REPRODUCTIVE BIOLOGY OF *NOTHAPODYTES NIMMONIANA* (GRAH.) MABBER.: AN IMPORTANT ANTI-CANCER DRUG YIELDING MEDICINAL TREE

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SEPTEMBER, 2002

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Thesis submitted to the
University of Agricultural Sciences, Dharwad
in partial fulfillment of the requirements for
the degree of

Master of Science (Forestry)

IN

FOREST BIOLOGY AND TREE IMPROVEMENT

By

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CERTIFICATE

This is to certify that the thesis entitled "**REPRODUCTIVE BIOLOGY OF *NOTHAPODYTES NIMMONIANA* (GRAH.) MABBER.: AN IMPORTANT ANTI-CANCER DRUG YIELDING MEDICINAL TREE**" submitted by **Mr. HOMBE GOWDA, H. C.,** for the degree of **Master of Science (Forestry)** in **Forest Biology and Tree Improvement**, to the University of Agricultural Sciences, Dharwad is a record of research work carried out by him during the period of his study in this University, under my guidance and supervision and the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship, or other similar titles.

Sirsi
September, 2002

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ACKNOWLEDGEMENT

It is with utmost respect and devotion, I place on record my profound sense of gratitude and indebtedness to **Dr. R. VASUDEVA**, Chairman of my advisory committee, Assistant Professor (Forest Biology), College of Forestry, Sirsi. His close counsel, erudite guidance, unstinted support, critical evaluation, unflagging enthusiasm, everlasting patience and constant encouragement made my task easy and has left indelible mark on this thesis work. A multitude of potential problems were solved through his guidance in the very right direction at the right time. I consider it as a privilege to work under his guidance. I owe a great deal to him and remain deeply indebted to him.

I avail this opportunity to express my sincere gratitude and thanks to **Dr. S. L. MADIWALAR**, Professor and Head, Dept. of Agroforestry, **Sri. S. G. HEGDE**, Associate professor (Stat.), **Sri. A. KRISHNA**, Assistant professor (Seed Tech.), and **Dr. RAMESH BHAT**, Assistant Professor (GPB), who acted in members of my advisory committee, for their encouragement, introspective suggestions, propitious support and benevolent guidance throughout this study. I also extend my great respect, devotion and sincere thanks to our beloved **Dr. A. M. CHANDRASEKHARAIAH**, former Director of Instruction (Forestry) for his constant encouragement.

It is my immense pleasure to express my deep scence of gratitude **Dr. R. UMA SHAANKER** Assoc. Professor (Crop physiology) and Dr. K. N. GANESHAIAH, Professor (Genetics and Plant Breeding), UAS, Bangalore, for their help, everlasting encouragement, and valuable suggestions through out this study.

I sincerely acknowledge the help rendered by **Dr. RADHAMANI. T. R., Dr. SURYANARAYANA, Dr. S.T. PRABHU, Sri. S. D. BHAT, V.M. DABGAR, Dr. K. MANJAPPA AND Sri. C.R. PATIL** who helped me in various ways during the course of this work.

I sincerely acknowledge the help rendered by **LAXMINARAYANA HEGDE**, Assit. Professor, College of Horticulture, Arabhavi, who introduced me to this system.

I sincerely acknowledge the help and constant support of my friends **BORAIAH, K.T., RAGHU, H.B., SURAJ, P. G., JOHN MATHEW, GEORGI, P. M., SUDHARSHAN, MANJUNATH, A., MURTHY, B. N. N., TARA CHAND, RAGHU, D., BALACHANDRA, H.C., MANJUNATH, L., NICOLEE, L SANTHOSH, G. R., MAHESH, V.M., SRINIDHI, H.V. AND SUNIL, H.**, for their help during field DATA collection and made this endeavour possible and also life lighter with good cheer during my stay in Sirsi.

In spite of all these, I lack in words to express my deep sense of gratitude to my beloved parents, **Sri CHIKKANARASIMHAIAH, AND Smt. RUKMINI, Brother, Mr. RAVI KUMAR, H.C., Sister Smt. SHYLAJA, H.C., Brother in law, CHANDRASHAKER** and family members, who encouraged me to undergo higher studies. Their selfless persuasion and sacrifices, heart-felt blessings and constant inspiration have made this manscript a little remuneration to translate their dreams into reality.

I take this opportunity to express my sincere thanks to my friends **PRAVEEN KUMAR, NANIAH, K.M, SRIDHRA AND ALL PONNAMPET FRIENDS** for their constant encouragement and support.

I take this opportunity with warm regards to extend my thanks to **JINDAL EDUCATION TRUST** for providing scholarship and ATREE for provided facility during my study.

I also express thanks to **Mr. N. DEVARAJ** Lab Assistant, Forest Biology & Tree Improvement for his help during the study.

I shall be failing in my duty if I forget to place on record the facilities offered by *alma mater*, College of Forestry, Sirsi and Ponnampet. The uninhibited and timely help by the office and library staff of College of Forestry, Sirsi is warmly acknowledged.

SIRSI

SEPTEMBER, 2002

(HOMBE GOWDA, H.C.)

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Introduction

I. INTRODUCTION

Nature has provided several useful plants for the welfare and prosperity of mankind. Plants have been one of the important sources of medicines ever since the dawn of human civilization. In spite of tremendous development in the field of allopathy during the 20th century, plants remain one of the major sources of drug in modern as well as traditional system of medicine throughout the World. Approximately one-third of all pharmaceuticals are of the plant origin. Out of nearly 3,00,000 species of higher plants available all over World, only 32,000 plant species have bean investigated for their medicinal properties which is a small proportion to total, still smaller number yield well-defined drugs (Farooqi and Sreeramu, 1996).

India has been known to be a rich repository of medicinal plants and it has a rich heritage of knowledge on plant based drugs. Even today India abounds a rich wealth of important medicinal flora, especially the Himalayan ranges, the Gangetic plains, the Eastern and Western Ghats and semi arid zones of the country provide an immense scope to procure the drugs for the pharmaceutical needs of the Indian system of medicine. In India different ethnic communities have recorded about 8000 plant species in medicinal use across the country. This constitutes 45 per cent of the 17,500 known flowering plant species of India and nearly 1/4th of the World (Ved *et al.*, 2000).

Western Ghats forests of India are regarded as, one of the twenty-five mega biodiversity hot spots in the World (Myers, *et el.*, 2000). These forests form a very unique ecosystem due to their rich plant and animal diversity. It is estimated that Western Ghats supports nearly 4,500 species of plants and about 1,500 animals including mammals, birds, reptiles and other invertebrates. It is estimated to harbours approximately 2,000 known medicinal plant species and many of these are endemic to this region (Ved *et al.*, 2000). Recently, several tree species of the Western Ghats are gaining international importance due to their newly identified pharmacological and curative properties. One such tree is the *Nothapodytes nimmoniana (*Syn:*Nothapodytes foetida; Mappia foetida).* The wood-extracts of this tree are used in cancer-fighting drugs. The active principle of the wood-CAMPTOTHECIN (CPT), is known as a potent drug to break single-strand DNA in the mammalian systems hence used in treating tumor (Wall *et al.*, 1966). CPT is also known to inhibit retroviruses such as human immuno-deficiency virus (HIV) and equine infectious anemia virus (Priel *et al.*, 1991). It is believed that CPT is the third most important alkaloid sought after by the pharmaceutical companies around the world.

Perhaps this has led to the large-scale exploitation of the species from its wild habitats in the recent years despite the ban on green felling. It is reported that from Mumbai port alone, about 54 tones of dry wood chips of this tree were exported during 1994. This figure must have been increased several times in the recent years. A world-bank survey suggests that the twigs of this tree are available for Rs 15-20 per kg, where as the extract after processing is sold by multinational pharmaceutical companies for US $ 15,000 per kg in the world market. Since the active ingredient is present in the wood, essentially the entire trees are chopped in the wild leading to their death upon harvesting. This has resulted in a sudden increase in the loss of populations. An estimated 20 per cent of the population of this species is believed to have declined over the last decade (Ved, 1997). Recently *N. nimmoniana* has been assigned the threat status of '*Vulnerable*' (Vu).

However, there is hardly any available literature on its occurrence and biology, except for the brief notes in the local floral descriptions (Ravi Kumar and Ved, 2000). Even now, we have to depend on the experience of the local botanists and tribesmen to identify good populations of this species. Understanding biology of rare species is a prerequisite for its genetic improvement and domestication (Vasudeva *et al.*, 2001). Hence there is an urgent need to study its breeding system and seed biology to standardize methods of propagation for ex *situ* conservation. Vegetative propagation serves as the primary method of reproduction of forestry species for commercial production on the majority of nurseries. In this background the present study was done with the following objectives:

- To understand a few aspects of reproductive and seed biology of *Nothapodytes nimmoniana*.
- To standardize a vegetative propagation technique.

Review of Literature

II. REVIEW OF LITERATURE

Literature pertaining to various aspects of biology and pharmacological properties of *Nothapodytes nimmoniana* is presented in this chapter. A few studies related to reproductive biology, standardization of seed germination and protocols of vegetative propagation in respect of other forest species are also reviewed.

2.1 Nomenclature, botanical description and distribution of *Nothapodytes nimmoniana*

2.1.1 Nomenclature

The genus *Nothapodytes* was earlier known as *Mappia*. Hooker in his monumental work on the Flora of the British India, (1872) has described four species of *Mappia viz. Mappia tomentosa, M. foetida, M. ovata* and *M. oblonga*. However, in 1921, Brandis clubbed these four *Mappia* species into one and recognized that inflorescence and leaf traits were the remarkable features for identification. Howard (1942a) studied the history, systematic position, geographical distribution and economic uses of the family Icacinaceae, to which both *Mappia* and *Nothapodytes* belong, and developed keys to the various genera. In a later report, he redefined *Nothapodytes* and *Mappia* as valid and distinct genera of the family Icacinaceae (Howard, 1942b). Currently the genus *Mappia* is confined to the American continent (Yoganarasimhan *et al.*, 1981).

2.1.2 Botanical description

Nothapodytes nimmoniana is a lower canopy tree of evergreen to moist deciduous forest types. It grows up to 12 m in undisturbed evergreen forest and appears shrubby, with a height of 3 to 4 m, in case of secondary forest or distributed forest (Plate 1). This species is also reported from shola forests of South India (Srivastava, 2000). Saldanha (1984) and Ravikumar and Ved, (2000)

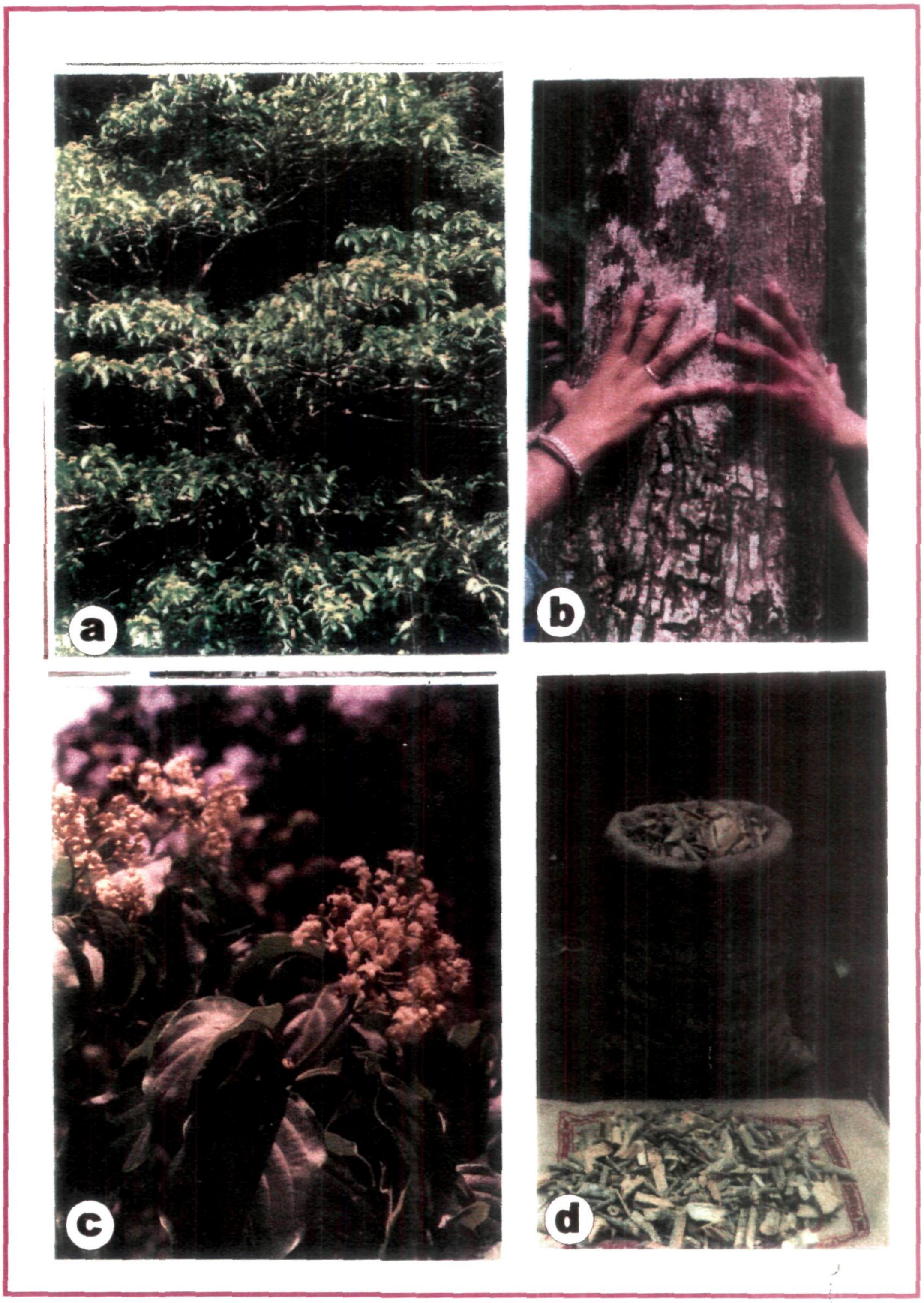

Plate 1

a. General habit of *N. nimmoniana* b. Close up of tree trunk

c. Flowering branch and foliage d. Dried wood chips ready for have described the traits of the species as follows: "leaves are usually alternate, slightly leathery, broadly egg shaped to elliptic-oblong; leaf base is often unequal, apex acute to acuminate, margin entire, hairless above, thinly hairy beneath, crowded at the ends of branch-lets; lateral nerves 8-10 pairs inside; bark smooth, gray, wrinkled, about 5mm thick; brachlets slightly angled, corky with prominent leaf scars; inflorescence is terminal, corymbose cyme; flowers are bisexual, white to creamy yellow, emitting an unpleasant odour, 5-merous, calyx toothed, about 5mm across petals hairy inside; ovary silky-tomentose, drupes oblong to ellipsoid, about 2×1 cm, smooth, purplish black when ripe seeds single".

2.1.3 Seed dispersal

Ganesh and Davidar, (2001) reported that fruits of this species are dispersed by small birds in Kalakad-Mundanthurai Tiger Reserve. Commonly occurring six frugivores birds *viz.*, Black Bulbul (*Hypsipetes leucocephalus*), Yellow-browed Bulbul (*Iole indica*), Red-whiskered Bulbul (*Pycnonotus jocosus*), White-cheeked Barbet (*Megalaima viridis*), Mountain Imperial Pigeon (*Ducula badia*) and Niligiri Wood Pigeon (*Columba elphinstonii*) were reported to be dispersers of this species.

2.1.4 Distribution of N. nimmoniana

It is distributed throughout tropical America and Asia and some part of China and Srilanka. In India, it is distributed widely in the North Eastern hills and the Western Ghats. In Karnataka it is reported in districts of Kodagu, Mysore, Chamarajanagara, Hassan, Chikkamagalore, Uttara Kannada, Dakshina Kannada, Udupi and Shimoga (Saldanha, 1984). In Karnataka it is commonly known as "*Durvasane mara*" (meaning foul smelling tree in Kannada) or "*Teku lakda*" (in Hindi). In some parts of Karnataka, it is also called *Haeladachi mara* or

Kodsa or *Hedare*. In fact, its earlier specific name *foetida* was derived from its foul smelling inflorescence.

2.2 Medicinal property of *N. nimmoniana*

Medicinal use of *N. nimmoniana* was recently discovered and it is not reported in any traditional medicinal system. Camptothecin (CPT), an alkaloid present in the plant, is responsible for its medicinal importance. CPT is a mono-terpene derived indole alkaloid with a α-hydroxylactone moiety and the conjugated rings, which are essential for the anti-neoplastic activity. Currently CPT and a few derivatives of CPT are the third most important alkaloid sought-after by the pharmaceutical companies worldwide. It was first isolated by Govindachari and Viswanathan (1972a) from wood chips of *N. nimmoniana* tree. CPT has previously been isolated from a rare Chinese tree, *Camptotheca acuminata* in low quantities, but *Nothapodytes* is most convenient source for large-scale production (about twenty folds higher concentration) of this pharmaceutically interesting alkaloid.

CPT has been extracted from most of the in plant parts of *N. nimmoniana*, like root, stem, leaves, flowers, fruits and seeds. But its content in different parts is effected by the rate of biosynthesis and catabolism and hence found to vary with respect to stage of development, diurnal variation and environmental factors (Zohara and Palevitch, 1982). Availability of CPT is maximum in root and bark (0.1% and 0.08%, respectively) followed by stem (0.06 %) and least in leaves (0.01%; Govindachari and Viswanathan, 1972b).

The anti-cancer property of CPT was first reported by Wall *et al.* (1966) and has been used for treatment of colon, head, neck, and breast cancer. Schultz (1973) has reported anti-leukemia and anti-tumoral activity of Camptothecin. Agarwal and Rastogi (1973) reported that alcoholic extract of *N. nimmoniana* possessed a powerful anticancer activity against human epidermoid carinoma of nasopharynax, walker carsinosarcoma 256 in rat (WM) and L-1210 lymphoid Leukemia in mice (PS).

Govindachari (1977) showed anti-cancer activity of CPT in P-388 lymphocytic leukemic (mouse) at a dose 0.5mg\kg of 9-methoxycamptothecin compared to the activity of CPT at a dose of 1mg\kg. Atherton and Burke (1978) reported that CPT induces production of interferon at concentration of 100μg. Aiyama *et al.* (1988) isolated another alkaloid (a Camptothecin derivative) known as (20S)-18,19-dehydrocamptothecin from *N. nimmoniana*.

The target of CPT in mammalian cell is topoisomerase I, the enzyme essential for DNA replication and transcription. CPT and its analogues, singly or combined with Cisplatin show efficacy against solid tumours, breast, lung and colorectal cancers which are unaffected by many other cancer chemotheraputic agents (Wall, 1996). Interestingly, CPT is also known to inhibit retroviruses such as human immuno-deficiency virus (HIV) and equine infectious anemia virus (Priel *et al.*, 1991). Han-Shung Wu *et al.* (1995) isolated a new naturally occurring alkaloid, acetylcamptothecin, together with 17 other known compounds. Among them, scopoletin, Camptothecin, 9-O-methoxycamptothecin and O-acetylcamptothecin showed significant cytotoxic activity.

