoxylum malabaricum, Garcinia morella, Vateria indica* (Pandurangan, 2003); *Popowia beddomeana, Goniothalamus rhynchantherus, Goniothalamus wightii* (Rajkumar, 2011); *Lagerstroemia speciosa* (Deepu, 2015); *Humboldtia bourdillo*nii (Rahul et al., 2020); *Atuna indica* and *Hydnocarpus longipedunculatus* (Subin et al., 2022).

Vegetative propagation techniques can solve the problem of seed unavailability for some species. The techniques mainly involved rooting different-aged branch cuttings and air-layering. The strategy is very important as it enables the reproduction of plants that show difficulty in seed setting or having sterile seeds (Morgubatul et al., 2017). The method also provides a sufficient supply of superior planting stock locally, at a cost-effective and quick rate (Baul et al., 2011). As reported by Phillip et al. (2014), these methods enable planting materials for the propagation of plants and recovery of wild populations.

Seed biological studies encompass various aspects, including fruit harvest periods, seed processing, categories, Critical Moisture Content (CMC), viability periods, seed dormancy, and storage practices. These studies contribute to germplasm conservation and seed management (Shyam et al., 2002; Rasmi & Jose, 2023).

Evaluating the nutritional elements of ripe fruits after toxicity tests from lesser-known threatened trees can effectively promote conservation through popularization and commercialisation. Many of these wild fruits exhibit nutrient levels comparable to or superior to commonly cultivated fruits. Developing *ex-situ* propagation strategies, especially for dioecious plants, facilitates the selective breeding of economically important trees where edible ripe fruits are a key consideration. The species such as *Flacourtia jangomas* (Hossain et al., 2011); *Garcinia indica*, *G. Xanthochymus*, *Eugenia uniflora* (Tripathi et al., 2022); *Baccaurea coutallensis* (Rasmi & Jose, 2023); *Flacourtia montana* (Rasmi & Jose, 2025) are a few case studies reported.

Environmental niche modeling, a computer-aided process, predicts species habitat distribution in similar forest landscapes, aiding in locating suitable areas for establishing new populations as part of restoration efforts. The realised ecological niche, defined by climatic and soil data, is accurately described through predictive

models. Such models enable identifying the required habitat conditions even with a limited number of species occurrences (Adhikari et al., 2012; Guo et al., 2017; Borthakur et al., 2018).

Among various biotic threats to plants, pathogenic fungi play a critical role in the natural dynamics of the species. The interactions affect various levels that will lead to seed damage/ loss, seedling wilt diseases, fruit rot diseases, etc. The associations are critical in the case of threatened plants where natural regeneration, seed propagation and seedling survival are significant to rescue the species from untimely endangerment (Agrios, 2004; Cannon et al., 2012; Rasmi et al., 2022; Subin et al., 2024; Praveena et al., 2023; 2024).

It is concluded that the distributional, ecological and biological studies of species are significant for developing conservation and management protocols *in- situ* and *ex-situ*. The standardisation of vegetative propagation methods for the selective breeding of desired individuals and optimum seed storage practices for the maintenance of viable seeds *ex-situ*. The assessment of the nutrient potential of lesser-known fruits will be a notable lead for the sustainable use of species *ex-situ*. The prediction of the distribution areas will be another strategy for the future management of threatened plants. A Species Distribution Model (SDM) is a tool that enables the present habitats of a species and possible habitats in the future. It uses the locations and the environmental conditions of species. Using certain computer applications, it helps us understand the required environments species need to survive. As research on protecting biodiversity has grown, SDMs are usually practised to support conservation efforts (Xie et al., 2023; Liu et al., 2024). The investigations on morphological and molecular levels in the identification of pathogenic fungi and disease management open up new avenues for identifying the responsible factors leading to the rarity of threatened

plants, *Hopea erosa* and *Cynometra beddomei* (Praveena et al., 2025); *Dendrobium nobile* and *Dendrobium officinale* (Sarsaiya et al., 2020); *Woodfordia fruticosa* (Kumar, 2021), *Citrus* stem rot disease (Wirya et al., 2025).

CHAPTER 3

MATERIAL AND METHODS

3.1. MATERIAL

Diospyros crumenata Thwaites (Ebenaceae), an Endangered, edible fruit tree of the Western Ghats, was selected for the present study.

Diospyros crumenata is an evergreen dioecious tree, growing up to 28m tall, belonging to the family Ebenaceae. The tree usually occurs in semi-evergreen, evergreen forests, up to 1200 m elevation. The species is distributed in the Western Ghats covering states of Maharashtra, Goa, Karnataka, Tamil Nadu and Kerala and in Sri Lanka (Singh, 2005). In Kerala, the tree was found to be at an altitude of around 500 m asl. The tree is locally known as Karimaram and Karimboodan (Sasidharan, 2011). The species included in the IUCN Red List Status, Critically Endangered C 2a (i) (Bachan & Devika, 2023). The ripened fruits are edible and eaten by local people. The wood is medium textured, hard and heavy with a yellowish or creamy white sapwood. The timber is of medium quality and used for making tools for agriculture and for household articles (Bhat, 1994). However, bark, fruit, seed, flower, and leaves of related species of the genus have been reported for treatments such as diarrhoea, dysentery, blood diseases, snake poisoning, etc. (Singh, 2005). Phenology pertains to flowering normally from June to August and to mature fruits from December to January. The germination of seeds is epigeal, and cotyledons get detached at an early date.



Figure 1. Male tree (inset) Inflorescence

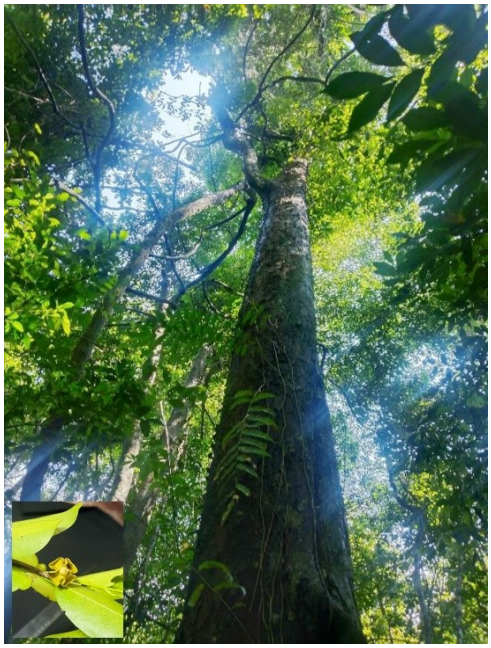


Figure 2. Female tree (inset) fruiting primordium

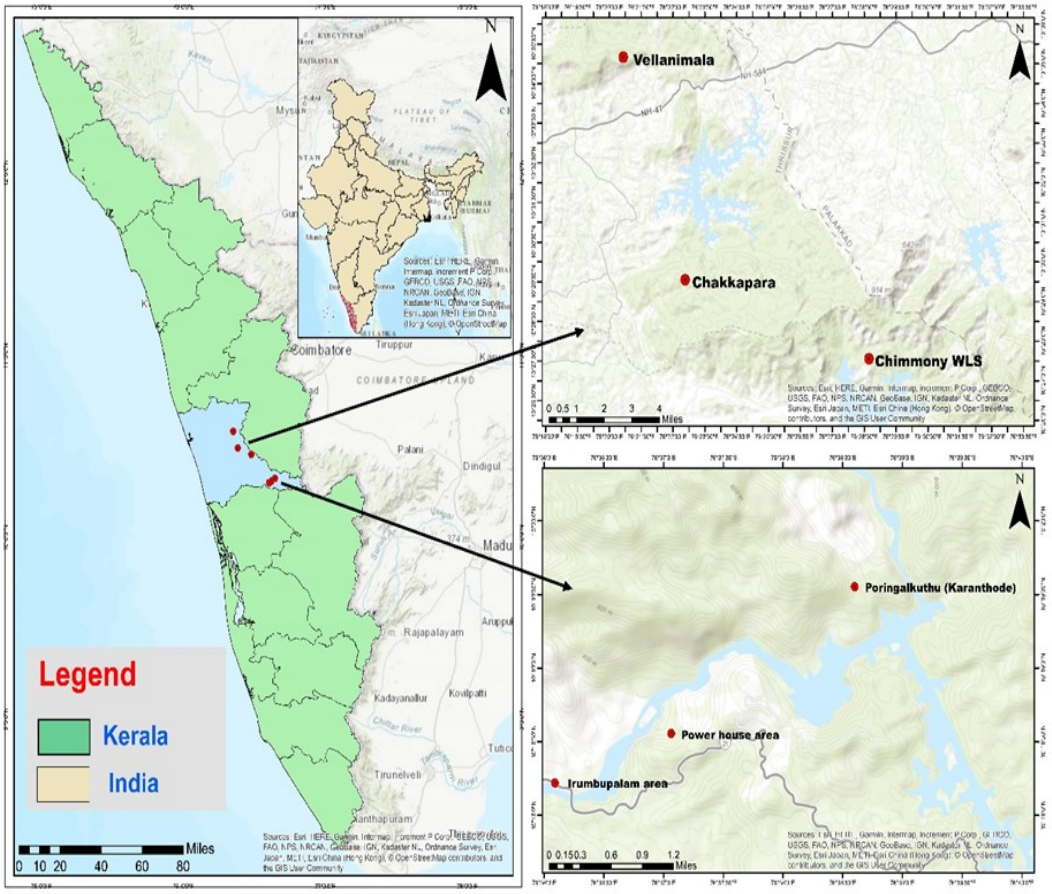
3.1.2. Taxonomic description of the species

The dioecious trees, mostly found in evergreen forests, as a canopy species up to 28 m in height (Figs. 1 and 2). The bark is greyish-black, thin, scaly, with small depressions all over the trunk. The blaze is whitish and yellowish in the xylem region. Leaves are simple, alternate, estipulate; petiole 7 to 13 mm and stout. Lamina 7.5-15 x 3.5-6.8 cm, oblong to elliptic, with obtuse base, apex bluntly acute or acuminate, margin entire, glabrous, glossy, coriaceous; lateral nerves 7 to 10 pairs. The male flowers many, emerge from the nodes of newly developed young shoots and are arranged in cymose inflorescences. The sepals are fused, four-lobed, and greenish. Female flowers are solitary, axillary; pedicel short, stout; calyx large, cupular, tomentose on both surfaces, corolla tubular, densely tomentose, yellowish-white; lobes 4, broad, acute; staminodes 8 to 12, epipetalous, unequal, ovary superior, pilose, 8-celled, ovule 1 in each cell; stigmas 4, fleshy, short. Fruit a berry, subglobose, 3 to 5 cm across, smooth; calyx much

enlarged, nearly flat, woody; seeds 1 to 8, 2.2 x 1.2 cm, oblong, compressed, glossy black; endosperm ruminate (Sasidharan, 2011).

3.1.3. Study site

The field study was conducted in the Western Ghats of Kerala region. Field trips were conducted to various forest areas, and four populations of *Diospyros crumenata* were identified. All these populations are located in the Thrissur district, such as Karanthodu, Irumbupalam area, Powerhouse area (Vazhachal Forest Range and Vazhachal Forest Division), Chakkapara and Vellanimala/Vellanipacha forests (Pattikad Forest Range and Thrissur Forest Division), and at Chimmony Wildlife Sanctuary in Thrissur Dist. (Map 1).



Map 1. *Diospyros crumenata*: Distribution in Kerala showing population sites

Voucher specimens:

During the field trips, vegetative, flowering, and fruiting specimens of the species were collected from the identified populations, and herbarium specimens were prepared following standard techniques (Bridson & Forman, 1998). The mounted specimens were deposited in the JTBGRI herbarium (TBGT).

3.2. METHODS

3.2.1. Ecology

3.2.1.1. Field Survey and Mapping

Repeated field surveys were conducted based on the literature and collection records of the species in the Western Ghats of Kerala. The Global Positioning System (GPS) data collected from the population areas were used to map the populations (Jose, 2001; Pandurangan, 2003). The criteria for assessing the conservation status are followed as per IUCN guidelines (IUCN, 2022), such as estimating the area of species occurrence (minimal continuous imaginary boundary encompassing the overall distribution of the species) and area of occupancy of the species (the area occupied by the species within its area of occurrence suited for its survival) by using the GeoCAT software (Vast, 2019).

3.2.1.2. Population Structure and Diversity Analysis

Quantitative analysis of vegetation data of each site was carried out as per the methods suggested by Richards (1952), Misra (1968), Halle et al. (1978), Pascal (1988), Swarupanandan & Sasidharan (1992), Jose (2001) and (Li et al. 2024). Population areas of the species were studied by adopting sample plots/quadrats of 50m length x 20 m breadth. Altogether, five plots were analysed in each forest area (0.1 ha. analysis). The total number of mature trees (≥ 30 cm GBH), saplings (10 to 30 cm GBH), established seedlings (≥1m height), and unestablished seedlings (<1m height) was enumerated

from the populations of the species, which include sampled and non-sampled population areas of the study site. An inverted J- shaped curve was adopted to describe girth class distributions of the candidate species, further to assess population trend (Sokpon &Biaou,2002). Vertical distribution/ arrangement (height range of *D. crumenata* among the associated tree species in the community), spatial distribution/ arrangement (ground level distribution of *D. crumenata*), age class distribution/ontogenic classes of *D. crumenata* (such as pre-reproductive (set of future), reproductive (set of present), and post reproductive (set of past) were analysed by direct observations among the identified populations areas during the period of study, 2021-2025 (Pascal, 1988; Parthasarathy & Sethi, 1997).

Stratification/ Vertical distribution

Measured by the vertical position of all the species (GBH 30 cm), including the candidate species, in the quadrat in height. Based on the height/ layer/strata, species were classified as the first layer (26 to 35 m, ht.), the second layer (16 to 25m ht.), the third layer (5 to 15 m ht.) and below this, which constitutes the shrubs and herbs. However, tree height variations in classes were noted as per site/ habitat characteristics. The height of the trees was measured using a Clinometer.

Horizontal/ Spatial distribution

It is the pattern of ground-level arrangement/ occurrence of the species in a particular quadrat. The horizontal perspective will also reflect the nature of the ground in the community, such as rocky areas, slopes, water sources, fallen and dead wood.

Age distribution

The individuals of the candidate species in the plot show different ages (Flowering status; flowering/ non-flowering individuals). They are categorised into

pre-reproductive (set of future), reproductive (set of present) and post-reproductive (set of past) phases.

Crown projections

The proportion of the ground occupied by the aerial parts indicates the canopy cover/ projections or basal cover of the species. It is measured in four perpendicular radii of the tallest individual of the species identified in the quadrat. The crown projections of both vertical and horizontal planes were measured.

3.2.1.3. Population Diversity Analysis

The community diversity and dominance of the candidate species were studied by laying sample plots of 50m L. to 20 m B. size as per basic ecological methods (Misra, 1968; Odum, 1959). A total of five sample plots (0.1 ha. analysis) were enumerated in order to accommodate the maximum associated species from each study site. All the species with a girth (GBH) above 30 cm were counted and measured. The density, frequency and dominance were calculated and found the Importance Value Index (IVI), which is the relative position of a species in its ecosystem. The IVI is calculated by the sum of relative frequency (rf), relative density (rd), and relative dominance (rD) (Philips, 1959; Curtis, 1959) of species using INVENT- NTFP software (Sivaram et al., 2006). The species importance values computed with respect to the stand's total IVI (300) are reduced to percentile values (Species IVI/3) for easy comparison (Narayanan & Swarupanandan, 1996).

3.2.1.4. Population Dynamics

Periodic dynamic changes in both vegetative and reproductive phenological phases were monitored and recorded during the period of study, 2021- 2025, on monthly basis as per the methods suggested by Faegri and Pijl (1979); Kershaw, (1973);

Armstrong & Drummond (1984); Armstrong and Irvine, (1989); Bawa and Hadley, (1991); Murali & Sukumar (1994); Swarupanandan & Sasidharan (1992).

Vegetative phenology

Vegetative phenology covers leaf phenological phases such as leaf initiation, growth, maturation**,** senescence and insect-pest associations. Each phase and duration was monitored directly by the individuals of the species among the populations identified. Apart from these, leaf infections during the period were also recorded.

Reproductive phenology

Reproductive phenology covers flower, fruit, seed and seedling regeneration phases. Data was collected similarly to the data collection made for the vegetative phenology. Inaddition, insect-pest associations were also recorded. Flower phenology includes bud initiation, blooming period, pollen fertility and pollen-ovule ratio.

Pollen fertility test

The acetocarmine staining method was suggested by Sharma and Sharma (1980). Fully matured anthers from different buds were crushed into 1ml of acetocarmine solution and kept for 1 hour to determine fertility. They were observed for staining or any other deformations. Those grains that are fully stained are considered fertile.

Pollen – Ovule ratio

The fully developed, unopened flower buds per flower were selected for pollen count. The anthers were crushed into a known volume of distilled water. The suspension was then loaded onto a Haemocytometer. The number of pollen grains per flower was counted and subsequently to single anther (Samuel, 1989; Shivanna & Rangaswamy, 1992). The ovules per ovary were counted by taking the cross-section of the ovary (Cruden,1977). The Pollen-Ovule ratio was calculated by using the formula:

$$\text{Pollen} - \text{Ovule ratio} = \frac{\text{Pollen per anther} \times \text{Number of anthers per flower}}{\text{Number of ovules per flower}}$$

Fruit phenology

The fruit phenology of the species was monitored and recorded, including fruit initiation, developing period, premature abscission, maturity, and pest incidence. The data were collected directly from the populations of the species during the study period.

Seed phenology

Encompasses seed number per fruit and seed characteristics. The data was collected from the fruit-harvesting period from the populations of the species.

Natural regeneration

The seedlings recorded from the populations during the study period were categorised into two classes, namely established seedlings of >100 cm height and unestablished seedlings of <100 cm. Accordingly, the seedlings' height in the above two classes was measured (Swarupanandan & Sasidharan, 1992). Further, an Inverted J-shaped curve was prepared at each population site to assess the age class distribution of species in the respective population site (Negi & Nautial, 2005; Raviraj et al., 2024).

Insect-pest associations

Larvae of the insect pest were collected from the candidate plant species and reared in glass bottles covered with cotton cloth under room temperature conditions. They were fed on the leaves of the species until they completed their life cycle. The duration of each moulting stage was recorded to identify the adult insect.

Lichen associations

The trunk of the *D. crumenata* trees has been noticed to have unique lichen associations across the populations identified in the study areas. The specimens were collected from the field, numbered and made into herbarium specimens as per the standard method. A chisel and hammer were used to remove the lichens from the bark.

The collected specimens were air-dried and mounted on cardboard (handmaid herbarium paper) and covered with thick brown packets. Then the specimens were labelled according to the international standard and were made into herbarium specimens. All the taxa thus studied were identified by comparing various keys and descriptions available in the published floras and monographs of lichen genera (Awasthi, 1991; Orange et al., 2001)

3.2.2. Conservation

3.2.2.1. Vegetative propagation protocol

Vegetative propagation experiments were conducted as per the methods suggested by Nagpal et al. (1982), Hartman & Kester (1983), Donald, (1987), Surendran & Seethalakshmi (1987), Gurumurthi et al. (1994), Jose et al. (1995), Subramanian & Sasidharan (1997), Praveena et al. (2025).

Stem cuttings

Stem cuttings of 12 to 15 cm in length, with 2–3 nodes and two or four leaves, were taken from young plants under 5 years old of *Diospyros crumenata*. Stem cuttings collected from the adult trees were also attempted similarly to that of young plants. The auxins (IAA, IBA, NAA) were prepared at 1000, 3000, and 5000 ppm in liquid form by dissolving them in absolute ethyl alcohol and made up to the required quantity with distilled water. Each set of cuttings (10 numbers per set) in duplicate was dipped in these solutions for one minute. A control set of cuttings dipped in distilled water was maintained for rooting response. The treated cuttings were then planted in clay pots containing coarse river sand and kept in a mist house at 28 ± 2°C and 80 ± 5% RH. (Nanda et al., 1970; Hartmann & Kester, 1983; Subin, 2023). After 45 days, rooting data were collected, and the rooted plants were transplanted into polybags, further kept inside a mist house for hardening, with their survival checked after three months. Data

on rooting, *viz.,* rooting per cent, number of roots per cutting and root length were collected and tabulated. The number of roots and the mean root length were compared across treatments using one-way ANOVA followed by Duncan's Multiple Range Test (DMRT). The survival performance of rooted plants (ramets) was recorded after three months.

Air-layering

Air-layering was conducted in saplings below 5 years old, maintained in the nursery, since physical vagaries (wildlife interventions) were found to disrupt the layering experiment *in situ.* Eight branches at each auxin concentration were used in the experiment. A small strip of bark was removed from the stem, approximately 15 to 20 cm below the shoot tip of lateral branches, using a sharp budding knife. The girdled sections were promptly treated with auxin solutions (IAA, IBA, and NAA) at 3000, 5000, and 7000 ppm concentrations. This area was then wrapped with white polythene sleeves filled with a moist rooting medium comprising coco peat, river sand, and dried, sieved cow dung in a 2:1:1 ratio. The sleeves were securely sealed at both ends to maintain moisture inside the rooting medium. Air layers were monitored in weekly intervals for root development, with observations conducted without opening the polythene sleeves (Jose et al., 2009; Subin, 2023).

$$\text{Rooting per cent} = \frac{\text{Number of layers}}{\text{Total number of shoots air layered}} \times 100$$

3.2.2.2. Seed propagation protocol

Ripe fruits of *D. crumenata* were harvested from natural populations in the Vazhachal forest areas in January-February 2023. The seed and fruit attributes, such as average weight, length, and thickness, were recorded. The Moisture Content (MC) of the processed fresh seeds was determined on a fresh weight basis following ISTA

guidelines (ISTA, 1976; Ellis et al., 1985). Five samples of 2 seeds each were chipped, and the fresh weight of each was determined. Later, the samples were dried in an electric oven set at 130±2 °C for one hour (based on previous trials conducted to determine the ideal drying method). The samples were then allowed to cool in a desiccator for half an hour, and then the dry weight, followed by moisture content, was determined by using the formula:

$$\text{Moisture content} = \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Fresh weight}} \times 100$$

(The mean value of the MC was calculated in each trial.)

The temperature response of the depulped seeds was examined for categorising seed types. For desiccation trials, seeds were kept open in a plastic tray under room conditions. Fresh seeds were kept in a freezer (0 °C – 5°C). The Critical Moisture Content (CMC) of the seeds was detected using the field germination method as well as the tetrazolium staining method, with respective MC on each sowing.

3.2.2.3. Seed pre-treatments

Seed pre-treatments were applied to processed seeds to improve the seed germination performance (Warakagoda & Subasinghe, 2015). Nine sub-treatments across six main treatments were conducted, viz., hot water treatment (T7), cold water treatment (T8), chemical application, plant growth regulator treatment (T1 and T2), scarification, and control. Each sub-treatment involved ten seeds, with three replications. For the hot water treatment, the seeds were soaked in 70°C water for 5 minutes (T7), and for the cold-water treatment (tap water), the seeds were soaked for 24 hours (T8). The chemical application treatment used was 2% Potassium nitrate (T4), 1% Disodium hydrogen phosphate for 24 hours (T5), and concentrated Sulphuric Acid for 1 minute (T3). The plant growth regulator (GA_3) treatment involved soaking seeds in GA_3 at 500 (T1) and 1000 ppm (T2) for 24 hours. Scarification was done by

scratching seeds with sandpaper before sowing, followed by soaking in tap water for 24 hours (T6), while the control set involved sowing fresh seeds without any treatment (T9) (Haider et al., 2014; Anaz et al., 2019). The seeds were sown in coarse river sand medium, both in beds and clay pots, and kept inside a low poly tunnel of the nursery, in which the climatic factors were maintained at 28±2°C; 80±5 % RH, and watered daily. Germination was monitored daily, and germinated seeds were counted and transferred until no further germination was observed. Germination was defined by the emergence of a primary root at least 2 mm in length, and seedlings were counted once all essential structures had developed. Upon the formation of two pairs of leaves, measurements were taken for seedling height, collar diameter, and number of leaves.

3.2.2.4. Seed storage practices

The temperature response of the depulped seeds was examined for categorising seed types. The Critical Moisture Content (CMC) of the seeds were determined through desiccation. The processed seeds were divided into six sets and stored in closed polythene bags and polycarbonate bottles in three different conditions, such as Ambient (27± 2°C; 50-60% RH); Seed bank (20± 2°C; 40% RH); Seed bank (16± 2°C; 45% RH). Ten seeds in duplicate from each condition were taken out every month, and their viability was tested using both the tetrazolium staining method and by sowing in nursery conditions. (Pandurangan 2003; Jose & Pillai, 2016). To assess the viability of *Diospyros crumenata* seeds, a 1% tetrazolium chloride (TZ) solution was prepared by dissolving 0.25 g of 2,3,5-triphenyl tetrazolium chloride in 25 mL of distilled water. The freshly prepared solution was protected from light to prevent degradation. Seeds were soaked in distilled water for 24 hours to soften the seed coat, then carefully bisected longitudinally to expose the embryo without causing damage. A total of 12 seeds, arranged in two replicates of six each, were immersed in the TZ solution and

incubated in the dark at 30°C for 24 hours. After incubation, staining patterns were observed, and seeds were classified as viable or non-viable following the ISTA Handbook for seedling evaluation (ISTA, 1999). Seed germination below 20% was treated as insignificant.

3.2.3. Utilization

3.2.3.1. Nutritional analysis of fruits

3.2.3.2. Acute oral toxicity test of ripened fruits

The fruit was deseeded, the pulp carefully extracted and finely ground to achieve a uniform consistency, and then weighed. It was then filtered for any coarse particles and was subjected to the study. An acute oral toxicity study of indoxacarb was performed as per the guidelines No. 420 of the Organisation for Economic Co-operation and Development (OECD).

