NAGALAND UNIVERSITY

(A Central University Estd. by the Act of Parliament No. 35 of 1989)

Lumami-798627, Nagaland, India

C E R T I F I C A T E

The thesis entitled **"REPRODUCTIVE PHENOLOGY OF TWO RARE, ENDANGERED AND THREATENED (RET) SPECIES OF GENUS *RHODODENDRON* L. OF NAGALAND"** submitted by Ms. Imtilila Jing, bearing Registration No. 472/2012 (Dated October 14, 2011) embodies the results of investigations carried out by her under our supervision and guidance.

Further, certified that this work has not been submitted for any degree elsewhere and that the candidate has fulfilled all conditions laid down by the University.

(Neizo Puro)
Supervisor

(S.K. Chaturvedi)
Co- supervisor

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DECLARATION

I, Ms. Imtilila Jing, hereby declare that the subject matter of this thesis is the record of work done by me, that the contents of this thesis did not form basis of the award of any previous degree to me or to the best of my knowledge to anybody else, and that the thesis has not been submitted by me for any research degree in any other University/ Institute.

This is being submitted to Nagaland University for the degree of Doctor of Philosophy in Botany.

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CONTENTS

CHAPTER-I

INTRODUCTION

The genus *Rhododendron* L. belongs to the family Ericaceae. Ericaceae consists of some 100 genera and 3000 species (Stevens, 1971). *Rhododendron* is one of the largest genus of the family with around 1000 species of evergreen, deciduous shrubs and trees (Chamberlain *et al*., 1996; Milleville, 2002; Fang *et al*., 2005; Tiwari and Chauhan, 2006; Sastry, 2010; Gibbs *et al*., 2011; Bhattarcharya and Sanjappa, 2014). The genus is divided into eight subgenera which include various sections and subsections (Sleumer, 1980; Chamberlain *et al*, 1996). The term '*Rhododendron*' comes from the Greek words 'rhodo' meaning 'rose' and 'dendron' meaning 'tree'. The genus *Rhododendron* was described by Carl Linnaeus in 1753 in Genera Plantarum.

Rhododendrons are reported to have ecological significance as well as immense horticultural importance for its beautiful flowers and foliage (Paul *et al*., 2005; Mao and Gogoi, 2012). It is distributed from the northern temperate zone, throughout tropical south-eastern Asia, to north-eastern Australia (Chamberlain *et al.*, 1996). According to Brown *et al*., (2006), the genus *Rhododendron* is widely distributed between the latitudes of 80°N and 20°S. They are considered Alpine native plants from North America to Europe, Russia and Asia; and from Greenland to Queensland, Australia and the Solomon Islands. The centres of diversification are in the Himalayas and Malaysia with the greatest species diversity in the Sino-Himalayan region, Southwest China and Northern Myanmar, from Uttarakhand, Nepal and Sikkim to North-western Yunnan and Western Sichuan and South-eastern Tibet, and with other significant areas of diversity in the mountains of Korea, Japan and Taiwan (Gao *et al*., 2002). According to Pradhan (1985), rhododendrons are found to be

inhabitants of higher elevations in Sino-Himalayan regions with highest concentration in Western China.

Generally rhododendrons prefer to grow in regions of high rainfall, high humidity and acidic soils. They vary from a few centimetres tall to large trees up to 30 metres tall (Gibbs *et al.,* 2011). They usually prefer mountainous areas that have a temperate climate and are found in humid and cool regions across the northern hemisphere (Milleville, 2002). In the lower altitudes, flowering starts from February to April and from May to June in the higher altitudes which gives an attractive appearance to the hills and mountain slopes with different shades like red, pink, white, yellow and purple depending upon the species (Paul *et al*., 2005; Mao and Gogoi, 2012). Rhododendrons have clustered inflorescence (trusses) at the tip of the branches and occur in all colours except a true bright blue. The flowers may be single or multicoloured with a contrasting throat blotches/spots which are always darker than the corolla and is one of the typical features of a *Rhododendron* flower (Paul *et al*., 2005). They serve as a guide for identifying different species either by their presence and their colour or by their absence (Milleville, 2002).

In India, the history of *Rhododendron* began in 1796 with the visit of Capt. Hardwich to the Siwalik mountain ranges in Kashmir where he discovered *Rhododendron arboreum* (Mao and Gogoi, 2012). Sir George Watt, the first Economic Botanist of British India was the first to collect *Rhododendron* species from Manipur and Nagaland during his survey from 1882-1885. He described four new *Rhododendron* taxa viz., *Rhododendron macabeanum, Rhododendron elliottii*, *Rhododendron triflorum* var. *bauhiniiflorum* and *Rhododendron wattii* from the Japfu hill ranges (Mao and Gogoi, 2012). Frank Kingdon-Ward, a British plant hunter and

explorer also contributed to the knowledge of rhododendrons in Nagaland. Scientists from Botanical Survey of India (BSI) had surveyed and published a few reports on rhododendrons of Nagaland but no detailed taxonomic accounts were given (Mao *et al*., 2001; Mao and Gogoi, 2007, 2010; Mao *et al*., 2009; Mao, 2010).

In the Indian Himalayan Region, Sekar and Srivastava (2010) have reported a total of 87 species, 12 subspecies and 8 varieties of rhododendrons. The maximum concentration was observed in Arunachal Pradesh (86%) with a total number of 75 species. Mao (2010) reported 121 taxa from India with a maximum concentration of 117 taxa (98%) in north-east India, where the highest number of taxa is reported from Arunachal Pradesh (106), followed by Sikkim (40), Manipur and Nagaland (10), Mizoram (4) and Meghalaya (2). 17 taxa of Indian rhododendrons are found to be endemic to north-east India. Arunachal Pradesh has maximum number of endemic taxa (9) followed by Manipur and Nagaland with 6 taxa each. As many as 43 *Rhododendron* species in India have been put into rare, endangered and threatened categories (Sastry and Hajra, 1983).

In Nagaland, rhododendrons are reported from higher subtropical to temperate regions. They are found in subtropical hills of Zunheboto and Wokha districts; temperate forests of Mt. Saramati, Mt. Japfu, Jakhama, Khonoma, Puliebadze and Dzulakie hills (Mao and Gogoi, 2012).

The rich growth of rhododendrons in the state has been severely degraded in recent times. Increase in human population and anthropogenic activities along with other natural factors are found to be the dominant factors of disturbances on rhododendrons growing in high altitudes (Mao *et al*., 2010). Natural threats include landslides and forest fires which affect the rich growth of rhododendrons.

Anthropogenic threats include agricultural activities, fuel wood collection, small scale extraction of timber and collection of plants by local people for their magnificent flowers. Mao and Gogoi (2012) reported that the genus *Rhododendron* is one of the most popular plants for their beautiful flowers, evergreen foliage and shapes. A great diversity in form and colour of flower is observed even within a species. It can be introduced as avenue trees along roadsides and residential areas in the hills between 2000 m and 4000 m. They are widely cultivated in Europe, America Australia, etc. *Rhododendron* is the state flower of West Virginia and Washington State; Georgia's official wildflower in USA and *Rhododendron arboreum* is the national flower of Nepal (Paul *et al*., 2005). In India, *Rhododendron* is the state tree of Sikkim and state flower of Nagaland.

Many *Rhododendron* species are crossed with each other to give beautiful hybrids through human intervention. Many hybrids have been created since the time Joseph Hooker and his companions took *Rhododendron* species to Europe. Rhododendrons are also crossed with azaleas to produce Azaleodendrons (Milleville, 2002). Mao and Gogoi (2012) have also reported that currently there are 28,000 cultivars of *Rhododendron* in the International Rhododendron Registry held by the Royal Horticultural Society, U.K. In many parts of the world both *Rhododendron* species and hybrids are valued as ornamental plants in landscaping. However, most of the species are not found to be popular in its native regions of South-east China and the Himalayan region.

The flowers of *Rhododendron* are also considered sacred and offered in temples and monasteries (Mao *et al.,* 2001). Apart from aesthetic and sacred values, rhododendrons also have medicinal and economic values. The dried flowers of

Rhododendron arboreum are considered to be very effective in treating diarrhoea and blood dysentery. The fresh and dried corolla that is acid-sweet in taste is given when fish bone gets stuck in the throat (Pradhan and Lachungpa, 1990). It is also reported that rhododendrons serve as antibiotics, anti inflammatories, and in some communities they are used for firewood and timber. They are also used as tea, wine and jams. They are also valued for their narcotic potential. Certain species are also sources of insecticides (Gibbs *et al*., 2011). Georgian and Emshwiller (2016) reported that the people in southwest China, belonging to Bai ethnic group eat the flowers of *Rhododendron decorum* Franchet for their taste. The flowers are cooked as a soup with flowers, beans and soup. They also reported that the Dulong ethnic minority uses rhododendron wood to carve implements used in weaving, saddles, plows, smoking pipes and other household items.

Rhododendrons play a vital role in ecosystem services. They grow in areas of high rainfall and high humidity on acidic soils; conditions under which few plants would survive. Therefore, they play an important role in slope stabilization and watershed protection particularly in the Himalayas where so many of Asia's major rivers start. They also provide the structure of plant communities which support a wealth of biodiversity (Gibbs *et al*., 2011) which when disturbed can degrade habitats that threaten associated biodiversity (Tiwari and Chauhan, 2006). Rhododendrons have extensive and dense root mat which helps in soil stabilization in the very steep terrain which prevents soil erosion (Colak *et al*., 1998). Majority of the *Rhododendron* species bear long lived evergreen leaves that serve as significant storage for nutrients (Monk *et al*., 1985). They provide favourable niche to several bird species and canopy lover insects. Thus they play a vital role in ecological stability (Badola and Pradhan, 2010). In the high elevations of the Eastern Himalayas,

rhododendrons act as keystone species. It is the only group of plants that extends broadly across the subalpine to alpine transition zone which is the most fragile ecosystem in the Eastern Himalayas (Menon *et al.*, 2012). The bud phenology of rhododendrons provide vital information on the timings of phenological events, necessary for tree improvement because the buds of rhododendrons are highly sensitive towards climatic variations (Badola and Palliwal, 1987; Badola, 2004; 2009). Many of the rhododendrons, especially of the high altitude zones, are very sensitive to specific climatic conditions and may be susceptible to slight change in temperature (Badola, 2010). Thus, rhododendrons have phenological sensitivity to climate change and play a vital role in ecological stability of ecosystems, as indicators of forest health (Mainra *et al.*, 2010).

Rhododendron macabeanum Watt ex Balfour f. and *Rhododendron elliottii* Watt ex Brandis are listed as 'Rare' and 'Endangered' species respectively as per IUCN category (Table: 1). Both species of *Rhododendron* are endemic to Nagaland and Manipur (Paul *et al*., 2005; Mao, 2010; Sekar and Srivastava, 2010; Mao *et al*., 2011, Mao and Gogoi, 2012; Bhattarcharya and Sanjappa, 2014). Therefore, the reproductive phenology of this two species is investigated in this study. These two species are found to be depleting in their natural habitat. This work is first of its kind in respect to *Rhododendron macabeanum* and *Rhododendron elliottii*. An attempt is made to provide data for their conservation strategies and to assess the factors responsible for the depletion of these two species.

Table 1: IUCN Status of *Rhododendron macabeanum* and *Rhododendron elliottii.*

Sl.No	Name of the Species	IUCN Status	Altitude (in metres)
1	*Rhododendron macabeanum* Watt ex Balfour f.	Rare, endemic	2500-2800
2	*Rhododendron elliottii* Watt ex Brandis	Endangered, endemic	2500-2700

Reproduction is the natural process by which new individuals are produced from their parents. It is one of the key processes in seed-plant life history which influences the evolutionary success of an individual. Thus reproduction is the basis of survival and sustenance of a species in natural habitats. However, the sustainability of biodiversity is threatened due to habitat degradation, overexploitation, and climate change. A large number of species have become rare, endangered and threatened (RET). The International Union for Conservation of Nature and Natural Resources (IUCN) has established the Rare (R), Endangered (E) and Threatened (T) categories to highlight the legal status of rare species for the purpose of conservation. According to the Red Data Book definition, Threatened (T) taxa are those taxa which are endangered, vulnerable and rare in IUCN categories. On the other hand Rare (R) taxa are taxa with small world population that are not at present endangered or vulnerable but are at risk. They are usually localized within restricted geographical areas or habitats or are thinly scattered over a more extensive range. The 'Rare' species has a small world population but is under no known or immediate threat. Any species with less than 20,000 individuals is considered 'Rare'. It is not endangered but is simply at risk because of the size of its population. According to Sharma (2007), one of the

important focus of conservation effort is the protection of rare species as they are considered to be especially vulnerable to extinction because they occupy only one or a few specialised habitats. Endangered (E) taxa are those taxa whose number has been reduced to a critical level and are in danger of extinction and whose survival is unlikely if the causal factors continue operating (Nayar and Sastry, 1983). A species is said to be endemic if it is found in only a single geographic area and nowhere else. Thus endemic taxa are those restricted to a defined geographic location such as a specific habit, region or continent (Singh, 2007). They occupy limited geographical ranges and are thus often the most vulnerable (Myers, 2003).

In India there are about 4900 endemic species of flowering plants concentrated in the Western Ghats, North-West Himalayas and Andaman and Nicobar Islands (Singh 2007). Flowering plants constitute about 33% of Indian endemic species and are mostly found in north-east India, Western Ghats, and Andaman and Nicobar Island (Sharma, 2007).

Reproductive failure due to constraint in one or several reproductive events is the major factor for species extinction. Using the IUCN categories, the World Conservation Monitoring Centre (WCMC) has described threats to about 60,000 plant and 2000 animal species in its series of Red Data Books (Sharma, 2007). Therefore to effectively manage, conserve and utilise biodiversity sustainably, a comprehensive knowledge in reproductive biology is required (Ramawat *et al.*, 2014). Reproductive phenology and pollination of plants are two important aspects of the reproductive biology (Rathcke and Lacey, 1985).

Phenology (Greek word *phainein* meaning to show or appear) deals with the timing of recurring biological events influenced by seasonal environmental factors

such as temperarture and precipitation etc. It is well acknowledged as one of the most preferred indicator of climate change (Badola, 2010). Plant phenological studies include the onset and period of leaf fall, appearance of vegetative buds (leaf flushing), appearance of floral buds, date of first flowering, duration and termination of flowering, fruit initiation and fruit development, fruit maturation and seed dispersal. Phenological observations are essential for studying the specific functions of plant in natural populations for their conservation (Aronson *et al*., 1994).

Studies on reproductive phenology have been undertaken with the following objectives:

i. To study natural distribution and morphological diversity of *Rhododendron macabeanum* and *Rhododendron elliottii.*
ii. To collect data on precise phenology in relation to climatic factors.
iii. To investigate development of anther, ovary, fruit and seed and identify barriers to seed development.
iv. To investigate fruit/seed dispersal mechanism, seed germination and seedling establishment.
v. To investigate seed-insect pests/predators/pathogens relationship, invasive species and other biotic factors that affect reproductive phenomena.
vi. To study natural regeneration- seedling recruitment, survival and causes of seedling mortality, impacts of invasive species.
vii. To study ecological services provided by the RET species to other organisms.
viii. To study the impact of human activities affecting reproduction, regeneration, and economic/ecological sustainability of RET species

STUDY AREA

The present investigation was carried out in the forest of Western Dzukou, Khonoma village of Kohima District in Nagaland. Nagaland is one of the hill states of north-east India which supports a very rich and luxuriant diversity of vegetation and wildlife. Geographically, Nagaland lies between 26°60' N and 27°40' N latitude and 93°20' E and 95°15' E longitude and has an area of 16,579 square kilometres. The geographical location, climate and topography support the rich species in the state. The state has a pleasant sub-tropical to sub-alpine with a typical monsoon climate.

The district of Kohima is one of the 11 districts in Nagaland located in the southern part of Nagaland. It covers an area of 1041 square kilometres. Kohima, the state capital has an altitude of 1444.12 m above sea level. It has a pleasant and moderate climate. It receives rainfall throughout the year and the average annual rainfall from May to October is between 200cm and 250 cm. Thus, Kohima enjoys sub-tropical type of rainfall.

Khonoma, the study area is situated 20 Km south-west of Kohima, at an altitude of 1500 m above sea level and is surrounded by hills that are as high as 3000 m. It is the first 'Green Village' in Nagaland. It is famous for its nature and Tragopan conservation. Terrace cultivation is practised and it is one of the oldest agricultural practises. The name 'Khonoma' is derived from a plant *Gaultheria frangrantisima* locally known as 'khwüno'. It is found in plenty in the higher altitudes. The village has a hilly terrain from gentle to steep slopes. Some of the regions of the forest are inaccessible because of the steep and rocky mountain slopes. Khonoma enjoys monsoon climate with little rainfall during April and May and heavy downpour from

June to September. The months of February and March are very windy. Winter is cold and chilly spreading from November to January. The maximum temperature in summer is 31°C and minimum temperature of 16°C whereas in winter 24°C and 4°C respectively.

The Khonoma Nature Conservation and Tragopan Sanctuary (KNCTS) was initiated to create environmental awareness among the inhabitants and preserves about 70 square kilometres of its village forest. The sanctuary is an ideal abode of many other rare and endangered species of plants and animals. KNCTS and the terrace fields are of outstanding value from a biodiversity conservation, water security and aesthetic point of view. The state bird of Nagaland, Blyth's Tragopan (*Tragopan blythii*) which is endangered according to IUCN category is found with very small population and is protected in the forests of Khonoma. KNCTS is recognised as one of the 465 Important Bird areas in India (Islam and Rahmani, 2004).

Preliminary ecological studies in Khonoma have recorded the use of about 250 plant species, including over 70 for medicinal purposes, 84 species of wild fruits, 116 species of wild vegetables, 9 species of mushroom and 5 species of natural dyes from the surrounding forests in the village. As many as 204 species of trees, 45 species of orchids, 11 species of cane and 19 species of bamboos have been recorded by the local people. They have also recorded many wild animals which include tiger, leopard, Serow, sloth bear, Asiatic black bear, common otter, snakes, amphibians and 196 bird species (Pathak and Kothari, 2006). The villagers rear mithun (*Bos frontalis*) in large numbers in the forest. These mithuns are allowed to graze in these forests openly and hence the ground vegetation is trampled or grazed by the mithuns.

In this area of study, it is found that a good number of rhododendrons adorn the forest vegetation. Some of the species like *Rhododendron arboreum, R. macabeanum, R. elliottii, R. triflorum* var *bauhiniiflorum, R. maddenii* subsp. *crassum,* and *R. formosum* var. *inequale* are found growing above 2000 m. *R. arboreum* is also found growing at an altitude below 2000m.

Sastry and Hajra (1983), Mao *et al* (2009), Mao (2010), Sekar & Srivastava (2010) and Mao and Gogoi (2012) have reported that *R. macabeanum, R. maddenii, R. triflorum* var *bauhiniiflorum* as rare, *R. formosum* var. *inequale* as threatened while *R. elliottii* as endangered species and all these mentioned species of rhododendrons are endemic to Nagaland and Manipur except for *R. arboreum*.

The two species of *Rhododendron* undertaken for the present investigaton *viz.*, *R. macabeanum* and *R. elliottii* belong to the subgenus Hymenanthes which include the elepidote (non-scaly) rhododendrons. According to Chamberlain (1982) most species in this subgenus are shrubs or trees and the leaves always persist through at least one winter. The leaves are glabrous at maturity, more or less densely hairy below but are rarely hairy on both surfaces. The leaves of rhododendrons in this subgenus are usually thick and the new leafy shoots emerge from the axils of shoots from the previous year's growth (Goetsch *et al*, 2005). The inflorescence is always a terminal raceme and few- to many-flowered. Generally the calyx is green and often reduced to a rim with tiny triangular lobes. The corolla is always zygomorphic. Majority of the species in this subgenus have 5-lobed corolla while some are 6-9 lobed. Tubular – campanulate corollas have nectar pouches which are intensely coloured, either deep red or purple. The number of stamens is usually twice the number of the corolla lobes and the filaments are glabrous or pilose towards the base. The ovary is 5- to 18-

locular and usually covered with a sparse or dense indumentums. The stigma is usually capitate or massive and discoid in some subsections. The fruit is a capsule which may be cylindrical, oblong to linear, straight or curve. The seeds are fusiform and usually with wings which may be irregular and are sometimes broken up into finger-like projections at the ends of the seeds (Chamberlain, 1982). The seeds are very minute and are dispersed by wind.