2.2.1 Alkaloid extraction through tissue culture

Roja and Heble (1994) for the first time isolated CPT and 9-methoxycamptothecin from callus. Immature embryos isolated from seeds were cultured on MS medium supplemented with 3 per cent sucrose and other growth regulators. Trace amounts of these alkaloids were detected in callus, but the quantity of 9-methoxycamptothecin in undifferentiated callus were very less

(0.0001%DW) compared to platelet (0.0007%DW). Fulzels *et al.* (2001) recently studied growth and production of Camptothecin by cell suspension cultures of *N. nimmoniana*.

2.3 Importance of *N. nimmoniana* as a Non-Timber Forest Produce (NTFP)

N. nimmoniana is also important as an NTFP, which yields oil and edible fruits. Nadkarni *et al.* (1944) extracted 48 per cent oil (consisting of 7.06 % palmitic acid, 17.69 per cent stearic acid, 38.45 per cent oleic and 36.8 per cent linolinic acid) from the kernels of *Nothapodytes nimmoniana* with benzene as a solvent. The oil is brownish in color and poorly florescent.

The fruits of this species, similar to *Syzygium cumini* in appearance and taste, are edible and also have medicinal properties. Hence it is being collected and marketed in Kerala as an NTFP. It is reported that Kerala state SC and ST federation received about 4,339 kg of fruits through its various collection centers during 1996-1997 (Nair, 2000), which was sold at a price of Rupees ten per kg of fruit.

2.4 Rarity and threatened status of *N. nimmoniana*

Understanding and analyzing botanical rarity has been an important task among plant ecologists. However, different scientists have ascribed fundamentally different meanings to this concept. The criteria used by scientists to assess and categorize plant species into classes of rarity vary considerably. For instance, Harper (1981) has considered rarity as a phenomenon in both time (related changes in the population size across time) and space (related to the sites on which the populations occur). In India, botanists have classified plants as rare, endangered, threatened based on their experience alone. In one of the finest treatment of concept of rarity, Rabinowitz (1981) recognized that geographic range, habitat specificity, and population density are the three axes on which botanical rarity can be defined. Species belonging to either small geographic range, occupying narrow habitat range or having lower population size merit to be called rare.

During 1995, Foundation for Revitalization of Local Health Tradition (FRLHT) initiated categorization of threatened medicinal plants through Conservation Assessment and Management Plan (CAMP) workshops. The CAMP workshop is an intensive, interactive and unique process, which facilitates objective and systematic prioritization of research and management action needed for species conservation both *in-situ* and *ex-situ.* It consists of 40-50 experts from different discipline *viz.* scientists, conservation manager, user group representatives, taxonomist *etc.*. A taxon is awarded a threatened status based on its population size, condition of its natural habitat, captive status, *etc.* For each taxon, an attempt is made to estimate the total population and distributional range and to compare these with the numerical threshold in order to determine the International Union for Conservation of Nature and Natural Resources (IUCN) Red lists categories. The Species Survival Commission (SSC) of the IUCN and Bird Life International have endorsed it as the logical first step towards the development of the taxon level action plans. Till date, three CAMP workshops have been conducted and a consolidated list of 112 threatened medicinal plants in Southern India has been prepared (Anon, 1997).

*N. nimmoni*ana is one such Red-listed medicinal plant, which was assigned a threat status of '**vulnerable/Regionally**', during third CAMP workshop conducted during 1997 in Bangalore. This categorization was based on its assessed decline of more than 20 per cent of it's natural population over the last

10 years (Ved, 1997). It is further reported that the reduction of this population was mainly because of destructive harvesting. Independently, Gowda *et al.* (1997) has also identified *N. nimmoniana* as one of the rare and endangered species of South India.

2.5 Studies on pollination biology of tropical trees

During the last decade, interest on reproductive biology studies has increased tremendously throughout the world. The Programme Advisory Committee on Plant Sciences, of Department of Science and Technology, has identified plant reproductive biology as one of the challenging areas of study (Shivanna and Parveen, 1994). Information on breeding systems of the Indian trees is slowly being documented although there was an initial lag. These reports basically cover the qualitative evaluation of floral biology, aspects of pollination biology *etc.* However, whether these studies cover threatened species or not is uncertain.

Sexual systems ranging from simple hermaphroditism to temporal dioecy have been reported for the Indian tree species (Borges, *et al.*, 1997). Androdioecy is a rare form of sexual allocation among flowering plants, wherein an individual bears either all staminate flowers (males) or all bisexual perfect flowers. Darwin (1877) identified that gynodioecy, where stamens are typically vestigial or absent from the pistillate flowers of female plants, functionless sterile androecioum may be lost evolutionarily. He conjectured that actual androdioecy is ephemeral transitional stage enroute to hermaphroditism to dioecy. The species have been found to undergo temporal gender changes, or else the species that appear to be androdioecious have proven to be cryptically dioecious (Liston *et al.*, 1990). These considerations are generally concerned with the loss of genetic diversity and how this loss affects persistence of small populations. Several theories such as avoidance of self-fertilization, optimal allocation of reproductive effort via maleness/femaleness, local mate competition, sexual selection, parent offspring conflict have been invoked to explain the wide array of sexual systems in plants. In a pioneering work, a team of scientists headed by Ganeshaiah and Uma Shaanker has successfully extended the concepts of sociobiology to explain sex allocation and other reproductive features of the plants. These include shaping of pollen grain to ovule ratios (Uma Shaanker and Ganeshaiah, 1984), floral sex ratios (Vasudeva *et al.*, 1987; Ganeshaiah and Uma Shaanker, 1991), initiation of mate competition (Uma Shaanker and Ganeshaiah, 1990), stigmatic regulation of pollen germination (Ganeshaiah and Uma Shaanker, 1988) and parent-offspring conflict (Uma Shaanker *et. al.*, 1988) in tree species. The rare and endangered plant species are shown to be unique in a few of their life history traits. Lokesha and Vasudeva (1997) have shown that a greater proportion of woody-lianas is rare and endangered in the South-Indian flora. Further animals more often disperse this group of plants, predisposing them for extinction.

Although, reproductive biology on *N. nimmoniana* is largely unknown, species is reported to be bisexual in various floral descriptions including the recent FRLHT publication (Ravikumar and Ved, 2000).

2.6 Seed germination studies in forest species

Seed germination is the resumption of active growth of the embryo that results in the emergence of the young plant. Seeds of many tree species remain ungerminated even when they were sown. Such kinds of dormancy in seeds may be due to presence of hard seed coat or some germination inhibitors or might be due to improper development of embryo *etc.* Recently, Srivastava (2000) reported that germination in *N. nimmoniana* starts at 25th day of sowing and completed by 80th day. A maximum of 60 per cent germination was reported even without any pre-treatment.

Since studies on aspects of seed germination on *N. nimmoniana* are minimum, a review of studies focusing on other forest species is presented the following section.

Soaking in water

Water is an important factor in the external environment in the stimulation of the seed germination. Soaking seeds in water helps in softening the seed coat, removal of inhibitors and reduction in time required for germination (Hartmamn and Kestar, 1976). Thomson (1981) recorded 90 per cent germination in *Terminalia calamonsonai* when seeds were soaked in cold water for 4 days. Soaking the seeds of *Terminalia chebula* in cold water for 24 hours along with cowdung stratification for 5 weeks enhanced the germination rate (77%) but seeds took 107 days for normal germination (Bhardwaj and Chakraborthy, 1984). More than 80 per cent of seed germination was achieved when *Terminalia catappa* seeds were soaked in water for 24 hours whereas, *Terminalia brassi* recorded only 60 per cent germination (Prins *et al.*, 1994).

Dayanand and Lohidas (1988) reported best germination (75%) from soaking pods with tap water for 8 days, followed by acid scarification (58.5%) in Red Sanders. Sarjan Bhagat *et al.* (1992) obtained 90.66 per cent germination by soaking *Dodonea viscosa* seeds in hot water for 24 hours compared to 24.75 per cent in control.

Growth regulators

Effect of growth regulators like Gibberlic acid, Cytokinin and Ethylene on germination and seedling growth have been recorded, since decades and numerous investigations are still going on. Among growth regulators Gibberlic acid has been frequently used to stimulate germination of dormant seeds in various crops. Karonda (*Carissa carandas*) seeds treated with GA3 at 25ppm for 24 hours recorded 67 per cent germination as compared to control (49.2%) and also GA3 treated seeds showed good seedling vigour (Bankar, 1987). Treating seeds of *Bursera pencillata* with 100ppm GA3 has found to have profound effect on germination per cent, seedling root length and shoot dry weight (Nagaraj and Farooqi, 1989).

Bhattacharya *et al.* (1991) reported that erratic seedlings emergence in most of the forest seeds which is either due to low seed viability or due to seed coat imposed dormancy. To overcome this problem, pre germination treatment with Gibberlic acid (100ppm) was given to *Eucalyptus citriodora* which resulted in better germination and seedling vigour in, FRI-4 and FRI-5 genotypes. Mishita and Ashry (1991) observed that higher seed germination (70%), seedling length and fresh weight in the seeds of *Magnolia grandiflora* when treated with 1000ppm of GA3. *Albizia odoratessima* seeds treated with 100ppm GA3 showed better germination (33.3%), minimum number of days for germination (17.90) and also recorded maximum plant height (13.09 cm) compared to control (Moktan *et al.*, 1993). Seeds without seed coat treated with 250ppm GA3 recorded 90 per cent germination compared to 75 per cent in seed with seed coat in case of *Garcinia indica* (Mathew and George, 1995). Swapna *et al.* (1999) reported that GA3 at higher concentration enhanced the germination rapidly, whereas biomass yield was higher at lower concentration in *Persea bombyciana.*

Treatment of seeds of *Givotia rottleriformis,* with 1000 and 2000ppm GA3, exhibited the higher percentage of germination compared to zero percentage control. But the germination is quicker (by 2 days) in case of 2000ppm when compared to 1000ppm (Kiran *et al.*, 2000).

2.7 Studies on effect of plant growth regulators in rooting of cuttings

It is almost evident that growth regulators play a significant role in controlling rooting ability. Auxins have a marked influence on root formation of stem cuttings. The most widely used auxins have been Indole acetic acid (IAA), Indole butyric acid (IBA) and Naphthalene acetic acid (NAA). However, there are no studies on their effect in *N. nimmoniana*. Hence an attempt was made to review the studies on rooting of stem cuttings in semi-hard woodcuttings.

Wood and Phillion (1985) observed that quick dips in IBA solution (1000 or 2000ppm) appears to promote early and maximum rooting in European larch cuttings. Rooting of semi hardwood cuttings of sweet lime was best (36.67%) with IBA at 100ppm, and it was only 16.67 per cent in untreated control (Sandhu and Zora Singh, 1986). Seethalakshmi, *et al.* (1983) reported that Coumarin, NAA and mixture of Coumarin and IAA are the effective chemicals and also reported that mixture of root promoting substance are known to be occasionally more effective than either compound alone, where as combination of Coumarin and IAA was more effective than Coumarin alone. The cuttings derived from branches of 15 months old *Gmelina arborea* plants did not root when treated with combination of 250ppm IBA plus 250ppm NAA or Seradox, and even though they were subjected to three misting frequencies (Sandum *et al.*, 1986).

Bahuguna *et al.* (1988) reported that best response (per cent sprouting, rooting and root length) was obtained in the cuttings treated with IBA for 24 hours in *Woodfordia fruitiscosa.* Barucha (1988) reported that growth regulator combination of IBA and NAA at the rate of 20000ppm was most effective in initiating rooting which produced 25 per cent rooting in custard apple. Adarsh Kumar *et al.* (1988) reported that *Bambusa tulda* showed 100 per cent sprouting and 40 per cent rooting in Coumarin at 10mg/lt. In Red Alder, best results (80%) were obtained with 10 seconds dip in 4000ppm IBA (Radwan *et al.,* 1989). Shameeth *et al.* (1989) found that IBA 3000ppm resulted 80 per cent rooting as compared to control (40%) in *Celtis australis.* Kesava Reddy *et al.* (1994) reported that *Avicennia officinalis* and *A. alba* showed a good response by producing rooting with 5000ppm IBA, followed by 10000ppm of IBA and 5000ppm of IAA.

Srivasuki *et al.* (1990) reported that IBA at 10000ppm concentration accelerated rooting (15 days) and rooting percentage (94.4) compared to control (25) in *Tamarindus indica.* Surendran (1990) found that exogenous application of IBA at 1000ppm recorded 60 per cent rooting followed by 53.3 per cent in Seradix as compared to control (26.7%) in *Gmelina arborea.* Adarsh kumar *et al.* (1990) reported that two nodal segments of *Bambusa tulda* showed maximum sprouting (100%) and maximum sprout length (50 cm) in Coumaron at a concentration of 20 mg/lt and 200 mg/lt respectively. Seethalakshmi *et al.* (1990) reported that, 10ppm and 100ppm Coumarin was most effective to *Ochlandra travencorica.* IBA resulted better rooting (50 to 70%) than IBA and IAA in *Acacia senegal* (Badji *et al.*, 1991). In *Amherstia nobilis*, stem cuttings treated with IBA at 6000 and 10000ppm resulted in 90 and 83 per cent rooting compared to 23.3 per cent in control (Bhattacharjee and Balakrishna , 1991). The hard wood cuttings of jamoon, rose apple and wood apple when treated with IBA 2000, 4000 and 6000ppm gave 26.25, 31.25 and 7.5 % rooting respectively under intermittent mist (Narayana Reddy, 1993).

Bhardwaj *et al.* (1993) reported that the cuttings of *Terminalia chebula* planted in July and October failed completely to produce roots. While, in March middle portion of the cuttings treated with 4000ppm IBA produced maximum rooting (24.3%) and sprouting (33.3%) and number of roots (4 per plant). However, at 6000ppm IBA concentration the rooting was only 3.5 per cent.

The IBA 2000ppm induced higher rooting both in terminal (60%) and middle (20%) cuttings of *Carallia brachita* compared to nil in control (Vijay Kumar *et al.,* 1993). Palaniswamy and Pramodh Kumar (1997) observed that 8000ppm IBA induced significant rooting during March in *Pongamia pinnata* cuttings. The application of IBA 5000ppm resulted in significant improvement in rooting per cent, primary root number, root length and root dry matter over control in Mapple (Bharadwaj and Mishra, 1998). In *Taxus bacata* an extreamly important cancer drug yielding plant, 67.5 per cent rooting was observed when cuttings were treated with 1000ppm IBA (quick dip) followed by 63 per cent in IBA 1000ppm (dip for 24 hours) compared to 2 per cent in control (Harsha *et al.,* 1999).

Rao *et al.* (1999) reported that IBA 4000ppm increased rooting per cent from 10 to 62.5 per cent in *Wrightia tinetoria* which is more than 6 times the control. Hareesh *et al.* (2001) reported that IBA 3000ppm recorded maximum sprouting (36%) followed by NAA 1000ppm plus IBA 1000ppm (27 per cent) and NAA 3000ppm plus IBA 3000ppm (27%) as compared to control (7per cent) in *Ougeinia oojensis.* However none of the treatments produced roots in cuttings, where as Seradix (3%) recorded 57.8 per cent sprouting with 35.85 per cent rooting. Karoshi and Hegde (2001) reported that IBA 2500ppm induced 60 per cent of rooting in apical and shoot cuttings and 50 per cent in mid cuttings of *Gymnema sylvestris*, as compared to control (5 and 0% respectively).

Material and Methods

III. MATERIAL AND METHODS

3.1 Study area

The present study was carried out in Joida and Sirsi forest ranges of Uttara Kannada district in Karnataka (Fig 3.1), which are parts of the Central Western Ghats. The vegetation in the study sites varied from disturbed moist-deciduous to secondary moist-deciduous forests, and was associated with, *Xylia xylocarpa, Terminalia paniculata, Terminalia belerica, Terminalia alata, Lagerstroemia lanceolata, Syzigium cumini* and *Ficus sp.* in the top canopy; *Holarrhena pubescens, Randia dumetorum, Bauhinia racemosa, Canthium spp, Careya arborea, Rauwolfia densiflora, Xantolis tomentosa, Zizyphus sp.* and *Pavetta indica*, in the ground and lower canopy.

3.2 Study site

Nothapodytes nimmoniana occurs in disturbed evergreen to moist deciduous forest types. A good population of *N. nimmoniana* was found in the secondary moist deciduous habitat near Joida town in Dandeli forest division and was the focal population of the study. This site is located 20 km South West of Dandeli, which is popular for plywood, pulp and paper industry in Karnataka. Additional observations on a few traits were also done on another population at Sirsi, which is located 100 km South of Dandeli. The exact latitude and longitude of these were presented in the Table 3.1.