The Oral Toxicity limit dose study at 2000 mg/kg body weight (OECD, 2022). The fruit pulp was administered to adult Wistar rats weighing 150-200g. Animals were fasted overnight before the day of dosing, and the animal body weight was recorded. The dose of the test substance, calculated as per the body weight, was administered orally through gavage. The rats were monitored for clinical signs, and changes in the various body systems like skin, mucous membrane, respiratory, cardiovascular, central nervous system and somatomotor functions. The mortality and clinical signs were observed for the first 10 min, 30 min, 1 h, 2 h, 4 h, and 6 h after dosing and thereafter twice daily for mortality and once a day for clinical signs. After the observation period of 14 days, all surviving animals were euthanised and subjected to a detailed gross pathology examination. The organs, such as the brain, heart, liver, spleen, kidney, lung, uterus and ovary, were collected, and the weight of the organs was taken for the

estimation of relative organ weight. The study was carried out at the College of Veterinary & Animal Sciences, Thrissur.

3.2.3.3. Nutritive analysis and evaluation of edibility of ripened fruits

The nutritional analysis of ripe fruits of *Diospyros crumenata* was carried out as per standard laboratory methods. Carbohydrates were estimated by the method given by Sadasivam and Manickam (2009), and protein content was quantified using the method by Lowry et al. (1951). The Ascorbic Acid (Vitamin C) was determined using standard procedures by Barkat et al. (1973). The mineral solution of the ground sample of *D. crumenata* was prepared as described by Shahidi et al. (1999), and Total Mineral Content was analysed using Atomic Absorption Spectroscopy (Allen et al. 1974). Sodium and Potassium were evaluated by a Systronics modiflame 127 flame photometer (Jones, 2001). Total sugar was determined by the anthrone method (Jayaraman, 1981). A comparative analysis of nutritional elements of *D. crumenata* was made with common fruits such as Jackfruit, Mango and Pineapple, as done by Nazarudeen (2010).

3.2.3.4. Evaluation of seed properties

The seed properties were analysed using standard procedures. Mineral content was determined using the Perkin Elmer-AVIO 200 instrument (Kingston & Jassie, 1988). The chemical composition included measurements such as moisture content (Hot air oven drying); crude protein (Kjeldahl method); crude fiber (Acid–Alkali digestion), ether extract (Soxhlet method) (Nancy et al., 2003; Nancy, 2009; Nancy et al., 2012) and Total Ash (Muffle furnace) (Nancy & Lawrence, 2012).

Phytochemical screening of seeds was carried out for various compounds using the following tests: alkaloids (Dragendorff's reagent), terpenoids (Salkowski reaction),

glycosides (Picric acid test), saponins (Foam test), tannins (Lead acetate test), phenols (Folin–Ciocalteu reagent), flavonoids (Shinoda test), and proteins (Biuret test).

Antioxidant activity was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay via UV spectrophotometric analysis, CKL/ ANL/UV-004 method (Ivan et al., 2024; Malik et al., 2017). Additionally, GC–MS analysis of the chloroform extract of seeds was performed following standard laboratory protocols (Afrin, 2019; Subin et al., 2021).

Experimental description of GC–MS analysis

The chloroform extract of seeds was analysed using a Shimadzu Nexis GC-2030 gas chromatograph equipped with an AOC-30/20i autosampler and coupled to a mass spectrometer. Separation was achieved on an SH-I-5Sil MS capillary column (30 m L× 0.25 mm inner diameter, 0.25 μm film thickness) using helium as the carrier gas. The oven temperature was programmed from 40°C to 300°C at 10°C min^{-1}. Data acquisition and processing were performed using GC-MS Solutions software, and compounds were identified by comparison with the NIST 20 mass spectral library.

3.2.4. Conservation protocol for *in situ* and *ex-situ*

3.2.4.1. Habitat distribution modeling

Twelve environmental variables were selected as potential predictors for modeling the habitat distribution of *D. crumenata*, based on their plant species distribution and insights from previous habitat modeling studies (Kumar et al., 2006; Guisan et al., 2007; Pearson et al., 2007; Murienne et al., 2009). The bioclimatic variables were obtained from the WorldClim dataset (http://www.worldclim.org/bioclim.htm), while elevation and slope raster layers were derived from the Digital Elevation Model (DEM) available on the same platform. All variables used were at a spatial resolution of 1 km. The MaxEnt software (Maximum

Entropy, version 3.4.1) was employed to model both current and projected potential distributions of *D. crumenata* in the southern Western Ghats (Elith et al., 2006; Benito et al., 2009; Hernandez et al., 2006; Papeş & Gaubert, 2007; Phillips et al., 2004; Wisz et al., 2008). Following the methodology detailed by Pearson et al. (2007), a jackknife procedure was implemented to evaluate model performance by testing its capacity to predict a single locality excluded from the training dataset.

3.2.4.2. Evaluation of seed infestation

The seeds showed infestation during germination and were found to affect the seedling production *ex-situ.* This was found significant conservation concern for the species. The infected seed samples were tested for detailed pathological examination, including morphological and molecular characterisation. The causal relationship between the pathogen and disease was confirmed by re-isolating the pathogen and verified through both micro and macro morphological characteristics, by the methods outlined in studies by Silva de et al. (2019), Luo and Jiang (2022), Sharma et al. (2022) and Praveena et al. (2025).

3.2.5. Statistical Analysis

The Importance Value Index (IVI), which is the relative position of a species in its ecosystem. The IVI is calculated by the sum of relative frequency (rf), relative density (rd), and relative dominance (rD), using INVENT- NTFP software (Sivaram et al., 2006). The species importance values computed with respect to the stand's total IVI (300) are reduced to percentile values (Species IVI/3) for easy comparison. The effect of different treatments on plant growth variables was analysed using One-way ANOVA and Duncan Multiple Range Test (DMRT) in SPSS software.

CHAPTER 4

RESULTS

4.1. Ecology

4.1.1. Population Survey, Identification and Mapping

Populations of the species were identified and located using GPS coordinates in three major forest areas in Thrissur District: Vazhachal within the Vazhachal Forest Division, Vazhachal Range; Vellanimala and Chakkapara in the Pattikkad Forest Range, within the Thrissur Forest Division. In the Vazhachal Range, populations were identified at Poringalkuthu, specifically in the Karanthode and Powerhouse areas, and in the Irumbupalam fringe forest areas. Additionally, a few individuals of the species were identified at Chimmony Wildlife Sanctuary (WLS), extending their distribution beyond the above sites (Table 1 and Map 1).

Table 1. *Diospyros crumenata*: Location details across population sites

Population sites	GPS coordinates	Altitude (m asl)
Vazhachal (Poringalkuthu /Karanthode area)	N 10° 19' 33.24" E 76° 38' 35.88"	476-514
Irumbupalam area	N 10° 18' 13.32" E 76° 36' 5.4"	254
Powerhouse area	N 10° 18' 33.48" E 76° 37' 3.72"	329
Vellanipacha / Vellanimala area	N 10° 29' 33" E 76° 22' 52.32"	312-506
Chakkapara area	N 10° 35' 10.32" E 76° 20' 55.68"	349– 431
Chimmony WLS	N 10° 27' 33.48" E 76° 28' 38.64"	202

The voucher specimens were deposited in the JNTBGRI herbarium (TBGT), viz., Thrissur Dist.., Chakkapara of Pattikkad Range; 99201; Rahul Jose, 20/09/2021; Thrissur Dist., Poringalkuth of Vazhachal Range; 99202; Rahul Jose, 27/10/2021; Thrissur Dist., Poringalkuth of Vazhachal Range; 99203; Rahul Jose, 27/10/2021; Thrissur Dist., Poringalkuth of Vazhachal Range; 99204; Rahul Jose, 29/12/2021; Thrissur Dist.., Chakkapara of Pattikkad Range; 99205; Rahul Jose 02/03/2022; Thrissur Dist.; Vellanimala/ Vellanipacha of Pattilkkad Range; 99216; Rahul Jose, 22 /03 / 2022; Thrissur Dist.; Chimmony WLS; 99220,99221; Rahul Jose, 26/05/ 2023.

Following IUCN criteria, the extent of occurrence and area of occupancy of the species was determined through the GeoCAT app, and found that the species has an Extent of Occurrence to be 251.67 km^2 and Area of Occupancy to be 56.00 km^2 (Fig. 3). Additionally, the population of mature individuals was less than 150, confirming the placement of species under the Critically Endangered (CR) category as per the 'D' criteria (estimate of the population size) of IUCN Guidelines within the study area of the Western Ghats of Kerala.

4.1.2. Population structural analysis

The population structure analysis in three study areas, namely Vazhachal (Poringalkuthu/Karanthode), Pattikkad-Chakkapara, and Pattikkad-Vellanimala, is detailed. The analysis was made by recording GBH, basal area, basal cover, age phases and height of each individual. An extended distribution of the species at Pattikkad-Chakkapara, along with a few isolated individuals from the Chimmony Wildlife Sanctuary (WLS), was identified through the present study. Scattered populations such as Poringalkuth (Karanthode), Irumbupalam, and Powerhouse areas were treated as major population sites under Vazhachal/ Karanthode.

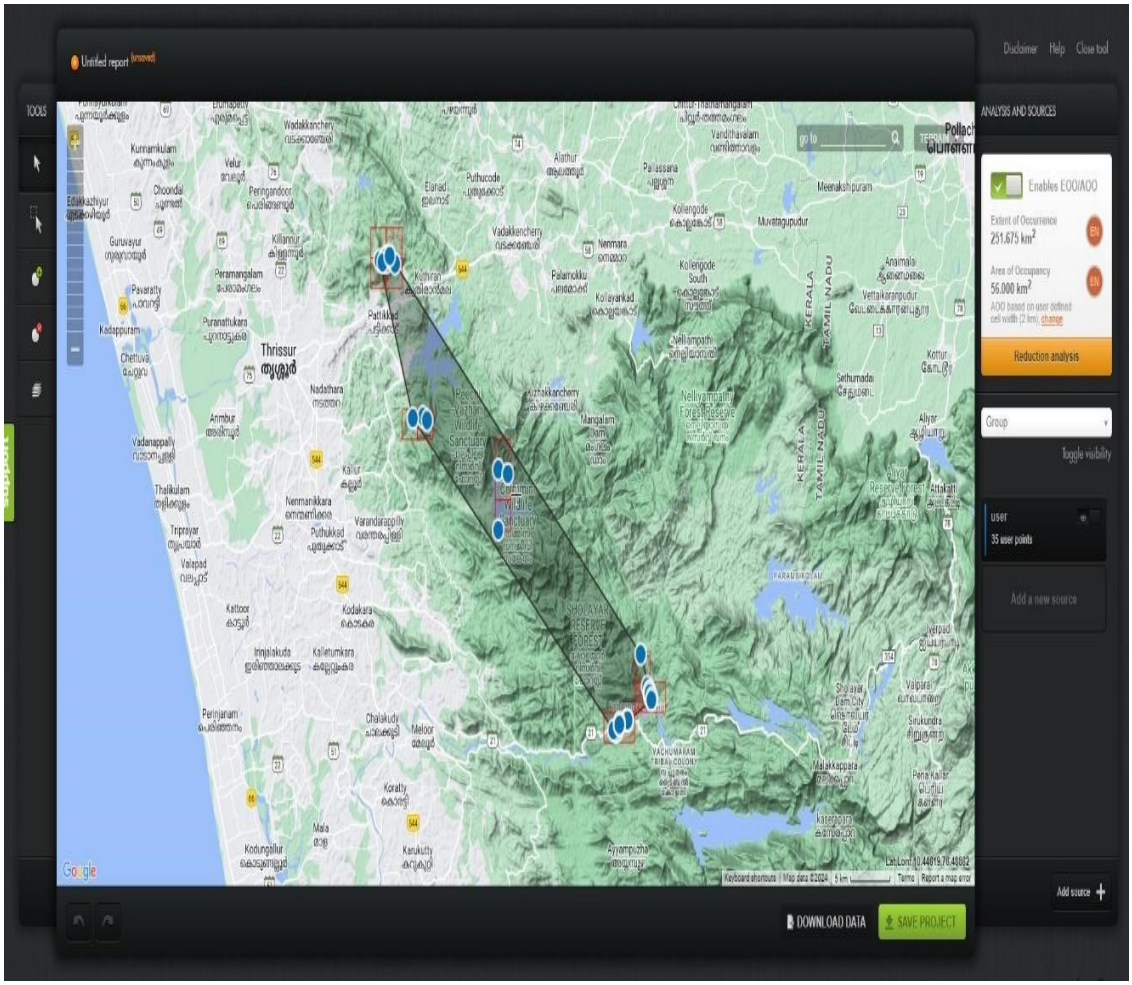


Figure 3. *Diospyros crumenata*: Estimation of the Extent of Occurrence and Area of Occupancy using the GeoCAT app.

Altogether, 138 mature individuals (≥30 cm GBH) fall in each GBH class were presented through an inverted J-shaped curve (Fig.4). Besides this, 35 saplings (10-30 cm GBH), 10 established seedlings (≥100 cm height), and eight unestablished seedlings (≤ 100 cm height) of *D. crumenata* were counted from all three major populations, which include Vazhachal, Chakkapara, Vellanimala and Chimmony WLS. No saplings and seedlings were recorded from the Chimmony WLS. All the mature trees are characterised by long straight boles, blackish-grey coloured, moderately rough bark and lichen associations in the main trunk. The trees are positioned as canopy species (1st layer) and occur in evergreen and semi-evergreen, and rarely in the fringes of semi–

evergreen–moist deciduous forests. The blaze is whitish and yellow in the xylem region (Plate 1).



Figure 4. *Diospyros crumenata*: Total individuals along with the Girth class from the four study sites

4.1.2.1. Population structure of Vazhachal- Karanthode

The Vazhachal population constitutes the Karanthode, Irumbupalam, and Powerhouse forest patches. Altogether, 94 mature individuals (≥30 cm GBH) fall in each GBH class were presented through an inverted J-shaped curve (Fig.5). Eighty-two mature trees, 15 saplings, five established seedlings and eight unestablished seedlings were enumerated from the Karanthode site at an altitude between 476 and 514 m. asl. The GBH of trees ranged from 47-380 cm (Fig. 5), and individuals' height ranged from 5-28m. Most trees are of the 151–200 cm GBH range class and are in reproductive age classes. Among the trees counted, 58 large trees are 20–28m in height. The pre-reproductive individuals range from 47 to 130 cm GBH. Among the individuals, the pre-reproductive individuals constitute below 10%. Natural regeneration was poor among the populations. Four individual trees of the species were recorded in the

Vazhachal- Irumbupalam areas at an altitude of 254 m. asl, along the fringe forests of Chalakudy- Malakkapara main road. These trees are at risk of habitat loss due to future road widening. The trees attained 140 – 220 cm GBH and 15–20 m in height. Another population of eight trees was recorded in adjacent Poringalkuth- Powerhouse Forest patches at an altitude of 329 m. asl. The population is also facing habitat loss due to Powerhouse developmental activities. The trees attained 150– 380 cm GBH and 15–25 m height (Table 2).

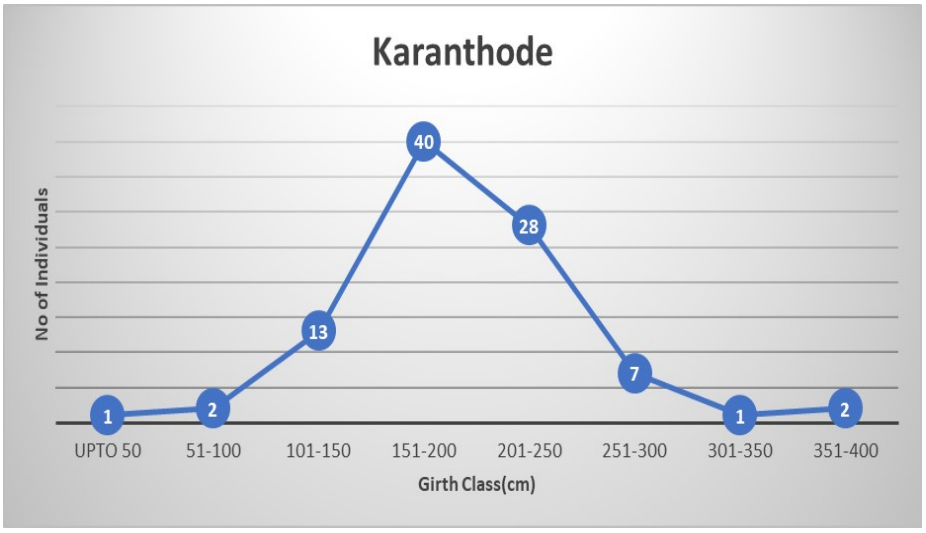


Figure 5. *Diospyros crumenata*: Total individuals along with the Girth class at Vazhachal/ Karanthode

Table 2. *Diospyros crumenata*: Population structure within the sampled and non-sampled area at Vazhachal/ Karanthode

Sl. No.	GBH (cm)	Basal Area (cm^2)	Basal Cover (m)	Age phase	Height of tree (m)
1	47	175.88	2.0	Set of future	05.0
2	68	368.15	2.5	Set of future	05.0
3	86	588.85	3.0	Set of future	16.0
4	123	1204.54	3.0	Set of future	20.0
5	150	1791.40	3.5	Set of present	25.0
6	113	1016.64	3.0	Set of future	13.0
7	109	945.94	3.5	Set of future	15.0
8	130	1345.54	3.0	Set of future	19.0
9	110	963.38	2.5	Set of future	18.0

10	140	1560.51	3.0	Set of present	18.0
11	150	1791.40	3.5	Set of present	15.0
12	140	1560.51	3.0	Set of present	08.0
13	150	1791.40	3.5	Set of present	20.0
14	150	1791.40	3.5	Set of present	20.0
15	150	1791.40	3.0	Set of present	15 .0
16	150	1791.40	3.5	Set of present	14 .0
17	153	1863.77	3.0	Set of present	22.0
18	172	2355.41	3.5	Set of present	20.0
19	199	3152.95	5.0	Set of present	25.0
20	160	2038.22	4.0	Set of present	25.0
21	164	2141.40	4.0	Set of present	21.0
22	155	1912.82	4.0	Set of present	24.0
23	169	2273.96	4.5	Set of present	20.0
24	190	2874.20	5.0	Set of present	18.0
25	160	2038.22	4.0	Set of present	15.0
26	160	2038.22	3.5	Set of present	18.0
27	180	2579.62	4.0	Set of present	17.0
28	190	2874.20	4.5	Set of present	18.0
29	170	2300.96	4.5	Set of present	22.0
30	180	2579.62	5.0	Set of present	14.0
31	160	2038.22	3.5	Set of present	16.0
32	160	2038.22	4.5	Set of present	24.0
33	180	2579.62	4.5	Set of present	18.0
34	190	2874.20	5.0	Set of present	16.0
35	190	2874.20	5.0	Set of present	20.0
36	153	1863.77	3.5	Set of present	18.0
37	172	2355.41	3.5	Set of present	22.0
38	199	3152.95	5.0	Set of present	25.0
39	160	2038.22	4.5	Set of present	22.0
40	164	2141.40	4.5	Set of present	17.0
41	180	2579.62	4.0	Set of present	16.0
42	190	2874.20	4.0	Set of present	17.0
43	190	2874.20	4.5	Set of present	16.0
44	190	2874.20	4.0	Set of present	25.0
45	199	3152.95	4.5	Set of present	23.0
46	160	2038.22	4.0	Set of present	20.0
47	164	2141.40	4.0	Set of present	23.0
48	155	1912.82	4.5	Set of present	28.0
49	169	2273.96	5.0	Set of present	14 .0
50	160	2038.22	4.0	Set of present	17.0
51	180	2579.62	4.5	Set of present	16.0
52	190	2874.20	4.5	Set of present	15.0
53	190	2874.20	4.5	Set of present	16.0
54	190	2874.20	5.0	Set of present	17.0
55	190	2874.20	4.0	Set of present	25.0
56	199	3152.95	4.5	Set of present	25.0
57	210	3511.15	4.5	Set of present	24.0

58	240	4585.99	5.0	Set of present	28.0
59	240	4585.99	4.5	Set of present	22.0
60	212	3578.34	5.0	Set of present	20.0
61	220	3853.50	5.0	Set of present	22.0
62	230	4211.78	4.5	Set of present	20.0
63	230	4211.78	4.0	Set of present	20.0
64	210	3511.15	3.5	Set of present	25.0
65	210	3511.15	4.0	Set of present	24.0
66	210	3511.15	4.0	Set of present	22.0
67	230	4211.78	4.5	Set of present	25.0
68	230	4211.78	4.5	Set of present	18.0
69	240	4585.99	5.0	Set of present	22.0
70	210	3511.15	5.0	Set of present	24.0
71	210	3511.15	3.5	Set of present	23.0
72	230	4211.78	4.0	Set of present	21.0
73	220	3853.50	4.5	Set of present	22.0
74	210	3511.15	4.0	Set of present	24.0
75	230	4211.78	4.0	Set of present	20.0
76	210	3511.15	3.5	Set of present	28.0
77	230	4211.78	4.0	Set of present	18.0
78	240	4585.99	4.0	Set of present	21.0
79	210	3511.15	4.0	Set of present	25.0
80	212	3578.34	5.5	Set of present	18.0
81	220	3853.50	4.5	Set of present	22.0
82	230	4211.78	4.5	Set of present	19.0
83	210	3511.15	3.0	Set of present	24.0
84	240	4585.99	5.0	Set of present	22.0
85	260	5382.17	5.0	Set of present	23.0
86	260	5382.17	5.5	Set of present	22.0
87	260	5382.17	5.0	Set of present	23.0
88	280	6242.04	5.5	Set of present	27.0
89	260	5382.17	5.5	Set of present	25.0
90	260	5382.17	4.5	Set of present	22.0
91	260	5382.17	5.5	Set of present	24.0
92	310	7651.27	6.0	Set of present	21.0
93	380	11496.82	5.5	Set of present	25.0
94	380	11496.82	5.0	Set of present	24.0

Stratification/ Vertical distribution

The vertical vegetation profile of the Poringalkuthu–Karanthode area reveals a distinct stratification of tree species. The uppermost layer or first storey, reaching heights of 26 to 35 meters, includes major tree species such as *Dipterocarpus indicus* Bedd., *Vateria indica* L., *Prioria pinnata* (Roxb. ex DC.) Breteler, and *Diospyros*

crumenata Thwaites. The second storey, ranging from 16 to 25 meters in height, comprises species such as *Myristica malabarica* Lam., *Atuna travancorica* (Bedd.) Kosterm., and *Diospyros bourdillonii* Brandis. The third and lowest storey, with a height range of 6 to 15 meters, is represented by species such as *Baccaurea courtallensis* (Wight) Muell. - Arg., *Xanthophyllum arnottianum* Wight, and *Litsea* sp., with a height range from 6 to 15 m. The shrubs/seedlings of small trees/ herbs of various species include *Cinnamomum malabatrum* (Burm. f.) Blume, *Otonephelium stipulaceum* (Bedd.) Radlk., *Ixora brachiata* Roxb. ex DC., *Aglaia* sp., *Calamus* sp., *Mallotus* sp., *Pandanus amaryllifolius* Roxb., *Thottea siliquosa* (Lam.) Ding Hou, *Smilax zeylanica* L., *Reinwardtiodendron anamalaiense* (Bedd.) Mabb., etc. (Fig.6).

Horizontal/ Spatial distribution

The spatial distribution of the population site displayed the scattered arrangement of individuals of *Diospyros crumenata* along with their associates, positioned near the watercourse in an evergreen forest on both sides of the forest trail. One side of the track shows an upward slope, and the other side slopes down to the perennial stream. The Perennial stream later drained to the Poringalkuth dam reservoir. The streamside and upper side featured big rock boulders. The individuals of the species were found distributed along the perennial stream side and adjacent annual stream on the upper side of the track (Fig.7).

Age-wise distribution profile

The age-wise distribution profile has shown seven individuals in a 50 x 20 m^2 plot, of which five individuals constitute a set of present (Reproductive class) and two individuals constitute a set of future (Pre-reproductive class), covering a height range from 5-28m (Fig.8).

Crown projections

The vertical crown projections illustrate the positioning of species excluding *Diospyros crumenata,* such as *Myristica malabarica*, *Atuna travancorica*, *Diospyros bourdillonii*, and *Ixora brachiata,* situated just beneath the tallest canopy-forming individual, *Dipterocarpus indicus* (Fig. 9). The horizontal crown projections reveal the overlapping crown cover of these species occurring beneath the canopy of *Dipterocarpus indicus* (Fig. 10).

4.1.2.2. Population diversity analysis at Vazhachal- Karanthode

The floristic diversity analysis encompassed 85 species with a girth at breast height (GBH) of ≥ 30 cm, totalling 390 individual trees within a 5000 sq m area. For each species, aggregated values of relative frequency (rf), relative density (rd), and relative dominance (rD) were calculated. Among these, *Dipterocarpus indicus* recorded the highest Importance Value Index (IVI) at 20.0932, making it the dominant species in the studied forest. In contrast, *Diospyros crumenata* ranked 41st, with an IVI of 1.8993 (Table 3).