The two species considered for this study is described below:

Rhododendron macabeanum Watt ex Balfour f. (Table 2)

Rhododendron macabeanum is named after Mr. McCabe who was a former British Deputy Commissioner of Naga Hills. It is a large tree which grows upto a height of 12-30 m. The leaves are broadly ovate to broadly elliptic, the upper surface is glabrous when mature, reticulate with impressed veins and the lower surface of the leave is covered with a dense whitish indumentum. The inflorescence is dense globose, 20-28 flowered; corolla is 8-lobed, ventricose-campanulate and lemon yellow with a purple blotch at base (Plate-3, figs. B-E). The stamens are 16-18 in number, filaments 3.5-4.5 cm long, glabrous to pubescent towards base. The ovary is densely rufous tomentose, style glabrous and fruit is a capsule which is woody and slightly curved.

Table 2: **Description of *Rhododendron macabeanum*.**

Predominant Flower Colour:	Yellow
Flower/Truss Description:	Tubular campanulate, yellow. Truss holds 20-28 flowers.
Fragrant:	No
Bloom Time:	March
Foliage Description:	Leaves broadly ovate to broadly elliptic, upper surface glabrous when mature.
Elepidote (E) or Lepidote (L)	Elepidote (E)
Plant Habit:	Upright, large tree.
Sub Genus:	Hymenanthes
Section:	Ponticum
Sub Section:	Grande
Geographic Origin:	North-East India

Rhododendron elliottii Watt ex Brandis (Table 3)

Rhododendron elliottii is named after Mr. Elliott, a friend of Sir George Watt. He collected the plant from Japfu Hills in 1882. It is an upright, often straggly, small tree growing to a height of 2-5 m. Leaves are oblanceolate to elliptic with apiculate apex, green and glabrous when mature on both surfaces. The flowers are tubular-campanulate, red colour with darker flecks. The inflorescence is 10-20 flowered and the corolla is 5-lobed (Plate-11, figs. B-D). Ovary is densely tomentose intermixed with stipitate glands and style is also tomentose (Plate-13, figs. A, B, D). Fruit is a capsule, straight and tomentose (Plate-14, fig. D).

Table 3: Description of *Rhododendron elliottii.*

Predominant Flower Colour:	Red
Flower/Truss Description:	Funnel campanulate, red. Truss holds 10-20 flowers.
Fragrant:	No
Bloom Time:	May
Foliage Description:	Leaves oblanceolate to elliptic, upto 4.5", both surfaces glabrous.
Elepidote (E) or Lepidote (L)	Elepidote (E)
Plant Habit:	Upright, often straggly shrub or small tree.
Sub Genus:	Hymenanthes
Section:	Ponticum
Sub Section:	Parishia
Geographic Origin:	North-East India

REVIEW OF LITERATURE

The genus *Rhododendron* was described by Carl Linnaeus in 1753 in Genera Plantarum. It is one of the largest family of the Ericaceae family occurring in the higher altitudes with more than 1000 species (Chamberlain *et al*., 1996; Milleville, 2002; Fang *et al*., 2005; Tiwari and Chauhan, 2006; Sastry, 2010; Gibbs *et al*., 2011; Bhattarcharya and Sanjappa, 2014) and the only genus in the family that reaches the height of a tree (Milleville, 2002). The genus is divided into eight subgenera which include various sections and subsections (Sleumer, 1980; Chamberlain *et al*., 1996). Rhododendrons are reported to have ecological significance as well as economic importance in addition to its graceful flowers (Paul *et al*., 2005). According to Chamberlain *et al*., (1996) the genus is distributed from the northern temperate zone, throughout tropical south-eastern Asia, to north-eastern Australia. According to Pradhan (1985), rhododendrons are found to be inhabitants of higher elevations in Sino-Himalayan regions with highest concentration in Western China.

According to Mao and Gogoi (2012), the history of Indian *Rhododendron* began in 1796 with the visit of Capt. Hardwich to the Siwalik mountain ranges in Kashmir where he discovered *Rhododendron arboreum* Smith Scarlet. Sir George Watt, the first Economic Botanist of British India described four new *Rhododendron* taxa viz., *Rhododendron macabeanum, Rhododendron elliottii, Rhododendron triflorum* var. *bauhiniiflorum* and *Rhododendron wattii* from the Japfu hill ranges of Nagaland. He was also the first to collect *Rhododendron* species from Manipur and Nagaland during his survey from 1882-1885. Frank Kingdon-Ward, a British plant hunter and explorer also contributed to the knowledge of rhododendrons in Nagaland and Manipur. Scientists from Botanical Survey of India (BSI) had surveyed and

published few reports on rhododendrons of Nagaland and Manipur but no detailed taxonomic accounts were given (Mao *et al*., 2001; Mao and Gogoi, 2007, 2010; Mao *et al*., 2009; Mao, 2010). Mao *et al*., (2009) studied the status and distribution pattern of *Rhododendron* species in temperate and sub-alpine hill ranges of Mt. Esii and surroundings in Manipur and Nagaland, India. They recorded eight taxa viz., *R. macabeanum*, *R. maddenii* ssp. *crassum*, *R. triflorum* var. *bauhiniiflorum*, *R. johnstoneanum*, *R. arboreum* ssp *arboreum*, *R. elliottii*, *R. fulgens* and *R. vaccinioides*.

Mao (2010) has reported 121 *Rhododendron* taxa from India with a maximum concentration of 117 taxa (98%) in north-east India. 17 taxa of Indian rhododendrons are found to be endemic to North-east India. Arunachal Pradesh has maximum number of endemic taxa (9) followed by Manipur and Nagaland with 6 taxa each. As many as 43 *Rhododendron* species in India have been put into rare, endangered and threatened categories (Sastry and Hajra, 1983). According to Sekar and Srivastava (2010) a total of 87 species, 12 subspecies and 8 varieties of rhododendrons have been reported from the Indian Himalayan Region (IHR). In Nagaland, rhododendrons are found in subtropical hills of Zunheboto and Wokha districts; temperate forests of Mt. Saramati, Mt. Japfu, Jakhama, Khonoma, Puliebadze and Dzulakie hills (Mao and Gogoi, 2012). Detailed taxonomic account of Rhododendrons of Manipur and Nagaland was given for the first time by Mao and Gogoi (2012).

Rhododendrons growing in high altitudes are facing the impact of disturbances due to various natural and anthropogenic factors (Mao *et al.*, 2010). Natural threats include landslides and forest fires which affect the rich growth of rhododendrons. Anthropogenic threats include fuel wood collection, small scale

extraction of timber and collection of plants by the local people for their beautiful, showy flowers. The main threats are habitat loss due to the increasing human population leading to the loss of rich floristic diversity (Mao and Gogoi, 2012).

Gibbs *et al*., (2011) assessed the red list of rhododendrons of the world where they indicated that 25 percent of all *Rhododendron* taxa are under threat of extinction in the wild. They reported that out of 1157 rhododendrons assessed, two species are no longer found growing in their natural habitats, 316 are considered threatened with extinction in the wild at the global scale and 483 taxa are of no current conservation concern.

Therefore, to effectively manage, conserve and utilise biodiversity sustainably, a comprehensive knowledge in reproductive biology which include phenology, floral biology, pollination and breeding system is required (Ramawat *et al*., 2014). Moza and Bhatnagar (2007) have also emphasized the importance of the studies of reproductive biology for successful cultivation and conservation of these rare, endangered and endemic species of *Rhododendron*. The evolutionary success and survival of plants is mostly determined by the efficiency of their reproductive performance (Kumar *et al*., 2011). To understand the causes and consequences of the species rarity, a comparison between the rare and common species is necessary to develop management strategies for their conservation (Kunin and Gaston, 1993; Kunin and Shmida, 1997). Studies on reproductive biology is necessary for systematic, evolutionary and conservation studies (Ornduff, 1969; Holsinger, 1991; Anderson, 1995), it also helps in estimating the genetic variation and the quality and quantity of seeds produced by a species (Costich, 1995; Nagrajan *et al.*, 1996).

Observations on floral morphology, phenology and pollination studies provide inferences into plant breeding systems (Nagrajan *et al*., 1998; Gituru *et al*., 2002).

Phenology is the art of observing the phases of the life cycle or the activities of organisms as they occur throughout the year (Leith, 1973). According to Fenner (1998), phenological events in plants involves bud-bursts, leaf expansion, abscission, flowering, fertilisation, seed set, fruiting, seed dispersal and germination which occur in due season. According to Sedgely and Griffin (1989), the relationship between the vegetative and floral phenology is essential for higher crop yield. Flowering phenology is of significance for both ecological and evolutionary reasons as flowers are important food resources in ecological time and provide a mechanism for reproductive isolation or speciation over evolutionary time (Kearns and Inouye, 1993). Plants interact with the environment at all times of the year, and flowering is often closely related to seasonal climatic changes. According to Badola (2010) phenology is well acknowledged as one of the most preferred indicator of climate change. Phenological observations are essential for studying the specific functions of plant in natural populations for their conservation (Aronson *et al*., 1994).

Many studies on phenology of tropical forests have been carried out in markedly seasonal climates (McClure, 1966; Janzen, 1967; Daubenmire, 1972; Medway, 1972 and Frankie *et al*., 1974). They reported several types of variations in the timing and duration of leaf, flower and fruit production. Flowering is seasonal in most tropical areas as phenology is affected by seasonal rains and temperature changes (Sinha, 1975; Menzel, 1984; Lord and Eckard, 1985; 1987; Scholefiled *et al*., 1986).

One of the most widely investigated aspects of the phenology of plant life cycles is the timing of flowering which has been studied on every scale, from the level of community (Murali and Sukumar, 1994) to that of the individual flower (Herrera, 1995). Hedegard *et al.,* (1975) observed that the phenological events were influenced by associated species coupled with climatic and seasonal changes in the soil conditions. Bisht *et al*., (1986) studied the phenology of various species in the Central Himalaya and reported that the dates of flowering, fruiting and seed maturity differed considerably which explains the latitudinal, longitudinal and temperature effects on phenophases.

In India, extensive studies have been made on the reproductive biology of trees which include ornamental plants and also endemic and endangered plants. Reproductive biology of *Butea monosperma* (Tandon *et al*., 2003) *Terminalia arjuna* (Chauhan *et al*., 2008), *Acacia nilotica* (Sekar and Ganesan, 2009), *Caesalpinia bonducella* (Singh and Chauhan, 2010), *Aegle marmelos* (Singhal *et al*., 2011), *Thottea barberi* (Femy *et al*., 2014), *Acacia senegal* (Tak and Jindal, 2014); floral biology of *Artocarpus heterophyllus* (Sinha, 1973), *Albizia procera*, *Cassia fistula* and *Delonix regia* (Balalia and Chauhan, 2003), *Mitragyna parvifolia (*Raghuvanshi and Singh, 2003), *Santalum album* (Chandra and Chauhan, 2003), *Clerodendrum splendens* (Gautam and Rohitash, 2012), *Alcea rosea* (Johri and Raghuvanshi, 2014); pollination biology of *Bombax ceiba* (Bhattacharya and Mandal, 2000), *Callistemon citrinus* (Sharanya *et al*., 2014) and reproductive ecology of *Terminalia pallida* (Raju *et al*., 2012) have been studied.

Rana *et al*. (2003) studied the reproductive biology of *Kigelia pinnata* and concluded that floral dimensions, floral architecture, colour intensity are influenced

by differences in environmental conditions, particularly temperature and relative humidity. A study on reproductive biology of ten species of *Cassia* and *Anthocephalus chinensis* was done by Bansal and Chauhan (2003), Dhakre and Singh (2003) respectively. They reported that the observations on phenological events help in describing and explaining seasonal aspects of ecological phenomenon.

Various workers have also studied the reproductive biology of endemic and endangered species of India. Pollination biology and floral biology of an endangered species *Salvadora oleoides* and a critically endangered species *Garcinia imberti* have been studied by Chauhan *et al*., (2003) and Kandasamy *et al*., (2015) respectively. They suggested that a detailed knowledge of reproductive biology is required for successful conservation efforts, particularly of endangered species where population is very few to supply propagules for future generations.

The reproductive ecology of *Terminalia pallid*a, an endemic and medicinal species in India was studied by Raju *et al*., (2012). The rate of seed germination and seedling establishment was observed to be closely related to the nutrient status of the soil. Raju *et al*., (2014) also studied the reproductive ecology of *Syzygium alternifolium* (Myrtaceae) which is an endemic and endangered tropical tree species in the southern part of Eastern Ghats, India. They observed that the rainy season was the prime determinant of seed germination and seedling establishment.

Reproductive biology of *Panax wangianus*, a critically endangered medicinal plant was studied by Venugopal and Ahuja (2014) where they investigated the phenology, pollination mechanism and breeding behaviour. Kandasamy *et al.,* (2015) studied the pollination biology and breeding system of *Eugenia discifera* which is an endangered species of Western Ghats, India. They reported that the decline of this

species is mainly due to habitat loss, overexploitation and fragmentation. They stressed on the importance of pollination biology for understanding the life history of this endangered plant for conservation practices.

Thus there are many published works on the reproductive biology and phenology of plants of the world in general and India in particular. However, there are very few reports available on the reproductive biology and phenology of rare, endangered and threatened taxa of *Rhododendron* in India though many species of *Rhododendron* have been described and recorded from India by various workers.

Naithani and Bahadur (1983) reported a new and rare taxa of *Rhododendron viz., Rhododendron tawagensis* from Arunachal Pradesh. Mao and Gogoi (2007) rediscovered a critically endangered and endemic *Rhododendron viz., R. wattii* from Japfu hills bordering Manipur and Nagaland. Rai and Adhikari (2012) reported *Rhododendron rawattii*, a new species from the Western Himalaya, India. The species has been recorded from only two localities in the Western Himalaya and has been put under the Endangered (EN) category of IUCN status. Mao and Bhaumik (2012) have also reported a new species, *Rhododendron pangeanum* from India. A new variety of *Rhododendron grande viz., Rhododendron grande* var. *singalense* was recorded by Rai *et al*. (2014). Mao and Bhaumik (2015) again reported *Rhododendron pseudomaddenii*, a new species from Arunachal Pradesh, India.

Preliminary enumerations of the genus at national and regional levels were made by Pradhan (1985, 1986); Ghosh and Samaddar (1989). Floristic diversity of the Indian rhododendrons was studied in detail by Mao *et al.,* (2001). A study on the phenology and climate responses in Himalayan rhododendrons was carried out by Badola (2010). Pradhan and Lachungpa (1990) provided a complete taxonomic

revision of all the 36 species of Sikkim Rhododendrons including sub-species and varieties.

Various publications on the conservation of *Rhododendron* including rare and endangered species in India and Sikkim in particular have been done by Tiwari and Chauhan (2006), Singh *et al*., (2003), Singh *et al.,* (2008), Singh *et al*., (2009), Sastry (2010), Singh and Rai (2010), Viraraghavan (2010). They suggested *ex-situ* and *in-situ* conservation methodologies for the conservation of rhododendrons. They also stressed on the importance of micro propagation as an ideal technique for the conservation of rare and threatened species. Contribution on rare and endemic Indian rhododendrons was made by Sastry and Hajra (1983) where they enumerated 43 species of *Rhododendron* and provided data on flowering time, plant description, ecological notes and distribution. Paul *et al.,* (2005) studied the biodiversity and conservation of rhododendrons in Arunachal Pradesh in the Indo-Burma biodiversity hotspot. They reported that the rhododendrons act as keystone species in the higher altitudinal region in western Arunachal Pradesh. They suggested that the *Rhododendron*-rich areas should be brought under protected area network in order to conserve the rare, endangered and endemic species from various anthropogenic factors. Tiwari and Chauhan (2006) presented a review on the conservation effort of *Rhododendron* in Sikkim and other parts of Himalaya with particular emphasis on the on ecology, growth studies, *ex situ* and *in situ* conservation initiatives. They also analysed and discussed the impact of land use and management on the conservation of rhododendrons.

Mao and Gogoi (2012) provided a detailed taxonomic account of 11 *Rhododendron* species of Nagaland and Manipur out of which five taxa viz., *R.*

elliottii, *R. formosum* var. *inaequale*, *R. macabeanum* and *R. wattii* are red listed under different IUCN categories. They gave a brief description of the taxa and their distribution within India. They also discussed the endemic species, economic importance of rhododendrons, major threats and the need for conservation.

The significant value of *Rhododendron arboreum* was analyzed by Lepcha *et al*, (2014) in order to promote the importance of this species in the state of Sikkim, India and also to raise conservation aspects in its natural habitat as many Himalayan Rhododendrons are now in definite danger of elimination (Singh *et al*., 2003; Kumar *et al*., 2004). Ranjitkar *et al*., (2012) monitored the flowering phenology of tree *Rhododendron* (*Rhododendron arboreum* Sm.) *in situ* along elevation gradients in two distinct ecological settings viz., in Gaoligong Nature Reserve (GNR) in China and in the Kanchenjunga Conservation Area (KCA) in Nepal. They reported earlier bloom at lower elevation in both study sites and shorter flowering periods with increasing altitude.

Ericaceous plants are known for their symbiotic association with ericoid mycorrhizal fungi (EMF) and this symbiotic relationship has been reported in rhododendrons by Peterson *et al*., (1980). Rhododendrons are known to benefit from mycorrhizal fungi infection in their natural habitats (Harley and Smith, 1983; Read, 1983; Read and Bajwa, 1985; Mueller *et al*., 1986). Ericoid mycorrhizal fungi are reported to influence growth, survival and competitiveness of their host species by enhancing nutrient uptake (Read, 1996; Read and Perez- Moreno, 2003). They also alleviate heavy metal toxicity (Perotto *et al*., 2002). According to Read and Bajwa (1985) mycorrhizal infection leads to enhancement of plant nitrogen content and this improves the amount of ammonium absorption at low concentration. For growth, the

mycorrhizal endophytes utilises amino acids, peptides and proteins as nitrogen substrates which are the predominant nitrogen sources in organic heathland soil which are acidic in nature. They have also suggested that the success of ericaceous plants in such soils is due to the ability of the mycorrhizal fungus to provide its host with access to these nutrients.

Chaurasia *et al.,* (2005) reported arbuscular mycorrhizal status of five species of *Rhododendron* viz., *R. anthopogon, R. arboreum, R. campanulatum, R. barbatum* and *R. lepidotum* distributed in Kumaun region of the Indian Central Himalaya. Their study confirmed the wide occurrence of arbuscular mycorrhizal fungi in members of Ericaceae. This extends the range of host plants of arbuscular mycorrhizae that plays an important ecological role in highly stressed environments like artic and alpine habitats where communities experience short growing periods, low temperature and low nutrient status. In north-east India, studies on the effect of ericoid mycorrhizal inoculum and plant growth regulators on the establishment of stem cuttings of *Rhododendron formosum* var. *inaequale* and 6-8 month old *in vitro* developed seedlings of *Rhododendron dalhousiae* var. *rhabdotum, R. elliottii* and *R. johnstoneanum* was done by Kaliamoorthy *et al*., (2012). They concluded that the ericoid mycorrhizal inoculum (EMI) obtained from the rhizosphere region is necessary for the establishment of *Rhododendron* plantlets for *in vitro* culture.

Rhododendron maddenii, an endemic and endangered species in North East India has been propagated through tissue culture methods by Singh and Gurung (2009) and some are under progress. They provided a protocol for rapid and large scale propagation of this endangered species. Mao *et al*., (2012) studied the *in vitro* micropropagation of three rare, endangered and endemic *Rhododendron* species of

North East India viz., *R. dalhousiae* var. *rhabdotum, R. elliottii* and *R. johnstoneanum*. They used nodal explants for multiple shoot induction studies. 60 % of the *in vitro*-raised plants of these three *Rhododendron* species transferred from lab to greenhouse condition were successfully established and were subsequently transferred to the field.