3.2.1 Climate

The study area falls under tropical climate. The climate is primarily monsoon and rainfall occurs by South West monsoon during June to September. The region receives an annual average rainfall of about 2500mm with a minimum of 1210 mm and a maximum of 3500mm. The region received a rainfall about

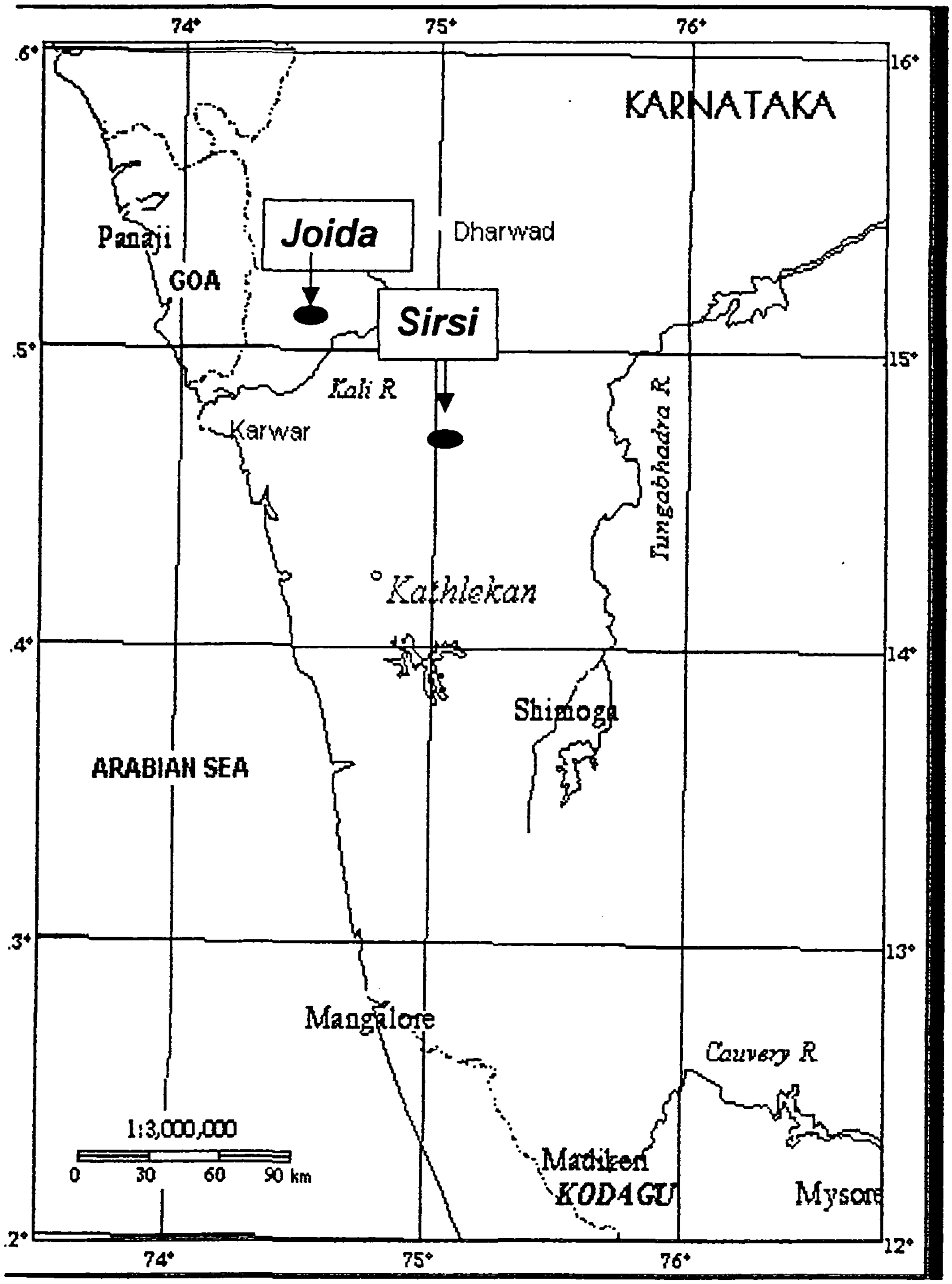

Fig. 3.1 Map showing study localities in Uttara Kannada.

Table 3.1 Latitude, longitude and altitude of study areas.

Site	Latitude North		Longitude East		Altitude In m (msl)
	From	To	From	To	
Joida	15° 5' 14.2"	15° 17' 38.2"	74° 25' 9.2"	74° 32' 32.1"	549-609
Sirsi	15° 44' 12.3"	14° 40' 21.3"	74° 53' 15.3"	74° 42' 15.3"	525-550

2135 mm during 2000. The mean annual temperature varied from 18° C to 31° C. April and May were the hottest months while December and January were the coldest.

3.3 Sexual system

All flowering individuals (n=66) were tagged and scrupulously observed for their sex expression during January to December, 2001. Additional observations *were done till August 2002. From every tagged individual tree, minimum of 200* flowers were examined and various flower traits (*viz.*, flower size, length of pistil, stigma & anthers, production of pollen grains) were considered to ascertain the gender of the flower as male, female and bisexual. For further confirmation, different inflorescence types (a minimum of five, in each type) were bagged and observed for fruit set following self and open pollination. Individuals in a population were classified into sexual systems based on flower types they borne. Number of inflorescences per individual and number of flowers per inflorescence were also recorded. Size of each plant and a few morphological characters were recorded for all flowering individuals in a population.

3.3.1 Flower description

Fifty flowers from each gender (male, female and hermaphrodite) were used for recording morphological character of flowers. Morphological characters *viz.* flower length, flower width, number of sepals, number of petals, length and width of petals, stamen length, stigma length, other stamen and stigma character were recorded with the help of microscope and scale.

3.3.2 Phenology

Phenological observations were recorded on naturally occurring, tagged trees (n=20) around Sirsi. Four phenophases identified were:

a. Initiation of leaf sprouting

b. Initiation of flowering and anthesis

c. Initiation of fruit setting, development and retention, and

d. Initiation of leaf and fruit drop

The observations on phenophases were made throughout the year at an interval of 15 days. A particular phenophase was considered to have started, when about 10 per cent of individuals were observed in that phase and considered to be completed when only less than 10 per cent individuals remained in that particular phase.

The observations pertaining to floral biology were started from the bud stage of the flower. The twigs were marked for counting various floral stages at different time intervals. The flowering was recorded by counting the average floral stages on different twigs and 10 replicates in each case were taken. The formation of first floral bud was considered as floral initiation. Full bloom stage was considered as that stage when more than 90 per cent flowers had opened.

3.3.3 Pollination

Five flowering individuals were observed every day for visitors during the blooming period. Pollinators were recorded during 0600 to 0700 and 1700 to 1800h and a time window of fifteen minutes was used to record kind and number of visitors. Observation was continued up to the end of flowering season. Every insect visitor was collected and identified to the family level.

3.3.4 Standardization of pollen germination media

Altering a basic media proposed by Vasudeva and Yeradoni (1998), an artificial pollen germination media was standardized. Pollengrains were collected from different individuals during bright sunny day and sprinkled on the media incubated under laboratory conditions.

3.3.5 Assisted pollination

Female flowers were bagged to avoid cross-pollination and controlled pollination was done for bagged flowers with fresh pollen grains from several male inflorescences. Pollen grains were dusted onto the stigmatic surface using a camel hair brush three to four times and it was done for a minimum of five trees. Number of fruits developing was counted after 30 days and per cent fruit set was computed.

3.3.6 Fruit Biology

Traits such as fresh fruit weight, seed weight, seed length, seed width, were recorded on individuals belonging to various sexual systems. Few observations on open pollinated and controlled pollinated fruits were to understand the effect of controlled pollination. A large bulk sample of fruits collected from various sexual systems was used to conduct studies on germination.

3.3.7 Seed parameters

Seed parameters such as, 1000 seed weight and seed density were recorded using bulk seed collection. 1000 seed weight was measured using electronic balance and seed density measured on volume basis.

$$\text{Seed density} = \frac{\text{Weight of 100 seeds in g}}{\text{Volume of water (ml) replaced by seed sample}}$$

3.4 Seed germination

3.4.1 Standardization of pre-germination treatment and germination studies

For the standardization of pre-treatments the following treatments were imposed on the seeds with four replications (n=100) under standard sowing conditions in the laboratory.

T_1 = control (sown directly along with seed coat)

T_2 = sown directly after mechanically removing the seed coat

T_3 = cold water soaking for 12 h, along with seed coat

T_4 = cold water soaking, after removing the seed coat, for 12 h

T_5 = soaking in GA 50 ppm for 12 h along with seed coat

T_6 = soaking in GA 50 ppm, after removing the seed coat, for 12 h

Seed germination was recorded on a daily basis for 21 days to compute per cent germination, germination rate and daily mean germination as follows:

$$\text{Germination rate/speed} = \frac{G_1}{D_1} + \frac{G_2}{D_2} + \frac{G_3}{D_3} + \ldots\ldots + \frac{G_n}{D_n}$$

G_1 = Number of seeds germinated on first day

G_2 = Number of seeds germinated on second day

G_3 = Number of seeds germinated on third day

G_n = Number of seeds germinated on n^{th} day

D_1 = Day one

D_2 = Day two

D_3 = Day three

D_n = Day n

$$\text{Mean Daily Germination} = \frac{\text{Cumulative germination percentage}}{\text{Number of days from germination}}$$

3.4.2 Biometric and biomass traits on seedlings

At the end of experiment (*i.e* after 90 days after sowing), shoot length, root length, number of leaves, dry weight of shoots and roots were taken on five randomly selected seedlings from each replication. The soil sticking to the roots were carefully removed by repeated washing in running water. The seedlings were then spread out for a brief period of drying and the following observation were recorded.

Seedling height

The seedlings were spread on a paper floor and the length of the shoot was measured from the tip of the growing point to the collar regions using a measuring scale and expressed in centimeters.

Collar diameter

The collar diameter of the seedlings was measured using vernier callipers and the two diagonally opposite readings were noted and the mean recorded in millimeters.

Tap root length

The length of the tap-root as measured from the collar region to the tip of the main root using a measuring tape and the mean length recorded in centimeters.

Dry matter production per seedling

The root and shoot parts were kept separately in a hot air oven maintained at 70 ± 2^0 c for 72 hours and dried to a constant weight. The weight was then determined using an electronic balance.

3.4.3 Seed viability studies

Hundred bulk seeds were sown at every fifteen days interval from their collection time and germination was recorded up to six months.

3.4.4 Statistical analysis

The data relating to each character were analysed using MSTATC programme.

3.5 Vegetative propagation

One-year-old stem cuttings of 15-20 cm length were used for standardization of vegetative propagation technique. These cuttings were treated with different plant growth regulators at different concentration and at different combination levels. Growth regulators such as IAA, IBA and Coumarin were used as detailed below.

Treatments are

T_1 -IAA 1000ppm
T_2 -IAA 2000
T_3 -IAA 3000
T_4 -IBA 1000
T_5 -IBA 2000
T_6 -IBA 3000
T_7 –COU 1000
T_8 -COU 2000
T_9 –COU 3000
T_{10} -IAA+IBA 1000+1000
T_{11}- IAA+IBA 2000+2000
T_{12} -IAA+IBA+COU 1000+1000+1000
T_{13} –control

This experiment was conducted in a small mist chamber in a glass house. Mist chamber was made from one-quintal capacity polythene bags. Sand and coir pith was used as a rooting media at 1:1 ratio. Different level of growth hormones was applied in solution and dust form separately. Same level of growth hormone was also treated with stem cuttings of different sex types Such as, male, female and bisexual and these experiments were done separately.

Treatment

Slant cut was made to each cutting before hormonal treatment and cut ends were treated with 0.2 per cent Bavistin for half an hour to avoid fungal attack. Quick dip method was used to treat cuttings with hormones. Later cuttings were kept in mist chamber to observe rooting and top of plastic bag were closed. The following observations were recorded.

Sprouting per cent and Number of sprouts

Sprouting per cent was calculated as follows

$$\text{Sprouting per cent} = \frac{\text{No of sprouted cuttings}}{\text{Total no. of cuttings}} \times 100$$

Maximum sprout length

The cuttings were spread on the floor and the length of the longest shoot was measured from the tip of the growing point of sprout to the bottom of the sprout. Using a measuring tape and the mean height in centimeters worked out.

Number of leaves

Total numbers of leaves were recorded for sprouted cuttings and mean was worked out.

Rooting per cent and number of roots

Number of rooted cuttings were counted by carefully removing the cuttings from bed and repeated washing with tap water. Also number of roots per cutting were recorded by counting the number of roots per cutting.

$$\text{Rooting per cent} = \frac{\text{No of rooted cuttings}}{\text{Total no of cuttings}} \times 100$$

Maximum root length

The cutting were spread on the floor and the length of longest root was measured from the tip of root to the basal attachment to the cuttings. Using a measuring tape and the mean length in centimeters worked out.

Statistical analysis

The data relating to each character were analyzed using MSTATC programme.

Experimental Results

IV. EXPERIMENTAL RESULTS

The results obtained in the study titled "**Reproductive biology of *Nothapodytes nimmoniana*** (Grah.) **Mabber. : An important anti-cancer drug yielding medicinal tree**" are presented in this chapter.

4.1. Breeding system

4.1.1 Sexual system

Through careful observation on a large population of *Nothapodytes nimmoniana* (n=60) in the forests of Joida region in Uttara Kannada district, it was identified that the species exhibits a wide array of sexual systems at various levels. All the three types gender of flowers *viz.* androceious, gynoceious and hermaphrodite (Plate 2) were observed in this population. As shown in the Table 4.1, at individual tree level, seven sexual types were identified *viz.* male, female, hermaphrodite, monoecious, andromonoecious, gynomonoecious and trimonoecious. The frequency of occurrence of these sexual systems in natural populations is also shown in the Table 4.1 and Fig 4.1, 4.2.

About 10 per cent of the total 60 individuals were pure males and 34 per cent were female flower bearing individuals; thus the population had 44 per cent dioecious type (Fig 4.1). One individual was found to be perfect monoecious, bearing male and female flowers separately on a single inflorescence. Individuals bearing perfect bisexual types constituted about 12 per cent. About 42 per cent of the population showed intermediate types such as andromonoecious(Fig 4.1), gynmonoecious and trimonoecious, with frequency, 30, 5 and 7 per cent, respectively (Fig 4.2).

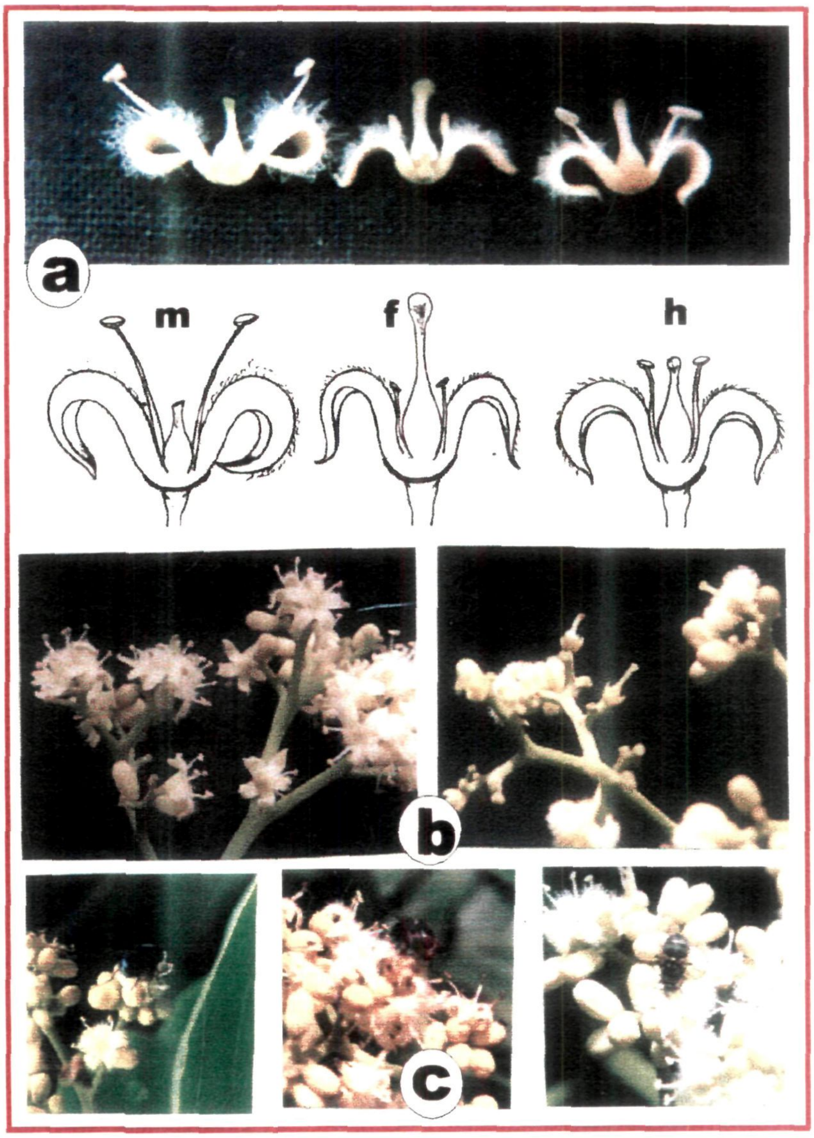

Plate 2

a. Close-up and schematic representation of male (m) female(f) and hermaphrodite (h) flower of *N. nimmoniana*

b. Close-up of male and female flowers

c. Different floral visitors

Table 4.1 Array of sexual systems at individual level in *Nothapodytes nimmoniana.*

Sl. no	Sexual system at individual level	Flower types borne by the individual plants	Per cent occurrence (n = 60)
1	**Male**	Only male flowers	10
2	**Female**	Only female flowers	34
3	**Monoecious**	Male and female flowers	2
4	**Hermaphrodite**	Only bisexual flowers	12
5	**Andromonoecious**	Male flowers with few bisexual flowers	30
6	**Gynomonoecious**	Female flowers with few bisexual flowers	5
7	**Trimonoecious**	Mixture of male, female and bisexual flowers	7

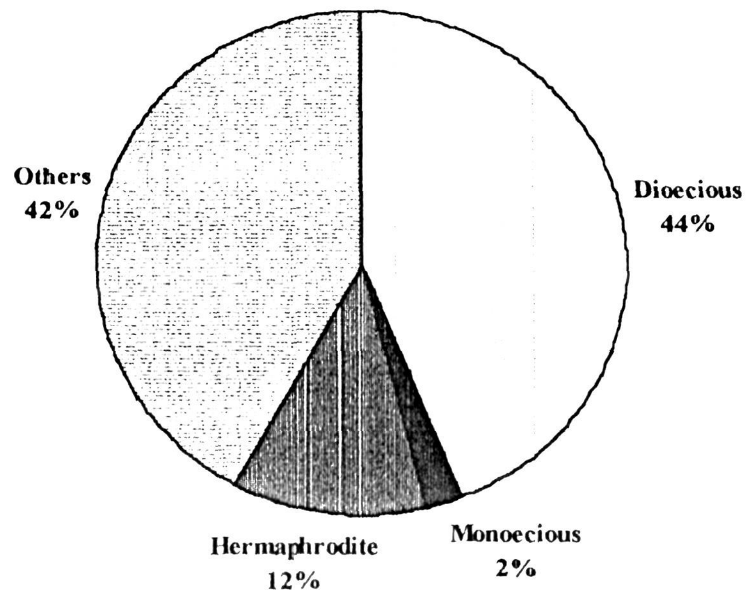

Fig.4.1 Broad categorization of sexual systems in *Nothapodytes nimmoniana*

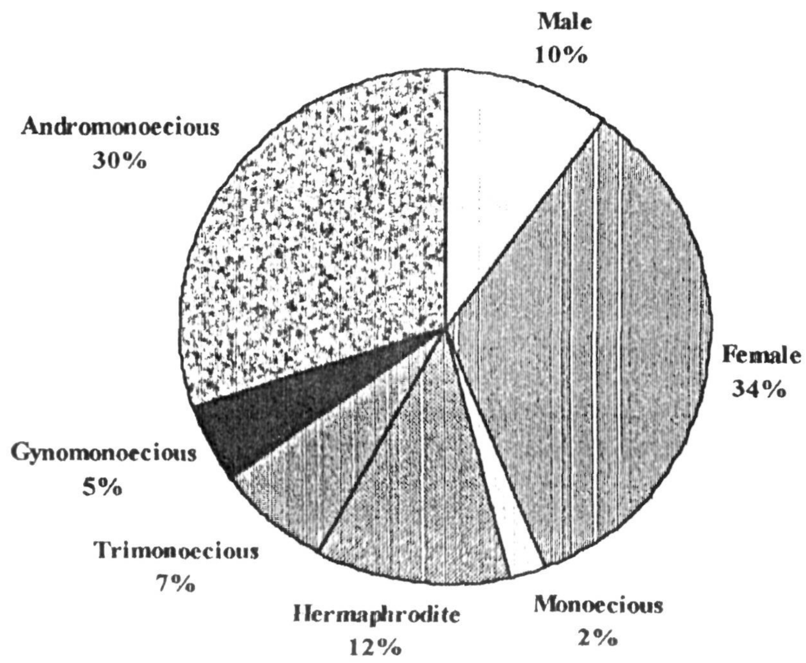

Fig.4.2 Sexual systems and their frequency in *N. nimmoniana*

4.1.2 Sex ratio at population

When a plant species exhibits a wide array of sexual systems individual trees can differentially contribute to male and female function. Hence it is desirable to estimate the sex ratio at population level. By considering the predominant gender exhibited by a tree, it was categorized as male and/or female as shown in the Table 4.2. All bisexual individuals were counted as both male as well as females. Interestingly, 36 individuals were functionally males and same number of individuals contributed to female function, thus the sex ratio at the population level could be considered as 1:1.