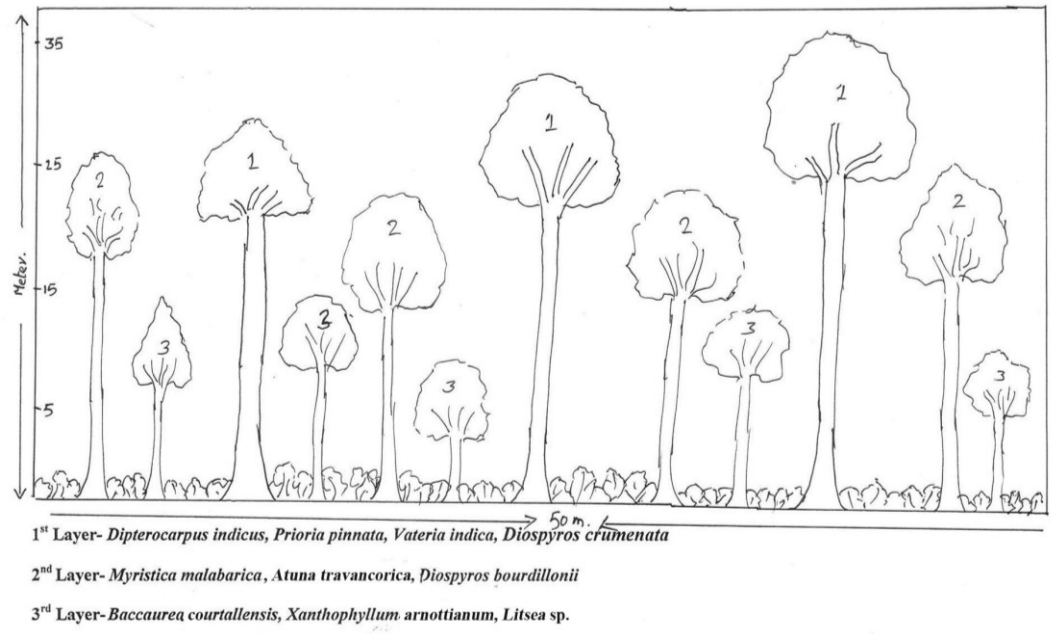


Figure 6: *Diospyros crumenata:* Vegetation profile (Vertical) at Vazhachal forest (Karanthode)

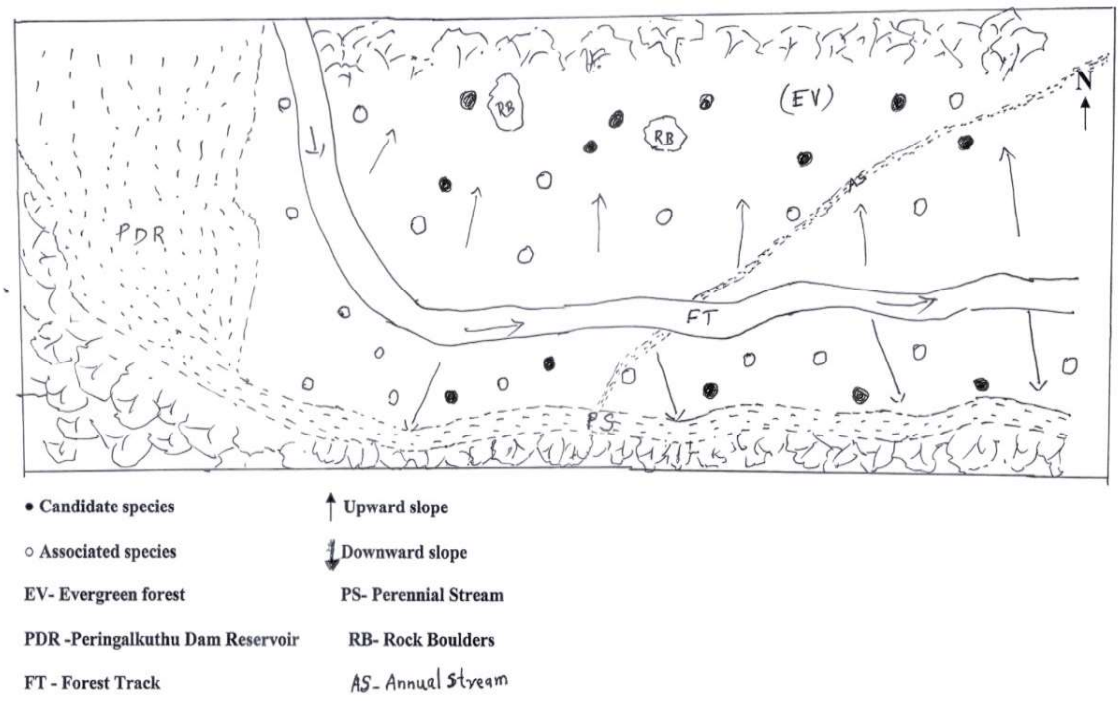
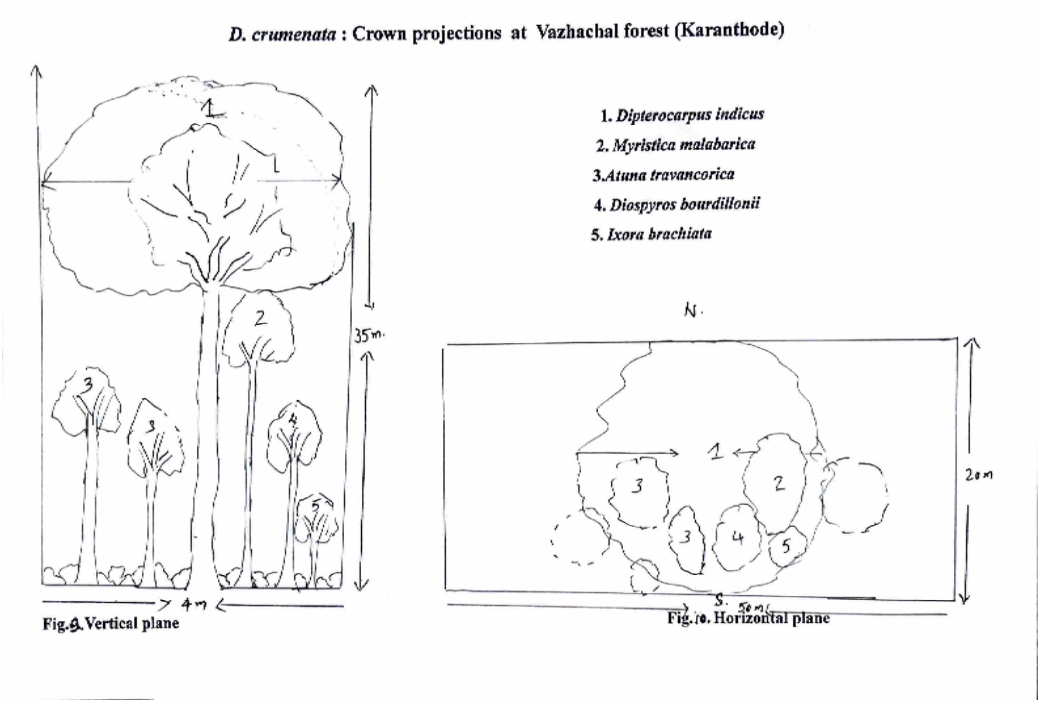


Figure 7. *Diospyros crumenata*: Vegetation profile (Horizontal) at Vazhachal forest (Karanthode)



Figure 8. *Diospyros crumenata*: Age -wise distribution profile at Vazhachal forest (Karanthode)



Figures 9 &10 *Diospyros crumenata*: Crown projections at Vazhachal forest (Karanthode (Fig.9. Vertical plane; Fig.10. Horizontal plane)

Table 3. *Diospyros crumenata*: Population diversity at Vazhachal/ Karanthode
(List of individuals with GBH≥ 30 cm)

Sl. No.	Species	Family	Relative density (rd)	Relative frequency (rf)	Relative Basal Area (rD)	IVI
1	*Dipterocarpus indicus*	Dipterocarpaceae	7.3134	2.625	10.1548	20.0932
2	*Prioria pinnata*	Fabaceae	6.1728	2.6262	8.537	17.336
3	*Vateria indica*	Dipterocarpaceae	6.7427	2.6263	7.3719	16.7409
4	*Atuna travancorica*	Chrysobalanaceae	7.4074	3.0303	3.1747	13.6124
5	*Litsea floribunda*	Lauraceae	3.1339	3.0303	3.9477	10.1119
6	*Syzygium gardneri*	Myrtaceae	1.9943	3.0303	4.7091	9.7337
7	*Sageraea thwaitesii*	Annonaceae	3.4188	1.8182	3.6072	8.8442
8	*Syzygium* sp.	Myrtaceae	3.7037	3.0303	1.4052	8.1392
9	*Vitex altissima*	Verbenaceae	2.2792	2.4242	3.4185	8.1219
10	*Diospyros bourdillonii*	Ebenaceae	2.849	2.4242	1.8366	7.1098
11	*Calophyllum polyanthum*	Calophyllaceae	2.849	2.4242	1.7239	6.9971
12	*Drypetes confertiflora*	Euphorbiaceae	1.7094	1.8182	3.1418	6.6694
13	*Palaquium ellipticum*	Sapotaceae	1.4245	1.8182	3.0255	6.2682

14	*Aglaia* sp.	Meliaceae	1.9943	2.4242	1.7187	6.1372
15	*Myristica malabarica*	Myristicaceae	1.9943	2.4242	1.6271	6.0456
16	*Aglaia malabarica*	Meliaceae	1.9943	2.4242	1.2614	5.6799
17	*Baccaurea courtallensis*	Phyllanthaceae	2.5641	2.4242	0.2641	5.2524
18	*Tetrameles nudiflora*	Datiscaceae	1.4245	1.2121	2.512	5.1486
19	*Canarium strictum*	Burseraceae	1.1396	0.6061	3.1962	4.9419
20	*Eugenia* sp.	Myrtaceae	1.1396	1.8182	1.6883	4.6461
21	*Aglaia* sp.	Meliaceae	1.1396	1.8182	1.6709	4.6287
22	*Elaeocarpus tuberculatus*	Elaeocarpaceae	1.1396	0.6061	2.5601	4.3058
23	*Knema attenuata*	Myristicaceae	1.4245	1.8182	0.9894	4.2321
24	*Olea dioica*	Oleaceae	1.4245	2.4242	0.3563	4.205
25	*Aglaia* sp.	Meliaceae	1.1396	1.8182	1.145	4.1028
26	*Toona ciliata*	Meliaceae	0.8547	1.2121	2.0167	4.0835
27	*Machilus glaucescens*	Lauraceae	1.9943	1.2121	0.6862	3.8926
28	*Syzygium* sp.	Myrtaceae	1.1396	1.2121	1.4482	3.7999
29	*Holigarna arnottiana*	Anacardiaceae	0.8547	1.2121	1.5471	3.6139
30	*Strombosia ceylanica*	Olacaceae	1.1396	1.2121	1.0778	3.4295
31	*Dysoxylum malabaricum*	Meliaceae	0.5698	1.2121	1.6118	3.3937
32	*Syzygium cumini*	Myrtaceae	0.8547	1.8182	0.6378	3.3107
33	*Diospyros buxifolia*	Ebenaceae	0.8547	1.2121	0.8918	2.9586
34	*Actinodaphne malabarica*	Lauraceae	0.8547	1.2121	0.8591	2.9259
35	*Cinnamomum malabatrum*	Lauraceae	1.1396	1.2121	0.5497	2.9014
36	*Otonephelium stipulaceum*	Sapindaceae	0.5698	1.2121	1.0525	2.8344
37	*Meliocopelunu-ankenda*	Rutaceae	1.1396	1.2121	0.2016	2.5533
38	*Terminalia travancorensis*	Combretaceae	0.5698	1.2121	0.562	2.3439
39	*Cinnamomum verum*	Lauraceae	0.5698	1.2121	0.3572	2.1391
40	*Neolamarckia cadamba*	Rubiaceae	0.2849	0.6061	1.227	2.118
41	***Diospyros crumenata***	**Ebenaceae**	0.5698	0.6061	0.7234	1.8993
42	*Holoptelea integrifolia*	Ulmaceae	0.2849	0.6061	0.8658	1.7568
43	*Cullenia exarillata*	Bombaceae	0.2849	0.6061	0.7853	1.6763
44	*Litsea bourdillonii*	Lauraceae	0.8547	0.6061	0.2048	1.6656
45	*Syzygium laetum*	Myrtaceae	0.8547	0.6061	0.181	1.6418
46	*Garcinia morella*	Clusiaceae	0.2849	0.6061	0.7087	1.5997

47	*Haldina cordifolia*	Rubiaceae	0.2849	0.6061	0.6939	1.5849
48	*Xanthophyllum arnottianum*	Polygalaceae	0.8547	0.6061	0.0963	1.5571
49	*Syzygium hemisphericum*	Myrtaceae	0.5698	0.6061	0.3702	1.5461
50	*Vepris bilocularis*	Rutaceae	0.5698	0.6061	0.3601	1.536
51	*Artocarpus hirsutus*	Moraceae	0.5698	0.6061	0.3292	1.5051
52	*Litsea coriacea*	Lauraceae	0.284	0.606	0.5541	1.4441
53	*Bischofia javanica*	Euphorbiaceae	0.5698	0.6061	0.1747	1.3506
54	*Hydnocarpus alpina*	Flacourtiaceae	0.5698	0.6061	0.1584	1.3343
55	*Terminalia chebula*	Combretaceae	0.5698	0.6061	0.1148	1.2907
56	*Atlantia racemosa*	Rutaceae	0.5698	0.6061	0.1018	1.2777
57	*Hopea parviflora*	Dipterocarpaceae	0.2849	0.6061	0.3848	1.2758
58	*Pterygota alata*	Sterculiaceae	0.2849	0.6061	0.3848	1.2758
59	*Ixora brachiata*	Rubiaceae	0.5698	0.6061	0.073	1.2489
60	*Chukrasia tabularis*	Meliaceae	0.2849	0.6061	0.3578	1.2488
61	*Macaranga peltata*	Euphorbiaceae	0.2849	0.6061	0.2827	1.1737
62	*Myristica malabarica*	Myristicaceae	0.2849	0.6061	0.2596	1.1506
63	*Lagerstroemia microcarpa*	Lythraceae	0.2849	0.6061	0.2463	1.1373
64	*Agrostistachys borneensis*	Euphorbiaceae	0.2849	0.6061	0.2376	1.1286
65	*Hydnocarpus pentandrus*	Flacourtiaceae	0.2849	0.6061	0.2376	1.1286
66	*Syzygium* sp.	Myrtaceae	0.2849	0.6061	0.159	1.05
67	*Pterospermum reticulatum*	Sterculiaceae	0.2849	0.6061	0.1555	1.0465
68	*Drypetes malabarica*	Euphorbiaceae	0.2849	0.6061	0.1555	1.0465
69	*Polyalthia fragrans*	Annonaceae	0.2849	0.6061	0.1385	1.0295
70	*Mesua ferrea*	Clusiaceae	0.2849	0.6061	0.1385	1.0295
71	*Mallotus atrovirens*	Euphorbiaceae	0.2849	0.6061	0.1075	0.9985
72	*Sapindus trifoliatus*	Sapindaceae	0.2849	0.6061	0.0638	0.9548
73	*Alseodaphne semecarpifolia*	Lauraceae	0.2849	0.6061	0.0572	0.9482
74	*Humboldtia vahliana*	Fabaceae	0.2849	0.6061	0.0551	0.9461
75	*Schleichera oleosa*	Sapindaceae	0.2849	0.6061	0.0531	0.9441
76	*Aporosa cardiosperma*	Euphorbiaceae	0.2849	0.6061	0.0511	0.9421
77	*Antiaris toxicaria*	Moraceae	0.2849	0.6061	0.0398	0.9308
78	*Gymnocranthera canarica*	Myristicaceae	0.2849	0.6061	0.0363	0.9273

79	*Orophea erythrocarpa*	Annonaceae	0.2849	0.6061	0.0314	0.9224
80	*Garcinia spicata*	Clusiaceae	0.2849	0.6061	0.0314	0.9224
81	*Croton malabaricus*	Euphorbiaceae	0.2849	0.6061	0.0241	0.9151
82	*Garcinia gummi-gutta* var. *gummi-gutta*	Clusiaceae	0.2849	0.6061	0.0214	0.9124
83	*Dalbergia latifolia*	Fabaceae	0.2849	0.6061	0.0154	0.9064
84	*Reinwardtiodendron anamalaiense*	Meliaceae	0.2849	0.6061	0.0064	0.8974
85	*Dysoxylum beddomei*	Meliaceae	0.2849	0.6061	0.0024	0.8934
			100	100	100	300

4.1.2.3. Population structure at Chakkapara forest

Population/ individuals were found distributed in a semi-evergreen to moist deciduous forest at an altitude between 349–431 m asl. A total of 10 mature trees of *D. crumenata* were recorded in this forest area. The trees are small to medium-sized and attain a height range of 6–17 m. The trees are 63–181 cm in GBH (Fig. 11), five of which are in reproductive age classes. The five pre-reproductive individuals fall between 63 and 95 cm GBH. The particular forest area recorded nearly 15 saplings and 5 established seedlings. All the mature trees showed short bole length, and the first branching was observed from 2–5 height (Table 4). Trees showed blackish-grey coloured, moderately rough bark along with lichen associations similar to the Vazhachal- Poringalkuth populations. The major associated tree species recorded in the population areas, such as *Tetrameles nudiflora* R. Br., *Toona ciliata* Roem., *Persea macrantha,* (Nees) Kosterm., *Holigarna arnottiana* Hook.f., *Dysoxylum malabaricum* Bedd. ex Hiern, *Polyalthia fragrans* (Dalz.) Bedd., and *Hydnocarpus pentandrus* (Buch. -Ham.) Oken



Figure 11. *Diospyros crumenata*: Total individuals along with the Girth class at Chakkapara

Table 4. *Diospyros crumenata*: Population structure within the sampled and non-sampled area at Chakkapara (List of individuals with GBH≥ 30 cm)

Sl. No.	GBH (cm)	Basal Area (cm^2)	Basal Cover (m)	Age phase	Height of Tree (m)
1	63	316.00	2.5	Set of future	06
2	77	472.05	1.5	Set of future	06
3	81	522.37	2.0	Set of future	06
4	93	688.61	3.0	Set of future	09
5	95	718.55	2.5	Set of future	13
6	106	894.58	3.0	Set of present	16
7	140	1560.51	3.5	Set of present	14
8	140	1560.51	3.0	Set of present	16
9	150	1791.40	4.0	Set of present	17
10	181	2608.36	05	Set of present	10

Stratification/ Vertical distribution

The vertical vegetation profile of the Chakkapara area reveals a stratified forest structure with distinct tree layers. The uppermost layer (first storey), reaching heights of 16 to 21 meters, is dominated by major tree species such as *Tetrameles nudiflora* R.

Br., *Dysoxylum malabaricum* Bedd. ex Hiern, *Hopea parviflora* Bedd., and *Diospyros crumenata* Thw. The second storey, with trees ranging from 8 to 12 meters in height, includes species like *Elaeocarpus tuberculatus* Roxb., *Toona ciliata* Roem., *Aglaia lawii* (Wight) Saldanha, and *Schleichera oleosa* (Lour.) Oken. The third and lowest layer, with a height range of 4 to 7 meters, is comprised of species such as *Otonephelium stipulaceum* (Bedd.) Radlk., *Strombosia ceylanica* Gard., and *Ixora brachiata* Roxb. ex-DC., with a height range from 4 to 7 m. The shrubs/seedlings of small trees/ herbs of various species include *Persea macrantha* (Nees) Kosterm., *Holigarna arnottiana* Hook. f., *Polyalthia fragrans* (Dalz.) Bedd., *Atlantia monophyla* (L)., *Macranga peltata* (Roxb.) Muell. -Arg., *Aporosa cardiosperma* Gaertn.) Merr., *Calamus* sp., *Mallotus* sp., *Pandanus amaryllifolius* Roxb., *Thottea* sp., *Smilax zeylanica* L., etc. (Fig.12).

Horizontal/ Spatial distribution

The spatial view of the population site exhibited the arrangement of individuals of *Diospyros crumenata* in a scattered manner along with their associates in a moist deciduous forest patch. The individuals of the species were seen on either side of the forest track. A perennial water source is drained into an extreme down surrounded by a semi-evergreen forest (Fig.13).

Age-wise distribution profile

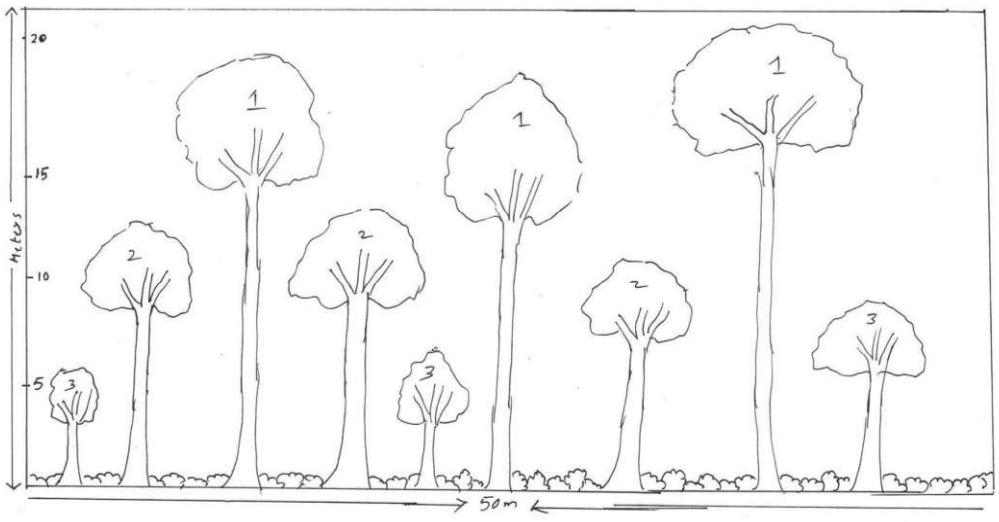
The age-wise distribution profile has shown ten individuals in a 5000 m^2 area (entire five plots), of which five individuals each constitute a set of present (Reproductive class) and set of future (Pre-reproductive class), covering a height range from 6-17m (Fig.14).

Crown projections

The vertical crown projections (Fig. 15) show the positioning of individuals excluding *Diospyros crumenata,* such as *Toona ciliata*, *Schleichera oleosa*, *Otonephelium stipulaceum*, and *Strombosia ceylanica,* situated just beneath the tallest canopy-forming tree, *Tetrameles nudiflora*. The horizontal crown projections (Fig.16) depict the overlapping crown coverages of these species beneath the dominant *Tetrameles nudiflora*.

4.1.2.4. Population diversity analysis at Chakkapara

The analysis of population diversity included 42 species with a girth at breast height (GBH) of ≥30 cm, totalling 210 individuals within a 5000 sq.m. The combined values of relative frequency (rf), relative density (rd), and relative dominance (rD) were calculated for each species across the quadrats. Among these, *Tetrameles nudiflora* recorded the highest Importance Value Index (IVI) of 25.5462, indicating it as the dominant species in the studied forest. In contrast, *Diospyros crumenata* ranked 26th with an IVI of 4.2818 (Table 5).