Knowledge in seedling germination strategies is necessary to understand the coexistence of species in high diverse environments and will assist those involved in forest management and restoration (Aud and Ferraz, 2012). Seed propagation is essential for protecting germplasm and to enrich genetic diversity of the species (Zhang *et al.,* 2010). Rhododendrons produce numerous seeds that are dispersed by wind which provide a great reproductive potential (Ng and Corlett, 2000). Seedling recruitment is poor in many *Rhododendron* species (Pornon and Doche, 1995) and for successful establishment the seeds require favourable microsites (Cross, 1981; Plocher and Carvell, 1987; Kohyama and Grubb, 1994). Most species of the genus *Rhododendron* and members of Ericaceae need light for germination (Singh *et al*., 2010). Many works on the influence of light and temperature on the seed germination of different species of *Rhododendron* has been carried out by Blazich *et al*., 1991; Cho *et al*., 1981; Blazich *et al*., 1993; Rowe *et al*., 1994; Arocha *et al*., 1999 and Singh *et al*., 2010. Arocha *et al*., (1999) studied the influence of light and temperature on seed germination of *Rhododendron chapmanii* and reported that they require light for germination regardless of the germination temperature. The influence of temperature and light on the seed germination of *Rhododendron niveum* a critically endangered *Rhododendron* of Sikkim Himalaya was done by Singh *et al.,* (2010). They observed that the seeds of *R. niveum* required light to trigger the germination

and no germination was observed in darkness. The optimum temperature for germination was found to be 21°C.

Gao-Lin *et al*., (2011) examined the correlation of seed size and germination percentage, germination rate and germination persistence time for 29 alpine shrubs in the eastern Qinghai-Tibetan Plateau, China. They reported that the smaller-seeded species show fast and concentrative germination strategy as compared to larger-seeded species which show slow and stochastic germination strategy. Their study concluded that the smaller-seeded species can germinate in a wider range of microsites than larger-seeded species (Pearson *et al*., 2003). The larger-seeded species of the shrubs studied presented a larger dormancy proportions because of thick seed capsule and they showed low germination rate and longer germinaton persistence time. Zhao *et al*., (2014) studied the seed germination of *Rhododendron calophytum* in response to temperature, light and gibberellin (GA_3). Temperature was found to be an important environmental factor affecting seed germination. Both seed germination percentage and vigour were found to be highest at 20°C, and lowest at 30°C. Seeds treated with GA_3 showed greater germination percentage and germination vigour.

Reproductive biology of Rhododendrons have been studied by Ng and Corlett (2000), Mejias *et al*., (2002), Ono *et al*., (2008), Escaravage *et al*., (1997), Williams *et al*., (2011) and Ling (2011). Ng and Corlett (2000) studied comparative reproductive biology of six *Rhododendron* species that are native to Hongkong, China. In their study they compared the reproductive phenology and the flower, fruit and seed characteristics of the six species. They identified potential pollinators and compared their visitation rates. They also identified the breeding systems and

measured fruiting success. Mejias *et al.*, (2002) observed that the pollination efficiency of *Rhododendron ponticum* visitors was very variable where the large and medium Hymenopterans and Dipterans were found to be the most effective pollinators. The moth *Noctua pronuba* was also recorded to be an efficient pollinator as pollen tetrads were found on their bodies. Palser *et al*., (1985) carried out a detailed study of the ovary, ovule and megagametophyte of the entire genus of *Rhododedron*. Schlussel *et al.*, (2000) studied the phenology of *Rhododedron ferrugineum* in correlation to temperature, frost, insulation, and snow cover duration in two sites in the Alps of Valais (Switzerland). They reported that the floral development of this species is mainly correlated with the sum of the mean daily air temperature. Frost, insulation and snow cover duration also showed significant influence on the floral development.

Detailed field studies of reproduction in *Rhododendron* species have only been done for a few species (Cooper and McGraw, 1988; Kudo, 1993; Pornon *et al*., 1997; Escaravage *et al*., 1997). Some earlier studies were carried out in gardens by Yamaguchi, 1980; Williams *et al*., 1984; Padrutt *et al*., 1992). A number of studies on post pollination events have been carried out by Williams *et al.*, 1982; Williams *et al*., 1984; Williams *et al.*, 1986; Kenrick and Knox, 1985; Rouse and Williams 1985; Padrutt *et al*., 1992.

Stout (2007) discussed the impact of bumblebee behaviour on reproduction and invasion by exotic *Rhododendron ponticum.* He examined how insect behaviour affects the pollination success of this ecologically damaging, exotic invasive shrub. Hirao *et al.*, (2006) reported that the seasonal changes in pollinator activity influence pollen dispersal and seed production of the alpine shrub *Rhododendron aureum.* They

observed that in the early season, pollinator visitation was low but seed production was high whereas in the late season, pollinator visitation was higher but seed production was reduced due to inbreeding depression.

Kudo *et al.*, (2011) studied the pollination efficiency of bumblebee queens and workers in the alpine shrub *Rhododendron aureum* in central part of the Taisetsu Mountains in Hokkaido, northern Japan. They reported that the flower visitation frequency and foraging behaviour differed between queen bumblebees and workers. They observed that the queen exhibit less frequent flower visits and longer flight distance between inflorescences than the worker bees.

Georgian *et al.,* (2015) investigated the pollination ecology of the ornithophilous-flowered *Rhododendron floccigerum* Franchet in Northern Yunnan Province, China. They determined the identities of visiting, potentially pollinating and robbing species and reported 13 species of visitors of *R. floccigerum* which includes two insects, two mammals, and nine birds. Their study provided the first empirical evidence of both bird and mammal visitors to *Rhododendron* species in support to the hypothesis given by Stevens (1985); Argent *et al.,* (1988); Steinheimer (1999); Kingdon-Ward (2007) and Cruttwell (1988). Escaravage and Wagner (2004) studied the role and effectiveness of insect visitors for pollination and their contribution as pollen vectors for gene dispersal in *Rhododendron ferrugineun*. They reported that honey bees and bumblebees were the most frequent and effective pollinators. Hyam (2010) has also reported that *Rhododendron* flowers are pollinated by a wide range of bees (*Bombus* sp.) and birds. Ling (2011) reported honey bees (*Apis cerana*) as the specialised pollinators of *Rhododendron excellens*. It was

observed that the honey bees were very active on sunny days but on cloudy or rainy days the visitation rate was very low.

Chwil and Chmielewska (2009) compared the morphology of nectaries and nectar secretion in flowers of *Rhododendron catawbiense* and *R. japonicum* in Poland. They reported that the nectaries in flowers of *R. catawbiense* were larger and they formed more distinctive protuberances at the base of the ovary base as compared to *R. japonicum*. They also observed that the sugar concentration in the nectar of both the species was dependent on temperature. The higher temperature was accompanied by higher content in the nectar of both the species. Cai *et al*., (2014) investigated the leaf anatomical structures and photosynthetic characteristics of three species of *Rhododendron* in Yunnan province, China viz., *R. yunnanense, R. irrriratum* and *R. delavayi* in order to identify performances relating to rhododendron's natural habitat. Their study concluded that these three *Rhododendron* species exhibit significant differences in leaf anatomical and physiological characteristics related to their natural habitats. When compared to *R. irrriratum* and *R. delavayi*, *R. yunnanense* had lower leaf dry mass per unit area (LMA), larger stomata, lower transpiration rate, light compensation point and light-saturated photosynthetic rate. Thus they concluded that *R. yunnanense* plants are vulnerable to moisture and light stress, while *R. irrriratum* and *R. delavayi* are better suited to dry and high radiation environments.

Nielsen (1986) compared the seasonal plant growth patterns for *Rhododendron maximum* L. in two contrasting subcanopy environments of the southern Appalachian mountains. The influence of canopy induced variation in microenvironment on the quantitative phenology of *Rhododendron maximum* was determined by measuring seasonal, within canopy, and site specific variation in

growth characteristics in south western Virginia. The study showed that the irradiance environment was the overriding factor influencing leaf survivorship and the subcanopy shoots have larger leaf survivorship than the canopy shoots.

Ma *et al*., (2015) studies the pollination biology of *Rhododendron cyanocarpum* which is an endemic species in NW Yunnan, China. They assessed the floral traits including the flowering time, floral morphology, petal colour and floral scents. They also recorded the associated pollinator assemblage and their foraging behaviour. They reported two *Bombus* species viz., *B. festivus* and B. *richardsiellus* representing 90% of total recorded visits as the only insect species. They were considered to be the effective pollinators of *R. cyanocarpum.*

Thus, many works have been carried out on the Rhododendrons of the world and India in particular but till date no reports are available on the reproductive phenology of RET taxa of *Rhododendron* in Nagaland as well as north-east India. In Nagaland, the pollination biology of *Rhododendron elliottii,* which is endangered and endemic to Nagaland and Manipur, was studied by Jing *et al*., (2015). They reported *Heterophasia pulchella*, commonly known as Beautiful Sibia as the bird pollinator and *Xylocopa* sp. (carpenter bee) as the only insect pollinator of this species. Pollination biology of a rare and endemic *Rhododendron* species of Nagaland *viz., R. macabeanum,* has also been investigated by Jing *et al.,* (2016). They reported the bird *Yuhina* sp. as the effective pollinator of this endemic species. *Heterophasia* sp. and *Yuhina* sp. have also been reported as bird pollinators of *Rhododendron floccigerum* by Georgia *et al.,* (2015).

The reproductive phenology of *Rhododendron macabeanum* Watt *ex.* Balfour f. and *Rhododendron elliottii* Watt *ex.* Brandis, listed as ‘Rare’ and ‘Endangered’

species respectively as per IUCN category is studied in this study. Both species of *Rhododendron* are endemic to Nagaland and Manipur. These two species are found to be depleting in their natural habitat. This work is first of its kind in respect to *Rhododendron macabeanum* and *Rhododendron elliottii*.

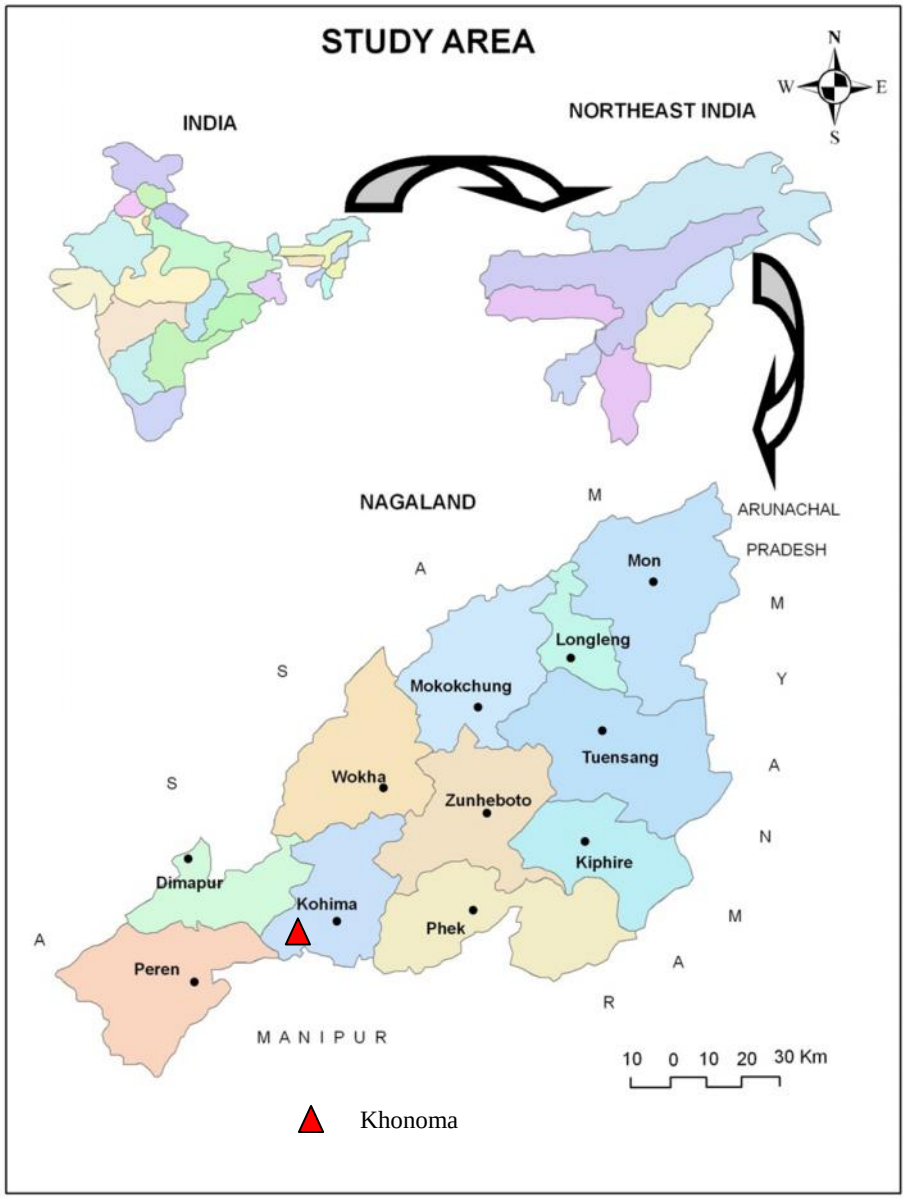
CHAPTER-II

MATERIALS AND METHODS

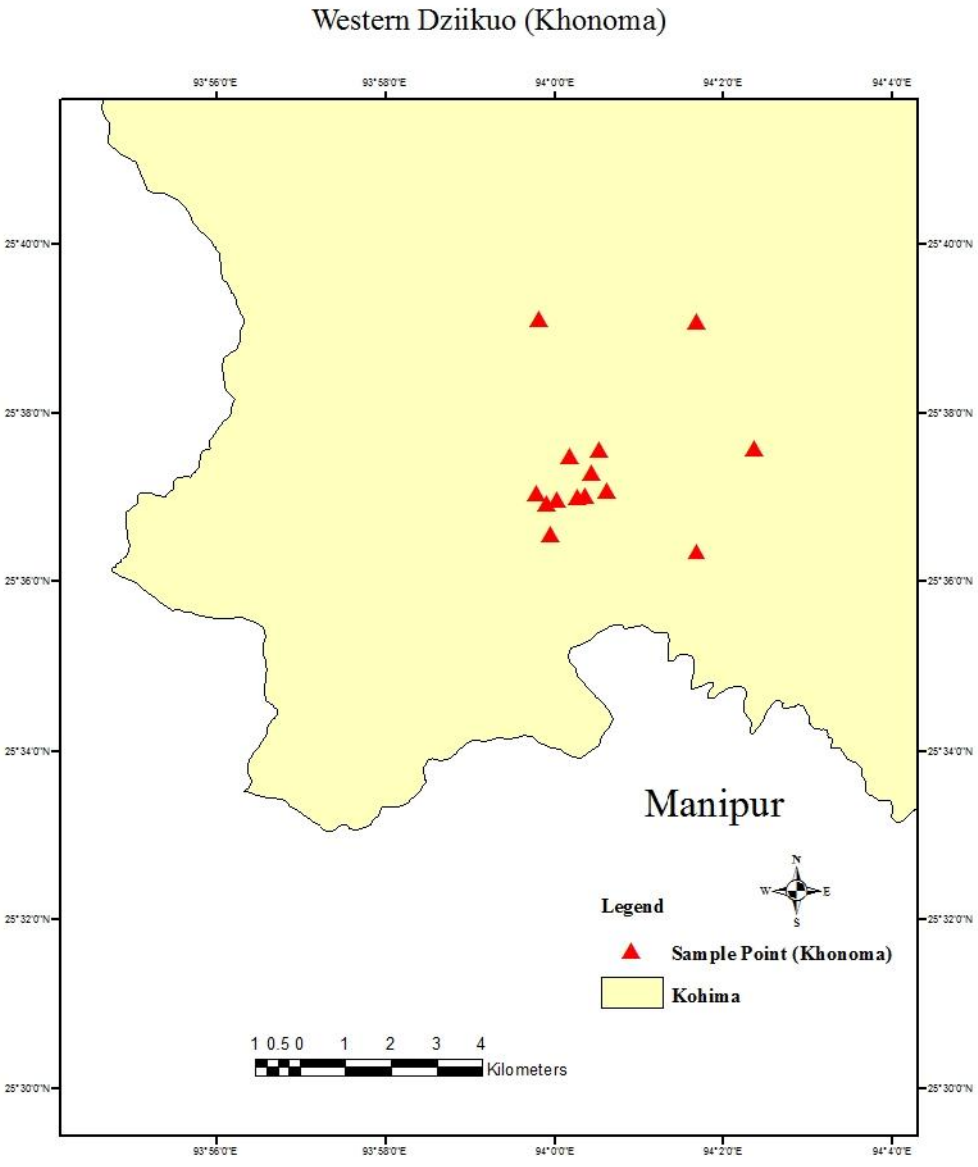
STUDY SITE

The present investigation was carried out in the forest of Western Dzukou, Khonoma village of Kohima District in Nagaland. Nagaland is one of the hill states of North-East India which supports a very rich and luxuriant diversity of vegetation and wildlife. Geographically, Nagaland lies between 26°60' N and 27°40' N Latitude and 93°20' E and 95°15' E Longitude and has an area of 16,579 square kilometres. The geographical location, climate and topography support the rich species in the state. The state has a pleasant sub-tropical to sub-alpine with a typical monsoon climate.

Khonoma is situated 20 Km south-west of Kohima, at an altitude of 1500 m above sea level and is surrounded by hills that are as high as 3000 m. It is the first 'Green Village' in Nagaland. The village has a hilly terrain from gentle to steep slopes. Some of the regions of the forest are inaccessible because of the steep and rocky mountain slopes. Khonoma enjoys monsoon climate with little rainfall during April and May and heavy downpour from June to September. The months of February and March are very windy. Winter is cold and chilly spreading from November to January. The maximum temperature in summer is 31°C and minimum temperature of 16°C whereas in winter 24°C and 4°C respectively.



Map 1: Map showing location of the study area.



Map 2: Map showing Khonoma Dzukou.

The present investigation was carried out during 2012- 2014 on the natural population of *Rhododendron macabeanum* and *Rhododendron elliottii* growing at Khonoma Dzukou which is situated 25°36' N Latitude and 93°59' Longitude at an altitude of 2500-2700 m above sea level.

The two species of *Rhododendron* undertaken for the present study viz., *R. macabeanum* and *R. elliottii* was identified and authenticated by consulting the Herbarium, Botanical Survey of India (BSI), Eastern Regional Circle, Shillong (ASSAM).

Regular field trips were conducted to study the biology of pollination, to observe timings of the onset and termination of flowering (duration of flowering), development of ovary as well as the success of fruit and seed set.

Field photographs were taken by using the Canon digital still camera with 8MP resolution and the photomicrographs were taken with the help of Leica L2 stereozoom and Leica DM 1000 microscope attached with the digital camera. For nectar quantification Digital Pocket Refractometer PAL-1 Atago (Japan) was used.

The density and abundance of the two *Rhododendron* species were carried out by randomly laying 10 numbers of 10×10m quadrats following Misra (1968). Density and abundance of the species were measured by the following formulae:

$$Density = \frac{Total\ no.of\ a\ species\ in\ all\ Quadrats}{Total\ no.of\ Quadrats\ studied}$$

$$Abundance = \frac{Total\ no.of\ a\ species\ in\ all\ Quadrats}{Total\ no.of\ Quadrats\ in\ which\ species\ occurred}$$

PHENOLOGICAL STUDIES

For phenological studies 20 plants were marked. Phenological data were recorded throughout the year for three years (2011-2013). The following phenological events were recorded from all the plants under observation (Table 4). Flowering phenology in the beginning, peak and end as well as the relative flowering intensity was registered from the marked plants following the procedure after Dafni (1992).

Table 4: Phenological Studies

Sl .No	Characters	Observations
i	Leaf shedding	Time of leaf fall and its duration
ii	Leaf renewal	Time of complete foliage development
iii	Bud initiation	Time of bud initiation
iv	Flowering	Time of flower initiation and its duration
v	Fruiting	Time of fruit formation
vi	Seed dispersal	Time of seed dispersal

REPRODUCTIVE STUDIES

FLORAL MORPHOLOGY

The floral density was evaluated by determining the number of inflorescence per branch from randomly chosen branches. The number of floral buds per inflorescence was recorded. Morphology of the flower and floral parts was studied by using magnifying hand lens. Flower longevity was determined by marking 50 buds on 50 branches/marked plants by the methods after Gill *et al*. (1998). The flowers were observed daily until the corolla withered.

FLORAL BIOLOGY

Anthesis of flower, anther dehiscence and stigma receptivity were studied by various methods given by Shivanna and Rangaswamy (1992).

Flower bud opening, anther dehiscence and stigma receptivity was determined by tagging a number of flower buds of different stages. The tagged buds were observed daily for anthesis. Some buds/flowers were excised each day to examine the anther dehiscence and the sigma for the presence of pollen tetrads.