4.1.3 Size of the plant and expression of sex

In order to test the effect of plant size on the expression of sex, the bloomed individuals were categorized into two classes as small and large based on their size. The frequency of individuals belonging to predominant gender was computed in each class. As shown in the Table 4.3, there was a tendency among smaller individuals to be more often males than other sex. A higher proportion of larger individuals tends to be females (Fig 4.3). However the chi-square test did not show significant deviation from random distribution.

4.1.4 Floral description

Male flowers

Male flowers, in whichever breeding system they occur, were large with an average length of 9.2 mm and 9.4mm diameter with yellowish hue and produced stronger foetid smell than other flower types (Table 4.4). The five united green sepals in male form a small toothed cushion at the base of flower. Petals are free, valvate, with acute tip and is much larger compared to female flowers. They possessed five antisepalous stamens that were 6.8mm in length, exserted,

Table 4.2 Number of plants contributing to male and female function at population level in *N. nimmoniana*.

Sl. No.	Sexual systems	No. of Plants contributing to	
		Male function	Female function
1	**Male**	6	0
2	**Female**	0	21
3	**Monoecious**	7	7
4	**Hermaphrodite**	1	1
5	**Andromonoecious**	18	0
6	**Gynomonoecious**	0	3
7	**Trimonoecious**	4	4
	Total	36	36

* grand total exceeds n=60 since a few individuals contribute to both the functions.

Table 4.3 Distribution of number of plants into classes of sex and relative size in *N. nimmoniana*.

Sl. No.	Size class	Functional /Predominant gender		
		Male	Female	Bisexual
1	**Small** **<15 cm dbh**	13	10	8
2	**Large** **≥15 cm dbh**	8	14	5

χ^2 test 2.67, NS, at 3 df, (Suggesting that the distribution is random).

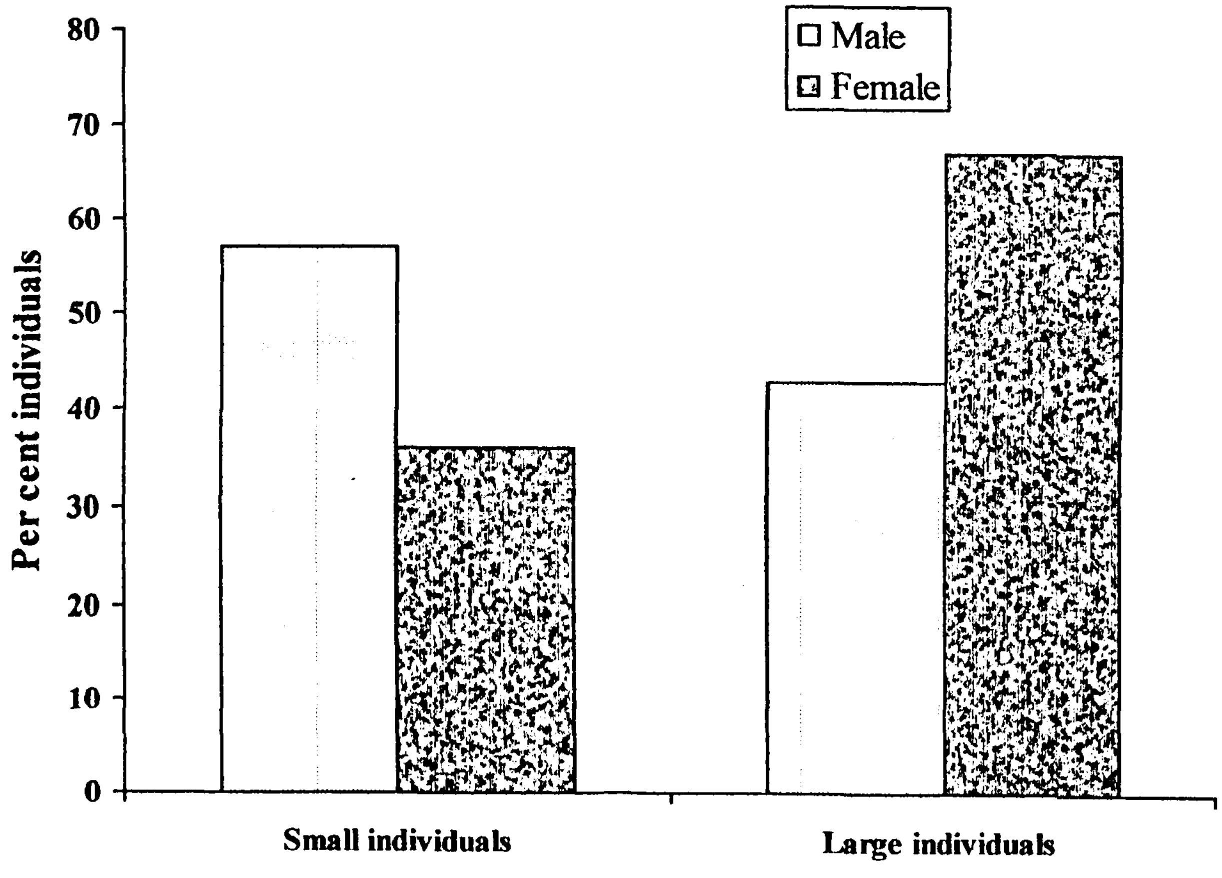

Fig. 4.3 Distribution of small (n=23) and large individuals (n=22) of *N. nimmoniana* into predominant gender.

Table 4.4 Variation in floral traits among male, female and bisexual flowers in *N. nimmoniana.*

Sl. No.	Character	Male flower Mean ± S.D.	Female flower Mean ± S.D.	Bisexual flower Mean ± S.D
1	Flower diameter (mm)	9.4 ± 0.05	6.90 ± 0.02	7.2 ± 0.02
2	Flower length (mm)	5.2 ± 0.31	3.52 ± 0.10	3.5 ± 0.15
3	Petals length (mm)	7.9 ±0.02	5.60 ± 0.20	6.5 ± 0.16
4	Petals width (mm)	2.8 ± 0.24	2.00 ± 0.13	2.4 ± 0.20
5	Length of stamen (mm)	6.8 ± 0.24	2.04 ± 0.07	3.0 ± 0.13
6	Length of pistil (mm)	3.5 ± 0.31	5.70 ± 0.40	5.0 ± 0.25
7	Petals	Five	Five	Four or Five
8	No. of functional Anthers	All	None	3-4
9	Stigma	Rudimentary	Active	Active

dorsifixed and introrse. All five anthers were functional, bithecous, dehiscing by longitudinal slits and produced copious amount of pollen. It also possessed a rudimentary pistil with a normal sized ovary and a short style not protruding outside. Male flowers dehisced during early morning hours (0600-0700 hrs) and maximum pollen production was observed at noon (1200-1400 hrs). Male plant bears about 230-350 flowers in corymbose cyme type inflorescence.

Female flowers

Female flowers were smaller than males with 6.2mm in length, 6.9mm in diameter and dull yellowish in color. The sepals are much larger than in males forming a toothed cup reaching up to half the length of the petals. Petals are short and narrower than in males. They possessed larger prominent pistils (5.7mm in length) with an exserted style and spatulate stigma. Stamens are rudimentary and non-functional (2.04mm in length). Anthesis of female occurred during early morning hours *i.e* between 0600 and 0800h. Female flowers produced detectable quantity of nectar than other flower types. Longevity of female flowers varied from two to eight days based on whether or not they are pollinated. Female plant bears about 70-185 flowers per inflorescence.

Bisexual flowers

Bisexual flowers were intermediate to male and female flowers in their size with 7.4mm in length, 7.2mm in diameter and were dull yellow in color. Both stamens and pistils were medium in size (3mm and 5mm respectively). Only three or four anthers out five, produced pollen grains and stigma was found to be functional. Bisexual plants bears about 125-160 flowers per inflorescence.

4.1.5 Phenology

Leaf sprouting and development:

The leaf initiation in *Nothapodytes nimmoniana* starts with the emergence of leaf buds during the second week of June. Male trees started sprouting a bit earlier followed by female and bisexual flowering individuals. Leaf sprouting was completed by the last week of July. The new leaves were light-green which later turns to dark-green by the end of July. During last week of December, leaves turn yellowish and were shed.

Flowering and anthesis

The phenogram of various events is shown in Fig 4.4. Generally male individuals were early to initiate buds, to initiate flowering and peak flowering compared to females and bisexual types. However, the duration of flowering and peak flowering were little longer among male individuals than in female and bisexual individuals. The average duration of flowering and peak flowering in males was 32 and 26 days respectively, whereas it was 24 and 19 days, respectively for females and 25 days and 21 days for bisexual flowering individuals. The phenogram shown in the Fig 4.4 suggests that male individuals started blooming during second week of July followed that of females during third week of July and bisexual during first week of August. The total period of blooming was 62 days for all the individuals in the population.

In monoecious trees, male flowers tend to initiate buds early, flower early than female flowers and flowers for a duration (28 days) and peak flowering duration (21days). No attempt was made to track the flowering phenologies of male and female flowers separately in andromonoecious, gynomonoecious and trimonoecious trees. These individuals tend to initiate flower and flowers over a

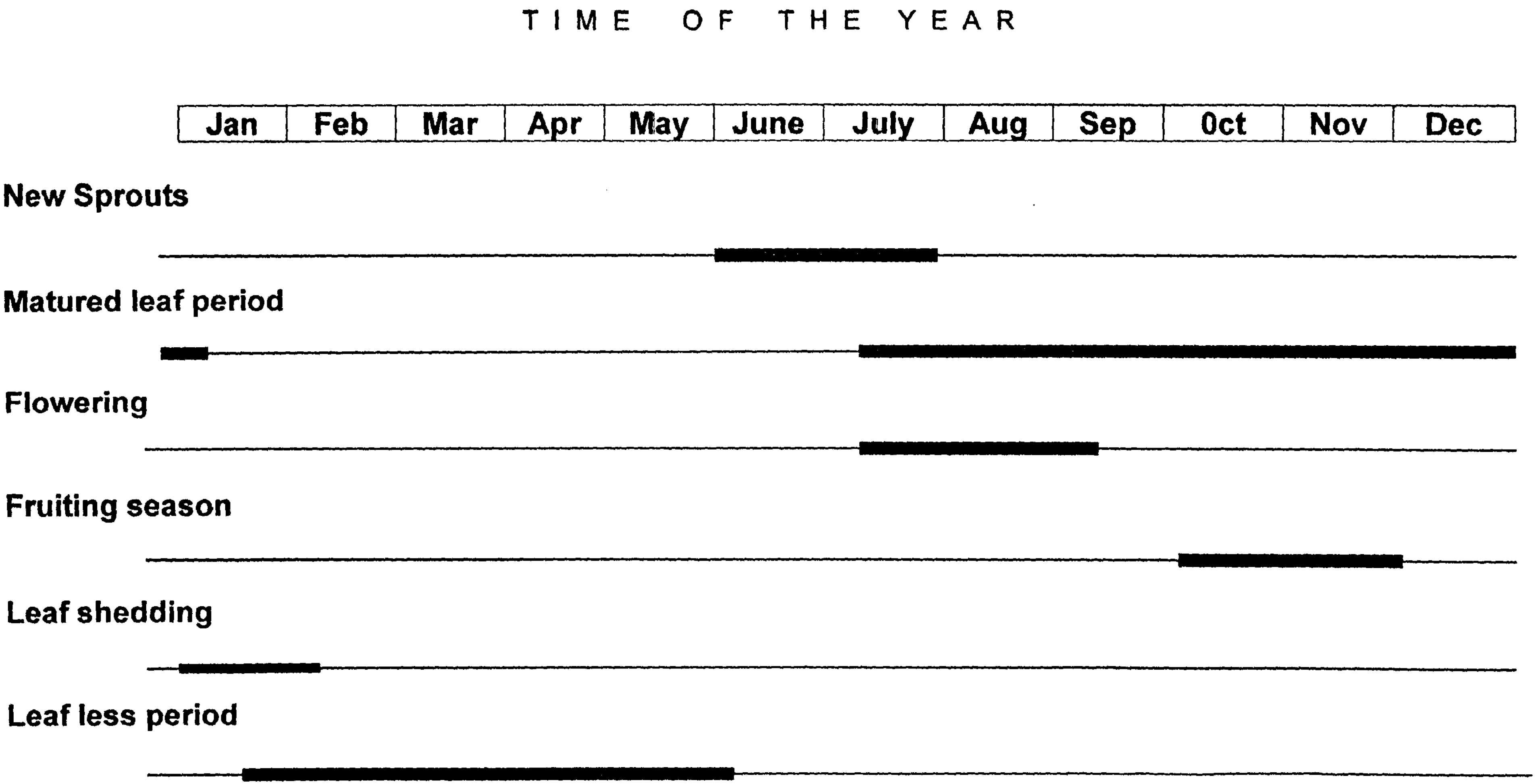

Figure 4.4 Phenogram showing various phenophases of *N. nimmoniana* in Sirsi forest area .

medium duration (28 days) with peak flowering duration (25 days). The anthesis percentage ranges from, 4.11 to 12.5 per cent of the total flowers marked was observed with in a single day.

Leaf and fruit drop

Fruits start maturing from first week of October and fruit maturing ends during last week of November. Birds like Red-Whiskered Bulbul, Red-Vented Bulbul and Pigeon were the major dispersers of the fruits. Leaves turned yellowish brown during December to January and leaf drop started from the second of January to third week of February. In March, April and May the tree remains leafless completely.

4.1.6 Mode of pollination

Bagging the unopened inflorescence suggested that *N. nimmoniana* is a self-incompatible and an obligate out-crosses. Observation on pollinators in bloomed inflorescence indicated that pollination is mainly fecilitated by insects. During flowering, the inflorescence emits a strong awful odour and produces little quantity nectar that attracts several species of insects predominantly flies.

The insect visitors included bees, wasps, ants, flies, butterflies and bugs (Plate 2). Generally male individual/inflorescence attracted more insect visitors than female during morning hours; however during evening hours, the insect visitation was higher on a female inflorescence. Flies were predominant pollinating agents, which includes 12 species belonging to different families, such as Calliphoridae (Blue Bot Flies), Tabanidae (Horse Flies) and 10 unidentified species. Butterflies were the second predominant pollinating agents, which included 7 species belonging to different families, such as Pieridae (Yellows), Hesperiidae (Skippers), Danaidae (Milk Weed Butterfly), Lycacnidae (Blues or Coppers two spp), Nymphalidae (Brush Footed Butterfly) and Satyridae.

Six species of wasps belonging to different families such as Vespidae (*Vespa cincta*), Apidae (*Apis cerana indica*), Anthophoridae (Carpenter bee) and three unidentified species were recorded on inflorescence.

Three species of ants belongs to different families such as Formicidae and two identified species were also recorded on the inflorescence. Two species of bugs visiting the flower were recorded and these are predators or phytophagous insects and also it includes one Diptera spp. (Tabanidae).

4.1.8 Pollen biology

Male flower and bisexual flowers produced abundant quantity of creamy white colored pollen grains during morning hours (0600 to 1100 hrs), when anthesis occurred. Diameter of pollen grains ranged from 58-72 micrometer. An *in vitro* pollen grain germination medium was standardized, which contained 15 per cent sucrose (Table 4.5).

4.1.7 Influence of assisted pollination on fruit set

Assisted pollination of female flowers with freshly collected pollen grains increased fruit set to a greater extent (about 16 times more; Plate 3). Among open pollinated inflorescence, the fruit set per cent was 4.9 per cent while the same in the inflorescence supplemented with pollen grains resulted about 79.5 per cent fruit set (Fig. 4.5).