1st Layer –*Tetrameles nudiflora,Dysoxylum malabaricum, Hopea parviflora, Diospyros crumenata*

2nd Layer -*Elaeocarpus tuberculatus, Toona ciliata, Aglaia lawii, Schleichera oleosa*

3rd Layer – *Otonephelium stipulaceum, Strombosia ceylanica, Ixora brachiata*

Figure 12. *Diospyros crumenata*: Vegetation profile (Vertical) at Chakkapara forest

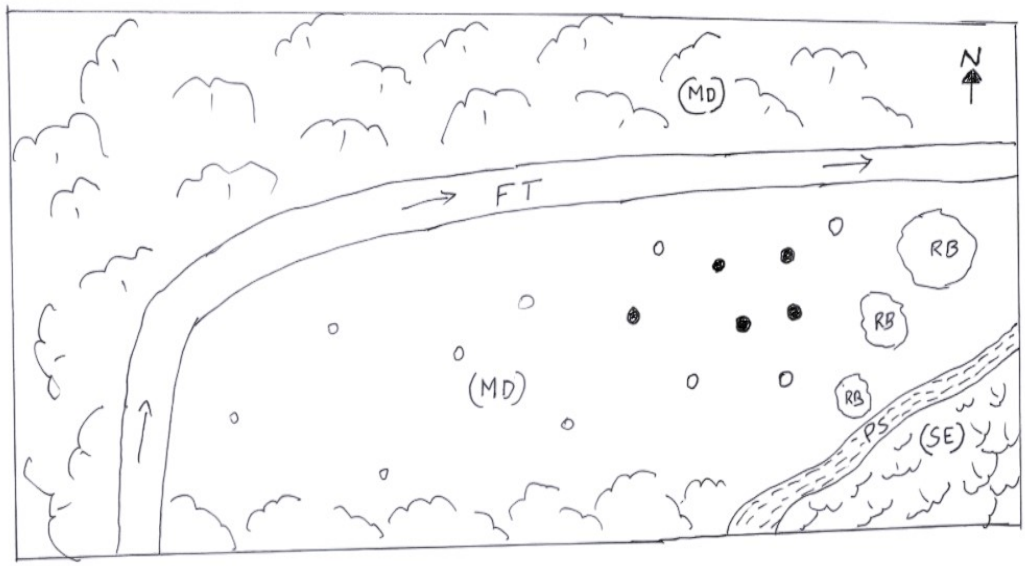
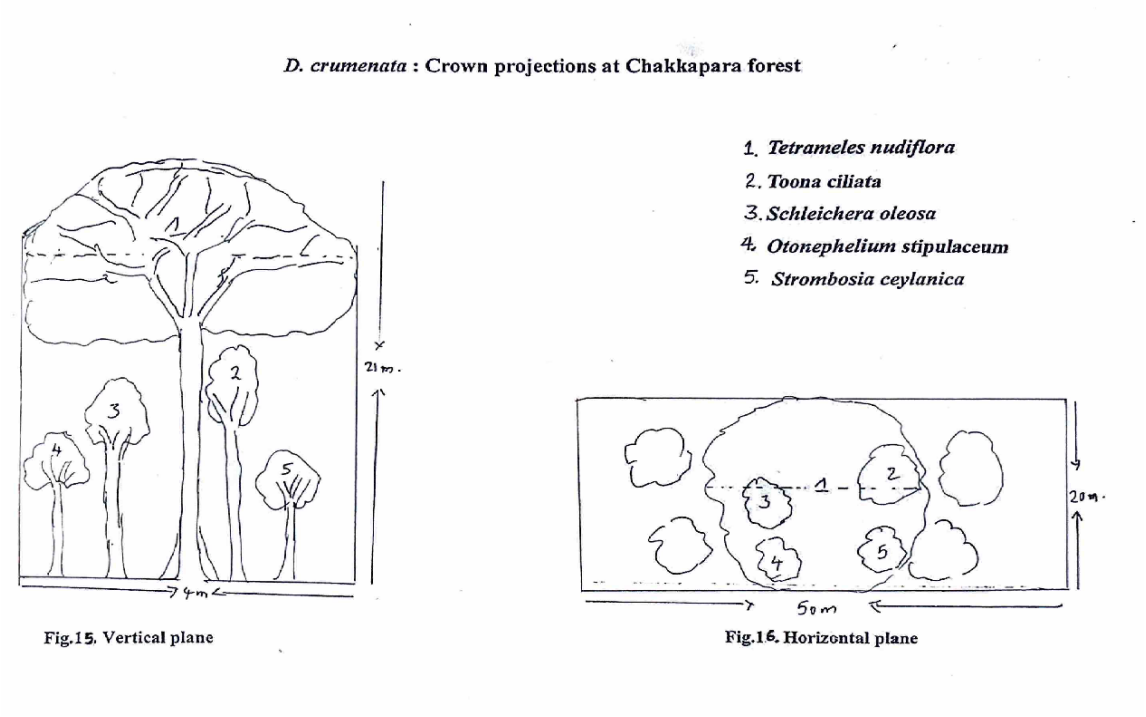


Figure 13. *Diospyros crumenata*: Vegetation profile (Horizontal) at Chakkapara forest



Figure 14. *Diospyros crumenata*: Age-wise distribution profile at Chakkapara forest



Figures 15 &16. *Diospyros crumenata*: Crown projections at Chakkapara forest (Fig.15. Vertical plane; Fig.16. Horizontal plane)

Table 5. *Diospyros crumenata*: Population diversity at Chakkapara (List of individuals with GBH≥ 30 cm)

Sl. No.	Species	Family	Relative Density (rd)	Relative Frequency (rf)	Relative Basal Area (rD)	IVI
1	*Tetrameles nudiflora*	Tetramelaceae	9.7667	6.6668	9.1127	25.5462
2	*Cedrela toona*	Meliaceae	5.9451	6.6668	7.5133	20.1252
3	*Hydnocarpus pentandrus*	Achariaceae	6.3694	5.2632	6.4679	18.1005
4	*Vitex altissima*	Lamiaceae	5.0956	5.2632	6.2636	16.6224
5	*Polyalthia fragrans*	Annonaceae	6.582	5.6141	2.9672	15.1633
6	*Knema attenuata*	Myristicaceae	5.0955	4.2105	2.617	11.923
7	*Baccurea courtalensis*	Phyllanthaceae	5.7325	4.2105	1.1242	11.0672
8	*Dysoxylum malabaricum*	Meliaceae	3.1847	4.2105	3.3502	10.7454
9	*Reinwardtiodendron anamalaiense*	Meliaceae	3.8217	2.1053	4.7936	10.7206
10	*Aglaia lawii*	Meliaceae	3.1847	4.2105	2.2281	9.6233
11	*Otonephelium stipulaceum*	Sapindaceae	4.4586	2.1053	2.469	9.0329
12	*Elaeocarpus tuberculatus*	Elaeocarpaceae	1.9108	2.1053	4.8677	8.8838
13	*Garcinia morella*	Clusiaceae	3.8217	3.1579	1.7296	8.7092
14	*Persea macrantha*	Lauraceae	3.1847	2.1053	2.9241	8.2141

15	*Lagerstroemia microcarpa*	Lythraceae	2.5478	3.1579	2.2239	7.9296
16	*Olea dioica*	Oleaceae	3.1847	3.1579	1.3582	7.7008
17	*Strombosia ceylanica*	Olacaceae	2.5478	3.1579	1.6916	7.3973
18	*Aporosa cardiosperma*	Phyllanthaceae	3.1847	3.1579	0.969	7.3116
19	*Grewia tilifolia*	Malvaceae	1.9108	2.1053	3.2322	7.2483
20	*Ixora brachiata*	Rubiaceae	1.9108	2.1053	1.588	5.6041
21	*Hopea parviflora*	Dipterocarpaceae	1.2739	1.0526	2.9998	5.3263
22	*Xanthophyllum arnottianum*	Polygalaceae	1.9108	2.1053	1.0371	5.0532
23	*Artocarpus hirsutus*	Moraceae	1.2739	2.1053	1.6652	5.0444
24	*Gmelina arborea*	Lamiaceae	0.6369	1.0526	3.0201	4.7096
25	*Syzygium gardeneri*	Myrtaceae	0.6369	1.0526	2.6764	4.3659
26	***Diospyros crumenata***	**Ebenaceae**	0.6369	1.0526	2.5923	4.2818
27	*Myristica malabarica*	Myristicaceae	0.6369	1.0526	2.5541	4.2436
28	*Clausena indica*	Rutaceae	0.6369	1.0526	2.4722	4.1617
29	*Zanthoxylum rhetsa*	Rutaceae	0.6369	1.0526	2.3635	4.053
30	*Schleichera oleosa*	Sapindaceae	0.6369	1.0526	1.8972	3.5867
31	*Sterculia guttata*	Malvaceae	0.6369	1.0526	1.4938	3.1833
32	*Messua ferrea* var. *ferrea*	Clusiaceae	0.6369	1.0526	1.2839	2.9734
33	*Dalbergia latifolia*	Fabaceae	0.6369	1.0526	1.1166	2.8061
34	*Poeciloneuron indicum*	Calophyllaceae	0.6369	1.0526	0.6433	2.3328
35	*Macaranga peltata*	Euphorbiaceae	0.6369	1.0526	0.5359	2.2254
36	*Aglaia barberi*	Meliaceae	0.6369	1.0526	0.5083	2.1978
37	*Walsura trifolia*	Meliaceae	0.6369	1.0526	0.5083	2.1978
38	*Archidendron* sp.	Fabaceae	0.6369	1.0526	0.4052	2.0947
39	*Holigarna arnottiana*	Anacardiaceae	0.6369	1.0526	0.3581	2.0476
40	*Atlantia monophylla*	Rutaceae	0.6369	1.0526	0.166	1.8555
41	*Holarrhena pubescens*	Apocynaceae	0.6369	1.0526	0.1182	1.8077
42	*Wrightia tinctoria*	Apocynaceae	0.6369	1.0526	0.0934	1.7829
			100	100	100	300

4.1.2.5. Population structure at Vellanimala

Population/ individuals were found distributed in a semi-evergreen forest at Vellani mala (Vellanipacha- Vellarikulam areas), at an altitude between 312–506 m asl. A total of 30 adult trees and 5 saplings were enumerated from this forest area. The medium to large-sized trees recorded from 13 to 25m in height (Table 6). The GBH ranged from 84–356 cm (Fig. 17). Trees showed blackish-grey coloured, moderately rough bark along with lichen associations similar to the other two populations. The major associated tree species recorded in the population areas, such as *Prioria pinnata*

(Roxb. ex-DC.) Breteler, *Aglaia lawii* (Wight) Saldanha, *Persea macrantha* (Nees) Kosterm., *Alseodaphne semecarpifolia* Nees., *Hydnocarpus alpina* Wight, *Dimocarpus longan* Lour., *Syzygium chavaran* (Bourd.) Gamble, *Polyalthia fragrans* (Dalz.) Bedd., *Tetrameles nudiflora* R. Br., and *Toona ciliata* Roem.

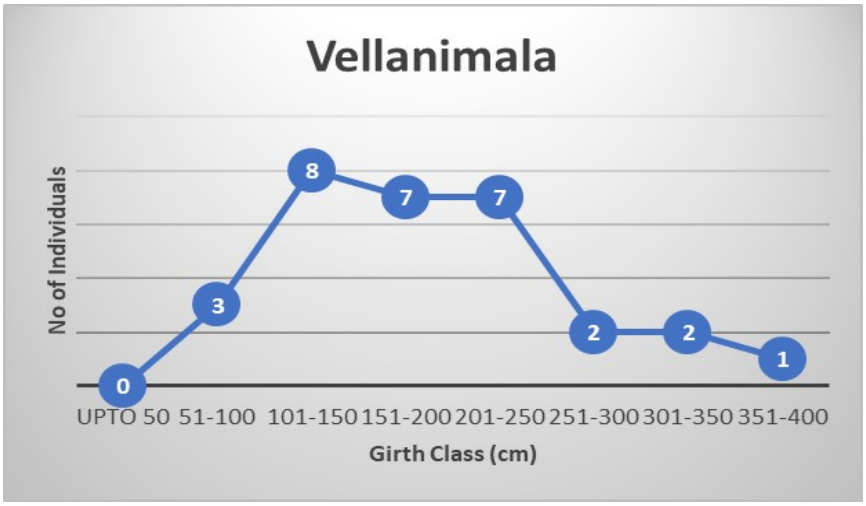


Figure 17. *Diospyros crumenata*: Total individuals along with the Girth class at Vellanimala

Table 6. *Diospyros crumenata*: Population structure within the sampled and non-sampled area at Vellanimala (List of individuals with GBH≥ 30 cm)

Sl. No.	GBH (cm)	Basal Area (cm^2)	Basal Cover (m)	Age phase	Height of tree (m)
1	84	561.78	2.0	Set of future	16
2	87	602.63	2.0	Set of future	13
3	97	749.12	2.5	Set of future	15
4	105	877.79	3.5	Set of present	14
5	107	911.54	4.0	Set of present	14
6	108	928.66	3.5	Set of present	13
7	110	963.38	3.0	Set of present	16

8	140	1560.51	3.0	Set of present	14
9	133	1408.36	3.5	Set of present	15
10	147	1720.46	4.0	Set of present	14
11	121	1165.68	4.0	Set of present	16
12	151	1815.37	4.0	Set of present	15
13	152	1839.49	4.0	Set of present	13
14	189	2844.03	4.5	Set of present	14
15	170	2300.96	5.0	Set of present	16
16	170	2300.96	4.5	Set of present	20
17	153	1863.77	4.0	Set of present	12
18	167	2220.46	3.5	Set of present	18
19	207	3411.54	5.0	Set of present	21
20	210	3511.15	4.5	Set of present	18
21	220	3853.50	4.5	Set of present	20
22	210	3511.15	4.0	Set of present	22
23	230	4211.78	4.5	Set of present	24
24	210	3511.15	4.5	Set of present	20
25	244	4740.13	5.0	Set of present	17
26	260	5382.17	5.0	Set of present	25
27	278	6153.18	4.5	Set of present	20
28	310	7651.27	5.0	Set of present	17
29	334	8881.85	5.5	Set of present	23
30	356	10090.45	5.5	Set of present	18

Stratification/ Vertical distribution

The vertical vegetation profile of the Vellanimala forest reveals a distinct stratification of tree species across three storeys. The uppermost layer, or first storey, includes major tree species such as *Prioria pinnata* (Roxb. ex DC.) Breteler, *Pterospermum reticulatum* Wight & Arn., *Dysoxylum malabaricum* Bedd. ex Hiern, and *Diospyros crumenata* Thw., reaching heights of 25 to 30 meters. The second storey comprises species such as *Aglaia lawii* (Wight) Saldanha, *Diospyros bourdillonii* Brandis, *Hydnocarpus pentandrus* (Buch. -Ham.) Oken, and *Dimocarpus longan* Lour., with a height range of 20 to 25 meters. The third and lowest storey features species such as *Xanthophyllum arnottianum* Wight, *Ixora brachiata* Roxb. Ex DC., *Baccaurea courtallensis* (Wight) Muell. -Arg., and *Cinnamomum malabatrum* (Burm. f.) Blume, grows between 10 and 15 meters in height. The shrubs/seedlings of small trees/ herbs of various species include *Aporosa cardiosperma* (Gaertn.) Merr., *Jasminum multiflorum* (Burm. f.) Andr., *Calamus* sp.*, Rauvolfia tetraphylla* L., *Leea indica* (Burm. f.) Merr.*, Smilax zeylanica* L.*, Glycosmis pentaphylla* (Retz.) DC.*, Macranga peltata* (Roxb.) Muell. -Arg.*, Myxopyrum smilacifolium,* (Wall.) Blume, *Litsea* sp., *Wrightia tinctoria* (Roxb.) R. Br. etc. (Fig.18).

Horizontal/ Spatial distribution

The spatial distribution of the population site revealed that individuals of *Diospyros crumenata* were scattered, along with their associated species, near the watercourse within semi-evergreen and evergreen forests flanking the steep ascending forest trail. Big rock boulders are the characteristic feature of the habitat. Annual or perennial water areas are microhabitats at the peak of the hill (Fig.19).

Age-wise distribution profile

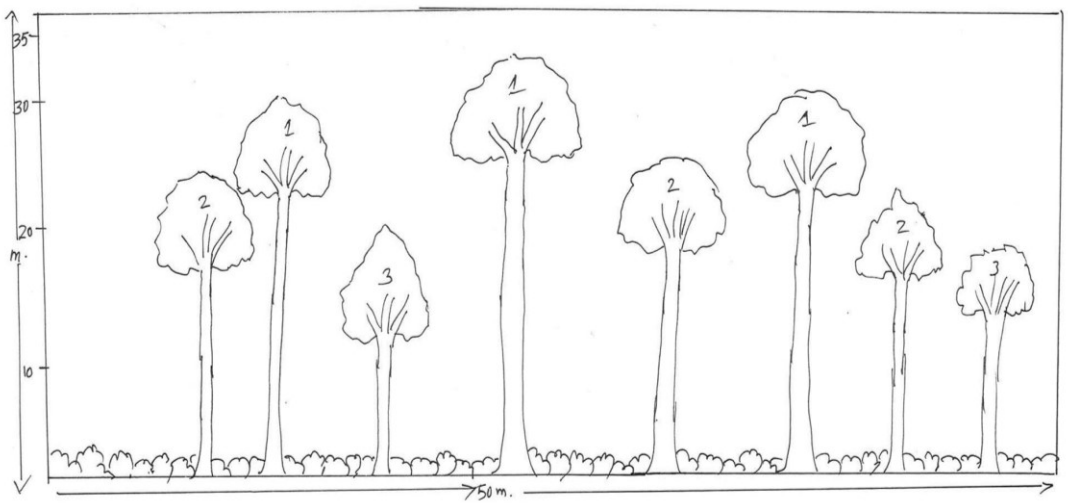
The age-wise distribution profile has shown thirty individuals in a 5000 m^2 area (entire five plots) of which twenty-seven individuals constitute a set of present (Reproductive class) and three individuals constitute as set of future (Pre-reproductive class), covering a height range from 13-27m (Fig.20).

Crown projections

The vertical crown projections show the placement of individuals excluding *Diospyros crumenata* such as *Hydnocarpus pentandrus, Xanthophyllum arnottianum, Ixora brachiata* and *Cinnamomum malabatrum* just below the tallest individual (canopy) of *Pterospermum reticulatum* (Fig.21). The horizontal crown projections showed the overlapping crown coverages of these species under the canopy of the tallest individual, *Pterospermum reticulatum* (Fig.22).

4.1.2.6. Population diversity analysis at Vellanimala

The analysis of population diversity (floristic diversity) included 59 species with a girth at breast height (GBH) of ≥ 30 cm, comprising a total of 240 individuals within an area of 5000 sq.m. The aggregated values of relative frequency (rf), relative density (rd), and relative dominance (rD) were calculated for each species across the quadrats. Among them, *Pterospermum reticulatum* recorded the highest Importance Value Index (IVI) of 20.07277, making it the dominant species in the studied forest. In contrast, *Diospyros crumenata* ranked 9th, with an IVI of 8.2055 (Table 7).

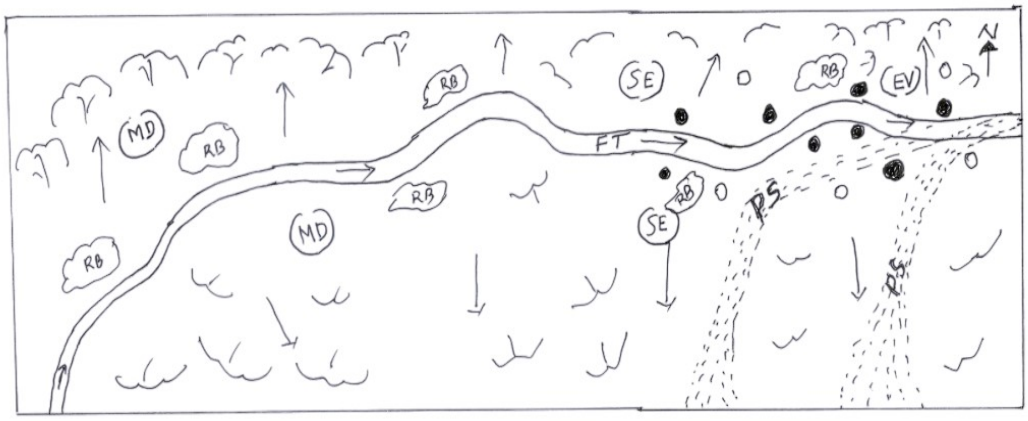


1st Layer –*Prioria pinnata, Pterospermum reticulatum, Dysoxylum malabaricum,* Diospyros crumenata

2nd Layer - *Aglaia lawii, Diospyros bourdillonii, Hydnocarpus pentandrus, Dimocarpus longan*

3rd Layer – *Xanthophyllum arnottianum, Ixora brachiata, Baccaurea courtallensis, Cinnamomum malabatrum*

Figure18. *Diospyros crumenata*: Vegetation profile (Vertical) at Vellanimala



- Candidate species
- Associated species

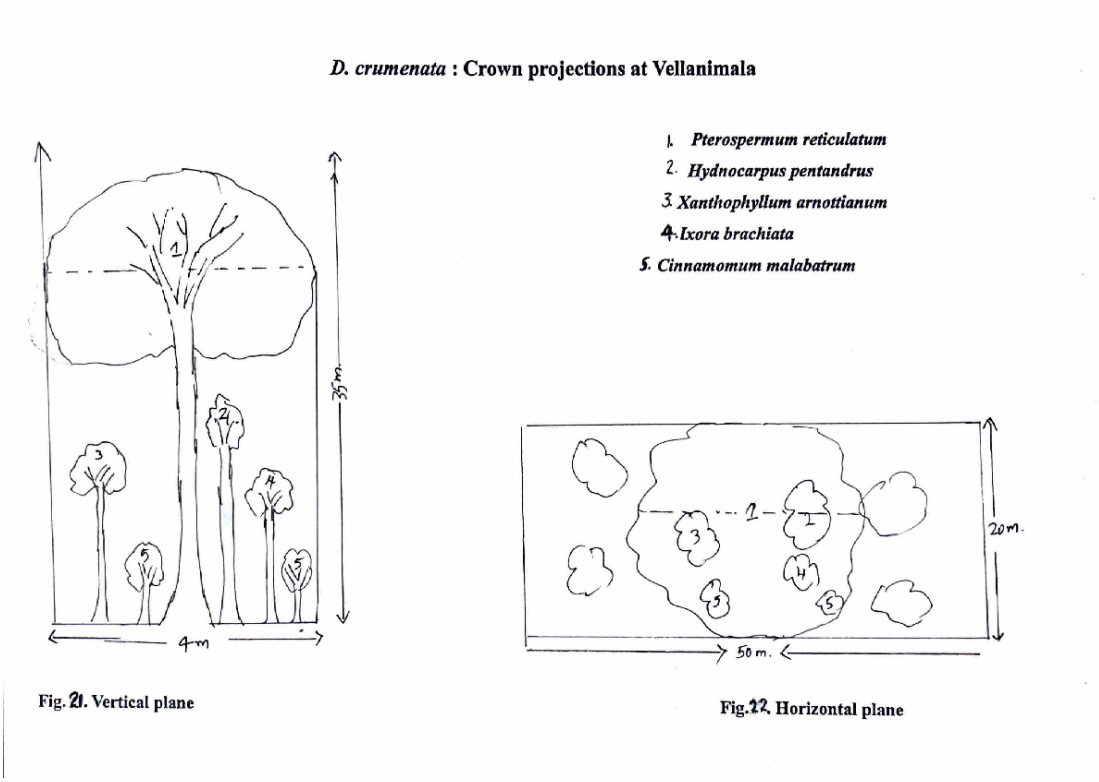
SE- Semi-Evergreen
MD- Moist Deciduous
EV Evergreen

RB- Rock Boulders
FT - Forest Track
PS- Perennial Stream
Upward slope
Downward slope

Figure 19. *Diospyros crumenata*: Vegetation profile (Horizontal) at Vellanimala



Figure 20. *Diospyros crumenata*: Age-wise distribution profile at Vellanimala



Figures 21 & 22. *Diospyros crumenata*: Crown projections at Vellanimala (Figure 21. Vertical plane; Figure 22. Horizontal plane)

Table 7. *Diospyros crumenata*: Population diversity at Vellanimala (List of individuals with GBH≥ 30 cm)

Sl. No.	Species	Family	Relative Density (rd)	Relative Frequency (rf)	Relative Basal area (rD)	IVI
1	*Pterospermum reticulatum*	Polygalaceae	9.40167	5.449	5.2221	20.07277
2	*Spondias pinnata*	Anacardiaceae	1.70937	2.5644	13.1888	17.46257
3	*Artocarpus hirsutus*	Moraceae	2.35037	3.5259	11.4604	17.33667
4	*Xanthophyllum arnottianum*	Sterculiaceae	1.9231	1.9231	9.5289	13.3751
5	*Aglaia lawii*	Meliaceae	5.1282	4.8077	1.7823	11.7182
6	*Prioria pinnata*	Papilionaceae	5.7692	3.8462	0.9163	10.5317
7	*Melia dubia*	Meliaceae	0.641	0.9615	7.939	9.5415
8	*Dimocarpus longan*	Sapindaceae	4.4872	2.8846	1.4126	8.7844
9	***Diospyros crumenata***	**Ebenaceae**	1.9231	2.8846	3.3978	8.2055
10	*Toona ciliata*	Meliaceae	1.2821	1.9231	4.9117	8.1169
11	*Aporosa lindleyana*	Euphorbiacea	3.8462	1.9231	2.2289	7.9982
12	*Macaranga peltata*	Euphorbiacea	3.2051	3.8462	0.8166	7.8679
13	*Terminalia bellirica*	Combretaceae	1.2821	0.9615	4.6881	6.9317
14	*Hydnocarpus alpina*	Flacourtiaceae	3.2051	2.8846	0.7281	6.8178
15	*Hydnocarpus pentandrus*	Flacourtiaceae	1.9231	0.9615	3.7784	6.663
16	*Syzygium chavaran*	Myrtaceae	1.9231	1.9231	2.5786	6.4248
17	*Drypetes oblongifolia*	Euphorbiaceae	3.2051	2.8846	0.3135	6.4032
18	*Ixora brachiata*	Rubiaceae	2.5641	2.8846	0.8782	6.3269
19	*Sterculia urens*	Sterculiaceae	3.2051	1.9231	0.4694	5.5976
20	*Diospyros bourdillonii*	Ebenaceae	1.9231	1.9231	1.7214	5.5676
21	*Holigarna arnottiana*	Anacardiaceae	1.9231	1.9231	1.6636	5.5098
22	*Nothapodytes nimmoniana*	Icacinaceae	1.9231	1.9231	0.9227	4.7689
23	*Lepisanthes tetraphylla*	Sapindaceae	1.9231	1.9231	0.9183	4.7645
24	*Madhuca* sp.	Sapotaceae	1.2821	1.9231	1.1714	4.3766
25	*Polyalthia fragrans*	Annonaceae	2.5641	0.9615	0.594	4.1196
26	*Dillenia pentagyna*	Dilleniaceae	1.2821	0.9615	1.8083	4.0519
27	*Aglaia elaeagnoidea*	Meliaceae	1.2821	1.9231	0.7651	3.9703
28	*Xylia xylocarpa*	Fabaceae	0.641	0.9615	2.0828	3.6853
29	*Lagerstroemia microcarpa*	Lythraceae	1.2821	1.9231	0.4582	3.6634
30	*Persea macrantha*	Lauraceae	1.2821	1.9231	0.4179	3.6231
31	*Mallotus philippensis*	Euphorbiaceae	0.641	0.9615	1.9271	3.5296
32	*Diospyros paniculata*	Ebenaceae	1.2821	1.9231	0.2271	3.4323
33	*Vitex altissima*	Verbenaceae	1.2821	1.9231	0.2137	3.4189
34	*Canthium travancoricum*	Rubiaceae	1.2821	1.9231	0.1157	3.3209
35	*Wrightia tinctoria*	Apocynaceae	1.9231	0.9615	0.348	3.2326
36	*Aglaia* sp.	Meliaceae	0.641	0.9615	1.4964	3.0989

37	*Dipterocarpus indicus*	Dipterocarpaceae	0.641	0.9615	1.0626	2.6651
38	*Sapindus trifoliatus*	Sapindaceae	1.2821	0.9615	0.177	2.4206
39	*Ormosia travancorica*	Fabaceae	0.641	0.9615	0.7982	2.4007
40	*Strychnos nux-vomica*	Loganiaceae	0.641	0.9615	0.7982	2.4007
41	*Mallotus* sp.	Euphorbiaceae	1.2821	0.9615	0.1297	2.3733
42	*Aporusa lindleyana*	Euphorbiaceae	1.2821	0.9615	0.103	2.3466
43	*Grewia tiliifolia*	Tiliaceae	0.641	0.9615	0.5715	2.174
44	*Strombosia ceylanica*	Olacaceae	0.641	0.9615	0.5715	2.174
45	*Archidendron bigeminum*	Fabaceae	0.641	0.9615	0.4914	2.0939
46	*Harpullia arborea*	Sapindaceae	0.641	0.9615	0.4723	2.0748
47	*Diospyros* sp.	Ebenaceae	0.641	0.9615	0.3825	1.985
48	*Alseodaphne semecarpifolia*	Lauraceae	0.641	0.9615	0.2448	1.8473
49	*Miliusa tomentosa*	Annonaceae	0.641	0.9615	0.2314	1.8339
50	*Schleichera oleosa*	Sapindaceae	0.641	0.9615	0.2314	1.8339
51	*Dysoxylum malabaricum*	Meliaceae	0.641	0.9615	0.1181	1.7206
52	*Baccaurea courtallensis*	Euphorbiaceae	0.641	0.9615	0.0833	1.6858
53	*Cinnamomum malabatrum*	Lauraceae	0.641	0.9615	0.0756	1.6781
54	*Ficus nervosa*	Moraceae	0.641	0.9615	0.0756	1.6781
55	*Litsea* sp.	Lauraceae	0.641	0.9615	0.0756	1.6781
56	*Sterculia guttata*	Sterculiaceae	0.641	0.9615	0.0756	1.6781
57	*Alangium salvifolium*	Alangiaceae	0.641	0.9615	0.0484	1.6509
58	*Psydrax dicoccos*	Rubiaceae	0.641	0.9615	0.0484	1.6509
59	*Stereospermum colais*	Bignoniaceae	0.641	0.9615	0.0425	1.645
			100	100	100	300

4.1.2.7. Chimmony WLS

Four scattered individuals were found distributed in a semi-evergreen forest at an altitude between 66–246 m asl. These medium to large-sized trees were recorded at 15–21m height. The GBH ranged from 73–158cm (Fig. 23). No seedlings/ saplings were recorded from the forest area. The major associated tree species recorded, such as *Hopea parviflora, Polyalthia fragrans* (Dalz.) Bedd., *Myristica malabarica* Lam., *Knema attenuata* (Hook. f. & Thoms.) Warb., *Diospyros* sp., *Baccaurrea courtallensis* (Wight) Muell. -Arg., *Xanthophyllum arnottianum* Wight, *Ixora brachiata* Roxb. ex-DC., *Bombax* sp., *Cinnamomum* sp., *Vitex altissima* L.f., *Memycelon* sp., and *Agrostachys* sp.