The sugar concentration in the nectar was analysed by using digital refractometer. The nectar was extracted by using 1 ml syringe according to the methods suggested by Dafni *et al*., (2005).

POLLEN –OVULE RATIO

Pollen- Ovule ratio was worked out according to the methodology proposed by Cruden (1977). The total number of pollen tetrads per flower was divided by the total number of ovules per flower to yield the pollen-ovule ratio.

The number of pollen grains present per anther as well as per flower was counted by teasing out the mature anthers (10 flowers per plant) in lactophenol-glycerine with aniline blue. A known dilution was placed on the grid and 10 replicate counts were made using a haemocytometer as suggested by Barret (1985). Pollen viability of all the marked plants was checked with 0.2 % TTC solution (2, 3, 5-triphenyl tetrazolium chloride) as suggested by Hauser & Morrison (1964).

Number of ovules per flower (20 flowers per marked plant) was recorded following Stelly *et al.,* (1984). The number of ovules was counted using a

stereomicroscope. Total number of fifteen trusses was observed for counting the number of pollen grains, ovule and seeds.

FRUIT-SET AND SEED-SET

Fruit set and seed set was calculated by marking and tagging inflorescence randomly. Percentage of fruit set and seed set was calculated by the following formulae given by Cruden (1977).

$$Fruit\ set\ \% = \frac{No.of\ fruits\ per\ inflorescence}{No.of\ flowers\ per\ inflorescence} \times 100$$

$$Seed\ set\ \% = \frac{No.of\ seeds\ per\ fruit}{No.of\ ovules\ per\ pistil} \times 100$$

POLLINATION BIOLOGY

Field trips were conducted daily to observe the types of floral visitors during the flowering season and the visitation rates were recorded.

SEED BIOLOGY

Matured fruit capsules of both the species were harvested before dehiscence. The capsules were air dried at room temperature. As the capsules dehisce by longitudinal slits, the seeds were collected, counted, put into paper bags and kept at room temperature.

SEED VIABILITY TEST:

Seed viability was tested by 2, 3, 5-triphenyl-2H- tetrazolium chloride (TTC) test as described by Kearns and Inouye (1993). 1 gram of 2, 3, 5-triphenyl-2H- tetrazolium chloride (TTC) was dissolved in 100 ml of distilled water to make a 1.0% stock solution. Seeds were softened by placing them between moist blotting paper overnight. Seeds were placed in staining dishes and covered completely with tetrazoliun solution. The seeds were stained overnight and were examined under magnification to evaluate staining patterns.

SEED GERMINATION TEST

Germination test was carried out in the laboratory at room temperature. 100 seeds were counted and put inside petri dishes containing moistened blotting paper. Again 100 seeds were put in petri dishes containing soil collected from natural habitat. The germination dishes were watered regularly to prevent drying up of the blotting paper and the soil. Visible emergence of radicle from seed coats was taken as the criterion for seed germination (Vieira and Silveira, 2010).

Germination percentage was calculated as:

$$Germination\ percentage = \frac{No.\ of\ germinated\ seeds}{No.\ of\ seeds\ tested} \times 100$$

SOIL ANALYSIS

Soil samples from 0-15 cm depth (Top soil) and 15-30 cm (Sub soil) were collected from the study area. These samples were air dried, crushed and sieved through a 2mm mesh screen. They were stored in plastic bags for determining the soil chemical properties.

DETERMINATION OF SOIL PH

Soil pH was determined by following the method of Allen *et al.*, (1974). About 20 grams of freshly weighed soil was taken in a flask. To it 50 ml of distilled water was added, stirred with a glass rod and allowed to settle for 30 minutes. The mixture was stirred again for 10 minutes and allowed to settle for five minutes. The supernatant solution was collected and the soil pH was determined electrometrically by a pH meter with the buffer which was standardized earlier.

DETERMINATION OF SOIL ORGANIC CARBON

The organic carbon content was determined by Walkley and Black's (1934) rapid titration method. 5 g of sieved and air dried soil is transferred to a dry 500 ml conical flask. To it 10 ml of 1 N $K_2Cr_2O_7$ is added followed by 20 ml of conc. H_2SO_4. The flask is shaken for 2-3 minutes and allowed to stand for 30 minutes. After 30 minutes, the contents of the flask are diluted with 200 ml of water. 10 ml phosphoric acid and 1 ml diphenylamine indicator are added. The mixture is titrated against 1N ferrous ammonium sulphate until the solution is purple or blue. A little more ferrous ammonium sulphate is added, until the colour flashes to green. 0.5 ml 1N $K_2Cr_2O_7$ is added to restore an excess of dichromate and titration is completed by adding ferrous ammonium sulphate drop by drop until the trace of blue colour disappears. Percentage of carbon is calculated by the following formula:

$$\% \, Carbon = \frac{V_1 - V_2}{W} \times 0.003 \times 100$$

Where,

V_1 = Volume of 1N Potassium dichromate (10.5 ml)

V_2 = Volume of 1N Ferrous ammonium sulphate (ml)

W= weight of soil taken

DETERMINATION OF AVAILABLE NITROGEN IN SOIL

Available (mineralizable) Nitrogen was estimated by the method described by Subbiah and Asija (1956).

5 g of soil sample was weighed in a micro-Kjeldahl tube. 20 ml of water was added by washing down the soil adhering to the neck. Few glass beads and 2-3 ml of paraffin liquid was added to prevent frothing and bumping during distillation. Measured 20 ml of 2 % boric acid containing mixed indicator in a 250 ml conical flask and placed it under the receiver tube. The receiver tube end was dipped in the boric acid. 50 ml of 0.32 % $KMnO_4$ and 50 ml of 2.5 % NaOH solution was added. The content was distilled until about 100 ml of distillate was collected. The distillate was then titrated against 0.02 N H_2SO_4 and taken in burette until pink colour starts appearing. Blank solution was then run with the sample.

Calculation

$$Available\ Nitrogen\ in\ soil\ (kg/ha) = \frac{(S-B) \times 0.00028}{20} \times 10^6$$

$$= (S-B) \times 31.36$$

Where,

S = volume of 0.02 N H_2SO_4 required for sample

B = volume of 0.02 N H_2SO_4 required for blank.

DETERMINATION OF AVAILABLE PHOSPHOROUS

For the estimation of available Phosphorous in soil, Olsen's method was followed (Olsen *et al*., 1954). 2.5 g of soil sample was weighed and transferred in 100 ml conical flask. A pinch of Darco G-60 and 50 ml of Olsen's reagent (0.5 M $NaHCO_3$) was added. The mixture was shook for 30 minutes on a mechanical shaker and filtered through Whatman No.1 filter paper. 5 ml of clear and colourless filtrate was transferred into a 25ml volumetric flask. 5 ml of ammonium molybdate solution containing 400 ml of 10 N HCL per litre was added drop by drop. It was shook slowly and carefully to drive out the CO_2 evolved. Distilled water was added to bring the volume to about 25 ml and 1 ml of freshly diluted $SnCl_2$ solution was added. The optical density of the solution was measured on a spectrophotometer and standard curve was drawn by plotting concentrations of P in µg against absorbance readings.

Available Phosphorous was calculated by the formula:

$$Available\ Nitrogen\ in\ soil\ (kg/ha) = \frac{Q \times V \times 2.24 \times 10^6}{A \times S \times 10^6} \times \frac{Q \times V \times 2.24}{A \times S}$$

Where,

Q = quantity of P in µg read on X-axis against a sample reading

V = volume of extracting reagent used (ml)

A = volume of aliquot used for colour development (ml), and

S = weight of soil sample (g)

DETERMINATION OF AVAILABLE POTASSIUM BY AMMONIUM ACETATE METHOD (HANWAY AND HEIDEL, 1952)

The procedure suggested by Hanway and Heidel, 1952 was followed to estimate the Potassium in the soil sample. 5 g of soil sample was weighed and transferred to 100 ml conical flask. 25 ml of neutral 1 N ammonium acetate solution was added, shaked for 5 minutes and filtered through Whatman No.1 filter paper. Potassium concentration was measured in the filtrate using flame photometer.

Available Potassium was calculated as:

$$Available\ Potassium\ (kg/ha) = C \times \frac{25}{5} \times 2.24 = C \times 11.2$$

Where,

C stands for the concentration (mg/ml) of potassium in the sample filtrate obtained X-axis, against the reading.

CHAPTER-III

OBSERVATIONS

The study was carried out from the period of 2011-2014. The species *Rhododendron macabeanum* and *Rhododendron elliottii* were found to be distributed in the temperate belt in Khonoma Dzukou area. Periodic observations were taken systematically. Different parameters were taken for observations as listed out in the synopsis. In the two species of *Rhododendron* studied the inflorescence is a terminal truss. *Rhododendron* flower have all the floral organs: sepals, petals, stamens and pistil. The flower is hypogynous and nectar pouches are present at the base of the corolla tube. The corolla has blotches or spots of darker colour which is the typical features of rhododendron flower and it act as nectar guides. The presence of nectary, which consists of a zone of nectariferous tissue at the base of the ovary, is characteristics of all *Rhododendron* species. Baker and Baker (1981, 1983) studied the nectar composition in a large number of angiosperms and reported that the most abundant component was sugar. Sucrose, glucose and fructose were most common and most concentrated while amino acids were found in lower concentration (Williams *et al.*, 2011).

The corolla is always zygomorphic in both the species. It was observed that as the flower first opens, the style curves downward separating the stigma from the dehiscing anthers which are protandrous. In *R. macabeanum* the corolla is 8-lobed whereas in *R. elliottii* it is 5-lobed. There are 18 number of stamens in *R. macabeanum* and 10 numbers in *R. elliottii*. The number of stamens is usually about twice the number of the corolla lobes. In both species stamens are usually declinate, arranged zygomorphically with filaments that are pilose towards the base. In both the species, the stamens were observed to have narrow filament with a shorter, more expanded anther at the top. The filaments had unicellular hairs particularly on the lower part and according to Leppik (1974) it protects the nectar against rain. The

anthers consist of four microsporangia. In rhododendrons the grains remain in permanent cluster of four to form tetrads. Each pollen grain is tricolporate with three germination pores that are located within furrows in the outer exine layer of the wall. Thus the pollens are present in tetrads in *R. macabeanum* and *R. elliottii* and are held together by viscin threads. The occurrence of viscin threads plays an important role in pollen removal from the anthers and its adhesion to pollinators. According to Hesse *et al*., (2000) the presence of viscin threads implies highly specific pollinators for accurate delivery of pollen to stigma which increases the efficiency of pollination.

The pistil is located in the centre of the flower with an enlarged basal region, the ovary with a lobed stigma. The ovary is usually covered with dense indumentums, 5 to 18 locular, and more or less abruptly contracted into style. They show axile placentation. The style may be capitate or discoid and usually glabrous. Rhododendrons have wet, non-papillate stigmas (Heslop-Harrison and Shivanna, 1977; Heslop-Harrison, 1981). Wet non-papillate stigmas have been observed in both the species. At receptivity, the stigmas are covered with a thick hydrophilic exudates where the pollen germinates and in the absence of this exudates pollen does not germination. The stigmatic grooves guide the growing pollen tubes down into the stylar canal. Ovules are small having a single integument (unitegmic) and a single-layered nucellus (tenuinucellate). They are anatropous for both species.

Fruit is a capsule in both species; it is oblong, grooved and they dehisce from the top by longitudinal slits. The seeds are fusiform and winged. The wings are irregular and broken up into finger-like projections. When stored at normal temperature and humidity, *Rhododendron* seed retain their viability for about one year (Williams *et al*., 2011). The same has been observed for seeds of *R.*

macabeanum and *R. elliottii*. Adequate warmth, water humidity and light are required for seed germination (Rouse, 1985; 1986). The cotyledons usually emerge within 2-4 weeks and the first true leaves are visible within 2 months. Rhododendrons are very slow growing plants and normally flowers 3 to 10 years after sowing the seeds (Williams *et al*., 2011). Both the species of *Rhododendron* produce considerable number of seeds due to the high number of flowers per plant. However, they show poor seedling recruitment, poor survivability but high seedling mortality.

The habit of the tree varied with altitude as observed in *Rhododendron macabeanum*. A mature tree is found to be approximately 30 m high but the trees were observed to become smaller in higher altitude. The tree was found to be growing mostly in hill slopes and usually show profuse branching. The bark is thin and smooth. The colour of the bark can be different shades of brown and generally of peeling kind. In areas where *R. macabeanum* were dominant, they were found to be 20 m tall but smaller stems compared to the same species growing amongst other *Rhododendron* species which has bigger stems and may grow up to 30 m however in *R. elliottii* these kind of difference in plant habit was not seen. The roots of rhododendrons runs on the surface of the ground and take their food from the upper part of the ground so they along very well with oaks whose roots go deep into the soil.

Both the species are evergreen and leaf renewal takes place throughout the year. It was observed that the same branch does not bear flowers consecutively for two years. The branch that bears flowers for the first year does not bear flowers the next year because the fruits stay on the branch for a very long time. The fruits dehisce when still attached to the branch. Both the species were usually found growing on the

rocky hill-slopes in association with some other species of the genus, such as *R. maddenii* and *R. triflorum* var. *bauhiniiflorum.* Other species like oaks (*Quercus* sp.), *Gaultheria fragrantissima*, dwarf bamboos, *Berberis* sp. and certain fern species are common in the habitat.

Rhododendrons in general are keystone species but specific to *Rhododendron macabeanum* and *Rhododendron elliottii* they were found to be rare. They provide nectar to the birds and bees. They stabilise the soil on which they grow because of their extensive rooting system. They also add to the soil fertility because their roots have mycorrhizal associations. Ericaceous plants are known for their symbiotic association with ericoid mycorrhizal fungi (EMF) and this symbiotic relationship has been reported in rhododendrons by Peterson *et al.,* (1980). Rhododendrons are known to benefit from mycorrhizal fungi infection in their natural habitats (Harley and Smith, 1983; Read, 1983). Read and Perez- Moreno (2003) have also reported that ericoid mycorrhizal fungi (EMF) influences growth, survival and competitiveness of their host species by enhancing nutrient uptake.

No direct human impact was observed as the study area Khonoma Dzukou is a conserved area. The villagers use to take the fresh and dried corolla of *Rhododendron arboreum* when fish bone gets stuck in the throat but in case of *R. macabeanum* and *R. elliottii,* there is no report of people using it for economic activity during the study period. The two specific plants are still conserved because of the fact that the community manages the *Rhododendron* forest. They forbid people from collecting the flowers and felling of the trees. This has helped the species to continue to survive and sustain in its natural habitat.

The village people rear mithun in plenty and their movement is not restricted thereby trampling the young seedlings of rhododendrons in the forests (Plate-8, fig. E&F). Some flowers were observed to be damaged by birds feeding on the larvae in case of *R. macabeanum* (Plate-5, fig. A&B) but in *R. elliottii* damaged flowers were not observed. Seedling damage by herbivory was not observed during the study period. Invasive species were not observed in the study area. Natural regeneration of the plants was found to be low. Only few plantlets were observed in the period of study though they produce lots of seeds. Seedlings have mainly been found on humid rocks or soils covered by blanket of bryophytes which retains moisture on the soil by reducing evaporation (Plate-8, fig. A&B; Plate-15, fig. D-F). They also reduce the impact of raindrops on the soil. The same observation has been reported by Cross (1981). The seeds does not require any particular treatment to break dormancy but germinate readily in the laboratory in the presence of light. This indicates that the recruitment failure does not result from non-viability of seeds. In nature, the growth rate of seedlings and young plants of rhododendrons is very slow which may jeopardize the effective seedling recruitment. Rhododendrons favour habitats with acidic, well-drained conditions and abundant rainfall.

Climatic parameters were taken and compared with the statistics given by the Directorate of Soil and Water Conservation, Kohima, Nagaland. The data is provided in the table format. Species density and abundance representing the numerical strength of species was carried out by using quadrat studies for both the species.

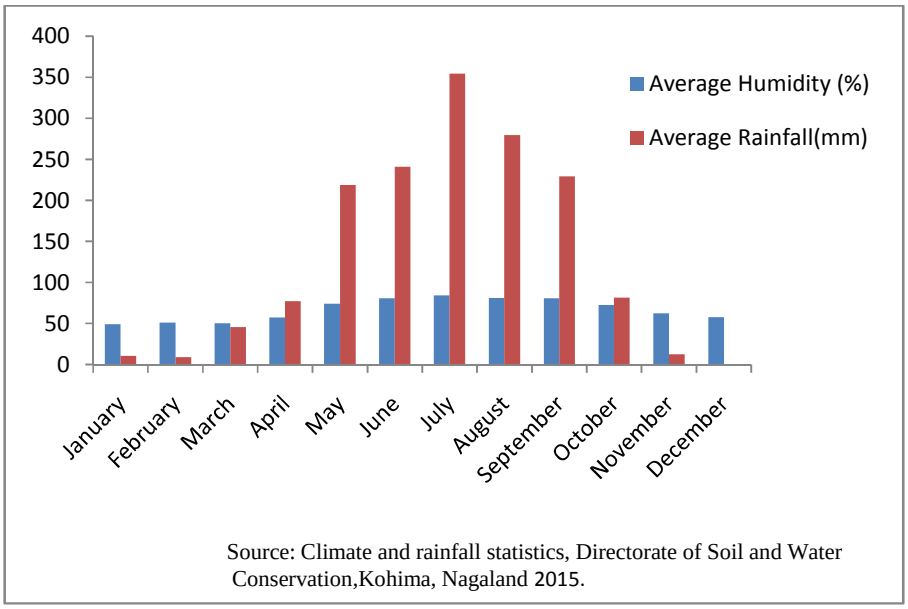
Phenology of both the species were carried out throughout the study period. Floral morphology, flowering phenology, floral biology, pollination biology, seed and fruit set of both the species were carried out in the field as well as in the laboratory.

The identification of the species was done with the help of flora and available literature. The authentic identification of the two *Rhododendron* species was done by consulting the herbarium Botanical Survey of India, ERC, Shillong (ASSAM). All possible observations on morphology was systematically carried out and confirmed by referring appropriate literatures. To understand on what type of soil these two species grow upon, soil tests were carried out on both the species. The samples from the top soil and sub soil were brought to the laboratory and the following parameters were analyzed (pH, Organic Carbon, available Nitrogen, available Phosphorus and available Potassium) following standard protocols. The different parameters are discussed in detail.

Kohima district has a pleasant and moderate climate. It receives rainfall throughout the year and the average annual rainfall from May to October is between 200cm and 250 cm. Thus, Kohima enjoys sub-tropical type of rainfall. Khonoma village enjoys monsoon climate with little rainfall during April and May and heavy downpour from June to September. The months of February and March are very windy. Winter is cold and chilly spreading from November to January. The maximum temperature in summer is 31°C and minimum temperature of 16°C whereas in winter 24°C and 4°C respectively.

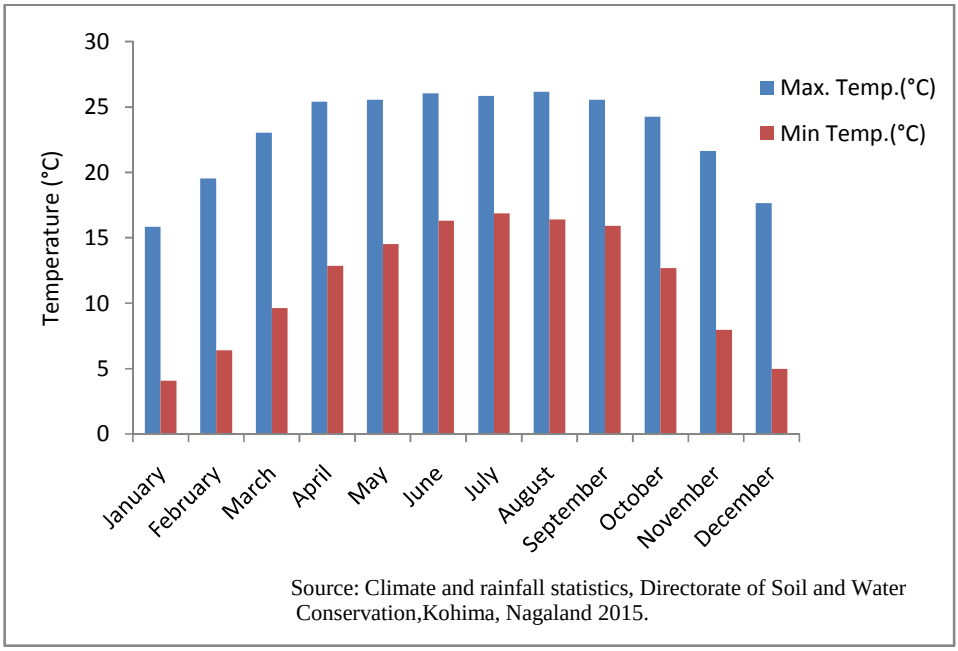
Table 5: Mean monthly maximum temperature (°C), minimum temperature (°C), humidity (%) and rainfall (mm) at Kohima district during the study period (2011-2014).