4.1.9 Variation in fruit set among different sexual systems

As shown in the Fig 4.6, per cent fruit set, significantly varied among the different systems. The highest fruit set (15.69 per cent) was observed among

Table 4.5 Composition of standardized medium for *in-vitro* pollen grain germination in *N. nimmoniana*

Sl. No.	Components	Concentration
1	Sucrose	15.0%
2	Agar-agar	0.5%
3	Calcium nitrate	300 mg/lit
4	Magnesium sulphate	150 mg/lit
5	Potassium sulphate	200 mg/lit
6	Boric acid	200 mg/lit

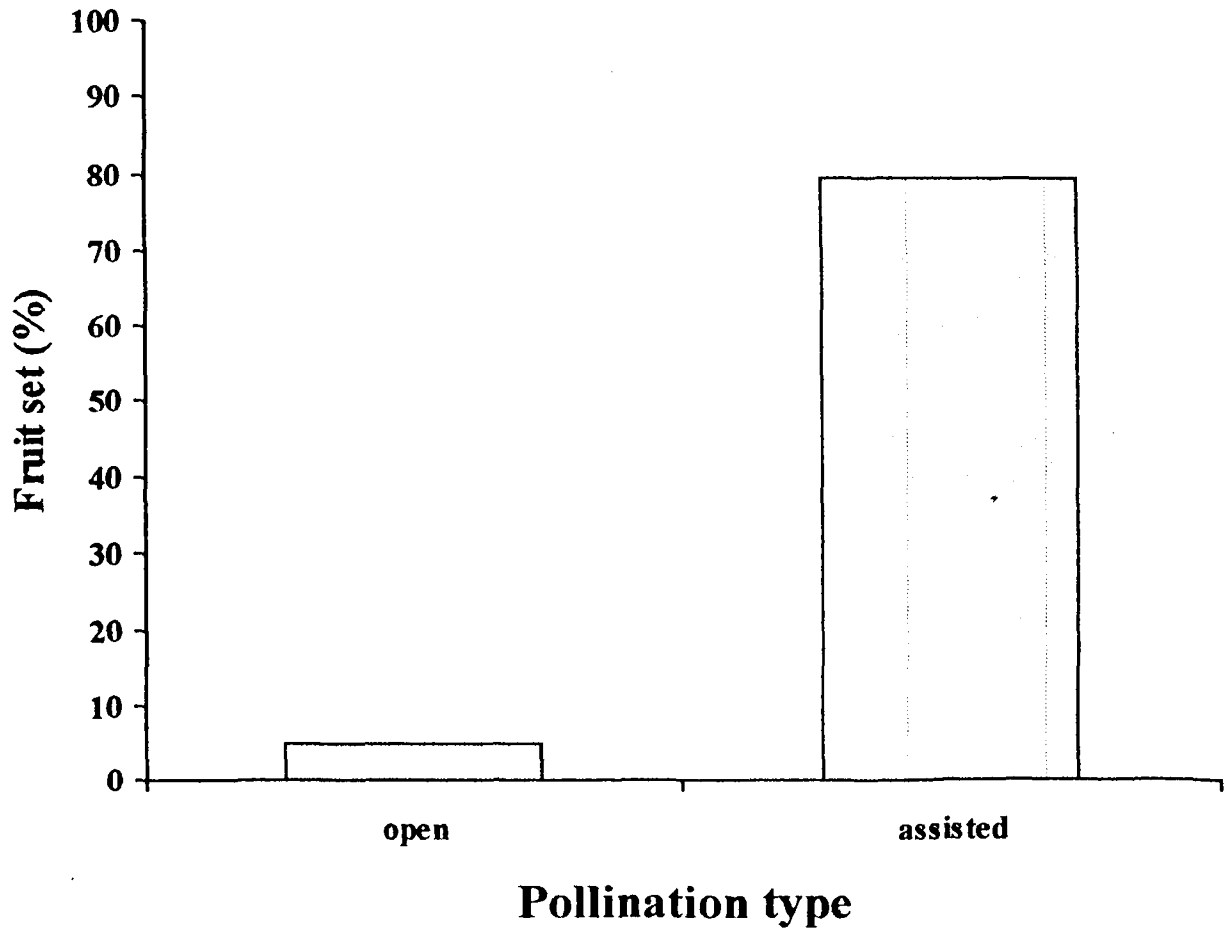

Fig.4.5 Effect of assisted pollination on fruit set (%) in *N. nimmoniana*.
(One way ANOVA, F Value= 739.6; P level <0.001 l)

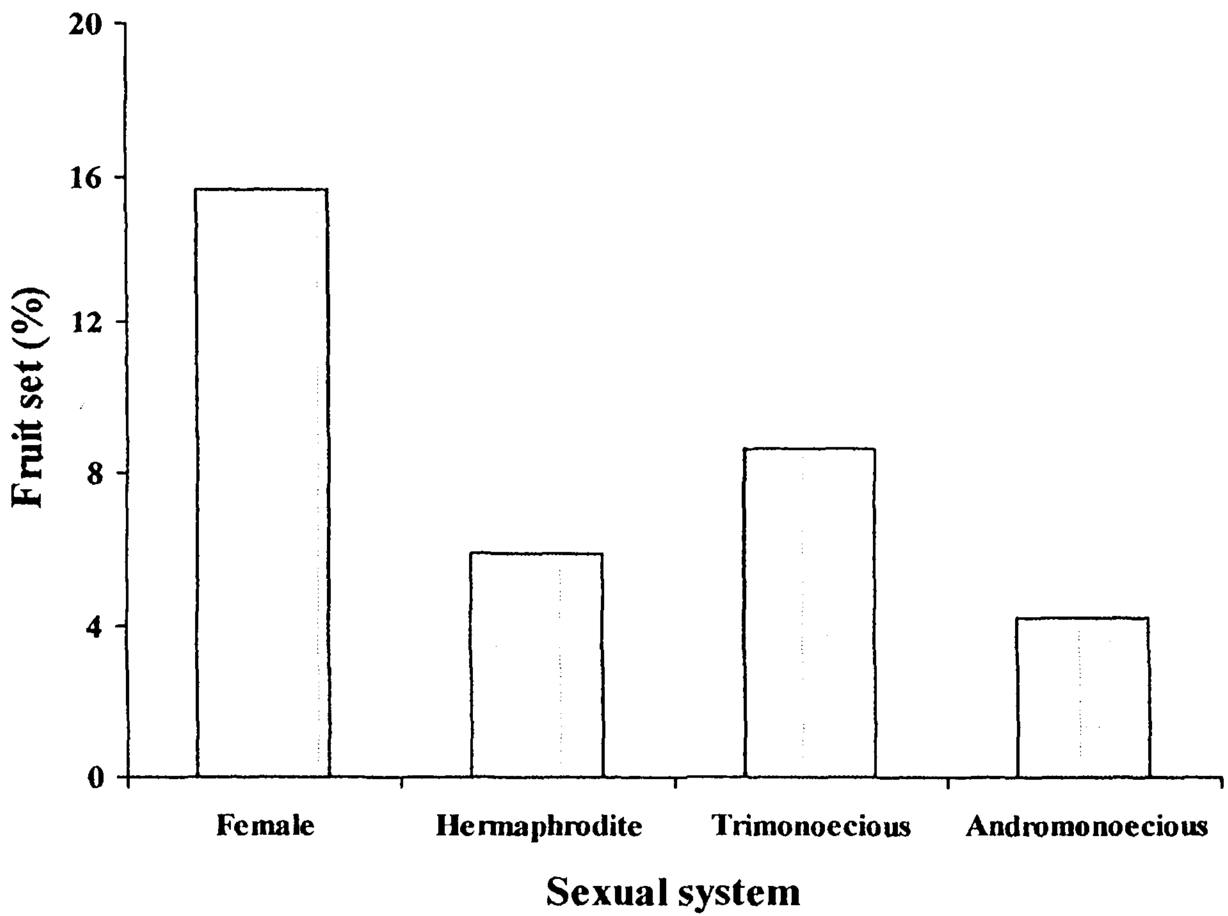

Fig.4.6 Fruit set (%) in different sexual systems of *N. nimmoniana.*
(One way ANOVA, F Value= 6.08; P level <0.001 l)

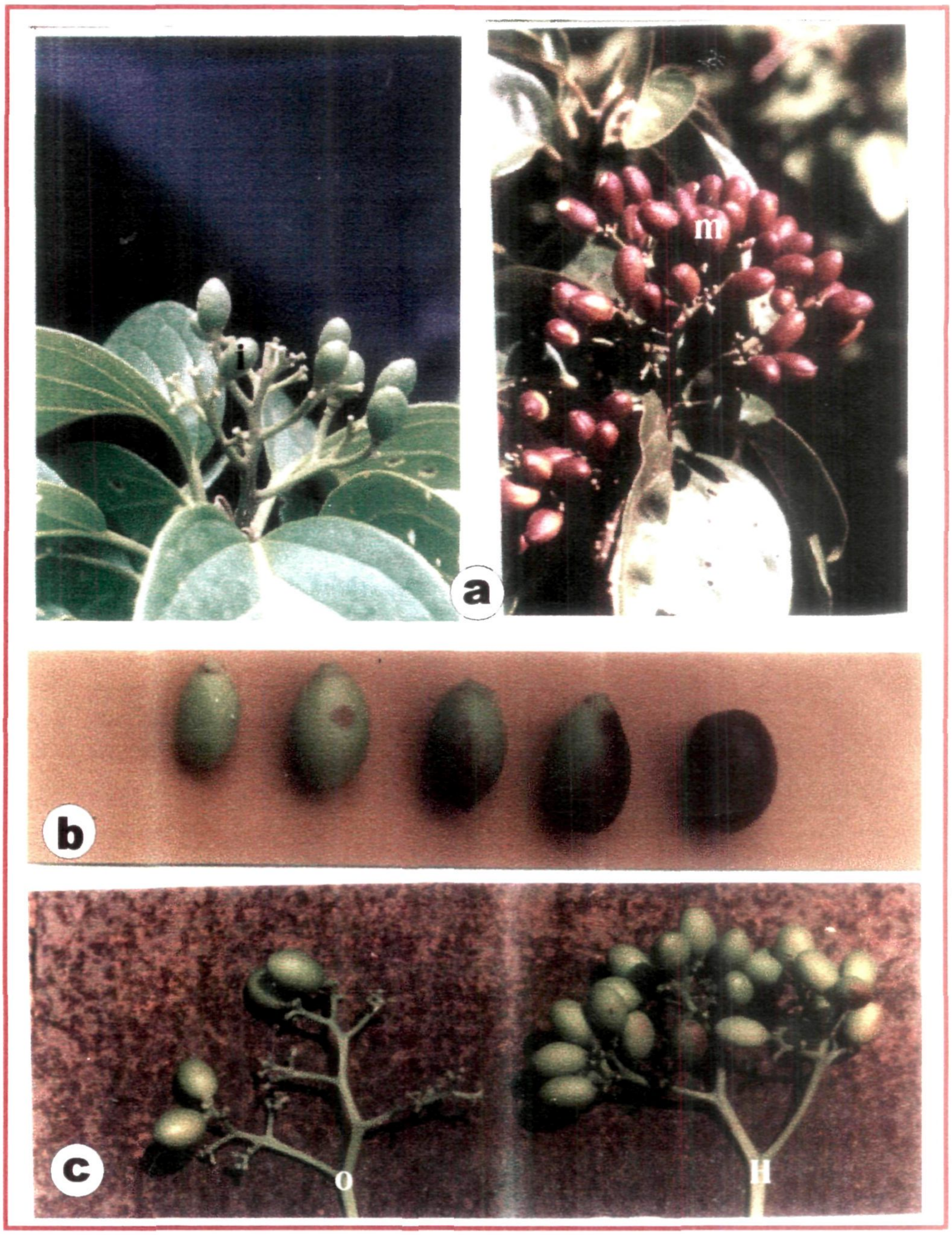

Plate 3

a. Immature (i) and mature(m) fruits of *N. nimmoniana*
b. Different stages of fruits maturation
c. Fruit set in open pollinated (o) and assisted pollinated (h) inflorescence

fruits of female plants. The least of 4.28 per cent was observed among andromonoecious plants.

4.2 **Seed Biology**

4.2.1 Test weight

Fruit of *Nothapodytes nimmoniana* is a single seeded drupe and it has edible pulp resembling that of *Syzygium cumini.* The seed has a seed coat, which is about 1 to 2mm in thickness. The fruit contains soft bluish pulp, which is rich in nutrients, hence many birds are attracted during fruiting season. Test weight (1000 seed weight) was around 242.5 g. The average seed weight with a range of 151 mg to 590 mg. One kg of *N. nimmoniana seeds* contains about 4124 seeds.

4.2.2 Seed length and breadth

Significant difference was observed with respect to seed length and seed breadth among the seeds derived from different sexual system. Seeds derived from andromonoecious system showed wide variation with respect to seed length and seed width. In general at population level seed length and seed width was 1.28 cm and 0.77 cm respectively.

4.2.3 Seed density

Seed density of *N. nimmoniana,* which is calculated by the method of volume basis, was about 0.843.

4.2.4 Effect of different pre-sowing treatment on germination and other seedling parameters

The results of standardization of pre sowing treatment in *N. nimmoniana* are presented in the Table 4.6 and 4.7. Although per cent germination did not statistically vary with the pre-treatment, the highest per cent germination was

Table 4.6 Effect of pre-treatments on seed germination in *N. nimmoniana* at the end of study period (80 days after sowing)

Treatments			Germination (%) Mean ± S.D.	Rate of germination Mean ± S.D
Control	With seed coat	T_1	89 ± 8.87	0.403 ± 0.050
	Without seed coat	T_2	94 ± 2.31	0.733 ± 0.048
Cold water 12 hrs	With seed coat	T_3	87 ± 8.25	0.337 ± 0.061
	Without seed coat	T_4	95 ± 6.00	0.627 ± 0.061
GA-50ppm	With seed coat	T_5	92 ± 7.30	0.650 ± 0.079
	Without seed coat	T_6	85 ±2.00	0.691 ± 0.040

One way ANOVA , F. ratio = 1.55, NS Co-efficient of variation = 7.08 %

Table 4.7 Effect of pre-sowing treatments on seedling parameters in *N. nimmoniana*.

Treatments			No of Leaves Mean±S.D	Collar Diameter (cm) Mean±S.D	Root length (cm) Mean±S.D	Shoot length (cm) Mean±S.D	Root dry weight (g) Mean±S.D	Shoot dry weight (g) Mean±S.D
Control	With seed coat	T_1	3.4±0.3	0.31±0.013	13.9±0.84	9.18±1.10	0.056±0.011	0.170±0.036
	With out seed coat	T_2	4.1±0.41	0.309±0.026	18.6±1.78	9.83±0.89	0.076±0.004	0.24±0.012
Water	With seed coat	T_3	3.9±0.19	0.324±0.011	18.1±0.88	10.95±0.23	0.085±0.016	0.249±0.024
	With out seed coat	T_4	3.7±0.38	0.322±0.011	16.7±1.0	10.08±0.95	0.085±0.006	0.226±0.017
GA_3-50ppm	With seed coat	T_5	3.9±0.2	0.313±0.007	19.5±2.9	9.19±0.59	0.081±0.028	0.258±0.036
	With out seed coat	T_6	4.4±0.54	0.289±0.009	14.1±1.7	8.93±0.33	0.059±0.003	0.212±0.019
F. test significance			*	NS	*	*	*	*
CD			**0.53**	---	**2.51**	**1.13**	**0.02**	**0.04**
SEM(±)			**0.18**	**0.01**	**0.85**	**0.38**	**0.01**	**0.01**
CV(%)			**9.28**	**4.88**	**10.86**	**7.83**	**19.08**	**10.95**

*** = significant at 0.05 P. level; NS= Non-Significant**

observed in T_4 (*i.e.* cold water treatment of seed coat removed seeds) and the least in T_6 (Gibberllic acid with seed coat removed seeds). However the pre-sowing treatment had an influence on the rate of germination. In general, in all the main treatments, removal of seed coat resulted in quicker germination of seed (Fig 4.7). Interestingly, a simple pre sowing treatment with removal of seed coat resulted in quicker germination as shown by highest value of rate of germination (0.733 day; Table 4.6). Seedlings derived from seed coat removal treatment also showed higher number of leaves per plant at 90 days after sowing (DAS), and possessed higher shoot biomass (Table 4.7). However this trend was not same for other growth parameters. For instance, collar diameter did not vary with the pre-sowing treatment. Longest root and highest root dry weight were observed among intact seeds treated with GA3 than those without seed coat. In general treatment of intact seeds with Gibberlic acid, rather than those in which seed coat removed, had beneficial effect on collar diameter, shoot length, root and shoot biomass.

4.2.5 Seed viability

In order to test the duration to which the seeds remain viable random samples of bulk seeds were sown at 15 day time interval. The data are shown in Table 4.8 and Fig 4.8. Clearly, seeds remained viable up to 150 days. Up to 45 days, the viability was excellent (near 100 per cent). In the third month, the viability reduced to 75 per cent and afterwards, it reduced drastically. While seeds, which were kept for 120 days, had 52 per cent and the end of five month the viability had crashed to 12 per cent.

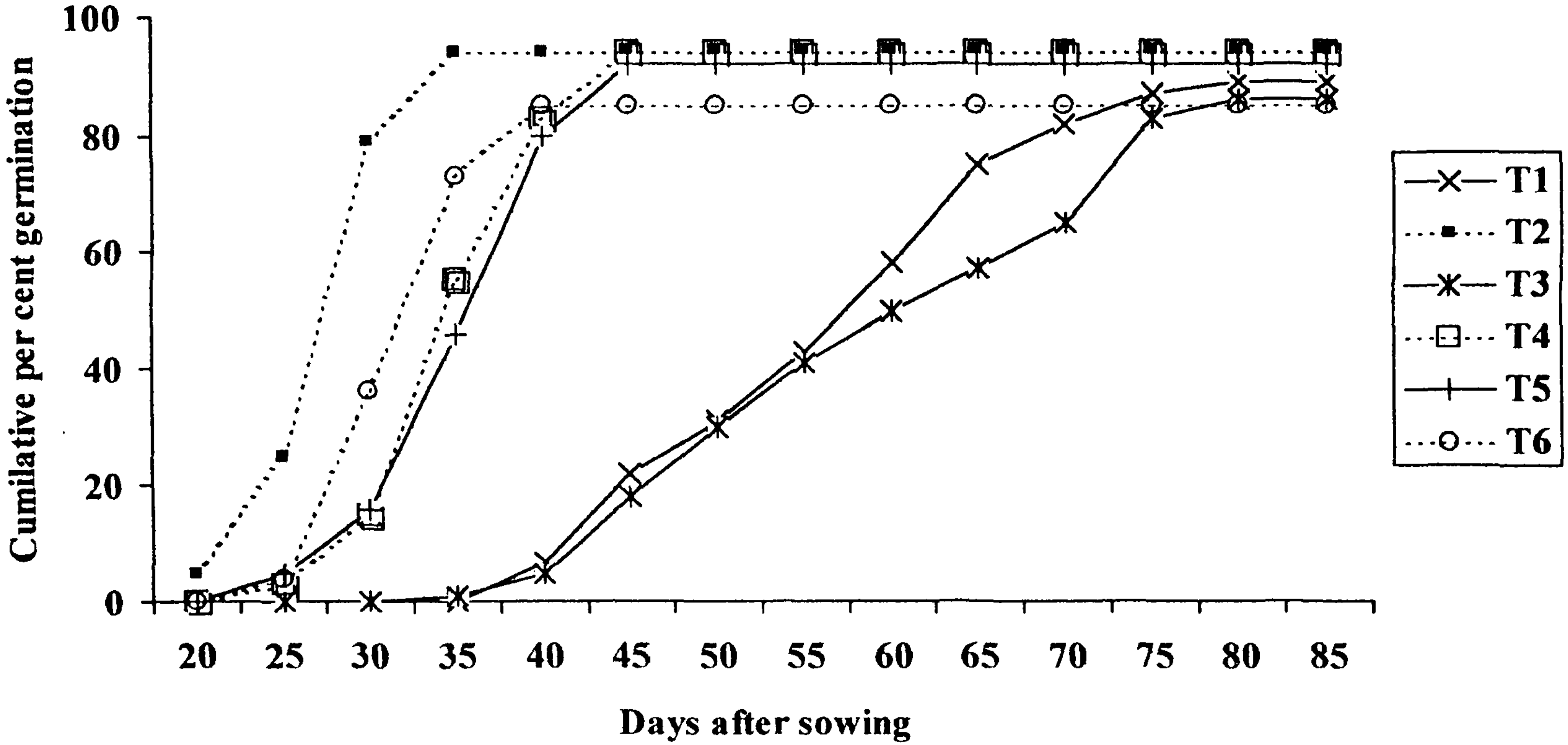

Fig.4.7 Effect of pre-sowing treatment on cumulative per cent seed germination in *N. nimmoniana*

T1= control; T2= seed coat removed; T3= cold water;

T4= cold water, seed coat removed; T5= GA 50 ppm; T6= GA 50 ppm, seed coat removed

Table 4.8 Seed viability as assessed through germination across storage period.