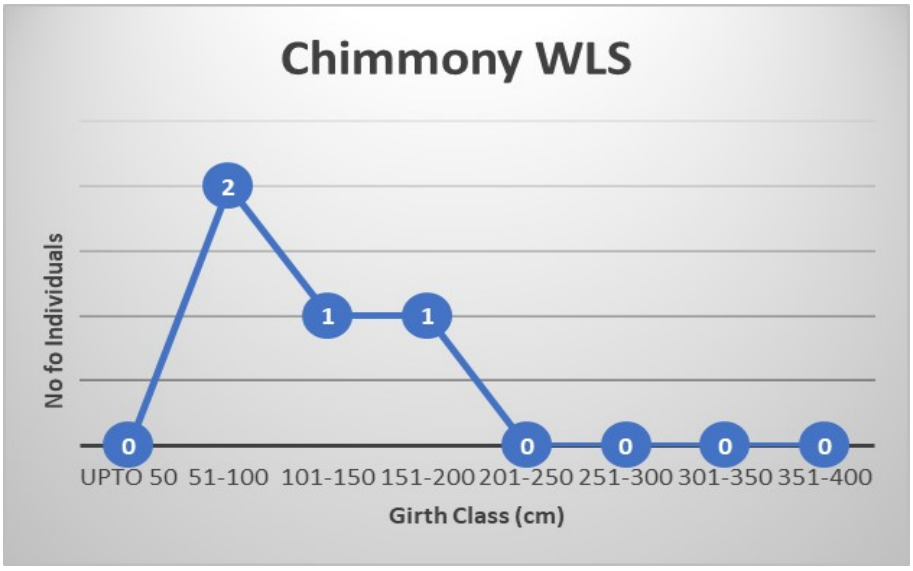


Figure. 23. Total individuals along with the Girth class at Chimmony WLS

Plate 1. *Diospyros crumenata*: Population Structure



A. & B. Habit ; C. Buttressing nature of the trunk ; D. E. F. Views of Habitats: D. Karanthode ; E. Chakkapara ; F. Chimmony WLS ; G. Closer view of bark; H. Blaze; I & J : View of population data collection

Population threats

Among the populations studied, at Vazhachal forests, the individuals located in Irumbupalam areas are prone to habitat loss due to the Chalakudy- Malakkapara road widening in the near future and man-made reasons such as forest fire. The habitats of the Powerhouse area are threatened due to the developmental activities of the power-generating plant. The Vellanimala location is found to be prone to forest fires. The lack of watercourses in the semi-evergreen/ moist deciduous population areas was found to be affecting the microhabitat at the Chakkapara study site.

4.1.3. Population Dynamics

4.1.3.1. Vegetative phenology

Both male and female trees begin to flush with rosy, pale green leaves, along with mature leaves during April-May months (Fig.24). Young leaves appear on new shoots arising from the axils of old terminal branchlets. These leaves gradually turn light green and eventually dark green. Both young and mature leaves are frequently fed upon by caterpillars and grasshoppers. The pupa is grass-green, and the adult moth is identified as *Arctornis marginata* Moore (Erebidae: Lymantriinae). This represents the first host record for *A. marginata.* The saplings with mature leaves are often fed upon by grasshoppers identified as *Xenocatantops humilis* (Acrididae: Catantopinae) (Plate 2).

4.1.3.2. Reproductive phenology

The species is dioecious, with male and female individuals occurring on separate plants. Based on observations of all mature trees during the flowering and fruiting phases across populations, female trees appeared to be slightly more dominant. An irregular flowering pattern was recorded for the species. Typically, male and female trees flower at the same time; however, the blooming period in male

and female trees is shorter. In some years, female trees do not produce flowers at all, but in such cases, early flowering is observed in the following year. Male trees, by contrast, flower consistently every year. Male flower bud initiation was normally observed along with flushing from the last week of April, and peak flowering was observed at the end of May and extended up to June (Fig.24). The male flowers emerge from the nodes of newly developed young shoots and are arranged in cymose inflorescences. The sepals are fused, four-lobed, and greenish. Each young shoot contains 2-4 cymes in a branched pattern, with one cyme containing 6-9 buds. Consequently, a young shoot has about 16 flowers, and a tertiary twig has been observed to contain 256 flower buds. During peak flowering, a single tree produces thousands of male flowers. It takes 40-45 days for a bud to bloom fully, with anthesis typically occurring at night. The blooming duration of a single flower lasts for only one day. A large number of flower buds fall off before blooming. Flower buds were also found to be infested by caterpillars. The tubular corolla has four petals, is creamy-yellow, and exhibits twisted aestivation. When fully open, the corolla spreads over the calyx (Plate 3). The flowers are slightly fragrant. During peak flowering, insects such as *Apis indica* (large honey bee), butterflies and moths are occasionally observed on the tree.

The development of female flower buds and blooming was observed along with mature leaves during June. In certain years, alternate-year flowering was recorded in such situations, an advanced flowering was followed in March (Fig. 24). Mass flowering of trees lasts for a few days only. The flowers are solitary, yellowish-white, and emerge from the axils of shoots. The corolla is tubular with four lobes. Ovary 8-celled; 2 to 6 ovules present in an ovary. Stigma 4 fid.

Plate 2. *Diospyros crumenata*: Vegetative Dynamics



A. Tree in peak flushing; B. C. & D. Stages of leaf development in sapling; E. Leaf infestation; F. Caterpillar; G. Pupae; H& I. Adult Moth- *Arctornis marginata* (Dorsal and ventral view); J.&K. Leaf feeding Grasshopper-*Xenocatantops humilis* (Nymph and Adult)

Plate 3. *Diospyros crumenata* : Reproductive Dynamics (Male tree)



A.B. & C. Male flower bud initiation; D. Stages of bud to bloom; E. Stages of anthesis; F. Section of buds; G. Dissected anthers; H. Abscissed buds; I. Abscissed flowers

Calyx persistent. A high degree of flower abscission (estimated to be 60±5%) was recorded within a fortnight of flowering, assumed due to ineffective pollination. The stalks of the abscised flowers could be seen in the flowering branches throughout the year. Each young shoot that arises from the old secondary/tertiary branches contains 3 to 4 flowers. An average of 4-5 young shoots is found in a secondary/ tertiary branch. Consequently, the young shoots in a tertiary branch contain about 14-16 flowers (Plate 4).

Pollen- Ovule ratio

A male flower typically has 8 to 10 anthers, which are linear in shape and arranged in clusters at the centre of the thalamus. According to haemocytometer measurements, each anther contains 362±10 pollen grains, meaning that each flower contains 2896 to 3620 pollen grains, with an average of 3,258 pollen grains. The fruiting primordium contains 2 to 6 ovules, with an average of 4 ovules. Hence, the P :O ratio for the species is calculated to be 814:1.

Pollen fertility

The Acetocarmine staining technique has demonstrated that, on average, 75-80% of pollen grains are fertile.

Fruit development

Fruit initiation and development started just after the withering of the corolla, which was normally noted from the month of July and in certain years during March, depending on the flowering period. The fruit development, maturity and ripening took 6-7 months (Fig.24). The young fruits of *D. crumenata* are initially pale green, gradually changing to a yellowish-green as they mature. The fruits are berry-like and subglobose in shape, varying in size, with an average length of 15.93 ± 1.81 cm and an average width of 43.73 ± 12.57 cm. Upon reaching maturity, the fruit exposes an

interior characterised by a whitish gel-like pulp which is sweet and delicious, along with nutritional and functional compounds.

During fruit development, the abscission of immature fruits was observed at various stages of maturity, and it was estimated to be 20 ± 5% (Plate 5). The fruit pulp, along with seeds at about maturity and on ripeness, was found to be predated by a large number of arboreal mammals, viz. Lion-tailed macaque (*Macaca silenus*), Nilgiri langur (*Semnopithecus johnii*), Giant squirrel (*Ratufa indica*), Flying squirrel (*Petaurista* sp.) and Palm civet (*Paradoxurus hermaphroditus*). The seeds of fallen fruits are consumed by the Indian brown mongoose or brown mongoose (*Urva fusca*) and rodents like the Malabar Spiny Dormouse (*Platacanthomys lasiurus*). The predation of fruits was estimated to be 70 ± 5% (Plate 5). The seed predation largely affected the soil seed bank and poor seedling regeneration. Though the ripened fruits are largely consumed by the arboreal mammals, the distribution of seeds to wider locations is significantly affected. Subsequently, the populations are restricted to specific niches. In general, poor natural regeneration by seeds was usually seen in and around existing natural stands (Plate 5).

4.1.3.3. Lichen associations

The trunk of the *D. crumenata* trees has noticed unique lichen association among the populations identified. The specimens were identified as *Phyllopsora parvifolia* (Ramalinaceae), *Porina glaucoflava* (Porinaceae), *P. subcutanea* (Porinaceae), and *Cryptothecia* sp. (Arthoniaceae) (Plate 6). The lichen associations are a characteristic feature of the species and are found throughout the year.

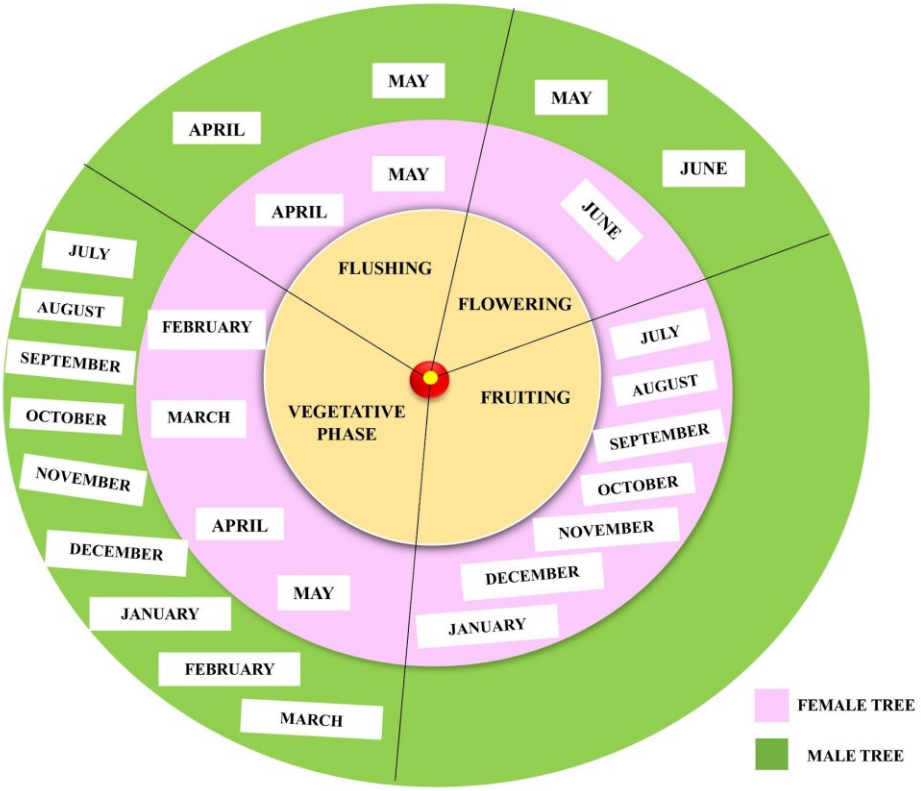


Figure 24. *Diospyros crumenata*: Phenology calendar

Plate 4. *Diospyros crumenata*: Reproductive Dynamics (Female tree)



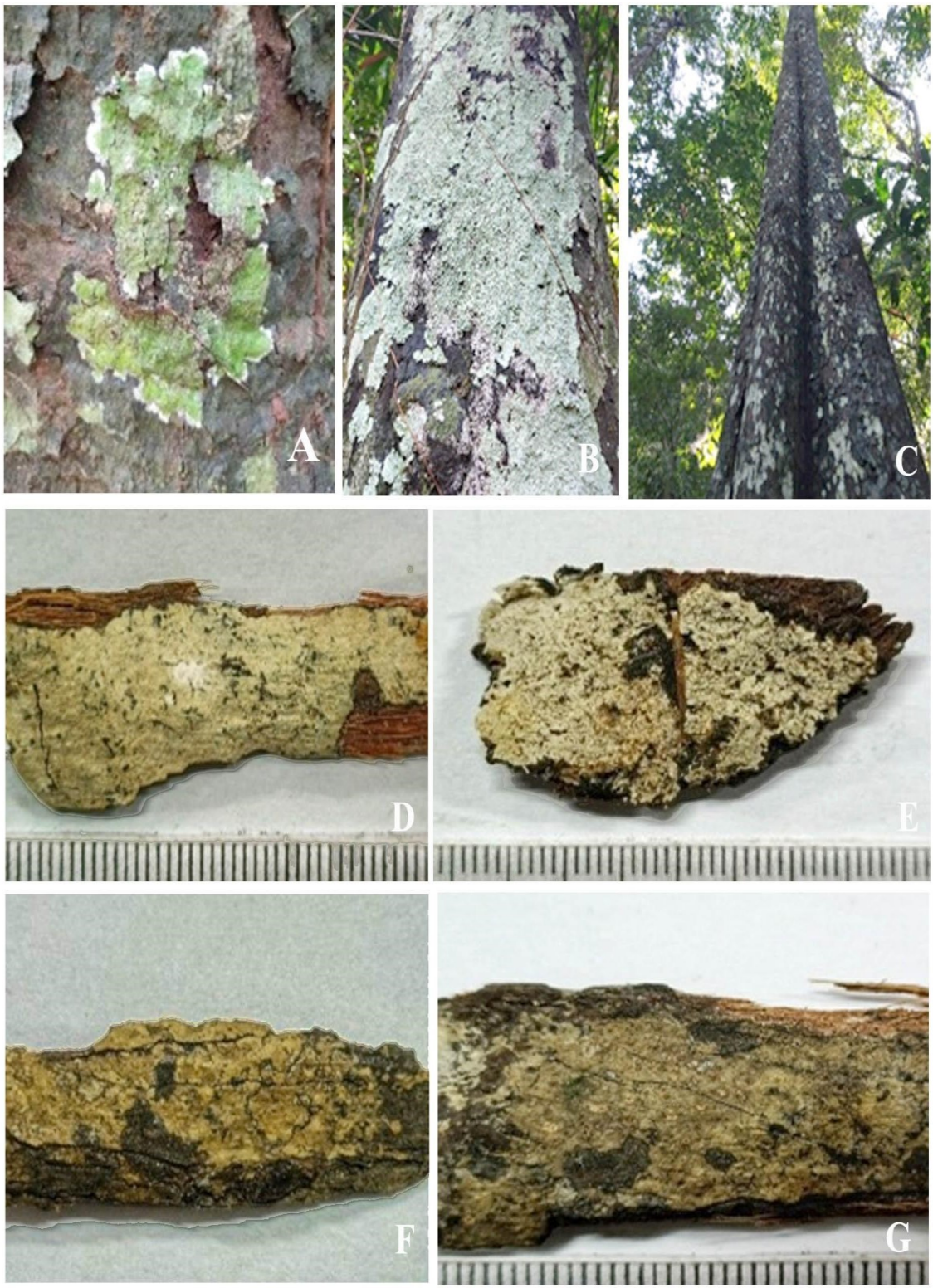
A. Female tree-leaf maturity stage before flower bud initiation; B. Twig showing stalk of abscissed flowers; C. Fruiting primordia; D.E.F. Closer view of development of fruiting primordia; G.H.I. Flowering branch showing different stages of fruiting primordia

Plate 5. *Diospyros crumenata*: Fruit Abscission and Predation



A.B.C. Abscission of fruiting primordia at various stages of development;
D.E. F. Abscission of immature fruits at various stages of maturity;
G.H.I. Views of predated fruits and seeds

Plate 6. *Diospyros crumenata*: Lichen Associations



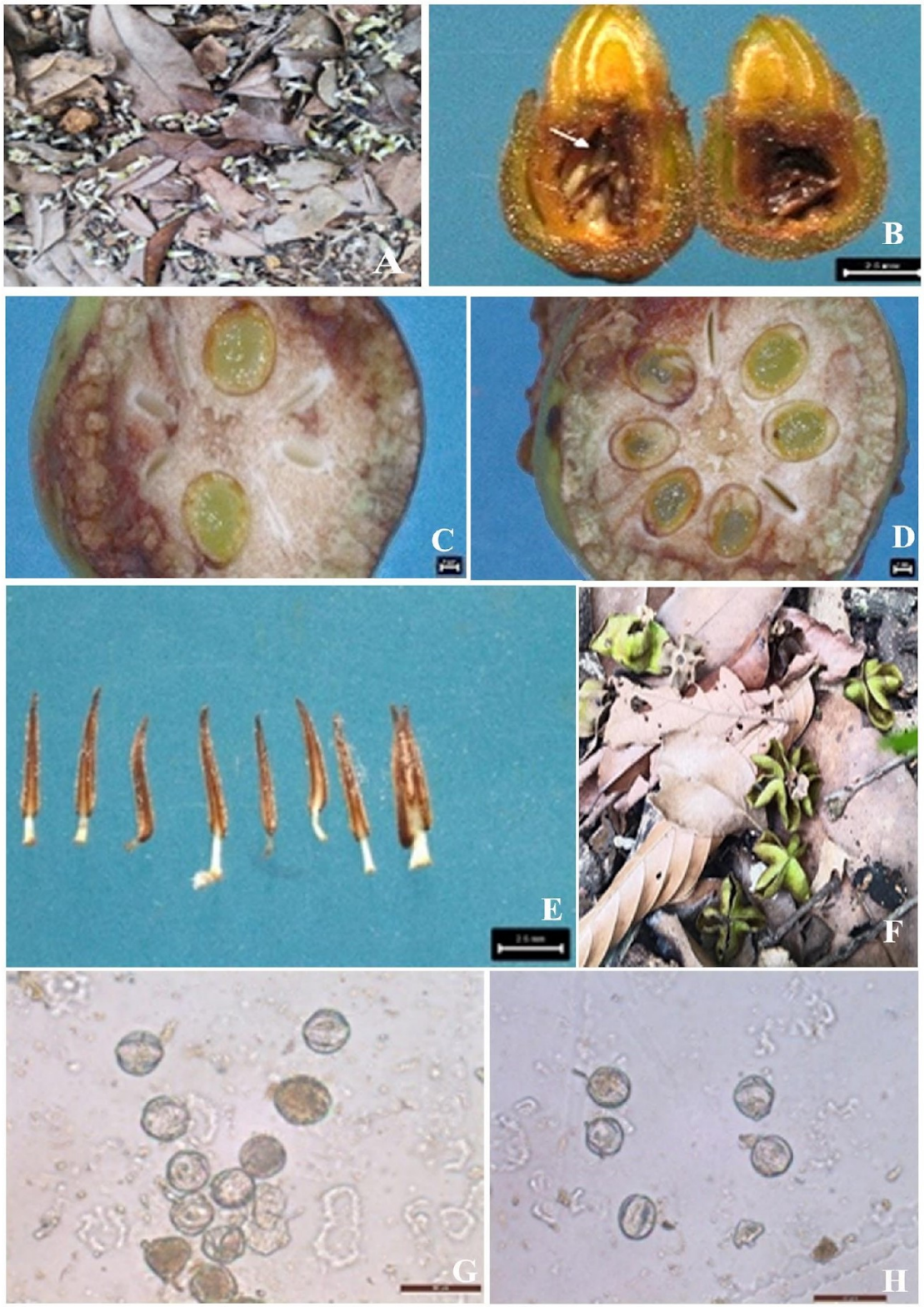
A.B.C. Tree trunk showing unique lichen associations ; D. *Creptothecia* sp. ; E. *Phyllospora parvifolia* ; F. *Porina subcutanea*; G. *Porina glaucofolia*

4.1.3.4. Ecological and biological constraints leading to the rarity of species

Ecological studies show restricted distribution of the species in the Thrissur District of the Western Ghats, Kerala. The number of adult individuals falls below the threshold for Critically Endangered status according to IUCN guidelines. The spatial distribution is displayed adjacent to the watercourse, indicating its habitat specificity. The age-wise distribution shows that only 10% of trees belong to the pre-reproductive stage, and a low number of seedlings and saplings across populations. Although an inverted J-shaped curve was constructed for each population site, the irregularity of the curve indicates poor regeneration and weak population growth. The habitats of the populations are vulnerable to threats such as forest fires, road expansion, and the development of powerhouses. The low Importance Value Index (IVI) within forest communities highlights its rarity among its associated species. Lichen associations in the trunks of the trees serve as indicators of habitat degradation in the ecosystems.

Several reproductive challenges were identified: male flower bud abscission, low pollen count and fertility, caterpillar infestations, and a short longevity of flowers. The species has a low pollen-ovule ratio, suggesting insect-mediated cross-pollination, but the occasional insect visits during the short blooming period have resulted in limited pollination efficiency. A large degree of female flower abscission was noted, indicating ineffective pollination. The fruit abscission at various developmental stages further reduces the yield of mature fruits. Additionally, heavy predation of ripe fruits and seeds significantly affects the soil seed bank and seedling recruitment. The recalcitrant seed behaviour limited the seed germination in exposed forest areas and rocky grounds. Multiple pathogenic fungal infections have been reported to cause seed rot both *in situ* and *ex situ*, as well as plumule decay in *ex situ*, thereby further hindering seed germination (Plates 7 & 8).

Plate 7. *Diospyros crumenata* : Reproductive Constraints



A. Abscised male flower buds and flowers; B. Caterpillar infestation in male flower buds; C, D. Section of young fruits showing ovule reduction; E. Low count of pollen in anthers; F. Abscised female flowers; G., H. Low fertility of pollen

Plate 8. *Diospyros crumenata* : Reproductive Constraints



A. Abscission of flowers/fruiting primordia B. Immature fruit abscission; C. Short viable seeds D. Germination inhibition by *Penicilliopsis clavariiformis* E. Close-up of *Penicilliopsis clavariiformis* F. Germination inhibition by *Penicilliopsis clavariiformis in- situ.* G. Germination inhibition by *Curvularia clavata* H. Degradation of fallen fruits I. Excreted and dried seeds on the rock surface

4.2. Conservation

4.2.1. Vegetative propagation through stem cuttings

The stem rooting ability of *D. crumenata* was found to be significantly affected by auxin treatment. The stem cuttings collected from adult trees failed to root, but young plants of 3-4 years old responded to rooting. The juvenility, along with the effect of auxins, played a major role in the rooting of the species. The percentage of root formation was significantly affected by both the concentration and type of auxins used. The root percentage was varied from 20 to 70% under different auxin treatments. The auxin IBA-3000 ppm resulted in maximum stem rooting of 70% within 60-70 days of planting compared to 20% in the control. The increase in concentration at 5000 ppm was found to decrease the rooting percentage to 60%. A moderate level of IBA concentration at 3000 ppm was found to be optimum for maximum rooting, @ 70%, whereas IAA and NAA resulted in a maximum rooting of 40 and 20%, respectively. The auxins significantly increased the root number of cuttings. The mean number of roots was higher, along with higher rooting performance in IBA 3000ppm than in the other auxin-treated sets, and root length was not significantly varied among auxins. (Table 8 & Plate 9).

Table 8. *Diospyros crumenata*: Vegetative propagation through stem rooting

Treatment	Rooting percentage	Mean ± SE	
		Number of roots	Mean length of roots (cm)
Control	20	2.50 ± 0.50	7.98^{a} ± 1.28
IAA 1000	40	2.00 ± 0.41	4.91^{b} ± 0.60
IAA 3000	30	2.33 ± 0.88	4.13^{b} ± 1.00
IAA 5000	40	2.00 ± 0.41	3.47^{b} ± 0.62
IBA 1000	50	2.80 ± 0.37	4.31^{b} ± 0.81
IBA 3000	70	2.71 ± 0.36	3.96^{b} ± 0.22
IBA 5000	60	2.00 ± 0.26	3.82^{b} ± 0.67
NAA 1000	10	2.00 ± 0	3.00 ± 0
NAA 3000	0	-	-
NAA 5000	0	-	-
F-value (P-value)		0.763^{ns} (0.606)	2.869* (0.030)

*** Significant at 0.01 level; ns non-significant*
Means having different letters as superscripts differs significantly within a column
Comparison of the mean number of roots and the mean length of roots is done, excluding the data related to the treatment of NAA1000 (as only one plant was rooted)

The number of roots and the mean root length were compared across treatments using one-way ANOVA followed by Duncan's Multiple Range Test (DMRT). The F-value for the number of roots was found to be non-significant, as the P-value exceeded 0.05. This indicates that there is no statistically significant difference in the number of roots among the various treatment groups. In the case of the Mean length of roots, the P-value (0.030) was found to be less than 0.05; hence, the F-value was found to be significant at a 5 per cent level of significance. Hence, post hoc analysis was done by using DMRT, and the results show that the control group has a significantly higher mean root length compared to all other treatment groups. All other treatments show no significant difference in mean root length.