Month	Mean Max. Temperature (°C)	Mean Min. Temperature (°C)	Mean Humidity (%)	Mean Rainfall (mm)
January	15.85	4.08	49.05	10.63
February	19.55	6.38	51.4	9.3
March	23.03	9.63	50.73	45.75
April	25.4	12.85	57.65	77.35
May	25.55	14.5	74.05	218.6
June	26.03	16.3	80.53	241.05
July	25.83	16.88	84.2	354.18
August	26.15	16.4	81.48	279.43
September	25.55	15.9	81	229.4
October	24.25	12.68	72.7	81.58
November	21.63	7.95	62.55	12.48
December	17.65	5	57.76	0



Source: Climate and rainfall statistics, Directorate of Soil and Water Conservation,Kohima, Nagaland 2015.

Fig.1: Mean monthly rainfall (mm) and humidity (%) at Kohima district during the study period (2011-2014).



Source: Climate and rainfall statistics, Directorate of Soil and Water Conservation,Kohima, Nagaland 2015.

Fig. 2: Mean maximum and minimum temperature (°C) at Kohima district during the study period (2011-2014).

The description of the two species of *Rhododendron viz., Rhododendron macabeanum* and *Rhododendron elliottii* taken for the study are given below.

Rhododendron macabeanum Watt ex Balfour f.

Rhododendron macabeanum is a large tree growing to a height of above 12 – 30 m (Plate-2, figs. A-C). Leaves are broadly ovate to broadly elliptic, 13-30 × 7-15 cm, apex is rounded to retuse, base rounded. The upper surface of the leaf is glabrous when mature and reticulate with impressed veins while the lower surface is covered with a dense whitish indumentum. The bark is reddish brown and peels off easily. The inflorescence bears 20-28 flowers (Plate-3, fig. C). The flowers are zygomorphic, the corolla is 8-lobed, tubular-campanulate and lemon yellow with a purple blotch at the base. The stamens are 16 in number and unequal, anther lobes are brown and dorsifixed. Ovary is conical, densely pilose and covered with brownish white indumentums while the stigma is cup-shaped, red in colour with a persistent style. The anthers dehisce through the apical pores. Pollen tetrads are held together by viscin threads (Plate-5, fig. F).

The species is usually found growing on the rocky hill-slopes in association with some other species of the genus, such as *R. maddenii, R. elliottii* and *R. triflorum* var. *bauhiniiflorum*. Plants like dwarf bamboos, *Gaultheria fragrantissima*, *Berberis* sp. and certain fern species are common in the habitat.

SPECIES DENSITY AND ABUNDANCE

Density represents the numerical strength of a species in the community. The density and abundance of *R. macabeanum* was carried out by using 10 numbers of 10×10m randomly laid quadrats in the study area. Both the density and abundance of

the species was recorded as 4.8 (Table 6 & 7) which indicates that there are 4.8 individuals per 100 square km area.

Table 6: *R. macabeanum*. Species Density

Quadrat No.	1	2	3	4	5	6	7	8	9	10	**Total**	**Density**
No. of Species	7	1	2	5	7	8	5	3	7	3	**48**	**4.8**

$$Density = \frac{Total\ no.of\ a\ species\ in\ all\ Quadrats}{Total\ no.of\ Quadrats\ studied}$$

$$Density = \frac{48}{10} = 4.8$$

Table 7: *R. macabeanum.* Species Abundance

Quadrat No.	1	2	3	4	5	6	7	8	9	10	**Total**	**Abundance**
No. of Species	7	1	2	5	7	8	5	3	7	3	**48**	**4.8**

$$Abundance = \frac{Total\ no.of\ a\ species\ in\ all\ Quadrats}{Total\ no.of\ Quadrats\ in\ which\ species\ occurred}$$

$$Abundance = \frac{48}{10} = 4.8$$

FLORAL MORPHOLOGY AND FLOWERING PHENOLOGY

The initiation of floral buds takes place in the month of November. The blooming takes place in the last week of February. The peak of the blooming was observed in the last week of March which becomes over by the second week of April. Inflorescence is ball shaped, dense, 20 – 28 flowered; corolla 8-lobed, tubular-

campanulate, lemon yellow with a purple blotch at the base. The flowers are zygomorphic and the ovary superior. Fruit initiation begins in May and matures by the month of November. Fruit is a capsule and it dehisces laterally producing numerous seeds which are dispersed by wind (Plate-7, fig. E&F).

FLORAL BIOLOGY

The flowers of *R. macabeanun* are lemon yellow, tubular-campanulate with a purple blotch at the base which serves as nectar guides. Generally 16 unequal stamens are present in a flower; anthers are dorsifixed, anther lobes are brown and dehisces through apical pores. Ovary is conical, densely rufous-tomentose; stigma is cup-shaped, red with a persistent style covered with stalked glands and hairs. The ovules are attached to the placenta and they show axile placentation (Plate-6, fig. D). They are anatropous and unitegmic. The stigmatic groove (Plate-5, fig. E) secretes sticky exudates (nectar). A single flower produces 0.1-0.4 ml of nectar and the percentage sugar concentration was found to be 3.3% - 4.0 %. The onset of nectar secretion was observed already at the opening bud stage. The same observation has been made by Chwil and Chmielewska (2009).

Table 8: Phenology of *Rhododendron macabeanum*

Sl.No	Parameters	Observations
1	Leaf fall	Evergreen
2	Leaf renewal	Throughout the year
3	Flowering period i. Minimum ii. Maximum iii. Decline	 Last week of February March Second week of April
4	Initiation of fruits	May
5	Fruit maturity	November
6	Seed dispersal	Last week of November-December
7	Mode of seed dispersal	Wind

POLLEN BIOLOGY

The pollen grains are present in tetrads and are held together by viscin threads. The pollen tetrads come out of anthers by vibration or shaking caused by the wind as well as the floral visitors. The anthers dehisce through the apical pores (Plate-5, fig. D) before the flowers open (Protandrous condition, i.e. stigma become receptive after the anther dehiscence of the flower). Each pollen grain is tricolporate with three germination pores located located within the furrow in the exine layer. The similar structure of pollen grains has also been described by Williams *et al*., (2011) for the pollen grains of several other species of the genus *Rhododendron.* The average pollen production per flower was 112362.90±29290.86 (Table: 9) and an average production of ovules per flower had been to be found as many as 2628.10±923.79 (Table: 10). So, the average pollen-ovule ratio becomes 43:1 (Table: 14).

Table 9: Pollen production in *R. macabeanum.*

Sl. No.	No. of Pollen Tetrads/flower	Mean	Standard Deviation (S.D)	Standard Error (S.E)
1	99837	112362.90	29290.86	9262.50
2	98048			
3	72176			
4	87536			
5	100197			
6	154960			
7	145872			
8	87223			
9	137540			
10	140240			

Table 10: Ovule production in *R. macabeanum*.

Sl. No.	No. of ovules /flower	Mean	Standard Deviation (S.D)	Standard Error (S.E)
1	1963	2628.10	923.79	292.12
2	1920			
3	1960			
4	2464			
5	3247			
6	4735			
7	3208			
8	2038			
9	1920			
10	2646			

POLLINATION BIOLOGY

The lemon-yellow flowers of *R. macabeanum* are foraged by birds of the genus *Yuhina* sp. (Plate-4, figs. A-C) for nectar by inserting their head within the corolla tube. The presence of viscin threads plays an important role in pollen removal

from the anthers and its adhesion to pollinators. Pollen tetrads were seen attached on their beak and dorsal as well as the ventral surfaces during sunny days between 9.00 am. - 2.00 pm. The duration of foraging visit lasts for 1 – 3 minutes. The beetles and flies also visit the flowers but their contribution in pollination cannot be ascertained. However, no visitor was observed during misty, windy and rainy weather.

Table 11: Visitor census *R. macabeanum*.

Forager Type	Forage Type	Duration of foraging per flower (Minutes)	Visiting hours	No. of Flowers foraged per Visit
Beetle	Nectar	1-3	09.00 am.-12.00 pm.	4-8
Yuhina sp.	Nectar	1-3	09.00 am.-2.00 pm.	6-9

FRUIT AND SEED-SET

There are 20 – 28 flowers in one inflorescence and an average of 17.4 fruits are produced in each infructescence and the fruit set percentage is 76.32 %. Capsules is grooved, rufous-tomentose, 16 – 18 chambered and dehisced from the top by longitudinal slits. The seeds are small, reddish brown having finger-like projections at the tip (Plate-7, fig. F) and dispersed by wind. The average number of seeds per fruit is recorded as 1628.80±320.17 (Table 12). Ovule-seed ratio is recorded as 2: 1 (Table 14). The seed set percentage was recorded as 61.98%. The seeds are very small in size (1-3 mm). The average weight of 100 seeds was 0.02gm. *In vitro* seed germination was 95% but seedling survivability was only 10 %. There is no requirement of any particular treatment to break seed dormancy but they germinate readily in the laboratory in the presence of light. This indicates that the recruitment failure does not result from non-viability of seeds. In nature, the growth rate of seedlings and young

plants of rhododendrons is very slow which jeopardize the effective seedling recruitment. The poor seedling establishment in the natural habitat has been assigned to the various climatic factors like heavy rains and the sloppy habitat around the population of *R. macabeanum* trees which could not provide the proper conditions for seedling establishment into the soil after the seed germination

Table 12: Seed production in *R. macabeanum*.

Sl. No.	No. of seeds/flower	Mean	Standard Deviation (S.D)	Standard Error (S.E)
1	1898	1628.80	320.17	101.24
2	1690			
3	1470			
4	1300			
5	1235			
6	1396			
7	1789			
8	1490			
9	2300			
10	1720			

Table 13: Fruit set percentage in *R. macabeanum*

Average number of Flowers /Inflorescence	Average Number of Fruits / inflorescence	Percentage of Fruit-set
22.8	17.4	76.32 %

$$Fruit\ set\ \% = \frac{No.of\ fruits\ per\ inflorescence}{No.of\ flowers\ per\ inflorescence} \times 100$$

$$Fruit\ set\ \% = \frac{17.5}{22.8} \times 100 = 76.32\%$$

Table 14: *R. macabeanum*. Pollen: Ovule ratio and Ovule: Seed ratio.

Mean no. of pollen tetrads/ flower	Mean no. of ovules/ flower	Mean no. of seeds/ flower	Pollen: Ovule ratio	Ovule: Seed ratio
112362.90	2628.10	1628.80	43:1	1.61

$$Seed\ set\ \% = \frac{No.of\ seeds\ per\ fruit}{No.of\ ovules\ per\ pistil} \times 100$$

$$Seed\ set\ \% = \frac{1628.80}{2628.10} \times 100 = 61.98\%$$

$$Germination\ percentage = \frac{No.of\ germinated\ seeds}{No.of\ seeds\ tested} \times 100$$

$$Germination\ percentage = \frac{95}{100} \times 100 = 95\%$$

BREEDING SYSTEM

Fruit set percentage in the natural population is 76.32 %. *R. macabeanum* is self-compatible and pollen-ovule (43:1) ratio indicates it is autogamous as reported by Cruden (1977) for many other species of flowering plants.

SOIL ANALYSIS

Rhododendron macabeanum prefers well drained, loamy and acidic soils. The mean soil pH was found to be 5.07±0.74 for top soil and 5.27±0.72 for subsoil (Table 19) which is found to be strongly acidic. Available nitrogen was found to be low for both top soil (250.50±50.15 kg/ha) and sub soil (232.17±16.39 kg/ha) (Table 15). The mean available phosphorus for top soil was found to be 40.70±21.49 kg/ha which is considered to be medium but showed only 18.53±0.70 kg/ha in subsoil which is low. (Table 16). The mean available potassium was also medium for top soil (129.61±84.29 kg/ha) and low for sub soil (66.15±11.76 kg/ha) (Table 17). Both the top soil and sub soil show high organic carbon content of 2.70±0.35 % and 2.35±0.48 % respectively (Table 18).

Table 15: Available Nitrogen (N) in *R. macabeanum*.

Particulars	Top Soil	Mean	Standard Deviation (S.D)	Standard Error (S.E)
Available Nitrogen (kg/ha)	200.60	250.50	50.15	28.96
	300.90			
	250.00			
	Subsoil			
	225.70	232.17	16.39	9.46
	250.8			
	220.00			

Table 16: Available Phophorus (P) in *R. macabeanum*.

Particulars	Top Soil	Mean	Standard Deviation (S.D)	Standard Error (S.E)
Available phosphorous (kg/ha)	52.50	40.70	21.49	12.40
	15.90			
	53.70			
	Subsoil			

	19.20	18.53	0.70	0.41
	17.80			
	18.60			

Table 17: Available Potassium (K) in *R. macabeanum.*

Particulars	Top Soil	Mean	Standard Deviation (S.D)	Standard Error (S.E)
Available potassium (kg/ha)	226.80	129.61	84.29	48.67
	76.44			
	85.60			
	Subsoil			
	77.00	66.15	11.76	6.77
	67.76			
	53.7			

Table 18: Organic Carbon (%) content in *R. macabeanum.*

Particulars	Top Soil	Mean	Standard Deviation (S.D)	Standard Error (S.E)
Organic carbon (%)	2.9	2.70	0.35	0.20
	2.9			
	2.29			
	Subsoil			
	2.1	2.35	0.48	0.28
	2.9			
	2.05			

Table 19: pH. *R. macabeanum.*

Particulars	Top Soil	Mean	Standard Deviation (S.D)	Standard Error (S.E)
pH	4.8	5.07	0.74	0.43
	4.5			
	5.9			
	Subsoil			

	4.8	5.27	0.72	0.42
	4.9			
	6.1			

Table 20: *R. macabeanum* show mean values available Nitrogen, available Phosphorous and available Potassium in kg/ha.

Soil Depth (cm)	Available Nitrogen (kg/ha)	Available Phosphorus (kg/ha)	Available Potassium (kg/ha)
0-15	250.50	40.70	129.61
15-30	232.17	18.53	66.15

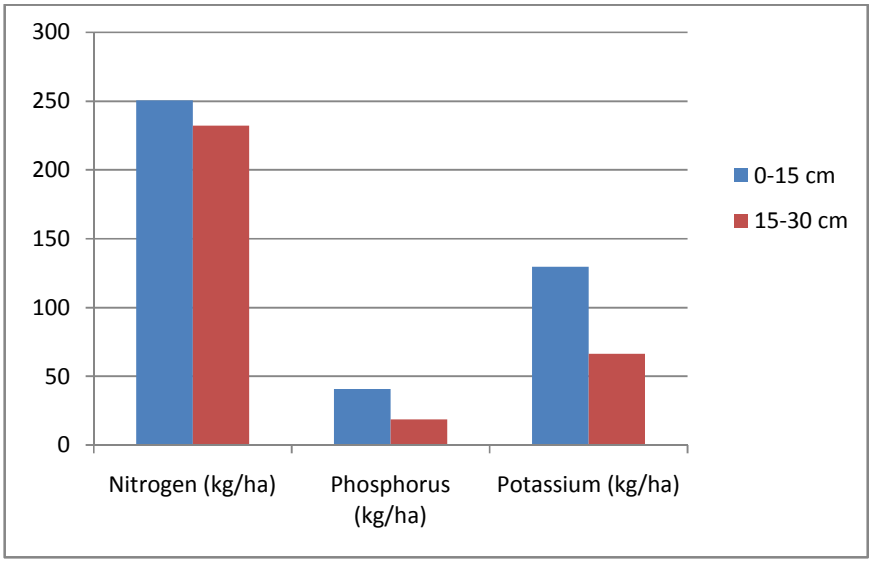


Fig. 3: *R. macabeanum* showing mean available Nitrogen, available Phosphorous and available Potassium in kg/ha.

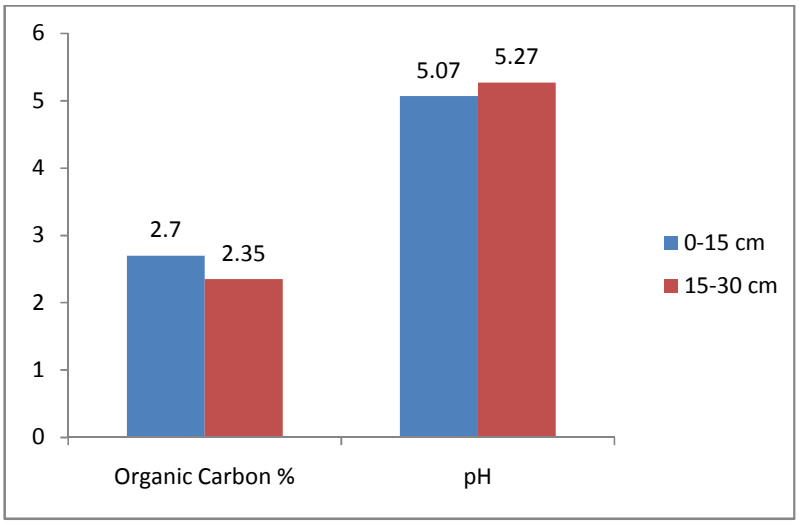


Fig. 4: *R. macabeanum* showing mean organic carbon (%) and pH.

Rhododendron elliottii Watt ex Brandis

R. elliottii is an upright, often straggly, small tree growing to a height of 2-5 m (Plate-10, figs. A&B). Leaves are oblanceolate to elliptic, 8.5-15×3.5-4.2 cm, apex rounded apiculate, base rounded and glabrous when mature on both surfaces. The flowers are tubular-campanulate, red colour with darker flecks. The truss holds 10-20 flowers, corolla has 5-lobes, stamens are 10 in number and unequal, anther lobes brown and dorsifixed. Ovary densely rufous stellate tomentose, intermixed with stipitate glands. Style is tomentose and glandular (Plate-13, fig. D). The anthers are dark brown in colour and they dehisced through the apical pores. Pollen tetrads are held together by viscin threads (Plate-13, fig. F).

The species is usually found growing on the rocky hill-slopes in association with some other species of the genus, such as *R. maddenii*, *R. macabeanum* and *R. triflorum* var. *bauhiniiflorum*. Also, plants like *Quercus* sp., dwarf bamboos, *Berberis* sp. and certain fern species are common in the habitat.

DENSITY AND ABUNDANCE OF THE SPECIES

Density represents the numerical strength of a species in the community. The quantitative study of *R. elliottii* was carried out by using 10 numbers of 10×10m randomly distributed quardrats in the study area. Both density and abundance of *R. elliottii* was recorded as 4.7 (Table 21 & 22).

Table 21: *R. elliottii*. Species Density.

Quardrats	1	2	3	4	5	6	7	8	9	10	**Total**	**Density**
No. of Species	6	2	5	4	2	4	8	5	7	4	**47**	**4.7**

$$Density = \frac{Total\ no.of\ a\ species\ in\ all\ Quadrats}{Total\ no.of\ Quadrats\ studied}$$

$$Density = \frac{47}{10} = 4.7$$

Table 22: *R. elliottii*. Species Abundance

Quardrats	1	2	3	4	5	6	7	8	9	10	**Total**	**Abundance**
No. of Species	6	2	5	4	2	4	8	5	7	4	**47**	**4.7**

$$Abundance = \frac{Total\ no.of\ a\ species\ in\ all\ Quadrats}{Total\ no.of\ Quadrats\ in\ which\ species\ occurred}$$

$$Abundance = \frac{47}{10} = 4.7$$

Table 23: Phenology of *Rhododendron elliottii.*

Sl.No	Parameters	Observations
1	Leaf fall	Evergreen
2	Leaf renewal	Throughout the year
3	Flowering period i. Minimum ii. Maximum iii. Decline	 Last week of April May June
4	Initiation of fruits	July
5	Fruit maturity	November
6	Seed dispersal	November-December
7	Mode of seed dispersal	Wind

FLORAL MORPHOLOGY AND PHENOLOGY OF FLOWERING

The flowers of *Rhododendron elliottii* are tubular-campanulate, deep red coloured with darker flecks (Plate-11, figs. B-D). Each floral truss holds 10-20 flowers, corolla is 5-lobed, stamens are 10 in number and unequal, anther lobes brown and dorsi-fixed. The pollens are present in tetrads and the nectar is secreted by the nectarines present at the base of the corolla lobes. The first appearance of floral buds was observed in the month of November. The initiation of flowering starts by the last week of April. The young floral buds take 7-10 days to grow into an open flower. Maximum flowering was observed in the last week of May. Flowering terminates by the end of June. Fruit initiation takes place in the month of July and matured fruits were observed in the month of November. The fruit capsule dehisces laterally and the seeds are dispersed by wind. Seed dispersal takes place in the month of November-December.