Days after fruit collection	Germination (%) Mean ± S.D.
0	88.00 ± 4.00 (69.90)
15	97.30 ± 2.30 (82.30)
30	98.60 ± 2.30 (86.20)
45	98.00 ± 2.00 (83.40)
60	89.30 ± 2.30 (71.00)
75	84.00 ± 6.00 (66.60)
90	73.30 ± 2.30 (58.90)
105	54.60 ± 3.05 (47.60)
120	52.00 ± 4.00 (46.10)
135	32.00 ± 1.15 (34.85)
150	12.00± 4.00 (20.09)
165	0 ± 0 (0)
F-value	137.88
P-level	<0.001
C.V	7.12
C.D	6.67

Numbers in the parentheses indicate the arc sine transformed values

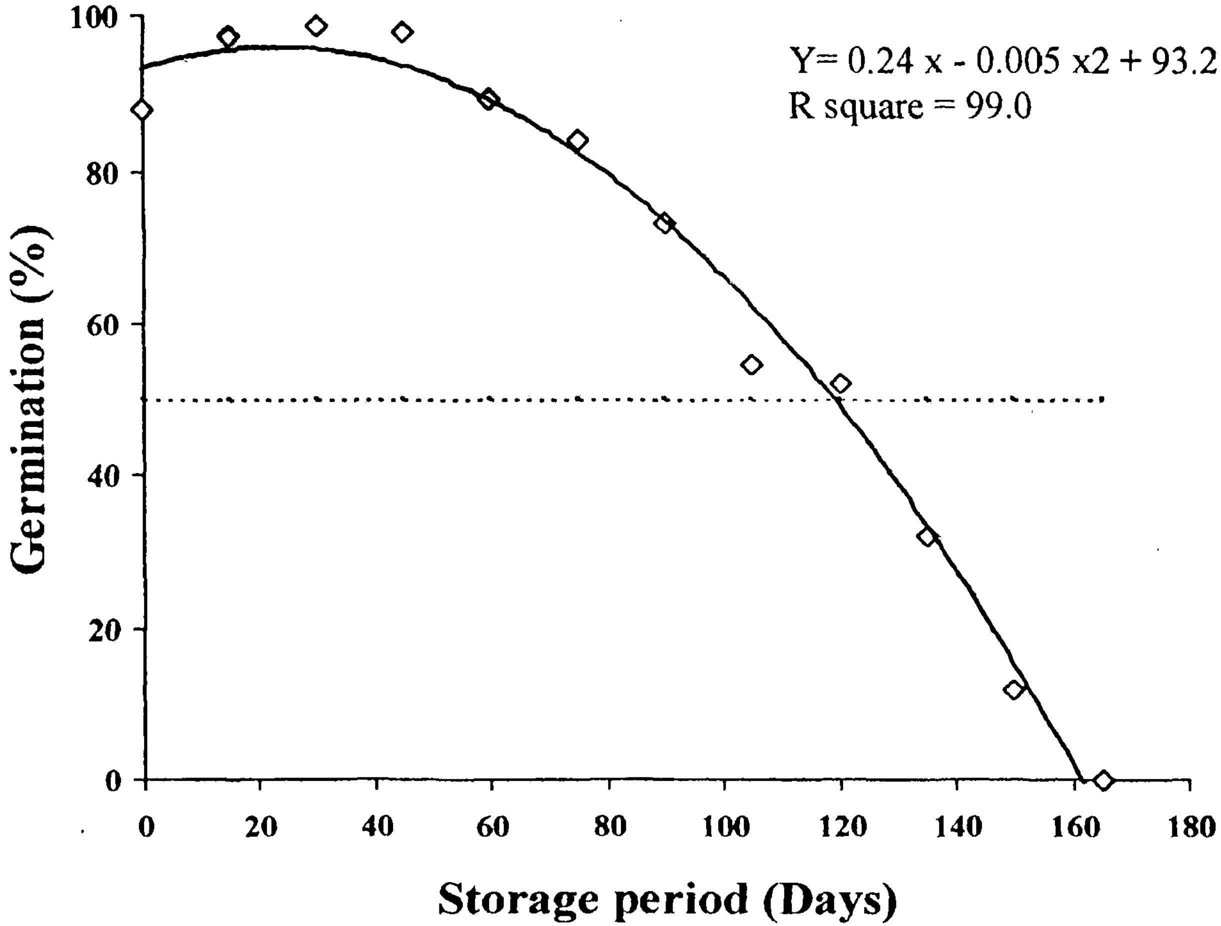

Fig.4.8 Seed viability (assessed through germination test) across storage period in *N. nimmoniana*. The dashed line represents 50 % germination. Trend line is the best fit of polynomial of degree two.

4.2.6 Effect of assisted pollination on seed parameters

Controlled pollination also influenced the fruit and seed parameters as well as germination parameters (Table 4.9). There was an increase in fruit weight, pulp weight, pulp to seed ratio and seed germination among fruits from assisted pollinated inflorescence when compared to those from open pollination. Thus difference were not statistically significant (Table 4.9 and Fig 4.9).

4.2.7 Effect of sexual systems on seed parameters

As shown in the Table 4.10 and Fig 4.10, fresh fruit weight, seed weight, pulp weight, pulp to seed ratio and per cent germination were significantly varied among the different sexual system. The highest fruit set (15.69 per cent) was observed among fruits of female plants. The least of 4.28 per cent was observed among andromonoecious plants.

Andromonoecious plants showed high fruit weight (1.22 g), high seed weight (0.413 g) and high pulp weight (0.806 g) compared to other breeding system. Whereas low fruit weight (0.832 g), low seed weight (0.274 g) and low pulp weight (0.558 g) were observed in trimonoecious plants (Fig 4.9). As shown in Fig 4.11 higher pulp to seed ratio and higher per cent germination were observed in trimonoecious and female sex individuals.

4.3 **Vegetative propagation**

4.3.1 Standardization of vegetative propagation technique through stem cuttings

The results of effect of different hormonal treatments on per cent rooted cuttings, per cent cuttings sprouted and other parameters in *N. nimmoniana* are presented in Table.4.11 (Plate 4).

One-way analysis of variance suggested that treatments significantly influenced per cent rooting, per cent sprouting and root length. However other

Table 4.9 Effect of assisted pollination on fruit and other seed traits in *N. nimmoniana*.

Treatment	Fruit weight (g) Mean±S.D	Seed weight (g) Mean±S.D	Pulp weight (g) Mean±S.D	Pulp/seed ratio Mean±S.D	Germination (%) Mean±S.D
Assisted pollination	0.951±0.115	0.336±0.03	0.615±0.13	1.82±0.32	86.2±10.73
Open pollination	0.946±0.090	0.353±0.03	0.593±0.09	1.70±0.36	94.0±8.24
F value	**0.004**	**0.873**	**0.899**	**0.348**	**1.68**
P level	**NS**	**NS**	**NS**	**NS**	**NS**
C.V	**13.04**	**8.65**	**18.6**	**19.33**	**10.63**

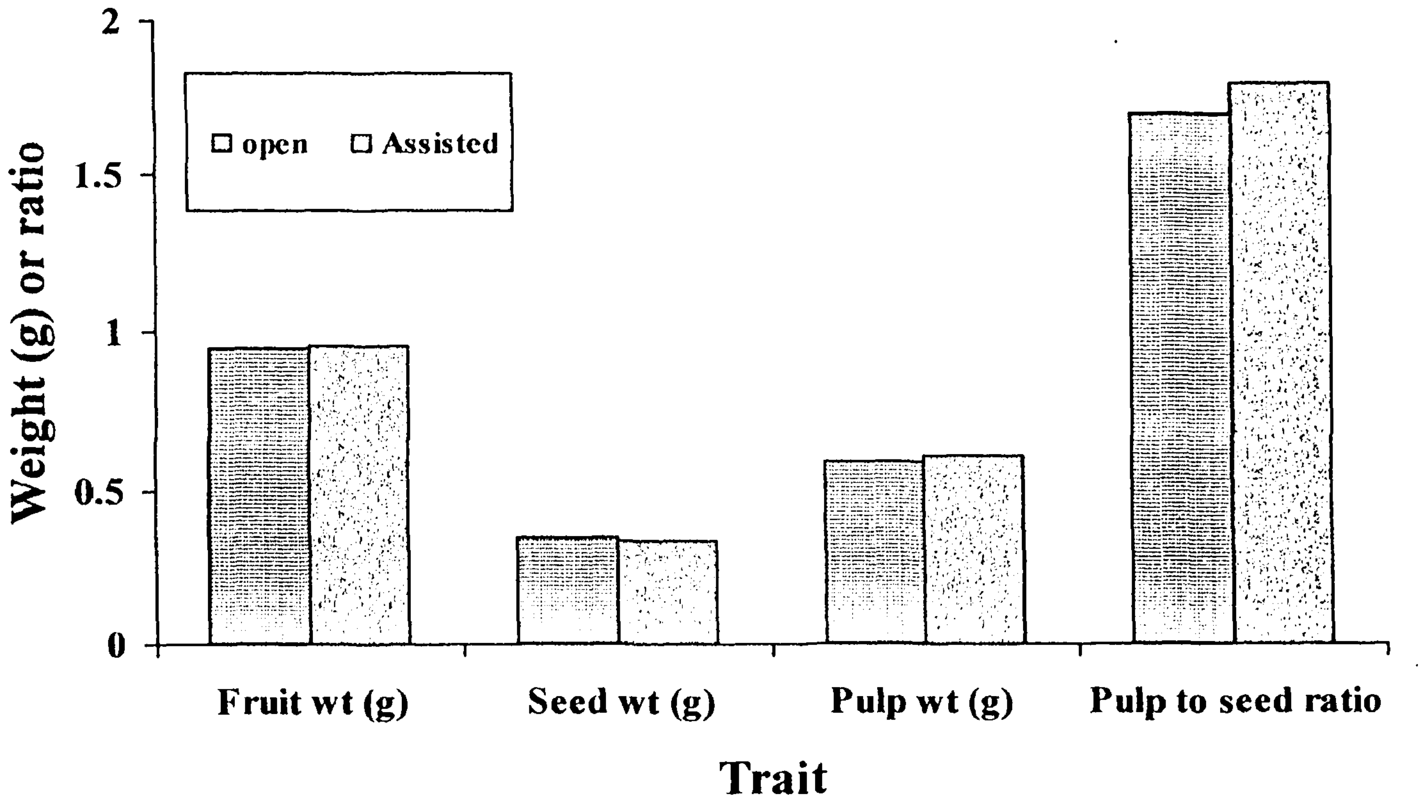

Fig.4.9 Effect of hand pollination on weight of fruits, seeds, pulp and pulp to seed weight ratio in *N. nimminiana*. (One way ANOVA, Non Significant)

Table 4.10 Influence of various sexual systems on fruit and other seed parameters in *N. nimmoniana.*

Sexual system	Fruit set (%) Mean ± S.D	Fresh fruit weight (g) Mean ± S.D	Seed weight (g) Mean ± S.D	Pulp weight (g) Mean ± S.D	Pulp/seed ratio Mean ± S.D	Germination (%) Mean ± S.D
Female	15.69±7.55	0.92±0.24	0.341±0.08	0.577±0.17	1.7±0.39	94±1.63
Hermaphrodite	5.96±4.34	0.98±0.14	0.333±0.06	0.664±0.11	2.03±0.42	73.8±7.36
Trimonoecious	8.65±6.67	0.832±0.14	0.274±0.05	0.558±0.16	2.11±0.67	62.8±3.77
Andromonoecious	4.28±5.3	1.22±0.27	0.413±0.08	0.806±0.26	2.03±0.78	78.5±4.12
F value	**6.08**	**15.5**	**16.3**	**10.66**	**4.35**	**30.53**
P level	**<0.01**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
C.V	**54.10**	**22.1**	**20.72**	**28.71**	**28.94**	**6.08**
C.D	**1.75**	**0.015**	**0.01**	**0.082**	**0.307**	**5.32**

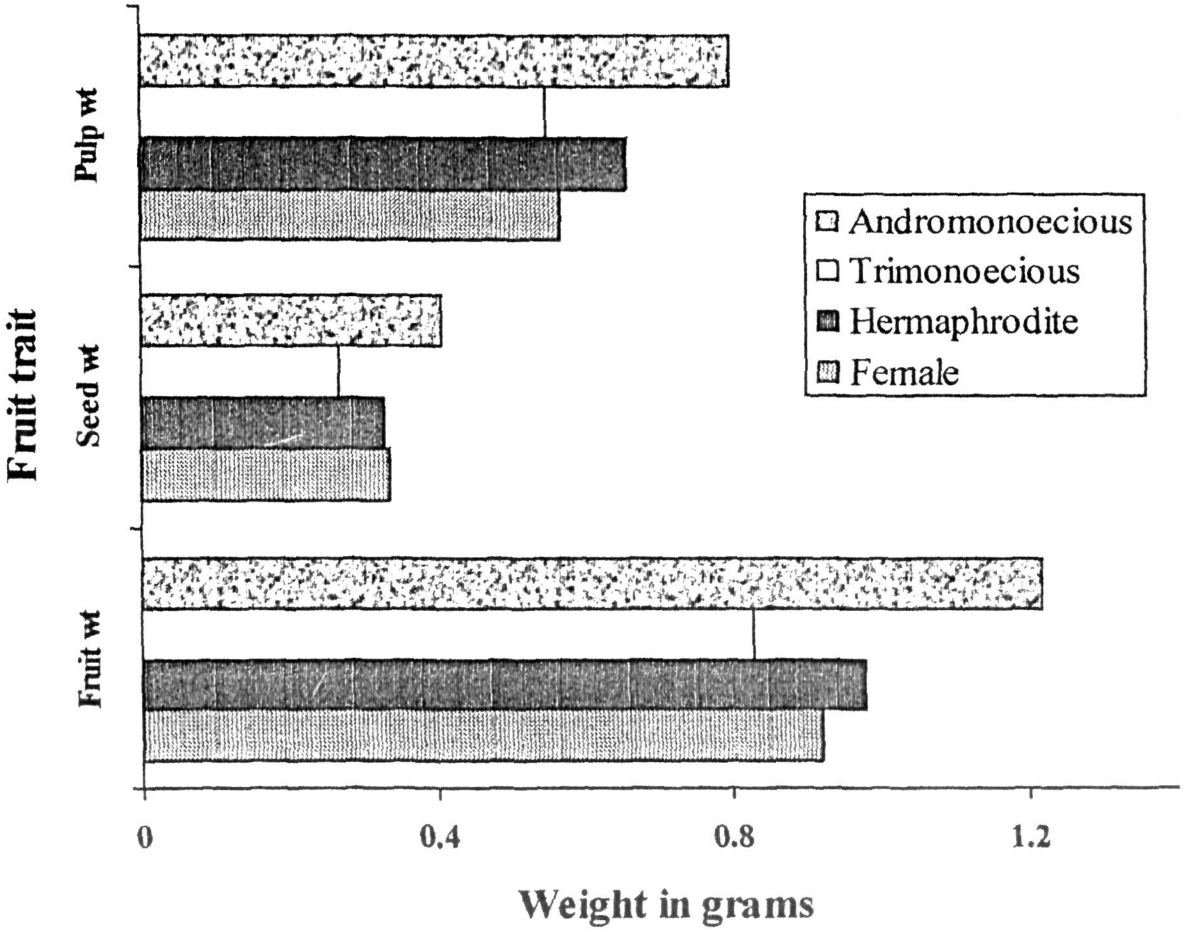

Fig.4.10 Weight of fruits, seeds and fruit pulp in different sexual systems of *N. nimmoniana*.

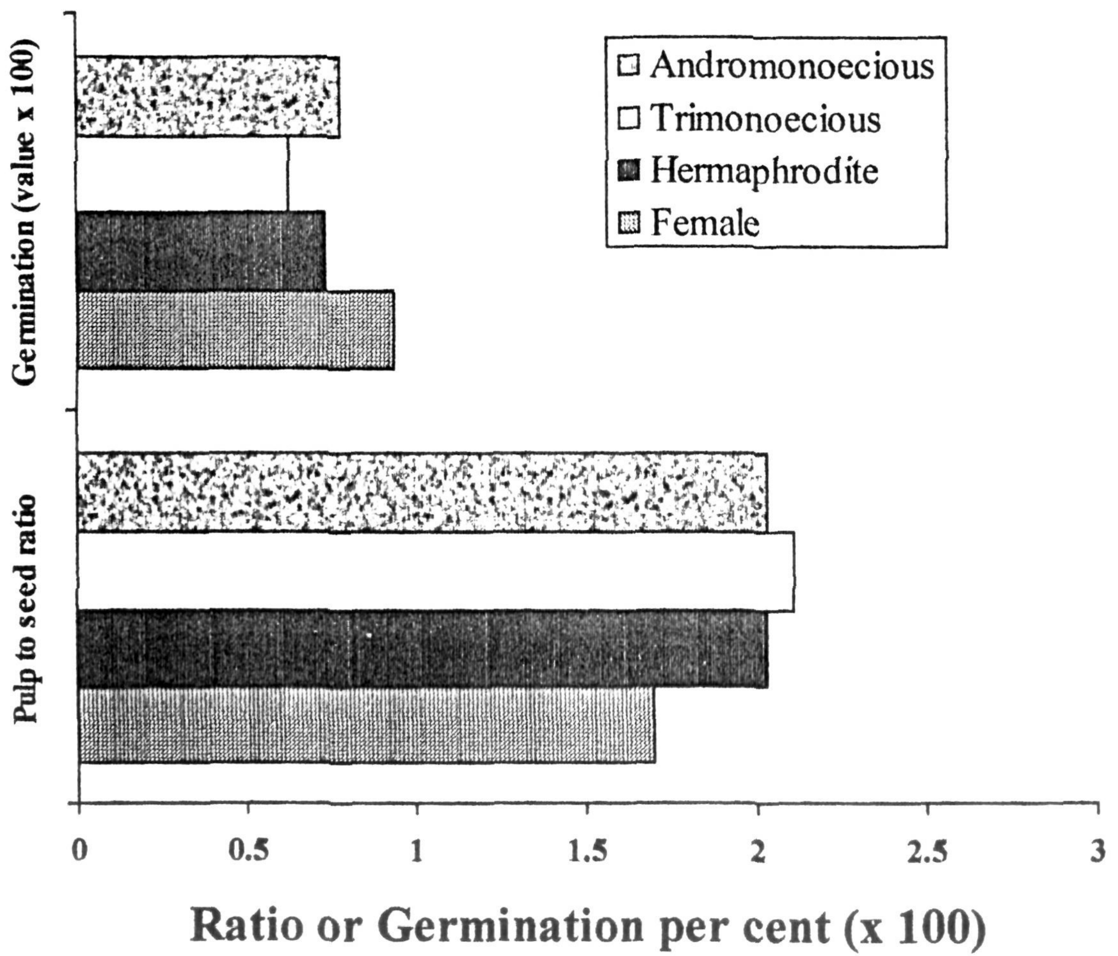

Fig.4.11 Pulp to seed weight ratio and seed germination (multiply value with 100) in different sexual systems of *N. nimmoniana*.

Table 4.11 Influence of different hormonal treatments on sprouting and rooting of stem cuttings in *N. nimmoniana*.