4.2.2. Vegetative propagation through air-layering

The success rate of air-layering varied between 25% and 100% across different treatments. The highest success rates, 100% and 75%, were recorded with Naphthalene acetic acid (NAA) at 5000 and 7000 ppm, respectively. Deviations from these optimal concentrations, either higher or lower, led to reduced rooting success. The air-layering achieved cent percent rooting with the application of NAA 5000 ppm within 40–45 days of girdling, followed by 75% with NAA 7000 ppm. In contrast, treatments with IAA and IBA at 5000 ppm resulted in no rooting, and 7000 ppm resulted in a significantly lower rooting rate of 25%. No root formation was observed in the control sets. The mean number of roots and the mean length of roots were calculated as per statistical methods. Cent per cent survival was recorded for both ramets and layers (Table 9).

Table 9. *Diospyros crumenata*: Vegetative propagation through air-layering

Auxin	Auxin concentration (ppm)	Number of branches layered	Rooting (%)	Average number of roots per layer (mean ± SE)	Average length of roots per layer (cm) (mean ± SE)
IAA	3000	12	Nil	-	-
	5000	12	Nil	-	-
	7000	12	25	4.71±1.496	11.27±1.49
IBA	3000	12	Nil	-	-
	5000	12	Nil	-	-
	7000	12	25	4.5±1.378	10.11±2.80
NAA	3000	12	Nil	-	-
	5000	12	100	6.5±1.195	16.67± 2.76
	7000	12	75	5.33±1.032	14.13±1.93
Control		12	Nil	-	-

Plate 9. *Diospyros crumenata* : Vegetative propagation



A, B. Rooted stem cuttings; C. Ramets; D. to G. Stages of ring air layering and root proliferation; H. Profusely rooted layers; I. Established layers J. Established ramets

4.2.3. Seed propagation

Seeds with an initial moisture content (mc) of 36% achieved a germination rate of 66% within 24 days of sowing. However, seeds on scarification followed by soaking in cold water (tap water) resulted in 100% germination within 15 days. The seedlings transferred in polythene bags were recorded with a 90-95% survival rate under the shade house of the nursery. Seeds on germination were occasionally affected by pathogenic fungi, leading to inhibition of germination and seedling decay due to plumule infection. The propagation method standardised could be used for large-scale multiplication of the species (Plate 10).

4.2.4. Seed pre-treatments

The seed germination performance in each set of pretreatments resulted in varying success rates (Tables 10 & 11). Among the treatments, the seed scarification followed by soaking in cold water (tap water), recorded 100% germination within 15 days of sowing, compared to 66% in 24 days in the control sets.

Table 10. *Diospyros crumenata*: Seed pre-treatments vs. germination

Sl. No.	Treatments	Dipping duration	Initiation of germination (days)	Germination (%)
T1	GA_3 500 ppm	24h	18	90
T2	GA_3 1000 ppm	24h	16	90
T3	Concentrated H_2SO_4 (90%)	1min	21	30
T4	KNO_3 (2%)	24h	18	80
T5	NA_2HPO_4	24h	16	80
T6	Scarification + tap water	24h	15	100
T7	Hot water (70°C)	(5min)	17	70
T8	Cold water	24h	19	60
T9	Control	Direct sowing	24	66

Table 11. *Diospyros crumenata*: Comparison of seed germination parameters in various seed pre-treatment

Treatment	Mean ± SE				
	Root girth (mm)	**Root length (mm)**	**Stem girth (mm)**	**Stem length (mm)**	**Leaf number**
1	$1.40^{ab} \pm 0.21$	$7.27^{ab} \pm 1.23$	$1.18^{abc} \pm 0.23$	$7.69^{ab} \pm 0.92$	$3.00^{ab} \pm 0.39$
2	$1.33^{ab} \pm 0.24$	$8.59^{ab} \pm 1.30$	$1.18^{abc} \pm 0.16$	$7.95^{ab} \pm 1.05$	$3.00^{ab} \pm 0.37$
3	$0.41^{c} \pm 0.21$	$2.60^{c} \pm 1.39$	$0.49^{c} \pm 0.25$	$2.75^{c} \pm 1.45$	$1.00^{d} \pm 0.52$
4	$1.53^{ab} \pm 0.39$	$8.20^{ab} \pm 1.46$	$1.29^{ab} \pm 0.27$	$7.19^{ab} \pm 1.27$	$2.70^{abc} \pm 0.50$
5	$1.15^{bc} \pm 0.22$	$6.92^{b} \pm 1.35$	$1.18^{abc} \pm 0.21$	$7.18^{ab} \pm 1.25$	$2.20^{abcd} \pm 0.42$
6	$2.03^{a} \pm 0.07$	$11.49^{a} \pm 0.3$	$1.81^{ab} \pm 0.06$	$10.50^{ab} \pm 0.56$	$3.50^{a} \pm 0.224$
7	$1.01^{bc} \pm 0.24$	$7.42^{ab} \pm 1.71$	$0.89^{bc} \pm 0.2$	$6.70^{abc} \pm 1.55$	$2.00^{bcd} \pm 0.47$
8	$1.13^{bc} \pm 0.34$	$5.72^{bc} \pm 1.58$	$0.80^{bc} \pm 0.23$	$5.79^{bc} \pm 1.64$	$2.00^{bcd} \pm 0.58$
9	$0.79^{bc} \pm 0.27$	$4.79^{bc} \pm 1.65$	$0.76^{bc} \pm 0.26$	$5.38^{bc} \pm 1.82$	$1.50^{cd} \pm 0.52$
F-value (P-value)	3.224** (0.003)	3.272** (0.003)	3.121** (0.004)	2.506* (0.017)	3.095** (0.004)

*** Significant at 0.01 level*
Means having different letters as superscript differ significantly within a column
Comparison between treatments was done by using one-way ANOVA followed by Duncan Multiple Range Test (DMRT)

P-values are less than 0.01 for all parameters except stem length. Hence, all the variables except stem length were found to show significant differences between treatments at a 1% level of significance. In the case of stem length, the p-value was found to be less than 0.05; hence, it was found to show a significant difference between treatments at the 5% level of significance. As there exists a significant difference between treatments for all variables, post hoc analysis was done by using Duncan Multiple Range test (DMRT) to find out which of the treatments shows significant differences and the same was also given in Table 11.

Root girth was significantly higher in the case of T6 compared to all treatments except T1, T2 and T4. The T1, T2 and T4 have significantly higher root girths compared to T3, where T3 has the least root girth. Root length is significantly higher for T6 compared to T3, T5, T8 and T9. The T3 has significantly lower root length compared to all treatments except T8 and T9.

Stem girth was significantly higher for T6 compared to T3, T7, T8 and T9. The T2 has significantly lower stem girth compared to T6 and T4. Stem length is significantly higher for T6 compared to T3, T7, T8 and T9. The T3 has significantly lower root length compared to all treatments except T7, T8 and T9. Leaf number is also significantly higher for T6 compared to all other treatments except T1, T2 and T4. The T1 and T2 have significantly higher leaf numbers compared to T3 and T9. The T3 shows significantly lower leaf number compared to T1, T2, T4 and T6.

4.2.5. Seed storage practices

Fresh seeds with an initial moisture content of 36% resulted in 66% germination and were classified as recalcitrant due to their sensitivity to temperature conditions. The critical moisture content (CMC) for these seeds was determined to be 12-13%, with a 50% germination rate; germination did not occur below this moisture content. The seeds exhibited varying viability periods when stored under controlled conditions in different containers. Seeds stored in closed polythene bags under ambient conditions remained viable for two months, with MC and germination rates at 28% and 33%, respectively. In contrast, seeds stored in closed polycarbonate bottles under the same conditions had a viability period of four months, with MC and germination rates of 26% and 33%, respectively. Seeds stored in controlled seed bank conditions at 16±2 °C and 45% relative humidity (RH) in closed polythene bags, maintained viability for four months,

with MC at 24% and germination at 33%. Under the same controlled conditions, seeds stored in closed polycarbonate bottles, viability was maintained for four months; however, 66% germination occurred at 27% MC. When stored at 20±2 °C and 40% RH in closed polythene bags, seeds had a four-month viability period with 83% germination at 24% MC. However, seeds stored in closed polycarbonate bottles under the same controlled conditions had a significant period of extended viability of up to 10 months, with 33% germination at 17% MC, compared to the normal viability period of just 12 days in open room conditions (Table 12 & Plate 10). Later, the seed viability was hindered due to the germination within the containers.

Table 12. *Diospyros crumenata*: Effect of storage conditions on the seed germination

Sl. No.	Container	Conditions (Temp./ Humidity)	Moisture/ Germination	Germination (%) after storage (months)											
				1	2	3	4	5	6	7	8	9	10	11	12
1	Closed polythene bag	Ambient (27± 2°C; 50-60% RH)	M	**34**	**28**	**22**	**18**	-	-	-	-	-	-	-	-
			G	**50**	**33**	**0**	**0**	-	-	-	-	-	-	-	-
2	Closed polycarbo-nate bottle	Ambient (27± 2°C; 50-60% RH)	M	**34**	**30**	**28**	**26**	**21**	-	-	-	-	-	-	-
			G	**83**	**83**	**16**	**33**	**0**	-	-	-	-	-	-	-
3	Closed polythene bag	Seed bank (16° C & 45% RH)	**M**	**34**	**32**	**30**	**24**	**21**	-	-	-	-	-	-	-
			G	**100**	**100**	**50**	**33**	**0**	-	-	-	-	-	-	-
4	Closed polycarbo-nate bottle	Seed bank (16°C & 45% RH)	**M**	**35**	**35**	**29**	**27**	**23**	-	-	-	-	-	-	-
			G	100	100	83	66	**0**	-	-	-	-	-	-	-
5	Closed polythene bag	Seed bank (20° C & 40% RH)	**M**	**36**	**34**	**28**	**24**	**23**	-	-	-	-	-	-	-
			G	100	83	83	83	**0**	-	-	-	-	-	-	-
6	Closed polycarbo-nate bottle	Seed bank (20 °C & 40% RH)	**M**	**36**	**35**	**30**	**28**	**24**	**22**	**20**	**20**	**18**	**17**	**15**	**14**
			G	**100**	**100**	**100**	**83**	**83**	**66**	**33**	**50**	**33**	**33**	**16**	**16**

Initial mc: 36%, initial germination: 66 %
M.: Moisture; G.: Germination

Plate 10. *Diospyros crumenata*: Seed propagation



A. Seeds with pulp B. Processed seeds ; C. Close-up of seeds ; D. Immature seeds E. Seed L.S. showing ruminate endosperm ; F. Seed viability test by tetrazolium stain; G. View of seeds sown; H. Germinating seeds; I. Seedlings; J. View of germinated seeds kept in storage containers

4.3. Utilization

4.3.1. Acute oral toxicity of ripened fruits

The acute oral toxicity test of ripened fruit pulp with respect to body weight, feed and water intake, clinical signs and gross morphological examinations in adult Wistar rats was tested.

Body weight:

There were no notable variations in the animals' body weight.

Feed and water intake:

The Wistar rats were found to be active, and no changes in feed or water intake were noticed during the entire experimental period.

Clinical signs:

No adverse clinical signs were noticed in any of the animals treated with the extract.

Gross morphological examination:

The animals were subjected to careful postmortem examination, and all the organs were examined for any gross lesions. None of the organs showed any specific lesions of toxicity.

4.3.2. Fruit edibility

Information about the edibility of fruits was obtained from tribes and local communities living near the natural habitats of the species (Plate 11). The total mineral content in fresh fruits (7.18%) was higher when compared to other fruits like Jackfruit (0.9%), Mango (0.4%), and Pineapple (0.4%). The overall carbohydrate content in fresh fruits was 5.0g/100g, appearing notably lower than that of other fruits in comparison. The fresh fruit has 1.05 g/100g protein. The total sugar content is less (4.51%) compared to three other cultivated fruits. The Vitamin-C content in *D. crumenata* is

notably higher at 12.50mg/100g when compared to mango (7.8mg/100g) and pineapple (7mg/100g). The fruits contain a higher amount of sodium (2.70mg/100g) compared to others. Additionally, the potassium content (219.90mg/100g) is significantly higher than that of mango (205mg/100g) and pineapple (109mg/100g) fruits. The nutritional analysis results of *D. crumenata* are presented in Table 13. A ggplot was generated using R Studio to visualise the concentrations of various nutrients across the fruit samples. This approach offers a more programmatic and flexible way to define the variables, display options, and overall visual aesthetics, effectively representing both numerical and categorical data (Fig. 25).

Table 13. *Diospyros crumenata*: Chemical composition of fruit - Comparative analysis with cultivated fruits

Sl. No.	**Parameters**	***Diospyros crumenata***	***Artocarpus heterophyllus* (Jackfruit)**	***Mangifera indica* (Mango)**	***Ananus comosus* (Pineapple)**
1	Total Mineral Contents (%)	7.18	0.9	0.4	0.4
2	Carbohydrate (g/100g)	5.0	19.8g	17.18g	13.52g
3	Protein (g/100g)	1.05g	1.9g	0.36g	0.54g
4	Total sugars (%)	4.51	13.80	11.5	10.8
5	Ascorbic Acid (mg/100g)	12.50	7.0	7.8	16.9
6	Sodium (mg/100g)	2.70	2.2133	1	1
7	Potassium (mg/100g)	219.90	254.9867	205	109

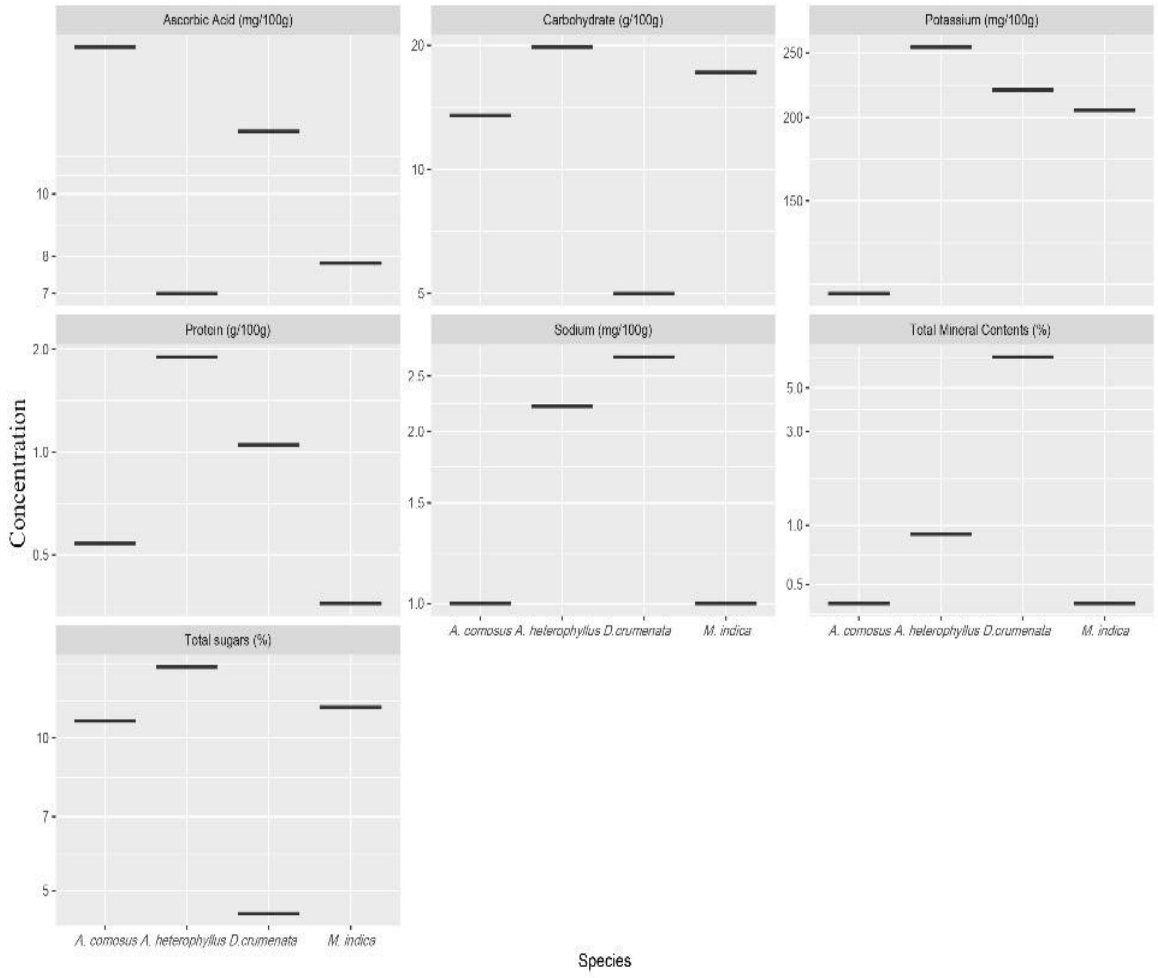


Figure 25. *Diospyros crumenata*: Chemical composition of fruit - comparative analysis with cultivated fruits generated by ggplot

Plate 11. *Diospyros crumenata*: View of fruit and pulp



A.B.C. Views of mature fruits; D. Ripened fruits ; E & F. Section of fruit showing pulp ; G.H.I. Views of edible fruit pulp

4.3.3. Seed properties

A fruit, on average, contains 4-6 seeds. The oblong, compressed, glossy black seeds are immersed and attached to the fruit pulp. The seeds exhibit a size range, with an average length of 11.038 ± 1.1388 mm and a width of 16.43 ± 0.2072 mm. The arboreal mammals usually consume fruit pulp along with the seeds. The ruminate type endosperm is also fed by ground-level rodents and mammals. The results of the seed properties evaluated are presented below.

Mineral composition

The dried seed powder showed in the presence of various elements in significant and below detectable limits (BDL). The elements such as Calcium (Ca), Magnesium (Mg), Sodium (Na), Phosphorus (P), Potassium (K), and Boron (B) are present at a significant level (mg/l). The elements such as Iron (Fe), Zinc (Zn), Manganese (Mn), Copper (Cu), Chromium (Cr), Cadmium (Cd), Nickel (Ni) and Polybdnum (Pb) are recorded as below detectable limits (Table 14).

Table 14. *Diospyros crumenata*: Mineral composition of seeds

SL. No.	Element Name	Sample (mg/100g)
1	Ca	10.43
2	Mg	5.12
3	K	61.87
4	Na	14.06
5	P	8.75
6	B	1.37
7	Fe	*BDL
8	Zn	BDL
9	Mn	BDL
10	Cu	BDL
11	Cr	BDL
12	Cd	BDL
13	Ni	BDL
14	Pb	BDL

* BDL: Below Detectable Limit

Chemical composition (Proximate)

A comprehensive overview of the nutritional value of the seeds was analysed by the components such as Moisture (The amount of water present in the seeds), Crude Protein (The total protein content, which includes all forms of nitrogen present), Crude Fibre (The indigestible portion of the seeds, mainly composed of cellulose, hemicellulose, and lignin), Crude Fat (Total fat content in the seeds) and Total Ash (The total mineral content left after burning the sample at high temperatures. The results are provided in Table 15.

Table 15. *Diospyros crumenata*: Chemical composition of seeds (Proximate)

Sl. No.	Parameters	Sample (%)
1.	Moisture	13.44
2.	Crude Protein	3.26
3.	Crude Fibre	17.77
4.	Crude Fat	1.13
5.	Total Ash	2.07

Phytochemical profile

The phytochemical profiling of powdered seed samples enabled the identification of various compounds and antioxidant properties of the species, as given in Table 16.

Table 16. *Diospyros crumenata*: Phytochemical composition of seeds

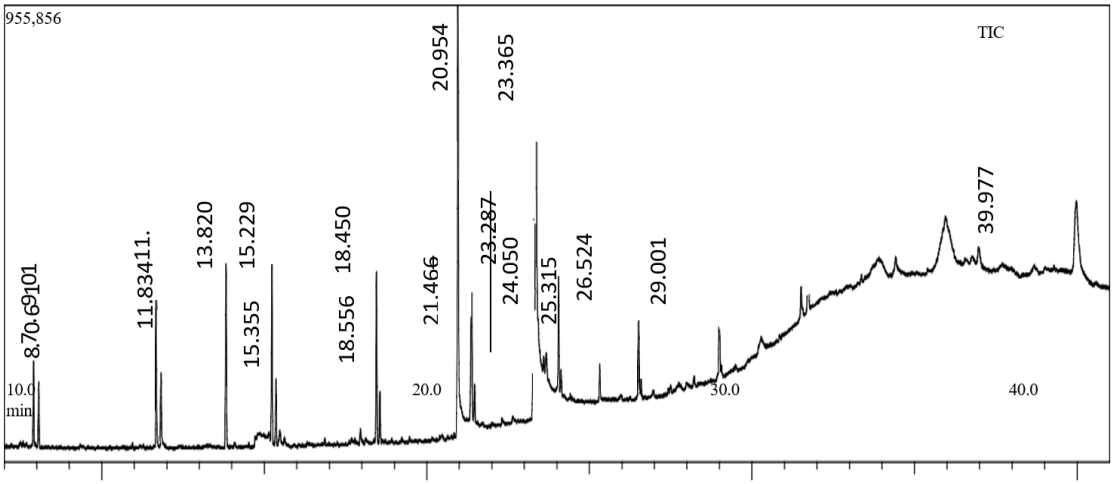
Sl. No.	Parameters	Status of compounds
1.	Terpenoids	Present
2	Tannins	Present
3	Phenol	Present
4	Flavonoids	Present
5	Antioxidant activity (IC 50 value)	2.78 ppm

Seed GC-MS

Gas Chromatography-Mass Spectrometry (GC-MS) analysis of the seed powder enabled the identification of the bioactive compounds, such as essential oils, fatty acids, alkaloids, phenolic and volatile compounds present in the seed (Fig. 26). The top five compounds with the highest area percentages (area %) in the chromatogram are given along with the activities of each compound (Table 17). The broad spectrum of biological activities of all phytocomponents identified in the chloroform extract is presented in Table 18.

