4.0. MATERIALS AND METHODS

The studies on the population ecology of *Hopea ponga* and *Syzygium travancoricum* were carried out in the natural habitat of Western Ghats of India.

4.1. Population Studies

The distributional status of *Hopea ponga* and *Syzygium travancoricum* was checked with extensive field trips conducted at various forest areas of Tamil Nadu and Kerala. The extent of occurrence, area of occupancy and the number of mature individuals were recorded by the guideline of IUCN (Mace *et al.*, 1992). The details of the above information were confirmed through various literature. For analysis of various lifeforms of both the tree species in the natural habitat, a detailed analysis on a range of seeds dispersal and mature individuals were calculated. To assess the proper dispersal mechanism of seeds, about 1x1 m plots were randomly created in the natural habitat and counted the average number of seeds per square meter plot. Also, the number of seedlings germinated per m$^2$ was calculated randomly at different study sites. The number of medium sized (4-6 years) trees grown per Km$^2$ and number of mature individuals available per square Km was also assessed for about 4 years from 2013-2016.

4.2. Ecological Analysis

The study site was divided into several rectangular plots of 100 x 100 m size and four of the 1 ha plots was selected by using simple random sampling technique (Fig. 1). Within each of the selected four sample plots, measurement and identification were limited to two candidate species and its woody associates with a diameter at breast height (DBH) of ≥10 cm. Most of the associated woody plants were measured at the breast height through the minimum diameter. The number and botanical names of all the tree species encountered in each field plot were recorded. When it was difficult to identify the species in the field, the common/ local name was recorded and plant specimens were collected for identification with the standard floras and herbarium. For the confirmation, identified specimens were compared by matching it with the specimens of the herbarium of the Botanical Survey of India (MH), Coimbatore and TBGT Herbarium, Jawaharlal Nehru Tropical Botanic Garden, Thiruvananthapuram, Kerala.
The following ecological parameters were used to analyse the candidate species and their associates.

4.2.1. Density

Density is an expression of the numerical strength of a species, where, the total number of individuals of each species in all the quadrats is divided by the total number of quadrats studied.

\[
\text{Density} = \frac{\text{Total number of individual species}}{\text{Total number of quadrats studied}}
\]

4.2.2. Frequency

This term refers to the degree of dispersion of individual species in an area and usually expressed in terms of percentage of occurrence. It was studied by sampling the study area at several places at random and recorded the number of candidate species that occurred in each sampling units. It is calculated by the following equation.

\[
\text{Frequency} = \frac{\text{Number of quadrats in which the species has occurred}}{\text{Total number of quadrats studied}}
\]

4.2.3. Abundance

It refers to the different number of individuals of the species in the community per unit area. The samplings are collected randomly by the quadrat method in different places and each species are summed up individually.

\[
\text{Abundance} = \frac{\text{Total number of individuals of the species in all the quadrats}}{\text{Total number of quadrats in which the species occurred}}
\]

4.2.4. Relative density

The numerical strength of a species in relation to the total number of individual species are studied by relative density.
Relative density = \frac{\text{Number of trees of each species}}{\text{Total number of trees}} \times 100

4.2.5. Relative frequency

The degree of dispersion of individual species in an area in relation to the number of all the species occurred.

Relative frequency = \frac{\text{Number of sampling units in which each species occurs}}{\text{Total number of sampling units of occurrence of all the species}} \times 100

4.2.6. Relative dominance

The dominance of a species is determined by the value of the basal cover. Relative dominance is the coverage value of a species with respect to the sum of coverage of the rest of the species in the area.

Relative dominance = \frac{\text{Total basal area of each species}}{\text{Total basal area of all the species}} \times 100

4.2.7. Basal area

Basal area is one of the grounds which is penetrated by the stem. The stem can be seen when the leaves and stems are clipped at the ground surface and it is one of the major characteristics to predict dominance. It can be measured to 2.5 cm above ground or actually on the ground level. It can be measured by calipers, line interception or point-centered quadrat method. Plants are entirely different in their growth form the relationship between herbage cover and basal area differs in different types of plants.

\text{Basal area} = \pi r^2

Where,

\text{Diameter of the stem} \quad r = \frac{\text{Diameter of the stem}}{2}

4.2.8. Shannon-Weiner’s index

Diversity is an important indicator of the status of an ecosystem. It consists of two components, the variety and the relative abundance of species. The higher value is
indicated through the higher diversity. The diversity of trees was estimated by using Shannon-Weiner’s index (Ludwig and Reynolds, 1988).

The formula for calculating the Shannon-Weiner’s index ($H'$) is

$$H' = -\sum p_i \ln p_i$$

Where,

$H' = \text{Shannon index of diversity}$

$p_i = \text{The proportion of important value of the } i^{th} \text{ species } (p_i = n_i / N)$

$N = \text{The important value index of all the species.}$

4.2.9. Simpson’s index of dominance

The dominance of the species is estimated by using Simpson’s index. It suggested that the diversity is related to the probability and the two individual are picked up at random which belongs to the same species. (Krebs, 1989)

The equation used to calculate Simpson’s index ($D$) is

$$D = \sum (p_i)^2$$

Where,

$D = \text{Simpson index of dominance}$

$p_i = \text{the proportion of the important value of the } i^{th} \text{ species } (p_i = n_i / N)$

$N = \text{the important value index of all the species.}$

As $D$ increases, diversity decreases and Simpson index was therefore usually expressed as $1-D$ or $1/D$.