Growth hormonal Treatments (ppm)		Per cent cutting rooted	Per cent cutting sprouted	No. of sprouts/ cutting	No. of leaves/ cutting	Length of shoots (cm)	No. of roots	Length of roots (cm)
T_1	IAA 1000	7.50 (9.96)	30.0 (29.1)	1.06	1.70	1.43	0.63	0.63
T_2	IAA 2000	40.0 (38.9)	62.5 (54.4)	1.33	3.30	2.86	2.58	2.07
T_3	IAA 3000	7.50 (8.20)	40.0 (38. 9)	1.34	3.75	3.64	1.13	0.38
T_4	IBA 1000	20.0 (19.5)	42.5 (40.2)	2.14	3.05	2.00	2.83	1.14
T_5	IBA 2000	22.5 (24.7)	30.0 (32.9)	1.50	3.10	3.70	2.61	2.41
T_6	IBA 3000	17.5 (21.5)	17.5 (19.8)	0.94	2.13	2.10	2.93	1.51
T_7	COU 1000	32.5 (32.6)	25.0 (26.2)	1.13	3.40	2.58	4.33	2.08
T_8	COU 2000	57.5 (51.3)	65.0 (54.0)	1.64	3.58	2.73	4.48	2.81
T_9	COU 3000	22.5 (22.8)	27.5 (27.6)	1.46	2.67	2.34	3.38	1.73
T_{10}	IAA+IBA 1000+1000	30.0 (31.0)	42.5 (38.6)	1.23	2.96	2.68	2.50	1.76
T_{11}	IAA+IBA 2000+2000	20.0 (21.3)	27.5 (29.6)	1.20	3.40	4.06	2.50	1.76
T_{12}	IAA+IBA+COU 1000+1000+1000	15.0 (14.5)	27.5 (25.8)	1.20	2.74	2.20	1.40	0.49
T_{13}	Control	2.50 (3.30)	40.0 (37.1)	1.29	2.07	1.88	0.50	0.50
F. test significance		*	*	**NS**	**NS**	**NS**	**NS**	*
CD		**17.67**	**18.03**	---	---	---	---	**1.27**
SEM(±)		**325.32**	**338.65**	---	---	---	---	**2.25**
CV(%)		**78.22**	**52.67**	**56.34**	**84.04**	**83.89**	**122.61**	**108.68**

Numbers in the parentheses indicate the arc sine transformed values.

*** = significant at 0.05 P. level; NS= Non-Significant**

Plate 4

a. General view of *N. nimmoniana* seedlings in a seed bed

b. Stages of seed germination and seedling growth

c. Sprouting and rooting of stem cuttings treated with growth regulators parameters such as, number of sprouts, number of leaves, length of shoot, number of roots were not statistically significant.

The stem cuttings treated with Coumarin 2000 ppm (T_8) showed better response, with maximum per cent rooting, per cent sprouting, number of roots and length of roots. Maximum number of sprouts per cutting, roots, leaves and shoot length were observed in IBA 1000 ppm (T_4), IAA 3000ppm (T_3) and IAA+IBA 2000+2000 ppm (T_{12}) respectively. The control had the least per cent rooting (2.5 per cent), number of roots (0.5) and length of roots (0.5cms).

4.3.2 Effect of different hormonal concentration and form of application of hormone on rooting and other parameter in N. nimmoniana

The results of effect of different form of hormonal treatment on rooting and other rooting parameters in *N. nimmoniana* stem cuttings are presented in Fig 4.12 and 4.13. Considering the mean values, the two-way analysis of variance suggested that form of hormonal application had influence on various parameters. Different forms of hormonal application showed statistically significant in number of sprouts, numbers of leaves and shoot length parameters, although the others parameter did not significantly differ.

Dust form of application of hormones showed maximum number of sprouts (1.52 per cutting), number of leaves (3.6 per cutting), and shoot length (3.03 cm per cutting) than solution form of application. Interaction of level of hormone and form of application did not differ statistically.

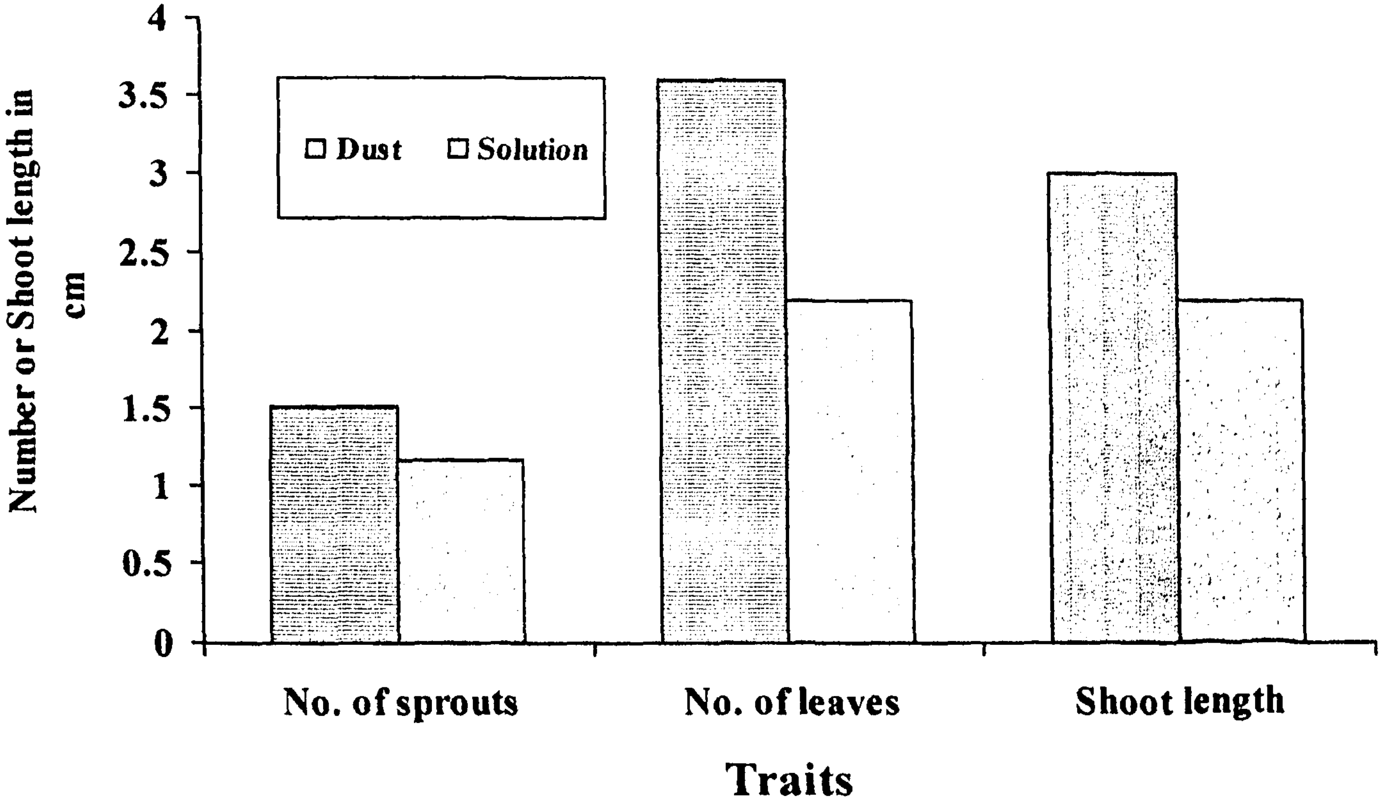

Fig.4.12 Effect of form of hormonal application on number of sprouts, leaves and shoot length in *N. nimminiana*.

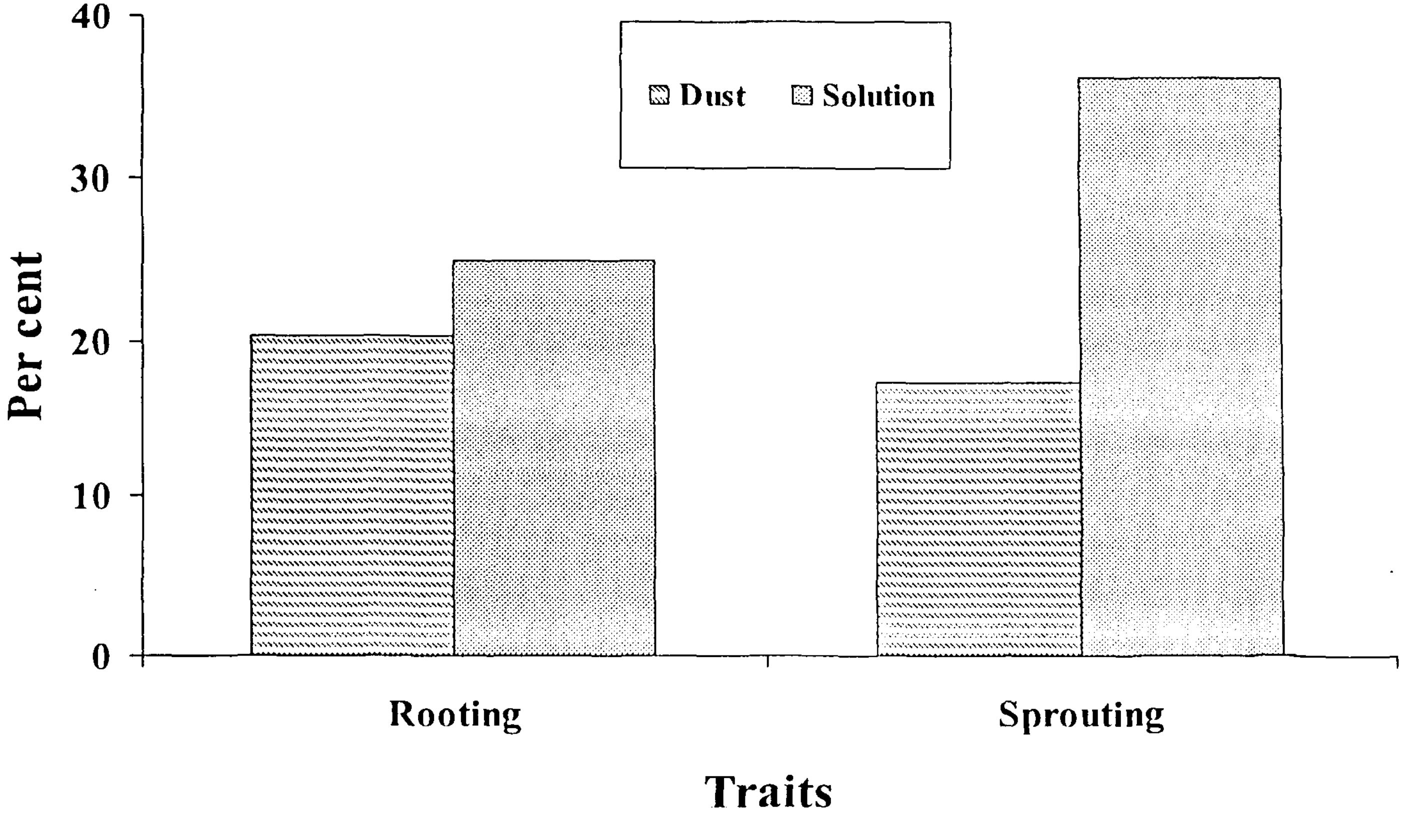

Fig.4.13 Effect of form of hormonal application on per cent rooting and sprouting in *N. nimminiana*.

4.3.3 Effect of different hormonal concentration and breeding system on rooting and other parameters in N. nimmoniana.

The results of effect of different breeding system, on rooting parameters in *N. nimmoniana* stem cuttings are presented in Fig. 4.14 and Fig 4.15. Interestingly, cuttings derived from male individuals showed maximum per cent rooting (27.69 per cent), number of roots (3.93) and root length (2.05 cm) followed by those from female and bisexual individuals. Least per cent rooting (13.08 per cent), number of roots (1.09) and root length (0.72 cm) were observed in bisexual individuals. Interaction of different sexual system and different hormonal level application was not statistically significant.

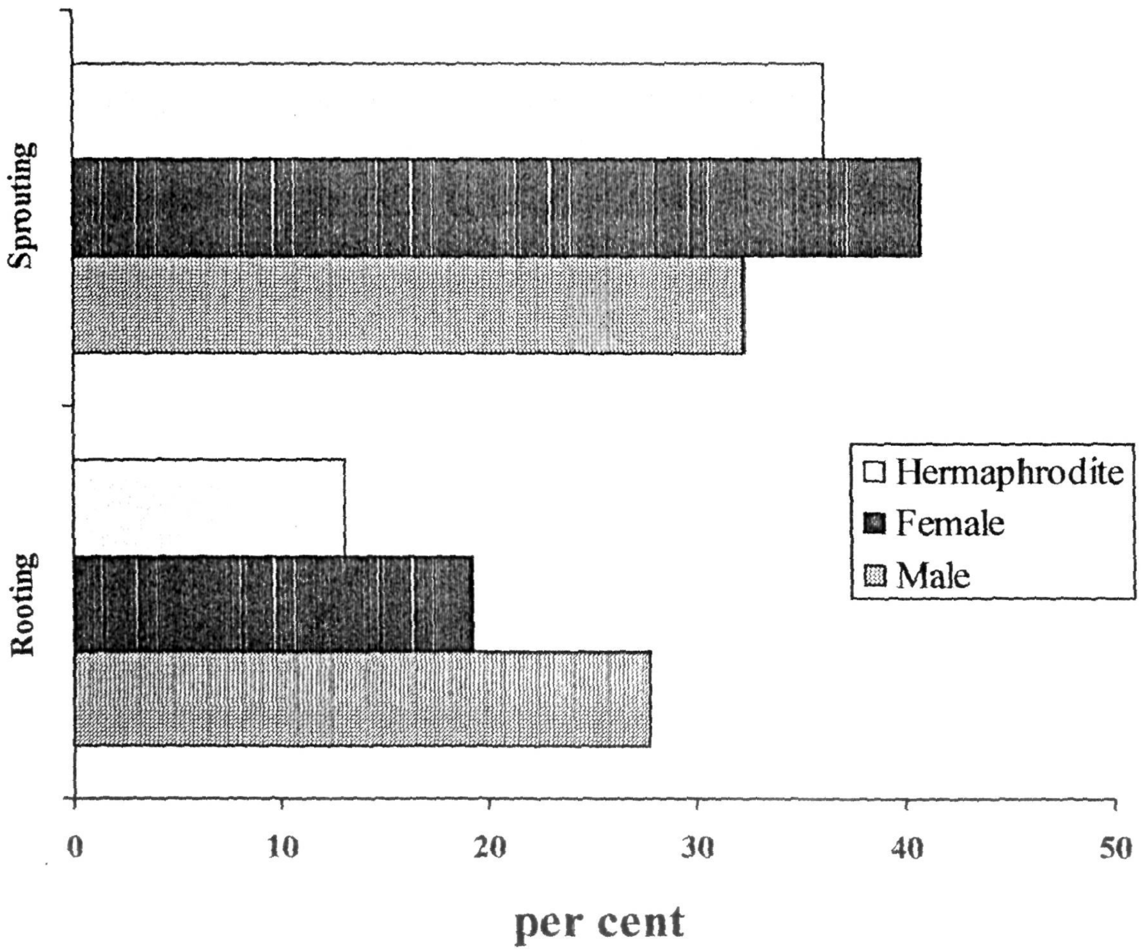

Fig.4.14 Rooting and sprouting of stem cuttings as influenced by different sexual systems in *N. nimmoniana*.

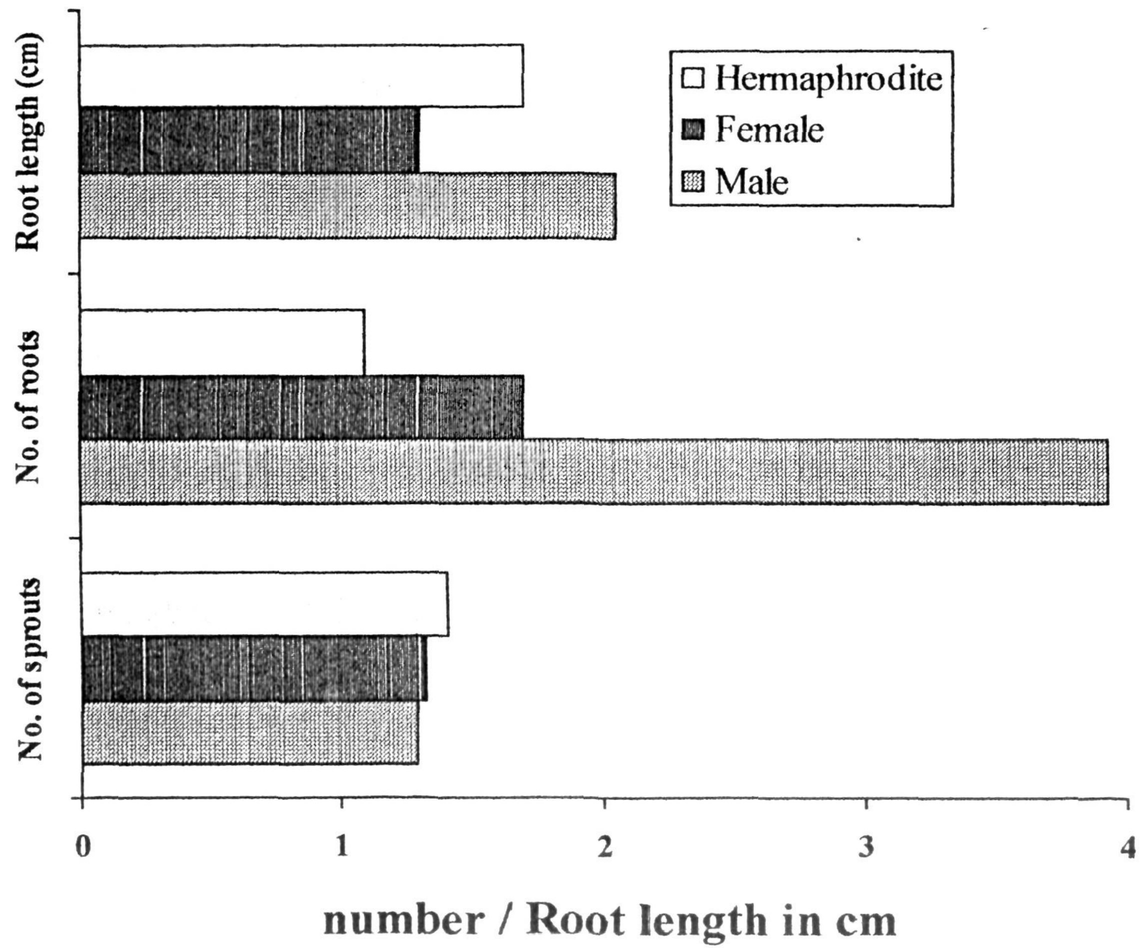

Fig.4.15 Number of sprouts, roots and length of roots (cm) of stem cuttings as influenced by different sexual systems in *N. nimmoniana.*

Discussion

V. DISCUSSION

Plant products and their modified analogues have been rich sources of clinically useful drugs, including anti-cancer agents. Prominent among them are the *Vinca* alkaloids, Camptothecin (CPT) and its derivatives (Wang HuiKang, 1997). Recent estimates on the incidence of cancer have indicated that about 10 million new cases of cancer and 6.5 million deaths annually occur due to cancer alone on a global scale (Vasantha Kumar and Asha Sankar, 2001). Hence there is a high demand for anti-cancer drugs such as CPT in the world market. Occurrence of CPT in *Nothapodytes nimmoniana* is a recent discovery (Govindachari and Viswanathan, 1972a). Earlier, CPT was extracted from a rare Chinese tree called *Camptotheca acuminata*. Currently, CPT is the third most important drug with an annual demand of about 15 kg. The CPT is present in all parts of the *N. nimmoniana*. Wood chips and the twigs are the chief raw material in local markets. A World Bank survey suggests that the twigs of this tree are available for rupees fifteen to twenty per kg, whereas the extract after processing is sold by multinational pharmaceutical companies for US$ 15000 per kg in the world market. It is reported that from Mumbai port alone, about 54 tonnes of dry wood chips of this tree were exported during 1994. This figure must have been increased several times in the recent years. Perhaps this has led to large-scale exploitation of *N. nimmoniana* from its wild habitat in the recent years despite the ban on green felling. The CPT is present in all parts hence, essentially the entire tree is chopped in the wild leading to their death upon harvesting.