Table 17. *Diospyros crumenata:* Biological activities of the top five phytocomponents identified in the chloroform extract of seeds

Sl. No.	(Area %)	Compounds	Molecular formula	Activities	Applications
1.	20.33	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	Antioxidant, antimicrobial, and anti-inflammatory (Bharath et al., 2021)	The use of medicated oils rich in n-hexadecanoic acid for treating rheumatic symptoms is a common practice in traditional medical systems (Hussain et al., 2023)
2.	10.78	(9E,11E)-Octadecadienoic acid (Isolinoleic acid)	$C_{18}H_{32}O_2$	Apoptosis inducer, an antineoplastic, anti-inflammatory, and antiatherogenic agent (Ramamoorthy et al., 2025).	A substance that inhibits or halts tumour growth. A cardiovascular drug that prevents the development of atherosclerotic plaques—fatty deposits accumulating on artery walls. (Ko et al., 2022)
3.	8.13	cis-9-Hexadecenal	$C_{16}H_{30}O$	Potential antimelanogenic antifungal properties (Shanu et al., 2020).	cis-9-Hexadecenal: A Natural Compound that targets cell wall Organisation and virulence in *Aspergillus fumigatus* (Marena et al.,2026)
4.	7.68	Stigmasterol	$C_{29}H_{48}O$	It functions as a plant metabolite (Zhang et al., 2022)	Anti-cancer Anti- inflammatory Anti-arthritis Anti-allergy Cardiovascular health (Bakrim et al., 2022)
5.	6.49	1-Hexacosene	$C_{26}H_{52}$	Used in cosmetics, skin conditioning, and functional fluids (Chandrasekar,2020).	As a solvent, paint thinner, and chemical reaction medium (NCBI,2026)



Peak Report TIC

Line#:1 R. Time:7.900(Scan n#:181) Mass Peaks:228
Raw Mode: Averaged 7.895-7.905(180-182) Base Peak:56(16099) BG Mode: Calc. from Peak Group 1 - Event 1 Scan

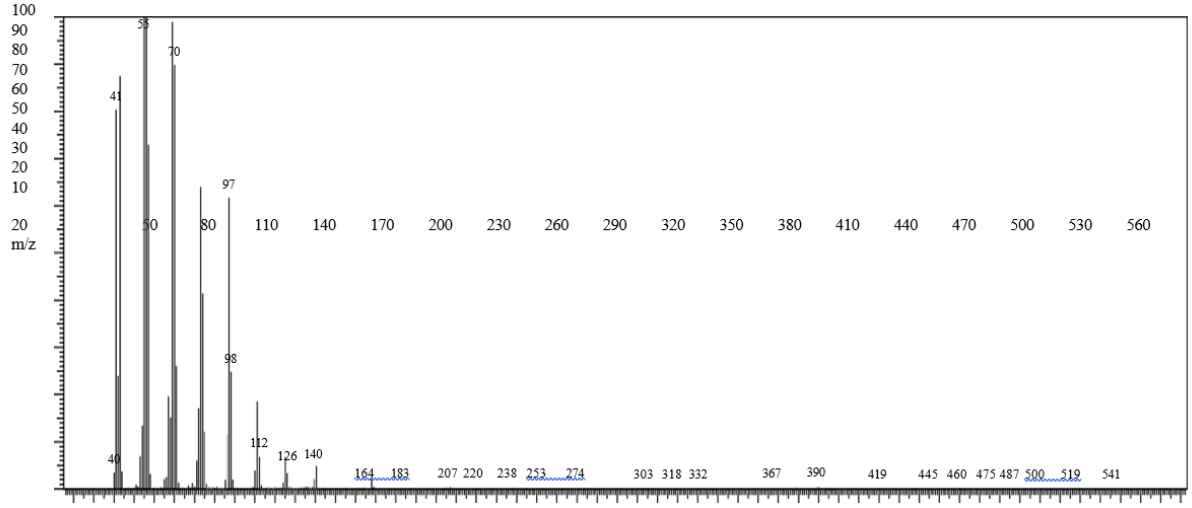


Figure 26. *Diospyros crumenata*: GC-MS spectrum of chloroform extract of seeds

Table 18. *Diospyros crumenata:* Biological activities of whole phytocomponents identified in the chloroform extract of seeds

Peak #	R.Time	Area	Area%	Height	Height %	A/H	Mark	Name
1	7.901	312949	2.41	176861	3.34	1.77	MI	1-Tridecene
2	8.061	250411	1.93	137855	2.60	1.82	MI	Dodecane
3	11.686	573407	4.42	306109	5.78	1.87	MI	1-Tetradecene
4	11.834	294079	2.27	151923	2.87	1.94	MI	Tetradecane
5	13.820	769892	5.94	385578	7.28	2.00	MI	2,4-Di-tert-butylphenol
6	15.229	720752	5.56	375261	7.09	1.92	MI	Tetradecyl trifluoroacetate
7	15.355	263762	2.03	138805	2.62	1.90	MI	Heptadecane
8	18.450	695614	5.37	357218	6.75	1.95	MI	1-Nonadecene
9	18.556	211592	1.63	107953	2.04	1.96	MI	Octadecane
10	20.954	2634745	20.33	903413	17.07	2.92	MI	n-Hexadecanoic acid
11	21.376	841664	6.49	329375	6.22	2.56	MI	1-Hexacosene
12	21.466	149996	1.16	78728	1.49	1.91	MI	Heneicosane
13	23.287	1397942	10.78	414876	7.84	3.37	MI	(9E,11E)-Octadecadienoic acid
14	23.365	1054316	8.13	489612	9.25	2.15	MI	cis-9-Hexadecenal
15	24.050	486047	3.75	239778	4.53	2.03	MI	Docosyl trifluoroacetate
16	25.315	169007	1.30	78403	1.48	2.16	MI	hexadecyl acrylate
17	26.524	314411	2.43	152216	2.88	2.07	MI	Octacosanol
18	29.001	190940	1.47	94505	1.79	2.02	MI	Tetracosyl pentafluoropropionate
19	31.510	202843	1.56	68293	1.29	2.97	MI	Octacosyl pentafluoropropionate
20	31.725	432809	3.34	168542	3.18	2.57	MI	Squalene
21	39.977	995171	7.68	137839	2.60	7.22	MI	Stigmasterol
		12962349	**100.00**	**5293143**	**100.00**			

4.4. CONSERVATION PROTOCOL FOR *IN- SITU* AND *EX- SITU*

4.4.1. Habitat distribution modeling

The model calibration test for *D. crumenata* produced excellent results, with an average test AUC of 0.999 and a standard deviation of 0.001 across replicate runs. Among the environmental variables, Bio19 (Precipitation of Coldest Quarter) emerged as the most influential factor, contributing 52% to the MaxEnt model (Table 19 and Fig. 27). Bio17 (Precipitation of Driest Quarter), Elevation, and Slope also play notable roles in the model, contributing 30.9%, 11.1%, and 5.5%, respectively, to the habitat distribution predictions. In terms of permutation importance, Bio19 also had the greatest impact on the habitat model, contributing 61.1%. The findings suggest that precipitation variables, especially Bio19, are critical in determining the potential habitat distribution of *D. crumenata*. Elevation and Slope also contribute significantly, highlighting the importance of topographical features.

Niche modeling suggests that the central part of Kerala, particularly Thrissur Dist. (Chimmony WLS, Idamalayar Dam reservoir adjacent areas, and Peechi WLS), is the optimal area for *D. crumenata* restoration. The model indicates that these central regions provide the most suitable environmental conditions for the successful reintroduction and rehabilitation of *D. crumenata* (Fig.28). Furthermore, the niche modelling highlights areas in the northern region of Kerala, including Kozhikode (Kakkayam and Anakkampoyil forests) and Wayanad (Manathavady forest), as moderately suitable for restoration efforts. While not as optimal as the central counterparts, the northern regions still provide favourable conditions for the growth and recovery of *D. crumenata*.

Table 19. *Diospyros crumenata*: Estimates of relative contributions and permutation importance of the predictor environmental variables to the MaxEnt model

Variables	Percent contribution	Permutation importance
Bio19 (Precipitation of Coldest Quarter)	52	61.1
Bio17 (Precipitation of Driest Quarter)	30.9	30.3
Elevation	11.1	4.9
Slope	5.5	1.9
Bio3 (Isothermality (BIO2/BIO7) (×100)	0.3	1.1
Bio18 (Precipitation of Warmest Quarter)	0.1	0.7
Aspect	0	0
Bio9 (Mean Temperature of Driest Quarter)	0	0
Bio11 (Mean Temperature of Coldest Quarter)	0	0
Bio1 (Annual Mean Temperature)	0	0
Bio12 (Annual Precipitation)	0	0
Bio15 (Precipitation Seasonality (Coefficient of Variation)	0	0

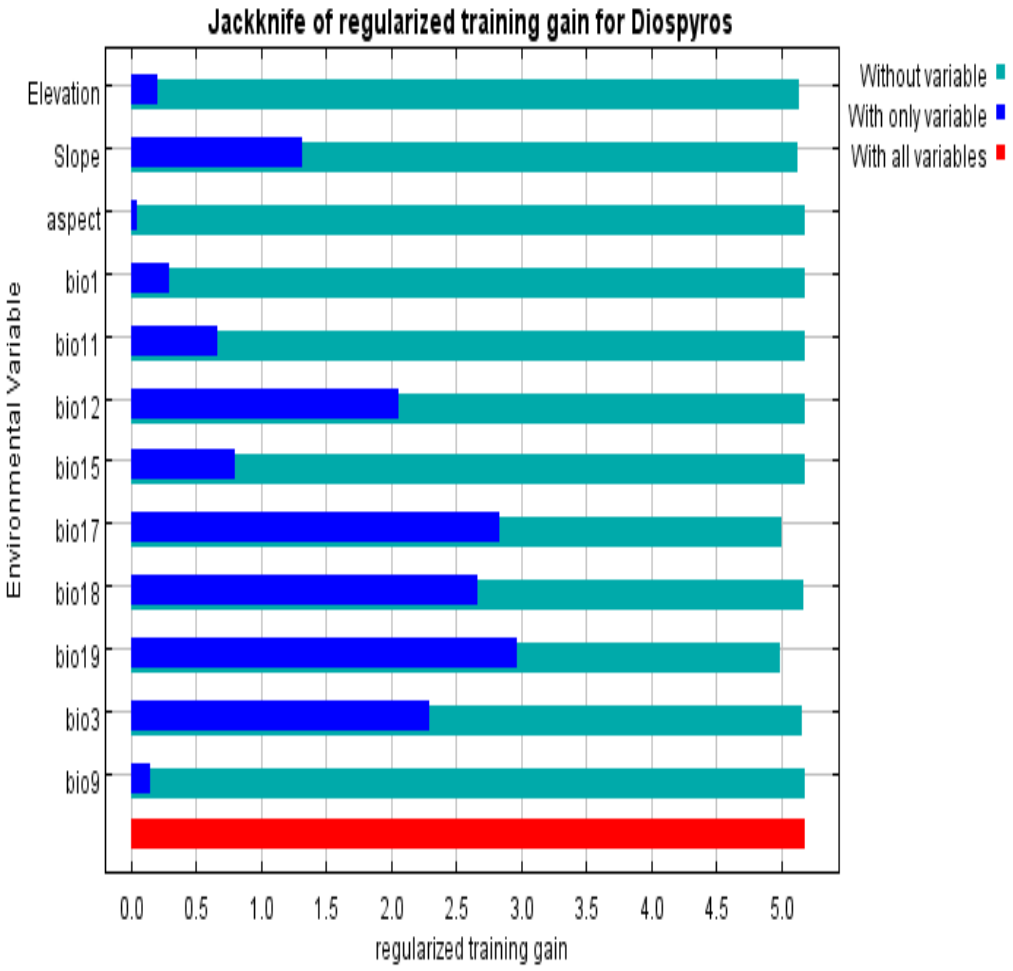


Figure 27. *Diospyros crumenata*: Habitat model

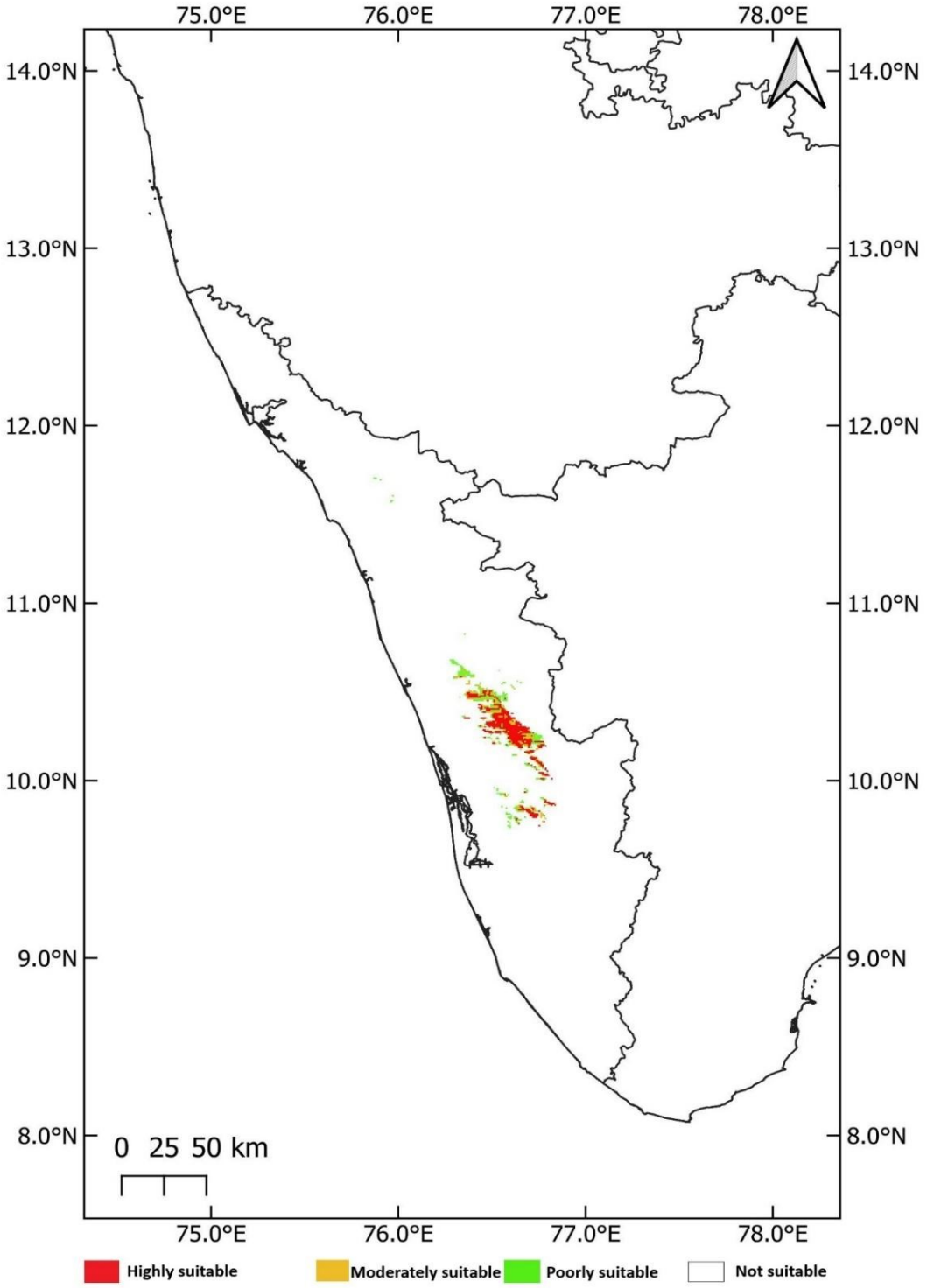


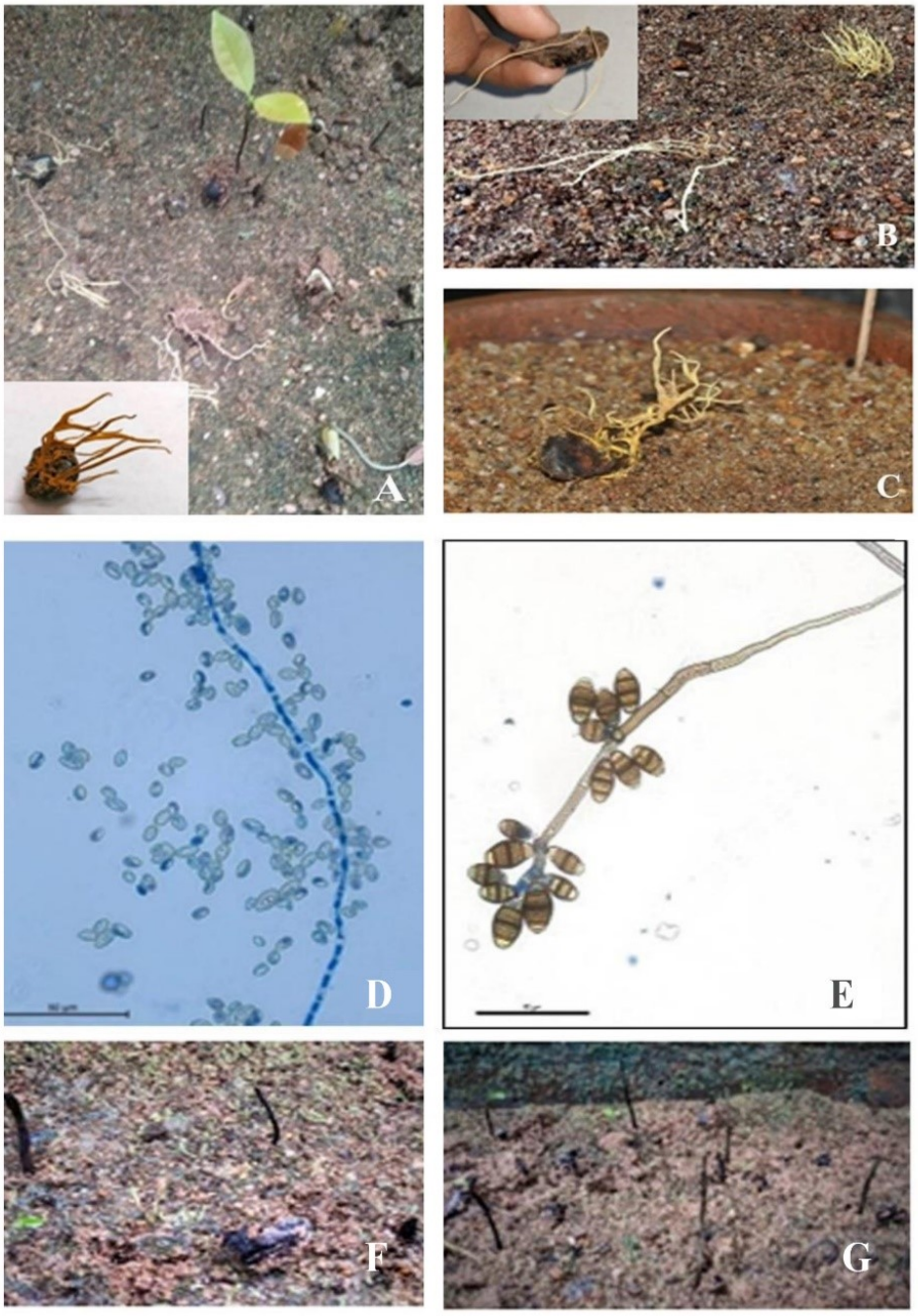
Figure 28. *Diospyros crumenata*: Predicted distribution emphasising favourable zones in both the southern and northern parts of Kerala

4.4.2. Evaluation of seed infestation

During the germination of *Diospyros crumenata* seeds, sulfur-yellow, clavarioid fungal fruiting bodies were observed emerging from the seeds. These fruit bodies, which also appeared during *in situ* germination, measured up to 9 cm tall and 2–3 mm wide. Initially bright yellow, they turned dull brown when dried, emitted an unpleasant odour, and produced a yellow spore print. Phylogenetic analysis of concatenated sequence alignment of Internal Transcribed Spacer (ITS) region, and beta-tubulin (TUB2) genes, alongside cultural morphological characteristics, identified *Penicilliopsis clavariiformis* (Trichocomaceae) as the causal agent. Pathogenicity tests conducted in vivo and in vitro, with subsequent re-isolation and identification of the pathogen per Koch's postulates, confirmed its pathogenic nature. This is the first report of seed damage caused by *P. clavariiformis* on *D. crumenata* in India and globally. A disease incidence rate of up to 5% was observed in seeds sown in the nursery.

Additionally, a pathogenic fungus was found infecting either the germinating seeds or the plumule of the seedlings. Identified through ITS and TEF molecular markers as *Curvularia clavata* (Pleurosporaceae), this fungus was recorded for the first time in *D. crumenata*. It initially produced whitish mycelium on PDA, which later turned grey to dark grey. Microscopic examination revealed that conidia were formed singly, with a clavate shape, straight or sometimes slightly curved. The base cell was pale brown, while the other cells ranged from brown to dark brown. The nursery-documented disease incidence rate reached up to 7% (Plate 12).

Plate 12. *Diospyros crumenata* : Seed pathogenic fungi



A.B.C. Emergence of pathogenic fungus during seed germination; Inset: *Penicilliopsis clavariiformis*-enlarged view; D. *Penicilliopsis clavariiformis;* E. *Curvularia clavata*- enlarged view; F & G. View of plumule damage on seed germination by *Curvularia clavata*

CHAPTER 5.

DISCUSSION

The Western Ghats is recognised as one of the world's biodiversity hotspots, hosting many endemic and threatened plant species. However, these species are experiencing a decline in their populations due to various factors. Many of these species lack comprehensive distribution and population data, making it difficult to assess their conservation status accurately. A thorough field study is essential to identify and map populations across their distribution ranges. According to the IUCN guidelines (2021), specific parameters, such as the Extent of Occurrence (EOO), Area of Occupancy (AOO) and the number of mature individuals, are studied for determining the conservation status of a species (Elias, 1986; Batianoff & Burgess, 1993; Bawa & Seidler, 1998; Scariot, 1999; Shapcott, 1999; Keith, 2000; Vormisto, 2002). Unfortunately, a significant proportion of tropical species lack this information.

EOO provides a broad sense of the geographical distribution of a species. A smaller EOO can indicate a species with a restricted range, which may be more vulnerable to extinction due to habitat loss, climate change, or other environmental changes. AOO provides a more refined estimate of the habitat availability and actual living space for a species. A smaller AOO often indicates higher vulnerability to threats, as there is less available habitat. Species with restricted AOO are more susceptible to localised threats, such as pollution, invasive species, and localised habitat destruction. A smaller number of mature individuals can signify a higher risk of inbreeding, reduced genetic diversity, and decreased adaptability to environmental changes. If the

population of mature individuals falls below a critical threshold, the species is at a greater risk of extinction.

By mapping the populations of threatened plant species, we can uncover distribution patterns and gain valuable insights into their habitat preferences and ecological requirements. This study can also provide data on population trends over time, help determine whether a species is declining, stable, or potentially increasing. Such information is crucial for prioritising conservation efforts. A population survey also helps identify the primary threats facing the species, such as habitat destruction, invasive species, climate change, or other factors. Understanding the population status and threats enables conservationists to prioritise species and habitats that require immediate attention.

In the current study, the target species, *Diospyros crumenata*, was extensively surveyed in the Western Ghats of Kerala. Three major population areas were identified in the Thrissur district. This population survey enabled the identification of new populations/ individuals for the species in the Chakkapara and Chimmony forests. The Extent of Occurrence and the Area of Occupancy were estimated to be 251.67 km^2 and 56.00 $km^{2,}$ respectively. Additionally, the population of mature individuals was estimated to be fewer than 150. As this number is below the threshold of 250 individuals, the species qualifies for classification as Critically Endangered (CR) under the 'D' criterion of the IUCN, based on population size estimates within the study area of the Western Ghats, Kerala.

The distribution and population mapping of *D. crumenata* align with IUCN guidelines for status assessment. The species' moderately low Extent of Occurrence (EOO) and Area of Occupancy (AOO) indicate a high risk of extinction, primarily due to its limited distribution range and reduced habitat availability. Additionally, the

decline in the number of mature individuals supports its classification as Critically Endangered, highlighting an increased risk of extinction driven by inbreeding, reduced genetic diversity, and diminished adaptability to environmental changes (Myers,1988; Nayar, 1996; Primack, 1994).

The population structure analysis of *D. crumenata* across the four forest areas revealed notable similarities in its distribution patterns, natural regeneration, and habitat preferences. Typically, this species is found in semi-evergreen forests at elevations below 500 meters above sea level. At all study sites, *D. crumenata* generally formed the first layer or canopy stratum, reaching heights of 25–28 meters. However, in Chakkapara, the trees were comparatively shorter, ranging between 6 and 17 meters in height. The species exhibited a moderately rough, blackish-grey bark and a straight bole. The blaze is a white and yellow colour in the xylem region. Across populations, the bole showed a characteristic association with lichens. In age-old trees, buttressing behaviour was seen. The reproductive trees normally exhibit a long straight bole and end up with a spread of branches forming a canopy into a rounded, irregular shape in open areas. The rounded or irregular-shaped canopy is due to the formation of a straight bole to receive sunlight, which thus led to low occupancy of crown coverage among other species in the community (Halle et al., 1978). The orientation of branches and leaves plays a great role in shaping the overall geometry of the tree crown. It can also be related to the adaptation strategy of the tree crown for light interception (Bruning,1970). Species such as *Diptercarpus indicus, Vateria indica* and *Prioria pinnata* are seen as major associated trees in the evergreen and at the fringes of semi-evergreen ecosystems. Whereas, *Tetrameles nudiflora, Toona ciliata, Dysoxylum malabaricum, Pterospermum reticulatum,* and *Polyalthia fragrans* were identified in the moist deciduous ecosystems and at the fringes of semi-evergreen ecosystems. The

associates of *D. crumenata* in evergreen/ fringes of semi-evergreen and moist deciduous/ fringes of semi-evergreen in the forests are different. This indicated that the species' growth and survival in the two ecosystems were equally successful.

The spatial distribution of *D. crumenata* exhibited a scattered pattern in the premises of the watercourse, highlighting the species' specific habitat preference. In the surveyed populations, a total of 138 adult trees were recorded, with only 15 individuals in the pre-reproductive stage, representing approximately 10% of the total population. The amazingly reduced number of young individuals among populations emphasises the poor regeneration potential, and seedling survival indicates the decline of population growth (Pandit & Basu, 1998). Among the four population sites, 35 saplings of below 30 cm GBH and 18 seedlings, of which 10 are established, and eight are under the unestablished category. This indicates poor regeneration performance of the species in all the sites. The size class distribution of whole individuals in the studied population sites through an inverted J- shaped curve revealed irregular curves indicating disturbed forest structure of the species due to various reasons (Bharathi & Devi, 2015; Raviraj et al., 2024; Li et al., 2024).

The population structure of *Diospyros crumenata* was assessed by recording girth at breast height (GBH), basal area, basal cover, age phase, and height of each individual. In the Vazhachal forests (Porigalkuthu–Karanthode area), floristic diversity analysis across five quadrats recorded 85 species. Relative dominance values were calculated for each species, revealing *Dipterocarpus indicus* as the most dominant. In contrast, *Diospyros crumenata* ranked 41st among the associated species. In the Chakkapara forest, 42 species were recorded from five quadrats. Here, *Tetrameles nudiflora* had the highest dominance index, making it the dominant species, while *Diospyros crumenata* ranked 26th among the associates. At Vellanimala forest, out of

59 species enumerated in five quadrates, *Pterospermum reticulatum* had the highest index and thus became the dominant species, whereas *Diospyros crumenata* attained 9th position among the associates.

The Importance Value Index (IVI) provides a comprehensive overview of a species' ecological status within a given community. A high dominance value suggests that the species exhibits strong biological competitiveness, often in a community with low diversity, creating favourable conditions for its growth and reproduction. The moderately lower dominance of *D. crumenata* suggests the minimum competitive behaviour due to an increase in diversity and overriding importance of physical difficulties existing in the respective habitats of the species (Kershaw,1973; Pascal, 1988; Jose, 2001).

Population dynamics of a species cover both the vegetative and reproductive dynamics, such as phenological phases, pollination, fruit development, dispersal, natural regeneration and insect-pest associations. The knowledge of phenological changes is important in understanding specific functions of plants in natural populations (Aronson et al., 1994), the evolution of species and communities as timing controls survival and reproductive success (Newstrom et al., 1994), *in-situ* management and conservation. It also affects animals which use young leaves, flowers and mature or immature fruits (Van Schaik et al., 1993).

Leaf phenological studies of tropical species have established that the leaf flush occurred with the onset of rain or after a spell of dry period (Proctor et al., 1983), in certain cases, seen early in the dry period (Whitmore,1984). In the present study, as a dioecious tree, the male trees initiated flushing along with mature leaves after a spell of dry period in April-May. Like in the majority of the forest trees, peak flushing is seen during January-April when temperature and sunshine are maximum (Murali &

Sukumar, 1994). Moreover, these environmental factors are suitable for maximising photosynthesis and vegetation growth (Salisbury & Ross,1974). Along with flushing, flower bud initiation was noted in April and May. A peak flowering was observed in May and extended to June. In certain years, flowering was advanced to March. The vegetative phase continues until the next flushing in April.