4.2.10. Important Value Index (IVI)

The index is used for determining the overall importance of each species in the community structure. When it is calculating the index, the percentage values of the relative frequency, relative density and relative dominance are summed up together and the value is designated as an Importance Value Index or IVI of the species (Curtis, 1959).

The important value index is equal to the sum of the relative density, relative frequency and relative dominance.

Where,
Relative density  = \frac{\text{Number of trees of each species}}{\text{Total number of trees}} \times 100

Relative frequency  = \frac{\text{Number of sampling units in which each species occurs}}{\text{Total number of sampling units of occurrence of all the species}} \times 100

Relative dominance  = \frac{\text{Total basal area of each species}}{\text{Total basal area of all the species}} \times 100

The index was calculated for each plot separately as well as for all the plots taken collectively.

4.3. Phenology

Phenology is the study of the functional rhythm of plants in relation to the seasonal and climatic factors. Phenological studies are most important for understanding the plant responses to various biotic and abiotic factors. For the present investigation, the selected individuals of both *Hopea ponga* and *Syzygium travancoricum* were marked in the natural habitat and observation were made by regular field visits in each month. The flowering phenology was observed on a day-to-day basis which included, flower initiation, development and maturation, anthesis, anther dehiscence, flowering and fruiting period, etc. The phenophase events were recorded as per the method suggested by Dafni *et al.* (2005).

4.4. Floral Biology

The morphology of flowers was studied through dissection microscope and hand lens to analyse the various floral parts of the flower. The size of the floral parts was measured with the help of measuring scale and Vernier caliper. The studies on male and female reproductive parts, time of anther dehiscence, mode of dehiscence and position and morphology of stamens and pistil were observed by using hand lens (40x).

The number of flowers produced per inflorescence and the average number of flowers produced per tree was calculated. The mature floral buds were continuously monitored for the time of anthesis. The lifespan of the flower was calculated by the observation of flower up to withering of floral parts. The position and arrangement of
anthers and stigma were observed with the help of hand lens and dissection microscope. The fully matured and unopened anthers were collected and observed for an average number of pollen per anther and per flower. The pollen-ovule ratio was calculated according to the method suggested by Dafni et al. (2005). The morphology, arrangements and position of stigma were observed through dissection and scanning electron microscope.

**4.5. Pollination Biology**

During the flowering period in general and peak flowering, in particular, the flowers were continuously monitored in day and night time to record the different type of pollinators visiting the flowers. The number of floral visitors, the percentage of the floral visit and stigma touch by the pollinators were recorded. The foraging behavior of the floral visitors was analysed by photography and visual observation through high resolution binocular (Olympus).

**4.6. Fruit and Seed Biology**

The mechanism of fruit initiation, maturation and seed dispersal were studied in the natural habitat on selected trees. The number of fruits developed per inflorescence, average number of fruits produced per branch and per tree was calculated. The mature fruits and seeds were collected at the time of detached stage and stored in seed containers and polyethylene bags for further studies.

**4.6.1. Analysis of moisture content of seeds**

The mature seeds were randomly collected to analyse the moisture content level. The seed moisture content was calculated by the formula developed by International Seed Testing Association (Bewley and Black, 1982).

\[
\text{Moisture content of seeds (\%)} = \frac{\text{Fresh weight of seeds-Dry weight of seeds}}{\text{Fresh weight of seeds}} \times 100
\]

**4.6.2. Analysis of viability of seeds**

The randomly selected seeds were collected at the time of detachment and analysed for viability at different time interval. The selected mature seeds were soaked for overnight in the distilled water for water absorption. The seed coat was slightly
pierced out from the cotyledon and to this few drops of tetrazolium-phosphate buffer solution (pH-7.5) was added and incubated at 30°C for 24 hours. After the incubation period, the seeds were washed thoroughly with water and observed under a microscope. The completely stained seeds were counted and consider as viable seeds whereas, partially stained and non-stained seeds were counted as sterile.

**4.6.3. Analysis of seed germination**

The viable seeds were collected from individual plants separately during the fruiting seasons and allowed to germinate in the mud pots filled with garden soil, sand and organic manure. Three replicates of 100 seeds were allowed to germinate at every 5 days to determine the optimal month for seed germination and seedling establishment. Quantitative features such as the number of days taken for seed germination and percentage of seed germinated in the laboratory conditions were also analyzed periodically.

In order to understand the fecundity and mortality rate of seedlings of both the species, about 100 seeds of five replicates were sowed at natural habitat during 2013-2016. The viable seeds of both the tree species were sowed at different climatic period especially during the seed germination period of both the tree species. The seed germination behavior of both the species was continuously monitored in the natural habitat and the parameters *viz.* a number of seeds germinated, the number of seedlings emerged, the number of seedlings died and the numbers of seedlings under survival for about 3 years were calculated. Based on the data generated, the percentage of mortality and survivability were calculated to assess the dynamics of seedlings.

**4.7. Insect and Pest Association**

Tropical trees have slow growth and any infestation or pathogenic effect on the tropical trees will lead to a drastic reduction of the tree population. Hence, it is important to observe the insect and pest association of the candidate species in order to assess their ability to sustain healthy individuals in the natural environment. The candidate species were continuously monitored for disease and pest infection. Seeds and fruits were also observed for the infection and the percentages of infected seeds, pathogens responsible for infection were also observed. Insect or microbes responsible for the infection in the tender shoots, the level of damage caused and percentage of seedlings infected were observed in the field.
Fig. 1. Sample plot for vegetation analysis: a-Shrubs, b-Trees, c-Herbs