FLORAL BIOLOGY

The inflorescence bears 10-20 flowers per truss. The flowers are tubular-campanulate, corolla 5-lobed, deep red with five nectar pouches at the base (Plate-11, fig. F). The amount of nectar produced per flower ranges from 0.3 ml to 0.6 ml. The percentage sugar concentration was found to be between 4.8%- 6.4%. The onset of nectar secretion was observed already at the opening bud stage. Chwil and Chmielewska (2009) have made the same observation. The ovary is densely rufous, stellate-tomentose, style stellately hairy and glandular with stalked glands, the hairs are more numerous towards the base. The stigmatic groove secretes sticky exudates. The ovary has six locules with axile placentation. (Plate-13, fig. C). The stamens are 10 in number and unequal, anther lobes are brown, dorsifixed, filaments glabrous and dehisce by apical pores. Ovary is densely pilose with brownish indumentums (Plate-13, fig. A). The ovules (Plate-13, fig. E) are anatropous and unitegmic.

POLLEN BIOLOGY

The pollens are present in tetrads and are held together by highly adhesive viscin threads. They are easily expelled from the anthers by vibrations caused by movements of flower visitors. The anthers mature before the flower opens and the pollen tetrads are observed inside the unopened flowers. Each pollen grain is tricolporate, with three germination pores located within furrows in the outer exine layer (William *et al*., 1990). The average pollen production per flower was found to be 52,662.90±13951.12 (Table 24). The average number of ovules per flower was 1614.30±555.52 (25) and the pollen-ovule ratio was 33:1 (Table 29**).**

Table 24: Pollen production in *R. elliottii.*

Sl. No.	No. of Pollen Tetrads/flower	Mean	Standard Deviation (S.D)	Standard Error (S.E)
1	37784	52662.90	13951.12	4411.70
2	58864			
3	59322			
4	81156			
5	66624			
6	36240			
7	49042			
8	49435			
9	43752			
10	44410			

Table 25: Ovule production in *R. elliottii.*

Sl. No.	No. of ovules /flower	Mean	Standard Deviation (S.D)	Standard Error (S.E)
1	2387	1614.30	555.52	175.67
2	1397			
3	2368			
4	1039			
5	1386			
6	1111			
7	1039			
8	1386			
9	2350			
10	1680			

POLLINATION BIOLOGY

The deep red flowers of *R. elliottii* attract the bird of the genus *Heterophasia pulchella* commonly known as Beautiful Sibia (Plate-12, figs. C-F). They were found collecting nectar from the flower by inserting their head in the corolla tube. Normally

there are five nectar pouches present at the base of the corolla. Pollen tetrads are found attached on the beak and neck of the birds. It is observed that the time spent by the visitor on the inflorescence was found to be 2-3 minutes. Among the insects *Xylocopa* sp. was observed to be the only visitor (Plate-12, fig. B). These bees forage each flower for 1-3 minutes. Pollen tetrads were found attached on the dorsal as well as ventral surfaces of neck as well as beak of the birds, whereas, on the ventral and dorsal surfaces of thorax, abdomen, fore limbs and hind limbs. However, during misty, cool, windy and rainy weather no visitors were observed over the flowers.

Table26: Depicting the Visitor Census of *Rhododendron elliottii* at Khonoma Dzukou.

Forager Type	Forage Type	Duration of foraging per flower (Minutes)	Visiting hours	No. of Flowers foraged per Visit
Xylocopa sp	Nectar	1-3	09.00 am-12.00 pm	4-8
Heterophasia pulchella	Nectar	2-3	09.00 am-2.00 pm	6-9

FRUIT AND SEED-SET

Fruiting initiation was observed in the month of July and fruits mature within six months. Mature fruits were observed during the last week of November. Fruit is a capsule and they dehisce by longitudinal slits (Plate-14, figs. C&D). The capsule is oblong blunt, grooved, rufous -tomentose and 6-chambered (Plate 14, Fig. E). Fruit-set percentage was found to be 75% and the seed set percentage was 42.68% (43%). The seeds are very small, flat with an oval shape and are reddish-brown (Plate-14, Figs. D&F). The average length and width was around 2mm and 1mm. The average weight of 100 seeds was found to be 0.004gm. These tiny seeds of *R. elliottii* are

dispersed by wind. At normal temperature and humidity the seeds of *R. elliottii* retain their viability for about one year. They show high seed germination rate in the laboratory but very low seedling survivability. The seeds require adequate temperature and light for germination. The radical emerges within 2-4 weeks and the germination is epigeal. *In vitro* seed germination test, the germination percentage was 90% but *in vitro* seedling survival has been observed to be 2 % only. In the natural locality, only few plantlets were observed in small number (Plate-17, figs. D-F). They were found growing among the litters and on soils covered by bryophyte carpets. They retain the soil moisture and reduce the impact of raindrops on the soil.

Table 27: Seed production in *R. elliottii.*

Sl. No.	No. of seeds/flower	Mean	Standard Deviation (S.D)	Standard Error (S.E)
1	698	689	150.72	47.66
2	748			
3	647			
4	566			
5	780			
6	1004			
7	635			
8	440			
9	762			
10	610			

Table 28: Fruit set percentage in *R. elliottii*.

Average number of Flowers /Inflorescence	Average Number of Fruits / inflorescence	Percentage of Fruit-set
20	15	75 %

$$Fruit\ set\ \% = \frac{No.of\ fruits\ per\ inflorescence}{No.of\ flowers\ per\ inflorescence} \times 100$$

$$Fruit\ set\ \% = \frac{15}{20} \times 100 = 75\%$$

Table 29: *R. elliottii*. Pollen: Ovule ratio and Ovule: Seed ratio.

Mean no. of pollen tetrads/ flower	Mean no. of ovules/ flower	Mean no. of seeds/ flower	Pollen: Ovule ratio	Ovule: Seed ratio
52662.90	1614.30	689	32.6:1	2.34:1

$$Seed\ set\ \% = \frac{No.of\ seeds\ per\ fruit}{No.of\ ovules\ per\ pistil} \times 100$$

$$Seed\ set\ \% = \frac{689}{1614.30} \times 100 = 42.68\ \%$$

$$Germination\ percentage = \frac{No.of\ germinated\ seeds}{No.of\ seeds\ tested} \times 100$$

$$Germination\ percentage = \frac{90}{100} \times 100 = 90\%$$

SOIL ANALYSIS

Rhododendron elliottii grows in acidic soil of pH ranging from 4.6-5.9. The average pH of top soil was 5.03±0.40 and 5.27±0.60 (Table 34) for sub soil which are considered strongly acidic. The mean available nitrogen content is medium for top

soil (312.63±58.S59 kg/ha) and subsoil (288.97±75.55 kg/ha) (Table 30). Low level of available potassium and phosphorous was observed (Table 31; 32). The soil has high organic carbon content. The average soil organic carbon content for top soil and subsoil are 2.58±0.46 % and 2.63±0.38 % respectively (Table 33).

Table 30: Available Nitrogen (N) in *R. elliottii.*

Particulars	Top Soil	Mean	Standard Deviation (S.D)	Standard Error (S.E)
Available Nitrogen (kg/ha)	376.20	312.63	58.59	33.83
	300.90			
	260.80			
	Subsoil			
	376.20	288.97	75.55	43.62
	245.70			
	245.0			

Table 31: Available Phosphorous (P) in *R. elliottii.*

Particulars	Top Soil	Mean	Standard Deviation (S.D)	Standard Error (S.E)
Available Phosphorus (kg/ha)	18.59	18.26	1.92	1.11
	16.2			
	20.00			
	Subsoil			
	20.64	18.18	2.58	1.49
	18.40			
	15.50			

Table 32: Available Potassium (K) in *R. elliottii.*

Particulars	Top Soil	Mean	Standard Deviation (S.D)	Standard Error (S.E)
Available Potassium (kg/ha)	105.00	93.96	15.85	9.15
	101.08			
	75.80			
	Subsoil			
	76.44	69.63	22.22	12.83
	87.64			
	44.80			

Table 33: Organic Carbon (%) content in *R. elliottii.*

Particulars	Top Soil	Mean	Standard Deviation (S.D)	Standard Error (S.E)
Organic carbon (%)	2.8	2.58	0.46	0.27
	2.9			
	2.05			
	Subsoil			
	2.8	2.63	0.38	0.22
	2.9			
	2.20			

Table 34: pH. *R. elliottii*

Particulars	Top Soil	Mean	Standard Deviation (S.D)	Standard Error (S.E)
pH	4.6	5.03	0.40	0.23
	5.1			
	5.4			
	Subsoil			
	4.7	5.27	0.60	0.35
	5.2			
	5.9			

Table 35: *R. elliottii* show Mean available Nitrogen, available Phosphorous and available Potassium in kg/ha.

Soil Depth (cm)	Available Nitrogen (kg/ha)	Available Phosphorous (kg/ha)	Available Potassium (kg/ha)
0-15	312.63	18.26	93.96
15-30	288.97	18.18	69.63

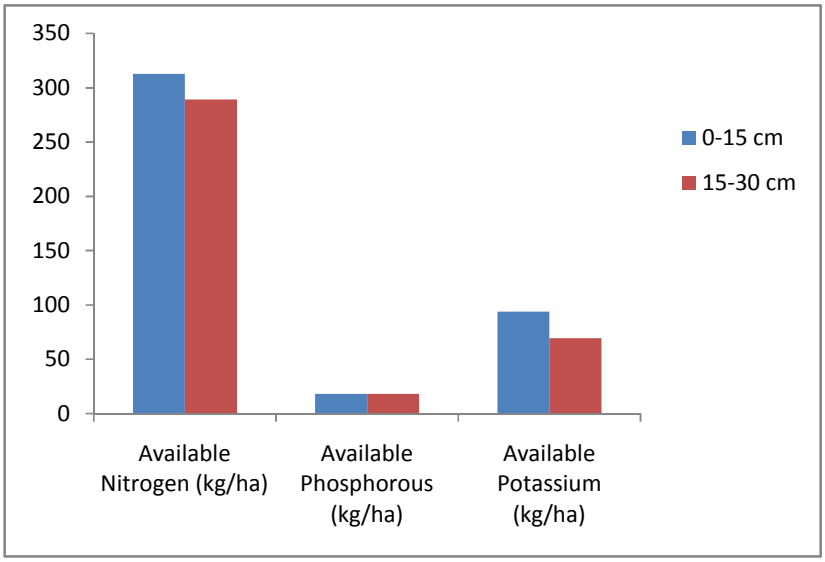


Fig. 4. *Rhododendron elliottii*. Available Nitrogen (kg/ha), Potassium (kg/ha) and Available Phosphorous (kg/ha).

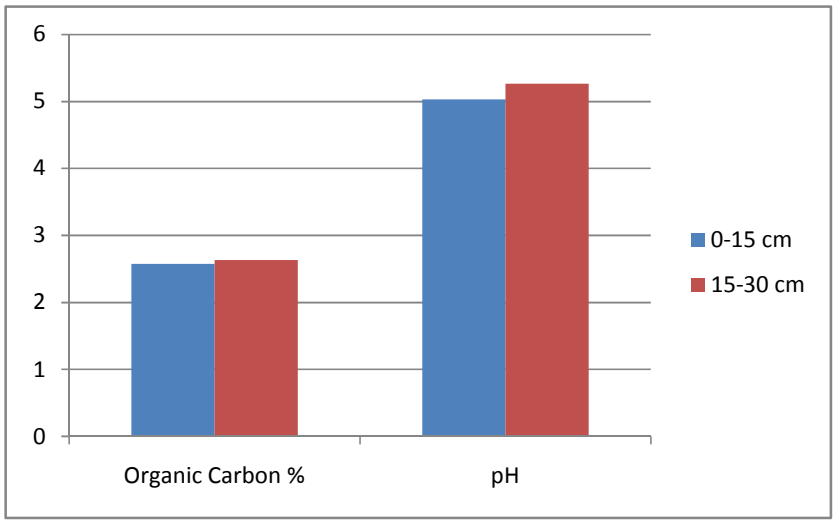


Fig. . *Rhododendron elliottii.* Mean Organic carbon and pH.

CHAPTER-IV

DISCUSSION AND CONCLUSION

Rhododendrons display a wide range of morphological characteristics. Their size varies from less than 10 cm high to trees taller than 20 m (Williams *et al*., 2011). They play a vital role in ecosystem services as they grow in areas of high rainfall and high humidity on acidic soils; conditions under which few plants would survive. They stabilize slopes in hilly areas and are useful in watershed protection particularly in the Himalayas where so many of Asia's major rivers start. They also provide the structure of plant communities which support a wealth of biodiversity (Gibbs *et al*., 2011). Rhododendrons have phenological sensitivity to climate change and play a vital role in ecological stability of ecosystems, as indicators of forest health (Mainra *et al*., 2010). Rhododendrons show symbiotic association with ericoid mycorrhizal fungi (EMF) and they benefit from mycorrhizal fungi infection in their natural habitats (Harley and Smith, 1983; Read, 1983; Read and Bajwa, 1985; Mueller *et al*., 1986) and this influence growth, survival and competitiveness of their host species by enhancing nutrient uptake (Read, 1996; Read and Perez- Moreno, 2003). According to Read and Bajwa (1985) mycorrhizal infection leads to enhancement of plant nitrogen content and this improves the amount of ammonium absorption at low concentration. For growth, the mycorrhizal endophytes utilises amino acids, peptides and proteins as nitrogen substrates which are the predominant nitrogen sources in organic heathland soil which are acidic in nature. They have also suggested that the success of ericaceous plants in such soils is due to the ability of the mycorrhizal fungus to provide its host with access to these nutrients.

The RET species of *Rhododendron viz., R. macabeanum* and *R. elliottii* taken for the present investigation were found growing in the temperate belt in Khonoma Dzukou area. According to IUCN category *R. macabeanum* is rare, endemic while *R. elliottii* belongs to endangered and endemic. The two species are listed as RET

species because their population is fragmented and their area of occupancy is estimated to be less than 500 km^2 (Gibbs *et al*., 2011). There is a continuing decline in their area of occupancy and in the number of mature individuals. Therefore, the reproductive phenology of *R. macabeanum* and *R. elliottii* has been taken for the present study. An attempt is made to provide data for their conservation strategies and to assess the factors responsible for the depletion of these two species.

Rhododendrons are reported to grow in acidic soils with high organic carbon (Milleville, 2002; Gibbs *et al*., 2011). Both species taken for the study grow in soil that is strongly acidic and high organic carbon content. The available nitrogen, available phosphorus and available potassium were found either in low or medium concentration for both the species. In *Rhododendron macabeanum* the pH ranges from 4.5-6.1 (Table 19) whereas in *R. elliottii* pH was found to be between the range of 4.6-5.9 (Table 34). The average organic carbon content in *R. macabeanum* was 2.70±0.35% in top soil and 2.35±0.48% in sub soil (Table 18). In *R. elliottii* the average organic carbon in top soil was 2.58±0.46% and 2.63±0.38% for subsoil (Table 33). In *R macabeanum* both top soil and subsoil have low available nitrogen content, top soil with an average of 250.50±50.15 kg/ha and subsoil 232.17±16.39 kg/ha (Table 15). The available phosphorus was medium for top soil with an average of 40.70±21.49 kg/ha and low in case of subsoil with an average of 18.53±0.70 kg/ha (Table 16). Available potassium was also found to be medium for top soil with an average of 129.61±84.29 kg/ha and low for subsoil with an average of 66.15±11.76 kg/ha (Table 17). In *R. elliottii*, the available nitrogen was medium for both top soil and subsoil with an average of 312±58.59 kg/ha and 288.97±75.55 kg/ha respectively (Table 30). The available phosphorus was low for both top soil and subsoil with an average of 18.26±1.92 kg/ha and 18.18±2.58 kg/ha respectively (Table 31**).** Both top

soil and subsoil show low amount of available potassium with an average of 93.96±15.85 kg/ha and 69.63±22.22 kg/ha respectively (Table 32). Chaurasia *et al*., (2005) have reported that the pH in acidic range support the colonization of arbuscular mycorrhiza fungi in rhododendrons. The arbuscular mycorrhiza fungi are obligate symbionts that are found abundantly in phosphorus and other mineral deficient soils. Phosphorus has been reported to be extremely essential for the proper growth of the *Rhododendron* seedlings (Bolan *et al*., 1984).

In the two species of *Rhododendron* studied *viz*., *Rhododendron macabeanum* and *Rhododendron elliottii,* the inflorescence is a terminal truss and further vegetative growth takes place by lateral branching. The flowers of both species are long- lived as they last approximately for eight days (Primack, 1985). *Rhododendron* flower have all the floral organs: sepals, petals, stamens and pistil. The flower is hypogynous and nectar pouches are present at the base of the corolla tube. The presence of nectary, which consists of a zone of nectariferous tissue at the base of the ovary, is characteristics of all *Rhododendron* species. In both the species the nectar secretion was observed already at the bud opening stage which has also been reported by Chwil and Chmielewska (2009). They reported that the sugar content in nectar of *Rhododendron catawbiense* falls within a range from 17% to 63%. But the sugar content in nectar of *R. macabeanum* and *R. elliottii* was less as compared to *R. catawbiense.* In *R. macabeanum* it ranges from 3.3% to 3.5% and in *R. elliottii* it ranges from 4.8% to 6.4%. The sugar content depends not only on the respective species, but also on the geographic location of plants (Martini *et al*., 1990; Chmielewska and Chwil, 2005).

The occurrence of viscin threads in the pollen tetrads was observed in both species of *Rhododendron*. The viscin threads play an important role in pollen removal from the anthers and its adhesion to pollinators. According to Hesse *et al*., (2000) the presence of viscin threads implies highly specific pollinators for accurate delivery of pollen to stigma which increases the efficiency of pollination. The presence of apically porose anthers and strongly adhesive pollen tetrads with viscin threads allow the efficient transfer of pollen loads to pollinators. This reduces wastage and the need for a large excess of pollen (Cruden and Jensen, 1979; Vasek and Weng, 1988, Neiland and Wilcock, 1995). The average pollen production per flower in *R. macabeanum* was recorded as 112362.90±29290.86 (Table 9) and the average number of ovules per flower was 2628.10±923.79 (Table 10), thus the pollen: ovule ratio becomes 43:1(Table 14). In *R. elliottii* the average number of pollen tetrads and ovules per flower are 52662.90±13951.12 (Table 24) and 1614.30±555.52 (Table 25) respectively. Pollen: ovule ratio was recorded as 32.6:1 (Table 29). According to Cruden (1977) the pollen: ovule ratio of plants whose pollen is dispersed in tetrads are substantially lower than those of species whose pollen is dispersed as monads. However Ng and Corlett (2000) has reported that the pollen: ovule ratio range from 176 in *R. simsii* to 1343 in *R. simiarum* which is comparatively higher than *R. macabeanum* and *R. elliottii*. The pollen-ovule ratio of both *R. macabeanum* and *R. elliottii* is low for xenogamous plants and comparable to the ratios reported for autogamous angiosperms (Cruden, 1977).

The flower visitors of the two species of *Rhododendron* viz., *R. macabeanum* and *R. elliottii* observed during the study were birds, bees, beetles, and flies. Stevens (1976, 1985), Argent (1985), and Argent *et al*., (1988) also observed a number of visitors to flowers of *Rhododendron* sp. (section Vireya) in Papuasia and Borneo

respectively. The visitors of flower included birds, bats, butterflies, moths, bees, caterpillars, beetles, mites and flies. Escaravage and Wagner (2004) have also reported bumble bees and honey bees as effective pollinators of *R. ferrugineum*. Mejias *et al*., (2002) reported *Bombus terrestris* and *Xylocopa violcea* as flower visitors of *R. ponticum*. *Xylocopa* sp. has been observed as pollinator of *R. elliottii* in the present investigation. Ng and Corlett (2000) also reported *Apis cerana*, *Bombus* sp. and *Xylocopa* sp. as major pollinators of *Rhododendron*.