In population dynamics studies, insect-pest associations during vegetative and reproductive phases play a key role. Their influence directly or indirectly affects the population growth of the species in the ecosystem. Economically, insects are very important to plants as they bring about the cross-pollination of flowers and thus assist in the setting of seeds and the development of fruits. On the other hand, insects injure many plant parts in the form of defoliators, leaf rollers, leaf miners, floral eaters, shoot/ stem borers, fruit/ seed feeders, sap suckers, etc. (Holloway,1987; Mathew & Rahmathulla, 1993). During the vegetative phase of *D. crumenata*, the caterpillars of *Arctornis marginata* (moth) and *Xenocatantops humilis* (grasshopper) cause damage by feeding on the leaves. *Arctornis marginata* is considered a pest species because it feeds on the leaves, while the grasshopper, *X. humilis,* primarily feeds on the leaves and tender shoots of young trees, especially seedlings and saplings. As insect larval activity had been noted at both flushing and maturity, the vegetative growth was temporarily arrested for a short period and later resumed vegetative growth.

As per various ecological studies conducted in the Western Ghats, 26% of tropical plants have shown the behaviour of simultaneous flushing and flowering (Murali & Sukumar,1994; Elourd et al., 1997). In *D. crumenata*, male flower bud initiation was observed in April and flowers were recorded from May to June. During peak flowering, a single male tree produces thousands of flowers borne in cymose inflorescences. It takes 40-45 days for a bud to fully bloom, with anthesis occurring at

night. The blooming duration of a single flower lasts for only one day. During flowering, casualties were encountered in the form of flower buds falling. Environmental influences like heavy summer rain, high humidity, and caterpillar infestation in the flower thalamus may be attributed to the abscission of flower buds. The flowers are slightly fragrant. During peak flowering, insects such as *Apis indica* (large honey bee), butterflies, moths, and skippers are frequently observed on the tree.

In 51% of tree species, flowering was seen after the flushing process (Murali & Sukumar,1994). In *D. crumenata,* the female flower buds were initiated after a spell of flushing during June. In certain years, flowering was advanced into March. Abscission of a large number of flowers during blooming or just after the pollination is found to be a significant episode in the reproductive dynamics. This may be attributed to ineffective pollination as a result of the low number of insect pollinators (Bawa, 1990). In general, the low fruit set is largely influenced by the limited resources of the flowers (Friedman & Barret, 2011). The Pollen - Ovule (P: O) ratio is a conservative indicator of breeding systems in flowering plants (Cruden, 1977). *D. crumenata* exhibited a P: O ratio of 814:1, indicating a facultative xenogamous breeding pattern. However, inadequate pollinator availability remains a limiting factor for sufficient fruit production (Cruden, 1990).

Abscission of immature fruits was observed at various stages of maturity, and it was estimated to be 20%. The immature fruit fall is due to many factors, which include ineffective pollination, pollinator limitation and Inadequate transfer of compatible pollen to the stigma (Bawa & Webb, 1984). In *D. crumenata*, an inadequate number of pollen per ovule (814: 1) and low insect incidence were assumed to be limiting factors for successful pollination and subsequent abscission of fruits.

The fruit and seed predation are the most important interactive relationships in the environment that enable the continuity of the ecosystem. Predation of fruits and seeds has positive and negative sides in the dynamics of a species. *D. crumenata,* owing to its fruit traits such as sweet pulp, aroma and fleshy nature, many mammals are found to predate the ripened fruits along with the seeds, and it is estimated to be 70%. Major consumers of the fruits of *D. crumenata* were arboreal mammals such as Lion-tailed macaque, Nilgiri langur, Giant squirrel, Flying squirrel and Palm civet. All these mammals except Palm Civet are neutral dispersers as mostly they dropped the seeds beneath the canopy of the parent tree and very rarely only they carry seeds to other areas. Nilgiri Langur, Giant Squirrel and Flying squirrels mostly feed on mature fruits and seeds and are seed predators rather than dispersers (Jothish 2016). Palm civet is the only long-distance disperser observed in the candidate tree. However, they deposit the seeds in clumps in mostly exposed or unfavourable conditions for germination, such as rock surfaces, fallen wood, and exposed paths. They are highly susceptible to secondary seed predators such as spiny dormouse, porcupine, and other terrestrial rodents in the area (Mohandas et al., 2016). One reason for the narrow distribution of *D. crumenata* may be attributed to the lack of effective seed dispersers mentioned here. Information on the frugivores visiting 82 tree species was reported from the Western Ghats (Ganesh & Davidar, 2001). The dispersal of fruits and seeds is a vital aspect of a plant's reproductive ecology. Typically, a majority of seeds fall beneath the parent plant, while a significant portion is carried away from the mother plant through various external agents. Usually, animals are considered major seed dispersers in tropical forests, in which seeds of over 70% of the species are dispersed by animals (Kasi & Ramasubbu, 2021). Vertebrates such as birds and mammals are the dominant seed dispersers of tropical rainforests (Jordano, 1995). Even though the ripened fruits of *D. crumenata*

are largely consumed by arboreal mammals, the distribution of seeds to wider locations is significantly affected. Subsequently, the populations are restricted to specific niches. Further, the removal of seeds through excretes facilitates rodents like Malabar Spiny Dormouse (*Platacanthomys lasiurus* Blyth) to eat the seeds *in situ* (Jayson, 2006). As per the studies conducted in the Western Ghats, various mammals, including porcupines, consume above-ground and below-ground plant materials (Praters, 1980; Shumpeikitamura & Pilaipoonswad, 2013). The authors, such as Mohandas et al. (2016) and Ganesh and Davidar (2005), recorded several potential fruit predators from wet evergreen forests of the Southern Western Ghats.

Species those having a heavy fruit predation nature and short viability of seeds (recalcitrant seeds) mostly show poor natural regeneration (Jose & Pillai, 2016). In such cases, the seeds are either dispersed to unsuitable habitat conditions, leading to a loss of seed viability quickly or will be consumed by ground-level predators within the short span of the viability period (Vidya & Jose, 2023). The sweet fruit pulp, along with seeds of *D. crumenata* at about maturity and on ripeness, were found to be predated by a large number of arboreal mammals such as Lion-tailed macaque, Nilgiri langur, Giant squirrel, Flying squirrel and Palm civet and ground-level mammal (Mongoose) and rodent (Malabar Spiny Dormouse) which largely affected the soil seed bank and poor seedling regeneration. The low seedling count indicates plausible impediments in the reproductive potential of the species as well as predicts a hampered future (Subin et al., 2022). Poor natural regeneration was reported in other threatened and recalcitrant seeds of the trees, such as *Humboldtia bourdillonii* (Rahul et al., 2020), *Hopea erosa* and *Hopea glabra* (Sanil et al., 2017) and affected population growth of the species.

The individuals of *D. crumenata* showed characteristic straight boles across populations identified, and the tree trunk displayed unique associations of lichens,

which were taxonomically identified. Lichens play an important ecological role in forest ecosystems by contributing to nutrient cycling, providing habitats and food resources, aiding in ecological succession, and serving as sensitive bioindicators of environmental conditions (Kristiina et al., 2026). The diversity of epiphytic lichens can be considered a characteristic of the host tree (Benítez et al., 2019). This diversity is also influenced by factors such as canopy cover and tree species richness across varying levels of forest disturbance. Lichens are sensitive indicators of environmental changes, including land use patterns, forest disturbances, fragmentation, succession, air pollution, nitrogen deposition, and climate change. As per Benítez et al. (2018), the epiphytic lichen diversity and distribution in humid tropical forests are the principal drivers of microclimatic changes associated with changes in forest structure, such as canopy cover and tree diameter. The study points towards the susceptibility of the microhabitats of the species towards habitat degradation and disturbances.

Development of propagation methods through vegetative means is an important way for multiplying genetically improved clones, providing planting materials of desired characters. The method of propagation is also considered the most important technical aid in modern conservation-oriented programmes where no alteration in the genetic system is desired (Jose, 2001; Benabise, 2023). Macro propagation by using stem cuttings and air- layering has been recognised as an important strategy in clonal forestry, particularly in tree improvement programmes. It is also noted that auxins play a significant role in rooting during vegetative propagation (Jose et al., 1995; Reddy et al., 2022). In *D. crumenata,* vegetative propagation through stem cuttings and air - layering is relevant as the seeds are largely predated and become limited in availability for multiplication, apart from the quick loss of viability. The young plants below 4 years old responded to rooting with the aid of auxins. The auxin IBA-3000ppm resulted

in maximum stem rooting of 70% within 60-70 days of planting, compared to 20% in the control. Whereas, the highest layering success of 100% and 75% was achieved with NAA 5000 and 7000 ppm, respectively. The increase or decrease in auxin concentration had a negative effect on rooting. Thimann (1956) observed that different plants respond uniquely to various types and concentrations of auxin. Each plant possesses distinct promotory and inhibitory thresholds, meaning that a certain type or concentration of auxin that promotes shoot growth might inhibit root growth, or vice versa. The results match the findings of Praveena et al. (2023) and Rasmi and Jose (2023a). The juvenility, ideal auxin, along with concentration, was optimised for the species as in the studies carried out in threatened trees of the Western Ghats, *Persea macrantha* (Praveena et al., 2023), *Canarium strictum* (Pandurangan, 2003), *Embelia ribes* (Saumya et al., 2014), *Salacia fruticosa* (Saumya et al., 2014a), *Madhuca diplostemon* (Shareef et al., 2025)

The seed is considered the major mode of propagation in plants. However, in some species, seeds require pretreatments to improve the germination performance. In *D. crumenata*, untreated seeds showed only 66% germination within a germination duration of 24 days. Among a set of pretreatments conducted, seed scarification followed by tap water soaking for 24 hrs. resulted in 100% germination within 15 days of sowing. The results of presowing treatments are similar to the studies conducted on *Acacia catechu* (Haider et al., 2014) and *Salacia gambleana* (Anaz et al., 2019).

Developing seed storage practices for recalcitrant seeds is still a challenge in tropical forestry (Jaganathan et al., 2019; Jha et al., 2022; Vidya & Jose, 2023). Recalcitrant seeds possess a remarkably short life span, particularly when kept in open or ambient conditions. Maintaining the Critical Moisture Content (CMC) of seeds is essential for retaining their viability (Tompsett,1992; Hay et al., 2023). The

prolongation of the seed viability period in controlled climatic conditions and different containers is generally practised for these types of seeds. The seeds of *D. crumenata* are recalcitrant and lose their viability within 12 days in open conditions. However, for seeds stored in closed polycarbonate bottles in controlled conditions (20±2 °C and 40% RH), viability was extended to 10 months with 33% germination. The optimum storage conditions of recalcitrant seeds were standardized in many endemic and threatened plants of the Western Ghats (Pandurangan, 2003; Sukesh & Chandrashekar, 2013; Umarani et al., 2015; Jose & Pillai, 2016).

Wild fruits are a vital part of the diet and are also a rich source of varied phytochemicals/bioactive compounds (Murthy& Bapat, 2020). Wild fruits are one of the significant bioresources of the Western Ghats. It also serves as an important component of the ecosystem of the area (Tripathi et al., 2015). In comparison to many cultivated species, especially those cultivated for generations, Wild Food Plants (WFP) often contain higher levels of nutrients and bioactive compounds (Martins et al., 2011; Getahun, 1974; Nazarudeen, 2010). Therefore, understanding the composition of wild fruits and their nutritional potential is essential for their popularization (Adepoju, 2009; Mariod, 2020).

Among the genus *Diospyros*, many species have been reported for edible fruits (Persimmon). They are versatile fruits with many health benefits. They contain vitamins, antioxidants, and fibre. *Diospyros kaki* (Butta et al. 2015), cultivated in East Asia, China, Japan and Korea, *Diospyros melanoxylon* (Sailakshmi et al., 2018), *Diospyros chloroxylon* Roxb., distributed in the Indian subcontinent (Murthy et al., 2022), *Diospyros malabarica* (Shrestha,2022), distributed in Western Nepal, are a few among these. *Diospyros crumenata* is an underutilised fruit tree, and the lack of nutritional information is a constraint to its popularization. The analysis showed that

the total mineral content, Vitamin C, Sodium and Potassium in fresh fruits were higher when compared to other fruits like Jackfruit, Mango, and Pineapple (Rahul et al., 2024).

Acute oral toxicity testing (OECD, 2022) is a crucial step to ensure that ripened fruits of a species are safe for consumption, especially when they are to be introduced to new environments, or when large-scale consumption is expected in domestic or agricultural settings (Atik et al., 2019; Muñoz et al., 2021). The acute toxicity test of the ripened fruits of *D. crumenata* revealed that the Wistar rats showed no significant changes in body weight, no changes in feed or water intake, no adverse clinical signs and none of the organs showed any specific lesions due to toxicity.

The fleshy fruits along with the endosperm-rich seeds of *D. crumenata*, were heavily consumed by various arboreal mammals and terrestrial seed predators, such as rodents and mammals. The traits or characteristics of the fruit can influence predator preferences in a forest environment. Likewise, seed size also influences seed predation (Ganesh & Davidar, 2005; Cheng et al., 2024). Larger seeds are prone to predation (Janzen, 1969). The ripe fruits of *D. crumenata* have a sweet smell that attracts many mammals. The seed size is also large, with an average length of 11.038 ± 1.1388 mm and a width of 16.43 ± 0.2072 mm.

The evaluation of seed potentials is highly significant in identifying the sources of biologically active components and nutritional sources (Pourghayoumi et al., 2024). Analysis of minerals, chemicals, phytochemicals, active compounds, and antioxidant potential plays a crucial role in the discovery of bioactive principles (Mundaragi & Thangadurai, 2015; Nna & Orie, 2023). The seeds of *D. crumenata* are rich sources of minerals, chemicals, phytoconstituents, bioactive compounds, and antioxidant activities.

The chloroform extract of *Diospyros crumenata* seeds demonstrated a rich and diverse phytochemical profile. The n-Hexadecanoic acid and isolinoleic acid likely contribute most to the extract's anti-inflammatory and anticancer effects (Bharath et al., 2021; Ramamoorthy et al., 2025). Stigmasterol and cis-9-Hexadecenal enhance the therapeutic scope, especially in terms of fungal infections, immune modulation, and cancer prevention (Shane et al., 2020; Zhang et al., 2022). 1-Hexacosene, while not pharmacologically potent, may aid in topical applications and product formulation. Overall, the extract shows significant medicinal potential, especially in developing anti-inflammatory, antimicrobial, and anticancer therapies, along with possible cosmeceutical and nutraceutical applications. Various researchers evaluated plant source extraction using different solvents and were further analysed using chromatographic techniques like GC–MS. Understand the presence of important constituents in seeds of Tamarind (Razali et al., 2012), Punica seeds (Huqail et al., 2015), *Caesalpinia bonduc* seeds (Pushpalatha &Abraham, 2022) and Cinnamon (Owrang et al., 2024) are a few among these studies.

Identifying suitable habitats for the restoration of threatened plants is essential for creating new populations of the species and subsequently minimising the threat status of a species (Bramwell, 1991; Volis, 2016). Habitat niche models are the well-known methods used in predicting suitable habitats of the species (Philips et al., 2006). With the help of the Maximum entropy model (MAXENT), suitable habitats of the species have been predicted in many species, such as *Elaeocarpus serratus* (Baruah et al., 2019), *Ilex khasiana* (Adhikari et al., 2012), *Mesua assamica* (Baruah et al., 2016), and *Calamus nambariensis* (Deka et al., 2018). The findings suggest that precipitation variables, especially Bio19, are critical in determining the potential habitat distribution of *D. crumenata*. Elevation and slope also contributed significantly, highlighting the

importance of topographical features preferred by the species. *D. crumenata*, is narrowly distributed in the Western Ghats of Kerala, with populations/ individuals identified in four forest areas in Thrissur district. Niche modelling suggests additional suitable areas for restoration in the northern region of Kerala, enabling an increase in the extent of occurrence and area of occupancy of the species, and in due course help minimise the endangerment of the species.

Effective disease management in nursery plant stock is crucial for the *ex-situ* conservation of plant species. The relationship between pathogenic fungi and plants is highly complex. In forest nurseries, a variety of diseases such as damping-off, root rot, stem rot, leaf curl, wilt, canker, rust, and decay are commonly observed. Among these, fungal pathogens are particularly prevalent and resilient, often leading to significant seedling losses (Mohanan & Yasodharan, 2005; Pathak et al., 2015). Fungal diseases pose a major threat to global food security, and gaining a deeper understanding of plant–fungus interactions is essential for effective disease prevention and control (Strange & Scott, 2005; Gai &Wang, 2024). Identification of fungal pathogens at the molecular level is the primary task, and the subsequent management of the diseases. Species of *Penicilliopsis* Solms are typically tropical and primarily inhabit seeds rather than soil. Currently, the genus includes 17 species (Index Fungorum, 2024). Several studies have documented the occurrence of *P. clavariiformis* on *Diospyros* species, including *Diospyros paniculata* (Krishnapriya et al., 2020), *Diospyros crassiflora* (Douanla-Meli & Langer, 2007), and *Diospyros macrophylla* (Solms-Laubach, 1887). *In D. crumenata*, during seed germination, fungal fruit bodies growing from the seeds were observed *ex-situ* and *in-situ*. The disease incidence impacts seed germination, and the fungal pathogen was identified as *Penicilliopsis clavariiformis* (Praveena et al., 2025). Apart from this, seeds on germination or the plumule of the germinated seedlings were

found to be infected by a pathogenic fungus, *Curvularia clavata*. Nearly 10% of the seedlings were damaged by these infections in *D. crumenata*.

This study emphasises the importance of adopting an interdisciplinary approach for the conservation, management, and sustainable utilization of threatened tree species in the Western Ghats of Kerala. Mapping the distribution of *Diospyros crumenata* populations provides a critical baseline for prioritizing conservation efforts. Understanding its ecological characteristics helps reveal the factors driving its rarity in natural habitats and clarifies its functional role within ecosystems. Considering the urgency, propagation and multiplication of the species is essential to prevent further endangerment and to ensure the availability of plant material for *ex situ* conservation. In addition, the development of habitat distribution models can guide future *in situ* restoration initiatives. Equally important is the scientific assessment and validation of the species resources, which can facilitate the introduction of new plant resources from the Western Ghats and promote their sustainable use for societal benefit.

SUMMARY AND CONCLUSIONS

The study on *Diospyros crumenata*, a threatened and wild edible fruit tree of the Western Ghats, explores the challenges it faces in imminent endangerment, propagation protocols for its conservation and the scientific validation of lesser-known fruit for its utilisation, along with the scope of seed potential aiming for the societal benefit. The population mapping was conducted following a comprehensive field survey of the species in the Western Ghats of Kerala, which served as the study area. The survey identified the species' occurrence in Vazhachal forests along with an extended distribution in the Pattikkad-Chakkapara forests and at Chimmony Wildlife Sanctuary. A total of 138 mature individuals were documented over an extended area of 251.67 km^2, with an area of occupancy of 56.00 km^2. Based on this population data in the study area, the species was assessed as 'Critically Endangered' under the 'D' criteria (population size) of the IUCN guidelines.

Diospyros crumenata predominantly occurs in evergreen and semi-evergreen forests, thriving as a canopy or first-layer species. The associated trees differ between these ecosystems, indicating the species' adaptability in both ecosystems. Its distinctive blaze, white and yellow in the xylem, helps distinguish it from the closely related *Diospyros bourdillonii* in its habitats. The survey also highlighted that among the trees enumerated, only 10% belong to the pre-reproductive class, indicating the declining trend of the populations. The Inverted J-shaped curve models at each study site displayed irregular curves, emphasising population reduction of the species. The species prefers wet soil and watercourse areas, which are found to be microhabitats. The moderately low IVI values reflect the relatively low abundance of the species among its associates in respective forest ecosystems. The habitats of *Diospyros crumenata* are threatened by activities such as the development of a powerhouse, road

expansion near forest edges, and the high risk of forest fires. The trunk of *D. crumenata* trees has a distinctive association with lichens; a notable feature consistently observed in all identified populations was confirmed through taxonomic identification. The lichen association in respective ecosystems indicates the susceptibility of the microhabitats of the species towards habitat degradation and disturbances. Flushing was observed simultaneously in both male and female trees, during which the trees served as hosts to the moth *Arctornis marginata*, representing a new host record for this species. Each male flower contained 3,620 pollen grains per flower. The development of the bud to bloom took approximately 40-45 days. Fertility tests using the Acetocarmine staining technique showed that 75-80% of the pollen grains were fertile. In female trees, flower bud initiation occurs after a spell of leaf maturity. During blooming and after pollination, a large number of flowers were found abscised (estimated to be 60-70%), indicating ineffective pollination taking place in the species. *D. crumenata* exhibited a P:O ratio of 814:1, indicating a facultative xenogamous breeding pattern.

Fruit development, maturation, and ripening took approximately seven months, with immature fruit abscission occurring at a rate of around 20%. Once mature and ripe, most of the fruits were predated by various arboreal and ground-dwelling mammals. The ripe fruit's pulp is edible and eaten by tribal communities. The whitish, gel-like pulp is sweet, delicious, and nutritious, containing functional compounds. Analysis shows that it has more nutritional and beneficial compounds than many commonly grown fruits. Various mammals relish the fruits along with seeds, which impacts natural regeneration. Fruit predation was estimated at 70%, which significantly impacted the soil seed bank and resulted in poor seedling regeneration. Seed analysis revealed the

presence of minerals and biologically active components, as well as phytochemicals and antioxidants, showing potential for the discovery of bioactive compounds.

Vegetative propagation using stem cuttings from young plants showed moderately good rooting when treated with auxins. The application of IBA at 3000 ppm resulted in a maximum rooting rate of 70% within 60-70 days, compared to just 20% in the control sets. Similarly, air-layering had the highest success rates of 100% and 75% with NAA concentrations of 5000 ppm and 7000 ppm, respectively. The ramets and layers demonstrated a 100% survival rate.

The seeds are categorised as a recalcitrant type. The seeds displayed 66% germination with a fresh MC at 36%. However, the seeds on scarification, followed by 24 hrs. soaking in cold water, resulted in 100% germination. The seed storage trials conducted in different climatic conditions and containers recorded 10 months of viability with significant germination of 33% at 17% m.c. compared to the normal viability period of just 12 days in open-room conditions

The acute oral toxicity test of ripened fruit pulp in adult Wistar rats resulted in no changes in feed or water intake, or adverse clinical signs and overall, none of the organs showed any specific lesions of toxicity. The tribal knowledge of the edibility of the fruits was reaffirmed, highlighting their nutritional value. The fruits were found to have a higher mineral content, particularly in sodium and potassium, compared to commonly cultivated fruits such as jackfruit, mango, and pineapple. Although their carbohydrate and protein levels are relatively low, the fruit contains a significant amount of Vitamin C.

A detailed analysis of the seeds' nutritional content revealed the presence of minerals like calcium, magnesium, sodium, phosphorus, potassium, and boron. The proximate analysis revealed the presence of moisture, crude protein, crude fiber, crude

fat, and ash content. Phytochemical analysis indicated the presence of terpenoids, tannins, phenols, and flavonoids, as well as antioxidant potential. Additionally, GC-MS analysis identified various bioactive compounds in the seed extract, including essential oils, fatty acids, alkaloids, phenolic and volatile compounds, emphasising significant medicinal potential, especially in developing anti-inflammatory, antimicrobial, and anticancer therapies and nutraceutical applications.

The use of MaxEnt for habitat distribution modeling highlights the significance of predicting suitable habitats for species reintroduction. By incorporating bioclimatic variables to identify optimal habitats for *D. crumenata*, these modeling techniques provide valuable insights into potential reintroduction sites, supporting the restoration of the species.

Seed and seedling infestations during seed propagation were found to be major obstacles to the *ex-situ* conservation of the species. Identifying fungal pathogens is essential for effective disease management and preventing their spread. Pathogenic fungi such as *Penicilliopsis clavariiformis* and *Curvularia clavata* have been identified at the molecular level and found to affect seed propagation to an extent in *D. crumenata*.

The study of *Diospyros crumenata* is highlighted as a model case from the Western Ghats of Kerala for the conservation and sustainable use of threatened tree resources. It encompasses population assessment, evaluation of habitat threats, analysis of natural regeneration, and biotic interactions to establish the ecological and conservation significance of the species. A phenology calendar developed for *D. crumenata* provides valuable guidance for foresters and researchers in planning *in situ* management. Protocols for vegetative propagation, extended seed germplasm storage, and identification of suitable habitat sites strengthen both *ex-situ* and *in- situ* conservation strategies. Monitoring seedling diseases and identifying associated

pathogens further contribute to effective *ex- situ* management. The analysis of seed traits highlights potential medicinal properties, while scientific validation of ripe fruit edibility supports its promotion among local communities. This enhances social relevance and encourages participatory conservation approaches. The research findings recommend comprehensive conservation measures for the species and posterity.

RECOMMENDATIONS

The multidisciplinary study on *Diospyros crumenata*, an Endangered and lesser-known fruit tree distributed in the Western Ghats of Kerala, was carried out for the first time. The research led to the identification and documentation of ecological factors contributing to the species' rarity and the development of propagation protocols aimed at its conservation. Furthermore, the study validated and evaluated the properties of ripe fruits and seeds, highlighting the species' potential for resource-based utilization to benefit society. In this context, the following recommendations are proposed:

- As the present investigation was confined to the Western Ghats of Kerala, an extended distribution study covering the entire Western Ghats and Sri Lanka is recommended. This would facilitate a comprehensive reassessment of the global IUCN conservation status of the species.
- The species exhibited irregular flowering behaviour during the study period. Female trees were found to be devoid of flowers in certain years and showed advanced flowering than the preceding years. Therefore, a detailed investigation into the possible influence of climatic and weather factors on flowering is needed.
- Although habitat distribution models have been developed and suitable planting sites identified, *in situ* reintroduction programmes should be undertaken to enhance the number of populations.
- As the ripe fruits have been scientifically validated for edibility, and the seeds analysed for nutrients, phytochemicals, antioxidants, and bioactive compounds, it is recommended to initiate participatory conservation and popularization programmes of the species. These should include awareness campaigns, large-scale production of planting materials, and the distribution of plants to the public and institutions.

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