In *Rhododendron macabeanum* Watt *ex* Balfour, the main pollinators were found to be the birds of genus *Yuhina* sp. (Plate-4, figs. A-C) as they forage for nectar and carry pollen on their beak, fore-head as well as on the ventral and dorsal surface of the neck. The other visitors like beetles and flies (Plate-4, figs. E&F) have not been found to be effective pollinators. The flowers are self-compatible and also exhibit geitonogamy and xenogamy due to frequent movement of these birds from one flower to another in search of nectar. The plants show high percentage (71.43%) of fruit set in open pollinated flowers. The ovule: seed ratio has been found as 1.61:1 and pollination experiments indicate that it is self-compatible and the pollen-ovule ratio indicates that it is autogamous as suggested by Cruden (1977). Although the seed-set percentage has also been very high, yet the seedling establishment in natural habitat is very low. Since, the seed germination in the laboratory has been found 95 % (Plate-9, figs. A&B), poor seedling establishment in the natural habitat has been assigned to the various climatic factors like heavy rains and the sloppy habitat around the population of *R. macabeanum* trees which could not provide the proper conditions for seedling establishment into the soil after the seed germination.

Whereas in *Rhododendron elliottii* Watt ex Brandis the flowers are foraged for its nectar by the carpenter bees of the genus *Xylopcopa* sp. and the birds of the genus *Heterophasia pulchella* only (Plate-12, figs. B-F). The same observations have been reported by Ng and Corlett (2000); Mejias *et al*., (2002) and Georgian *et al*., (2015). The transfer of pollen tetrads from anthers to stigma takes place during the process of foraging. The pollen- ovule ratio has been found 33:1 which is comparable to the ratios reported for autogamous angiosperms (Cruden, 1977). The plant also shows high percentage of fruit- set. The tiny seeds in *elliottii* are dispersed by wind. They show as high as 90% of seed germination *in-vitro* but *in-vitro* seedling survival is as low as 2% only. The poor survival rate of seedling has been assigned to the improper habitat for the seedling establishment which is the probable cause of the low population of *R. elliottii* at its natural locality.

Fruit is a capsule in both species of *Rhododendron* (Plate-7, figs. B-E; Plate-14, figs. C&D). It is oblong, grooved and dehisce from top by longitudinal slits. The seeds are fusiform and winged. They retain their viability for about one year at normal temperature and humidity (Williams *et al*., 2011). The seeds of *R. macabeanum* and *R. elliottii* require light for germination. They germinate readily in the laboratory in the presence of light and do not require any particular treatment to break dormancy. Most species of the genus *Rhododendron* and members of Ericaceae need light for germination. Blazich *et al*., 1991; Cho *et al*., 1981; Blazich *et al*., 1993; Rowe *et al*., 1994; Arocha *et al*., 1999 Singh *et al*., 2010 have reported influence of light and temperature on the seed germination of different species of *Rhododendron*. Arocha *et al*., (1999) studied the influence of light and temperature on seed germination of *Rhododendron chapmanii* and reported that they require light for germination regardless of the germination temperature.

Natural regeneration of both *Rhododendron* sp. was found to be low. Only few plantlets were observed in the period of study though they produce numerous seeds. Seedlings have mainly been found on humid rocks or soils covered by blanket of bryophytes which retains moisture on the soil by reducing evaporation. They also reduce the impact of raindrops on the soil. The same observation has been reported by Cross (1981). This indicates that the recruitment failure does not result from non-viability of seeds. In nature, the growth rate of seedlings and young plants of rhododendrons is very slow which may jeopardize the effective seedling recruitment. Pornon and Doche (1995) have also reported that seedling recruitment is poor in many *Rhododendron* species and for successful establishment the seeds require favourable microsites (Cross, 1981; Plocher and Carvell, 1987; Kohyama and Grubb, 1994). Because of poor seedling survivability and recruitment failure, the population of two species of *Rhododendron viz., R. macabeanum* and *R. elliottii* is less and becoming rare and endangered in their natural habitats.

Most species of the genus *Rhododendron* and members of Ericaceae need light for germination (Singh *et al.,* 2010). Many works on the influence of light and temperature on the seed germination of different species of *Rhododendron* has been carried out by Blazich *et al*., 1991; Cho *et al.,* 1981; Blazich *et al.,* 1993; Rowe *et al*., 1994; Arocha *et al.,* 1999 and Singh *et al*., 2010. Arocha *et al*., (1999) studied the influence of light and temperature on seed germination of *Rhododendron chapmanii* and reported that they require light for germination regardless of the germination temperature. The influence of temperature and light on the seed germination of *Rhododendron niveum* a critically endangered *Rhododendron* of Sikkim Himalaya was done by Singh *et al.,* (2010). They observed that the seeds of *R. niveum* required

light to trigger the germination and no germination was observed in darkness. The optimum temperature for germination was found to be 21°C.

No direct human impact was observed as the study area Khonoma Dzukou is a conserved area. There is no report of people using *Rhododendron macabeanum* and *R. elliottii* for different economic activity. The two species are still conserved because of the fact that the community manages the *Rhododendron* forest and forbid people from collecting flowers and felling of trees which have helped the species to survive and sustain in its natural habitat. However, the villagers rear mithun (*Bos frontalis*) in plenty and their movement is not restricted but allowed to graze in the forests openly. Hence the ground vegetation is trampled or grazed by the mithuns. Seedling damage by herbivory was not observed in the study area. In *R. macabeanum* it was observed that some flowers were damaged by birds while feeding on the larvae and this leads to poor fruit development. But in *R. elliottii* damage flowers were not observed during the study period. Invasive species were not observed in the study area.

CONSERVATION STRATEGIES

The rich growth of rhododendrons in the state has been severely degraded in recent times. Increase in human population and anthropogenic activities along with other natural factors are found to be the dominant factors of disturbances on rhododendrons growing in high altitudes (Mao *et al*., 2010). Natural threats include landslides and forest fires which affect the rich growth of rhododendrons. Therefore to counteract the possibility of full scale destruction of *Rhododendron* habitats through such natural calamities, rare and endangered species should be grown in similar favourable habitats.

Anthropogenic threats include fuel wood collection, small scale extraction of timber and collection of plants by local people. The main threats are habitat loss due to the increasing human population leading to the loss of rich floristic diversity (Mao and Gogoi, 2012). Pradhan and Lachungpa (1990) have suggested that the fast growing species like *Alnus nepalensis* should be grown extensively as a source of firewood for the local people. *Alnus nepalensis* also enrich the soil as it has the ability to fix atmospheric nitrogen.

Moza and Bhatnagar (2007) emphasized the importance of the studies of reproductive biology for developing effective strategies for *in situ* and *ex situ* conservation of the RET species. Such studies will be useful in determining the factors responsible for the depletion of these species in the natural habitat.

The RET taxa of rhododendrons require immediate conservation. In order to conserve the rare, endangered and threatened species it is necessary to protect their natural habitats. Conservation can be effected through *in-situ* (conservation in its natural habitat) and *ex-situ* (conservation outside the natural habitat) conservation (Sharma, 2007).

In-situ conservation is brought about by the conservation of genetic resources through their maintenance within natural ecosystems such as biosphere reserves, National parks, Wildlife sanctuaries etc. and by creating public awareness of the importance of the species. For an effective conservation measures educating the local people on the ecological significance and the beauty of the species is very necessary. Their cooperation is required to implement different conservation measures. The school children should also be taught at the primary level about the important local plants. Conservation programs will be successful when it is linked in a sustainable

manner to the economic upliftment of the local people (Pradhan and Lachungpa, 1990).

Ex-situ conservation is effected through Botanical Gardens/ Arboreta/herbal gardens, Seed/Germplasm Banks under suitable climatic conditions and *in vitro* tissue culture techniques.

The Botanical Survey of India, Eastern Regional Circle, Shillong has successfully mass propagated 8 species of *Rhododendron* through tissue culture technique. *Ex situ* conservation of some species of *Rhododendron* through tissue culture techniques have been taken up by G.B. Pant Institute of Sikkim Himalaya, Gangtok (Mao and Gogoi, 2012).

In the west many countries have societies or organisations, such as Royal Horticultural Society, London, American Rhododendron Society, Rhododendron Society of Canada & Australia, Rhododendron Species Foundation, International Rhododendron Union, etc. to popularise the plant for promoting conservation (Mao and Gogoi, 2012). Sikkim is the only state in India having Rhododendron Society patronage by the state government for the purpose of conservation. These organisations or societies should organise conferences and lecture programmes in important *Rhododendron* areas in order to monitor the status of *Rhododendron* polulation in their habitats. Therefore, considering their economic importance and vulnerability there is an urgent need for conservation of rhododendrons in Nagaland.

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PLATES

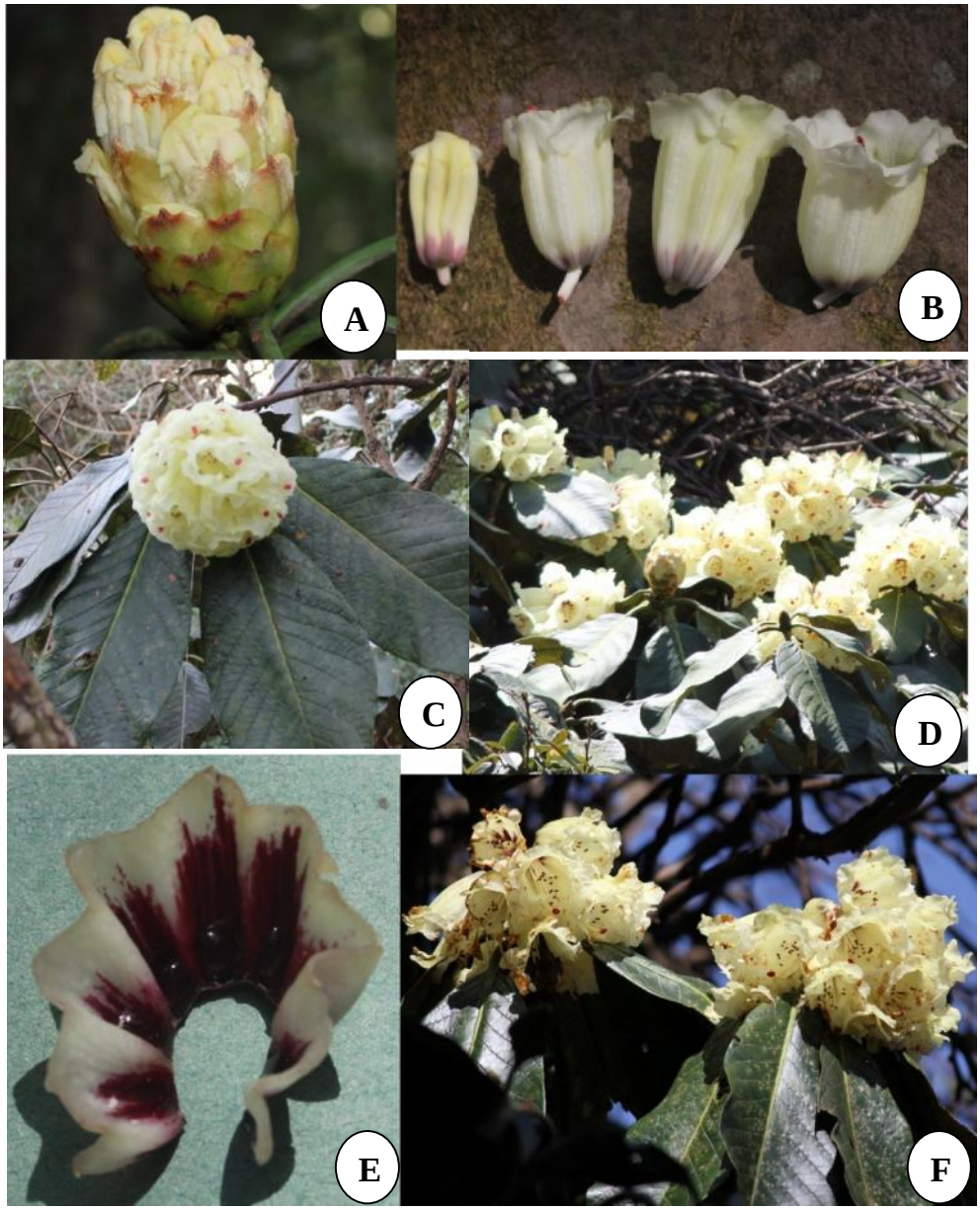
Plate -1

Figs A-F: Study Area. A. Khonoma village gate; B. View of village; C-F. Khonoma Dzukou forests.

Plate -2

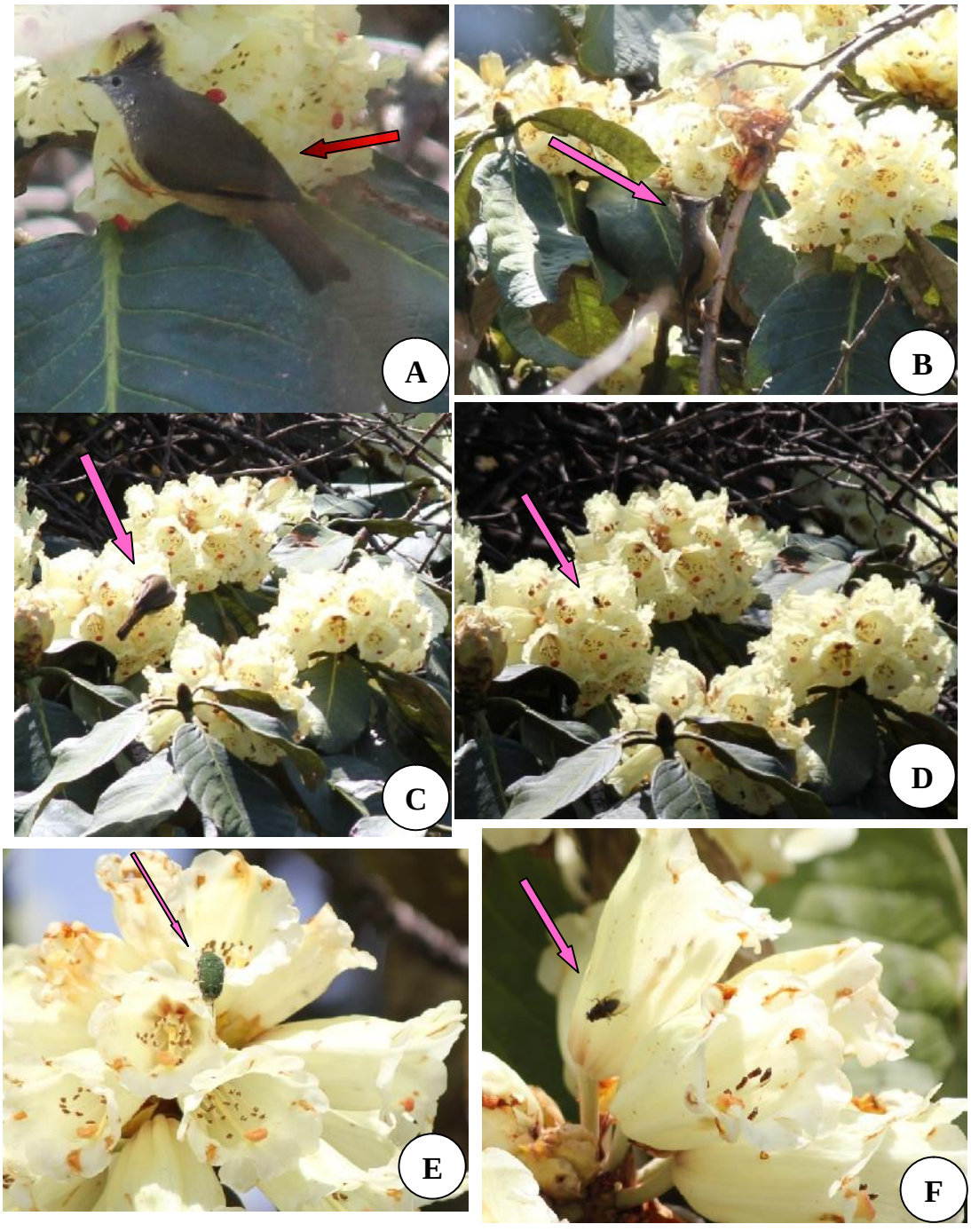
Figs. A-F: *Rhododendron macabeanum* showing the habit of the plant. A-C. Tree; D. Small tree; E. New leaf shoots; F. Branch with mature leaves.

Plate -3



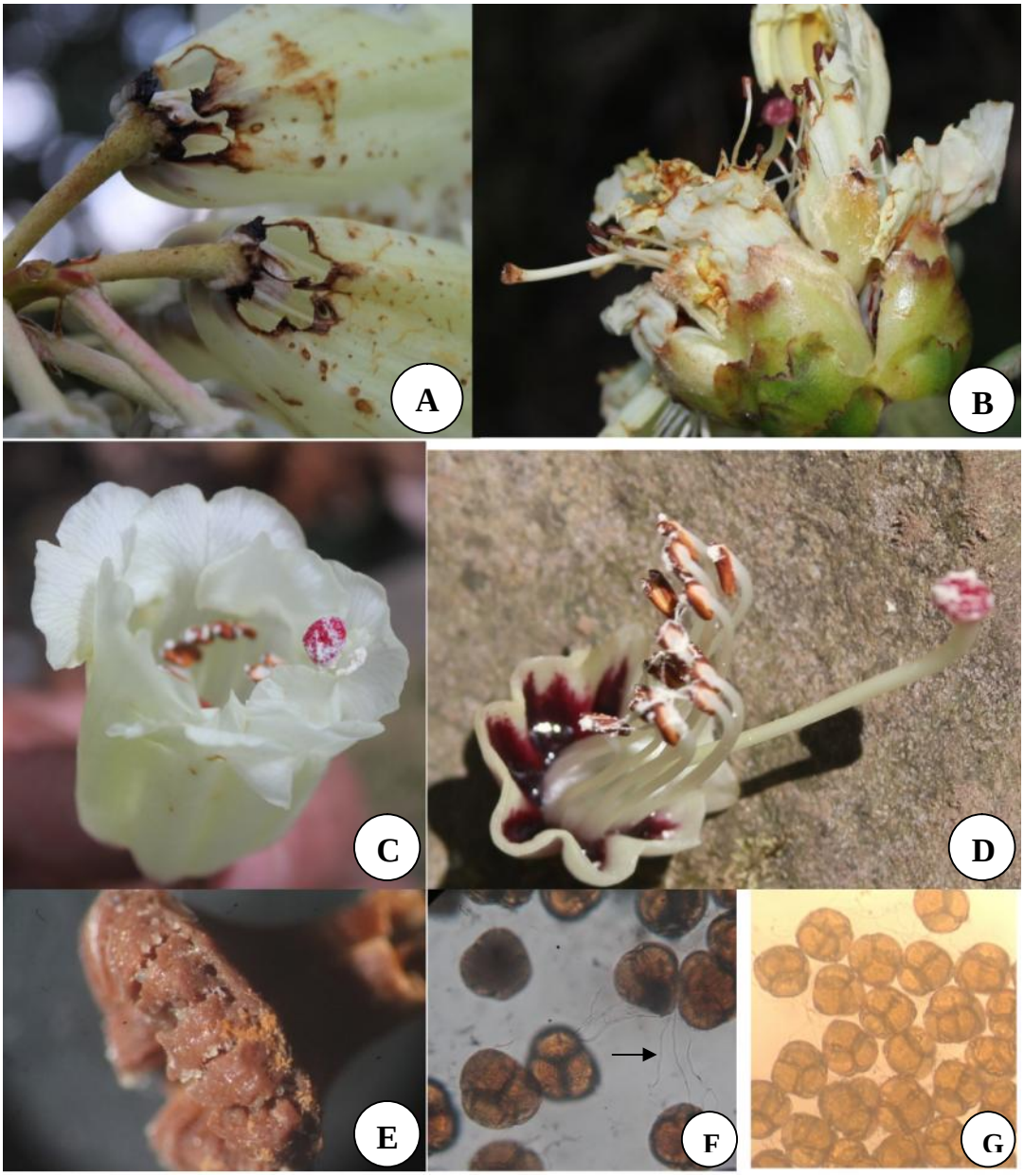
Figs. A-F: *Rhododendron macabeanum* showing different stages of flower. A. Flower bud; B. Different stages of bud development; C. An inflorescence; D. Flowers in full bloom; E. Dissected corolla showing nectaries; F. Mature flowers.

Plate -4



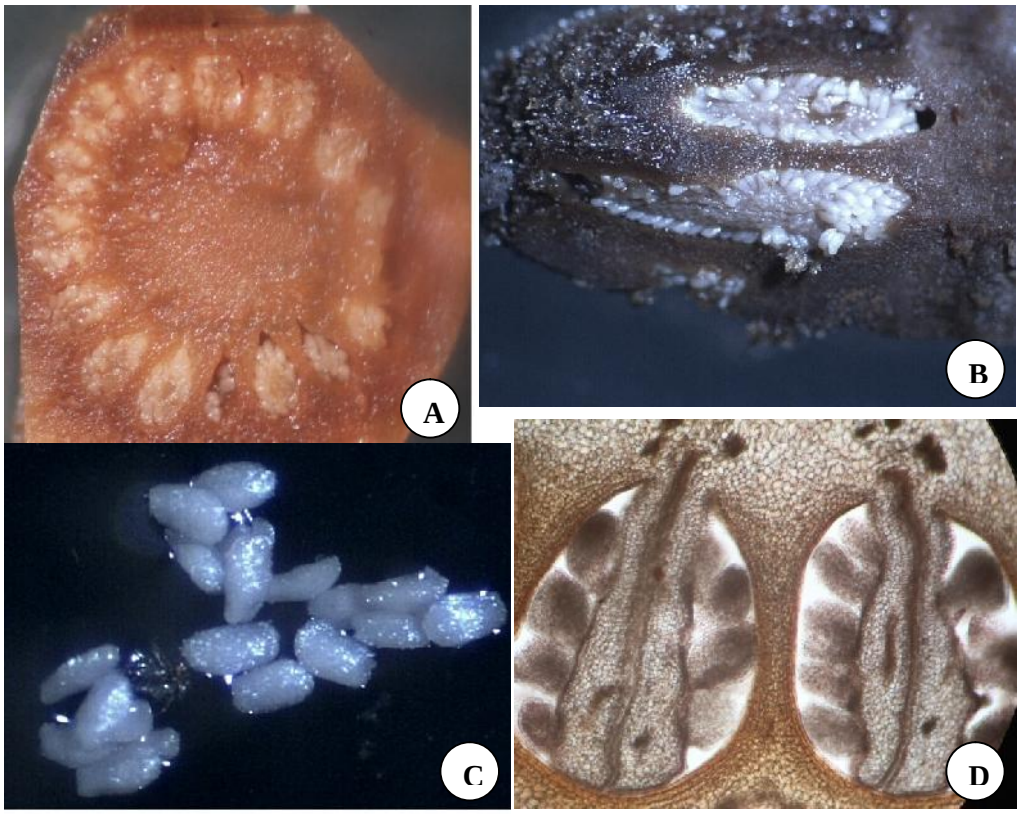
Figs. A-F: *Rhododendron macabeanum* flowers showing floral visitors. A-C. *Yuhina* sp. collecting nectar from the flowers (pointed by arrows); D & E. Beetle and a bee foraging the flower; F. A fly.

Plate-5



Figs. A-G: *Rhododendron macabeanum* showing damaged flowers and pollen tetrads. A&B. Flowers damage by birds feeding on the larvae; C&D. Pollen tetrads deposits on stigma; E. Stigmatic grooves with pollen tetrads; F-G. Pollen tetrads with viscin threads (arrow).

Plate-6



Figs.A-D**:** *Rhododendron macabeanum* Ovary. A. T.S. of ovary showing ovules; B. Dissected ovary showing ovules; C. Ovules; D. T.S. of ovary showing axile placentation.

Plate-7

Figs. A-F: *Rhododendron macabeanum* capsules A. Young fruits; B. Matured capsules; C. Infrutescence showing dehisced capsules; D. T.S of a mature capsule; E. Capsule dehiscing from the top by longitudinal slits; F. Small seeds showing finger like projections at the ends.

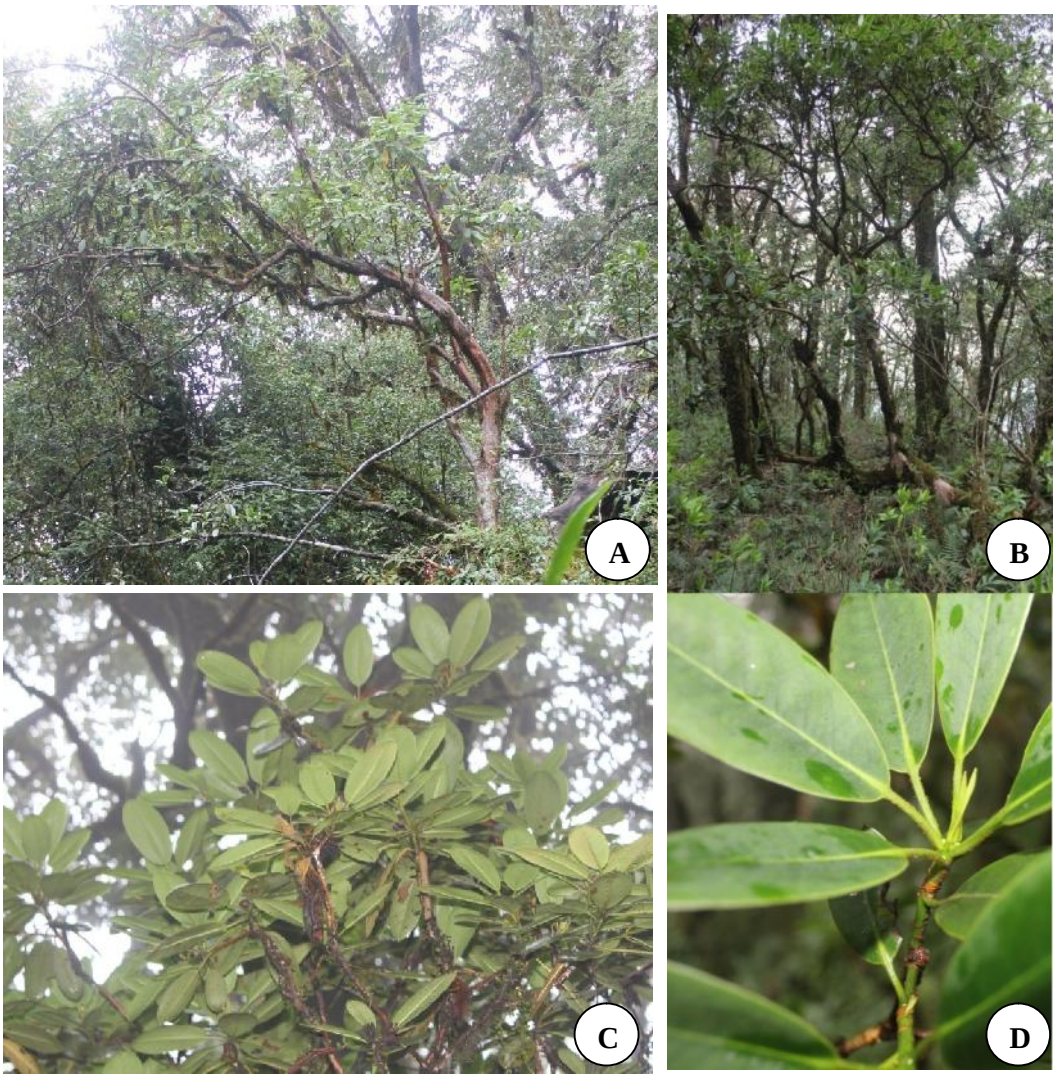
Plate -8

Figs. A-F: *Rhododendron macabeanum*. Natural regeneration of plants and factors affecting plant growth. A&B. Plantlets growing among the bryophytes; C&D. Landslides observed in the *Rhododendron* forest; E. Mithuns (*Bos frontalis*) grazing in the forest; F. Footpath destroyed by mithuns.

Plate -9

Figs. A-D: *Rhododendron macabeanum* seed germination. A. Seeds germinating on moist blotting paper; B-D. Seeds germinating on soil collected from the natural habitat.

Plate -10

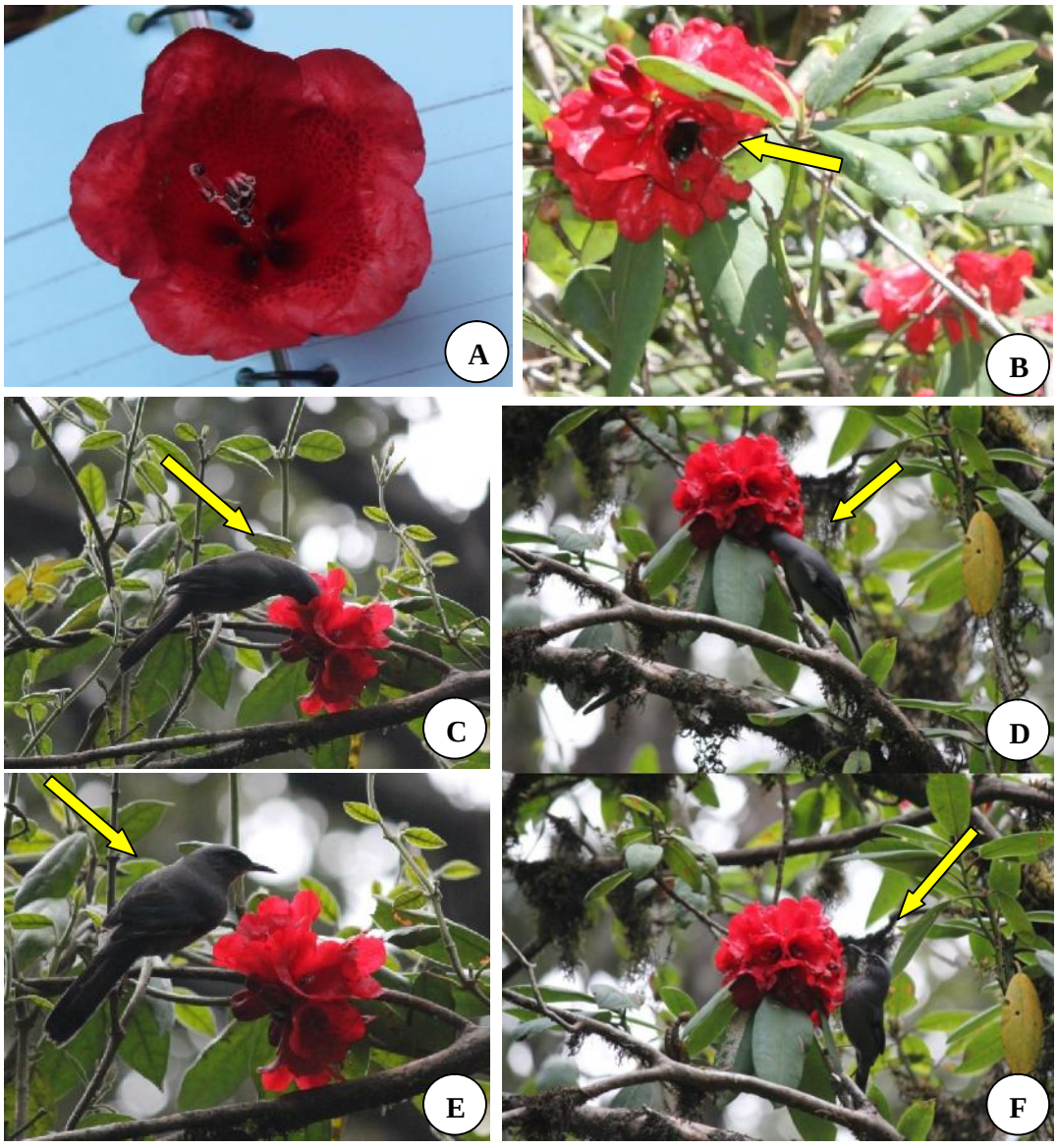


Figs. A-D: *Rhododendron elliottii* showing the habit of the plant. A&B. Tree; C&D. Branches with mature and young leaves.

Plate -11

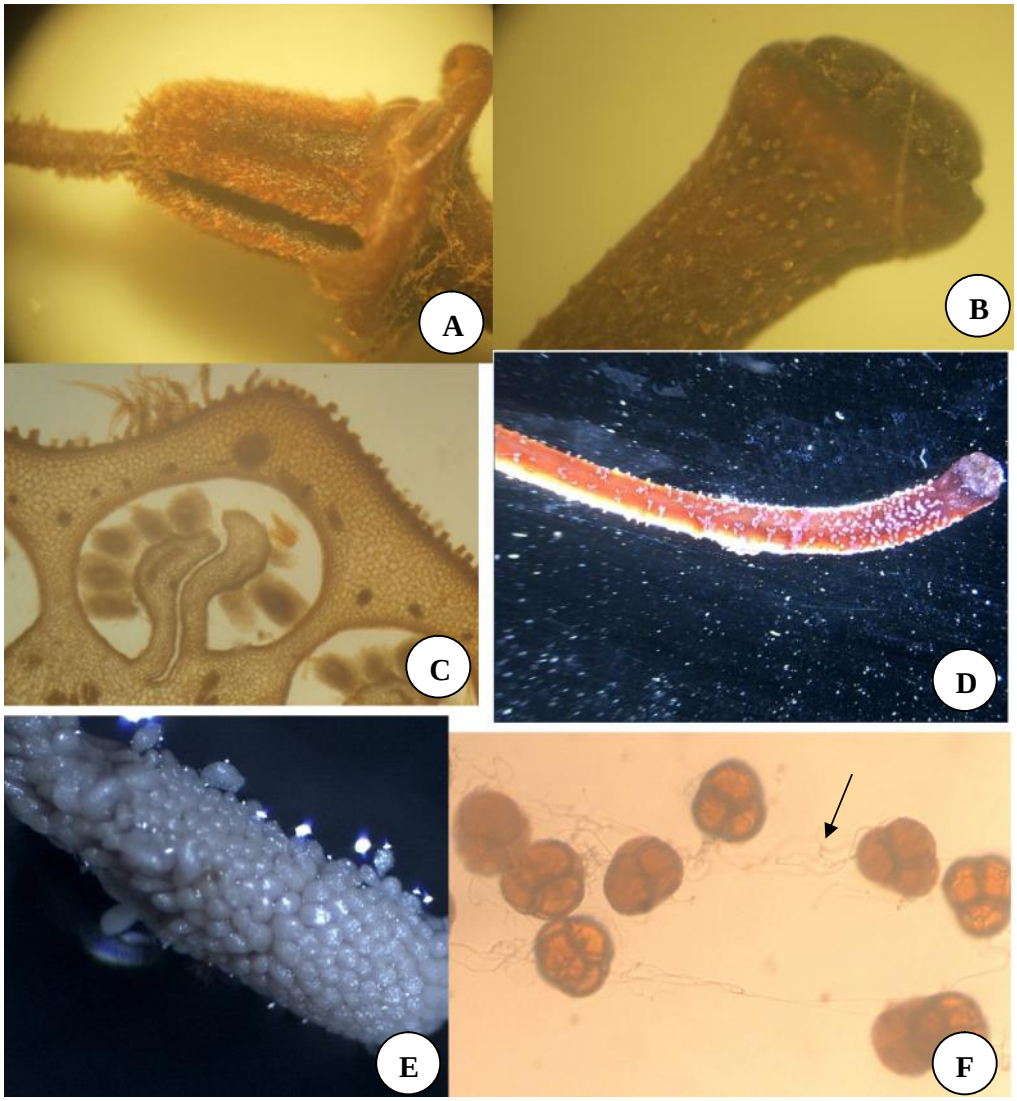
Figs. A-F: Flowers of *Rhododendron elliottii*. A&B. Flower buds; C&D. Inflorescence; E. Dissected flower showing pistil and stamens; F. Dissected flower showing five nectaries.

Plate -12



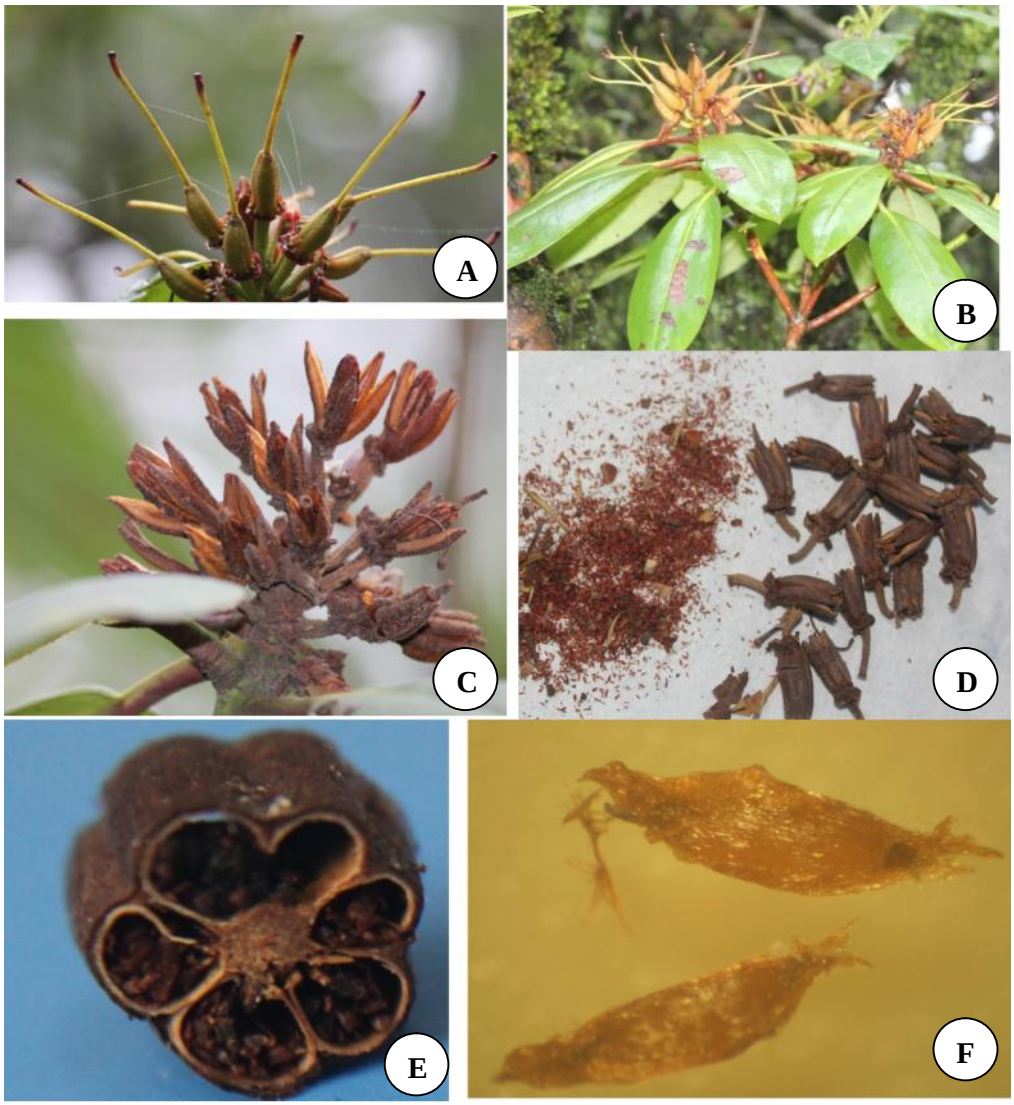
Figs. A-F: *Rhododendron elliottii* flower and the floral visitor. A. A tubular-campanulate flower; B. *Xylocopa* sp. foraging for nectar (arrows); C-F. Birds (*Heterophasia pulchella*- arrows) on flowers.

Plate-13



Figs. A-F: *Rhododendron elliottii* ovary and pollen tetrads. A. Ovary; B. Stigma; C. T.S. of ovary; D. Style; E. Ovules; F. Pollen tetrads with viscin threads (arrow).

Plate -14



Figs. A-F: *Rhododendron elliottii* fruits and seeds. A&B. Infrutescence; C. Dehisced fruits; D. Matured dehisced fruits and seeds; E. T.S. of capsule; F.Seeds (magnified).

Plate -15

Figs. A-F: *Rhododendron elliottii* showing seed germination and natural regeneration of plants. A. Seeds germinating on moistened blotting paper; B&C. Seeds germinating in soil collected from the natural habitat. D-F. Plantlets growing in the natural habitats among the bryophytes.