Ved (1997) estimated that 20 per cent of the population of this species is believed to have declined over the last decade. Recently it has been assigned the threat status of "*vulnerable*" by Foundation for Revitalization of Local Health

Tradition (Anon, 1997). Gowda et al. (1997), Swaminath and Dasappa (2000) have also reported that populations of *N.* nimmoniana in natural forests has substantially decreased and also reported on its poor regeneration in wild. There are several reports carried in leading newspapers and journals regarding the increased threat to this species due to the un-sustainable harvest from it's natural habitats. According to Ved (1997) if same trend of exploitation continues in future years, it might seriously affect the existence of the species.

A complete knowledge of reproductive biology and breeding system is a prerequisite for developing an appropriate conservation strategy for any species. Till date, no studies are reported on *N. nimmoniana* focusing on the reproductive biology and propagation. Further, detailed data on biology and autecology of this rare species are also important for its genetic improvement and domestication. Hence the study was aimed at following aspects:

- to understand the autoecological details of *Nothapodytes nimmoniana*, such as reproductive biology and seed biology.
- to standardize a vegetative propagation technique.

The results of the investigation are discussed in this chapter. The implications of the results to the genetic improvement of *N. nimmoniana* are also discussed under a separate sub heading.

5.1 Breeding system in *Nothapodytes nimmoniana* and evolution of dioecy

Breeding systems include all aspects of sex expression in plants that affect the relative genetic contribution to the next generation of individuals within a species (Wyatt, 1983). Darwin (1859) in his theory of evolution proposed that sexual traits have evolved to enhance the reproductive success of the possessor by increasing the probability of gaining access to mates. Plants are known to show a bewildering array of sexual systems ranging from pure unisexual systems to mixtures of male, female and bisexuals.

A plant's gender is a function of how it devides its reproductive resources between male and female structures. In the present investigation all the three types of genders, *viz.* male, female and bisexual, were identified among flowers (plate 2). This is the first report of its kind since in all early floras *viz.* Flora of Karnataka, Flora of British India, Indian Trees *etc*, *N. nimmoniana* is reported to bear only bisexual flowers. In this investigation, seven sexual systems were identified at individual level, which includes hermaphroditic, monoecious, gynoecious, androecious, andromonoecious, gynomonoecious and trimonoecious following the categorization proposed by Frankel and Galun (1977). We are not aware of any plant species that shows all the seven systems in a single population. In a few genera, occurrences of many breeding systems have been reported. For instance, genus *Erythrina* (Arroyo, 1981) and *Salix* (Gustafson, 1942) constitute three types of sex system *viz*, hermaphoridte, monoecy and dioecy.

In *N. nimmoniana*, 44 per cent of the population possessed dioecious sexual system, while 2 per cent recorded monoecy (Fig 4.2). Proportion of dioecious species among angiosperms varies from 1 to 40 per cent, with the highest proportions found in the tropical forests (Bawa and Opler, 1974; Sakai and Weller, 1999). About 12% of flowering plants are reported to be hermaphrodite, where there is a chance of selfing at intra and inter floral level. Individuals with andromonoecious, gynomonoecious and trimonoecious systems constitute about 42 per cent of the population in *N. nimmoniana*. Several theories such as avoidance of self-fertilization, optimal allocation of reproductive effort *via* maleness/femaleness, local mate competition, sexual selection, parent-offspring conflict have been invoked to explain the wide array of sexual systems in plants (Uma Shaanker *et al.*, 1988), which is beyond the scope of this study. However, it should be emphasized here that *N. nimmoniana* offers an elegant system to test some of the (still controversial) theorical models on evolution of mating systems such as lottery model.

Occurrence of variation among individual plants in the extent to which they function as male versus female has rekindled an interest in evolutionary transition between breeding system. Darwin's (1877) original suggestion that dioecy has evolved gradually from distly in several groups was supported by the observation of Baker (1958), Ornduff (1966) as well the present study. Bawa (1980) has reviewed several alternative pathways leading to the evolution of dioecy including evolution directly from hermaphroditism (Ross, 1978), *via* gynodioecy (Webb, 1978), *via* androdioecy (Lloyd, 1975a) and *via* monoecy (Lloyd, 1975b). The occurrence of seven sexual systems with self-incompatible bisexual flowers in *N. nimmoniana* suggests that the species may be in a evolutionary mid stage wherein the species is evolving from hermaphrodite towards complete dioecy. The possible route of this evolution may be schematically traced as shown in the Fig. 5.1.

Based on the contribution of different individuals to male and female function, the population sex ratio of *N. nimmoniana* was shown to be almost 1:1, which is a balanced in sex ratio. The occurrence of different sex flowers at equilibrium is necessary for balanced male and female functioning in a population and their relative contribution to the next generation.

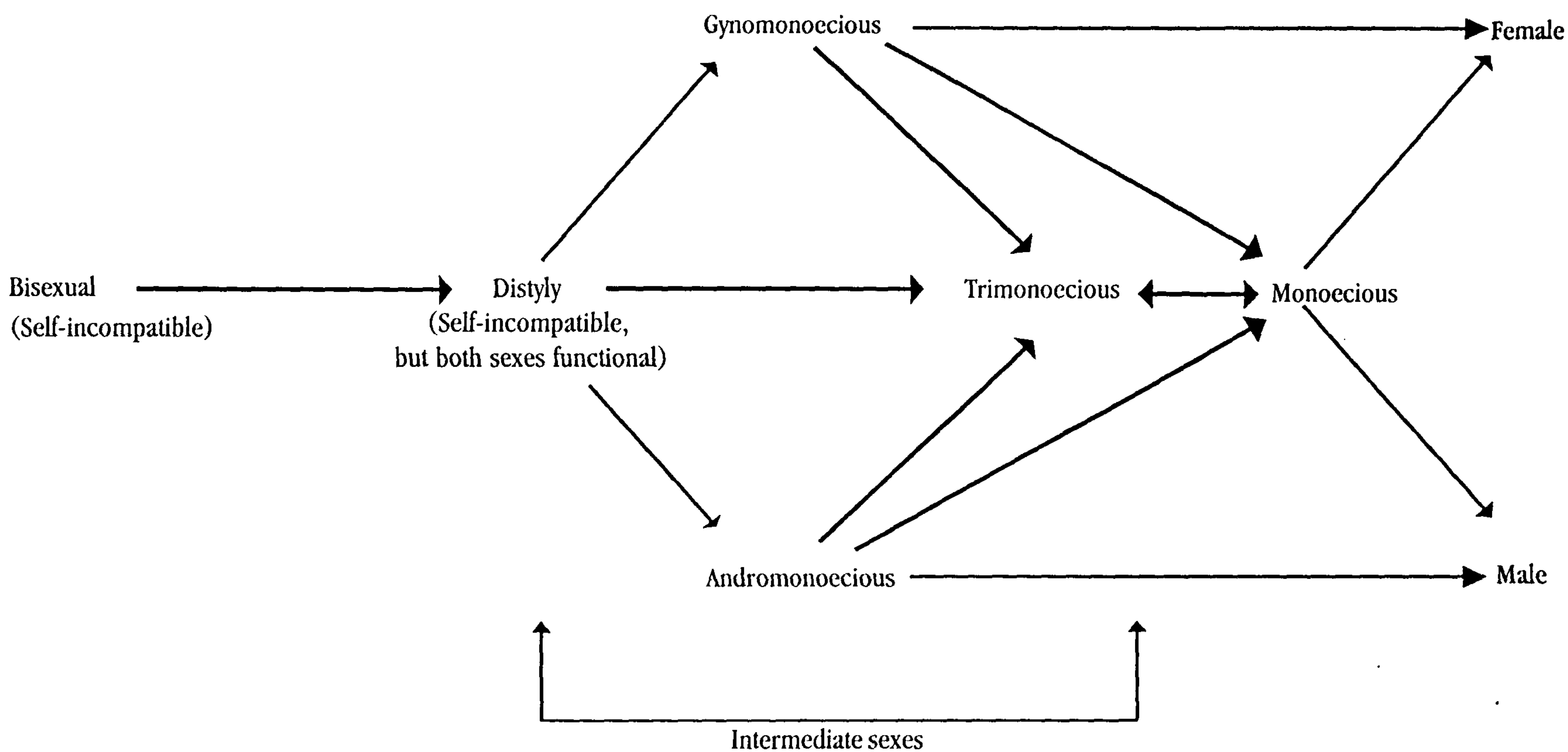

Fig 5.1 Path way of evolution of dioecy in *Nothapodytes nimmoniana*

5.2 Pollination in *N. nimmoniana*

N. nimmoniana flowers show perfect fly-pollination syndrome. About 12 species of flies were attracted mainly because of characteristic foetid odour emitted by flowers and other rewards such as pollen and nectar. Also many other species of insect have been observed. About 7 species of Lepidopterans, 6 species of wasps, 3 species of Hymenopterans, and 2 species of bugs were recorded in the present study. It appears that *N. nimmoniana* is a generalist in terms of insect vector. Pollen transfer from ant's may not be viable because of presence of inhibitory chemicals on ant body that renders the pollen sterile (Beattic *et al.*, 1984). Bugs are mainly visiting flowers for the predation of other insects and some are phytophagous.

Bagging experiments suggested that *N. nimmoniana* is a cross-pollinated species. It strictly requires insect visitation for pollination. Assisted pollination experiment showed (Fig. 4.5) that fruit set among open pollinated flowers were limited by the lack of pollen transfer, which was lower (4.9%) when compared to that of assisted pollinated flowers (79.5%). Thimmaraju and Shivananda (1993) also obtained similar results in case of *Artocarpus heterophyllus*. Radhamani *et al.* (1998) have reported pollination limitation in *Tamarindus indica*. It is observed that pollen limitation is mainly because of lack of floral visitors and also may be a consequence of pollen-wash by rains. These results suggest that fruit production can be enhanced by artificial pollination or by encouraging pollinators. However, fruit parameters such as fruit weight, seed weight, pulp weight, pulp to seed ratio and germination percentage did not differ among assisted and open pollinated flowers.

5.3 Phenology

5.3.1 Vegetative phenology

Tropical deciduous forests grow under a wide range of annual rainfall and dry season. The knowledge of ecophysiology of forest trees is scanty and their phenology is not well-understood (Murphy and Lugo, 1986). Seasonal water stress is likely to determine the timing of phenological events in forest, however, the mechanism of its action remains obscure. Most trees in tropical deciduous forest initiate leaves before the onset of rains and shed their leaves during dry season; further many species flower soon after leaf shedding. *N. nimmoniana* being a deciduous tree, initiated leaf flushing in the month of June (Fig. 4.4) coinciding with the onset of rainfall. The mean life span of leaves in *N. nimmoniana* (from fully expanded to initiation of leaf drop) lasts for approximately 225 to 250 days. Murali (1992) reported that in *Ougenia dalbergioides* duration of leaf retention was 210 to 240 days. Similar pattern was observed in *Tectona grandis* and *Anogeisus latifolia* in Mudumalai dry deciduous forests. The duration of leaf less period ranged from 95 to 110 days. In summary, *N. nimmoniana* seems to behave perfectly like a late successional species of deciduous forest with respect to vegetative phenology.

5.3.2 Reproductive phenology

Results of the present study indicate that flowering in *N. nimmoniana* begins soon after leaf sprouting. Flower buds were initiated on young branches, at the terminal position and are exposed to full sunlight. Usually flowering initiation time coincides with monsoon season *i.e.* during July and completes in the first week of September. In summary, *N. nimmoniana* flowers during rainy season and in a narrow time window (Fig. 4.2) this perhaps also limits its reproductive success due to low pollinator activity. There was a synchronized blooming among individuals of different sex, perhaps to facilitate gamete exchange.

In *N. nimmoniana* complete development of fruits, takes about 65 to 70 days after fertilization. Fruits start maturing from first week of October and ends during last week of November. Birds like red Whiskered Bulbul, Red vented Bulbul and Pigeon were the major agents of fruit dispersal observed during the study. Generally small birds attracted by the fruits because of high pulp content which is sweet in taste. The results of present study is in line with that of Ganesh and Davidar (2001) who reported that six commonly occurring frugivores birds are the fruit dispersal agents *viz*. Black Bulbul, Yellow-browed Bulbul, Red-whiskered Bulbul, White-cheeked Barbet, Mountain Imperial Pigeon and Nilgiri Wood Pigeon in *N. nimmoniana* .

5.4 Sexual system and life history traits

5.4.1. Sex expression and size of the plant

Several life history traits in *N. nimmoniana* seem to be influenced by sex expression and vice-versa. Interestingly, there was a tendency among larger individuals to be females while smaller individuals tended to be males. Sex change with size is a fundamental feature of the life history of a number plants. Borges (1998) reported that small individuals of snake lilies are male biased and later change their sex and became female biased in large individuals. This adaptation is also often assumed that an individual plant has a resource budget demarcation for reproduction. This may be fixed or a variable proportion of its total resource budget, the rest of which would be spent on many other function (Borges, 1998).

5.4.2 Differential reproductive phenology of sex types

In *N. nimmoniana* always male individuals bloom early followed by female and bisexual individuals. Among andromonoecious and gynomonoecious individuals, unisexual flowers bloom first followed by bisexual flowers. This is perhaps is to enhance the reproductive success, through increased out crossing and regulate the gene transfer in population (Waser and Price, 1991).

5.4.3 Fruit traits and sex expression

The results of our study showed that fruit set among female individuals was highest (15.69%) and lowest among andromonoecious types (4.28%). There are no comparative studies on these issues till today. Higher fruit set among female individuals may be a consequence of benefit of out-crossing and/or because of predominance of femaleness among larger individuals.

Fruits borne on andromonoecious individuals were larger and had higher pulp weight, which may be a consequence of less number of fruits per inflorescence compared to that in female individuals. Lowest fruit weight, seed weight, pulp weight and per cent germination were observed in trimonoecious types Sex expression in trimonoecious requires more resource to exhibit all sex flowers. Hence low resource allocated towards fruit development, this may be the cause for low fruit weight, seed weight, pulp weight and per cent germination.

Seeds from female individuals showed highest germination per cent may be because of strict cross pollination. Darwin (1877) reported that out-crossing progeny were usually more vigorous than those produced by self-fertilization. This indirectly indicates that cross-pollination encourage fertilization with vigorous pollen that helps in healthy seedling production. In summary, female's sexes occurs in larger individuals and are completely cross-pollinated, hence they may have higher fruit set and good germination compared to other cosexual types.

5.5 Seed germination studies

As shown in Fig 4.7, total percent seed germination was not significantly affected by pre sowing treatments. However speed of germination did vary. Simple removal of seed coat helps in quicker germination of *N. nimmoniana*. This may be facilitated by the easy absorption of water. However the best results were obtained by treating coat removed seeds with 50 ppm GA. This technique can be employed in large-scale production of seedlings. This is in sharp contrast to the results of Srivastava (2000) where 60 days were taken for germination.

Seedling parameters like number of leaves, root length, shoot length, root dry weight and shoot dry weight were also directly affected by pre-sowing treatment. Results of our study showed higher speed of germination where seedling growth parameters were higher. Results reported by Nagaraj and Farooqi (1989) in *Bursera paniculata*, Bhattacharya *et al.*, (1991) and Moktan *et al.*, (1993) in *Albizia odoratessima* were found to be similar to that in our study.

Viability studies showed that *N. nimmoniana* seeds belong to sub orthodox group of seeds. The germination was maximum only upto 90 days after seed maturation and at 165th day, the viability was zero (Fig. 4.8). This study clearly suggests that *N. nimmoniana* seeds were viable only upto 150 days. Perhaps because of higher amount of fatty acids present in the seed. Such association of lower viability and higher oil content has been reported in *Azadiracta indica, Madhuca latifolia* and *Shorea robusta* (Bonner, 1990).

5.6 Standardization of vegetative propagation

5.6.1 Vegetative propagation

Vegetative propagation serves as the primary method of reproduction of forestry species for commercial production on the majority of nurseries. Rooting of stem cutting is one of the important means of vegetative propagation which is generally practiced in forestry to obtain the plants of desired genetic constitution of economic important and multiplication with in a short period. It also helps in mass multiplication of species and some times in bringing out flowering much earlier from seedlings and also to conserve the species which possess the problem of natural regeneration in their habitat.

Results of the vegetative propagation in *N. nimmoniana* through stem cuttings indicated that treatment with Coumarin at 2000ppm induced maximum per cent rooting and sprouting. Stem cuttings of *N. nimmoniana* responded poorly to all the treatments. In contrast to other auxins, Coumarin causes considerable cell elongation and proliferation in the cortex, phloem and cambium region. Hence it may be concluded that to induce rooting and shooting in the cuttings of *N. nimmoniana*, Coumarin at 2000ppm can be followed as a standard practice.

5.6.1Influence of different form of hormonal application

Application of growth hormone in the form of dust gave better results in *N. nimmoniana*. Number of sprouts, number of leaves and shoot length showed significant difference in this form of hormonal application. This may be because of longer retention of hormone in cuttings, this may influence better results in the production of number of sprouts, number of leaves and shoot length.

5.6.2 Influence of sex on vegetative propagation

The results of our study on influence of sex on rooting and sprouting varied significantly (Fig 4.14; Fig 4.15). Stem cuttings from male tree showed higher per cent rooting, number of root length followed by female and bisexual cuttings. Auxin levels were higher in female than males in the floral structures, which is also reported in *Salix carpea* and many of conifers (Heslop- Harrison, 1964; Hashizume, 1969; Pharis and Kuo, 1977). Auxins are utilized during flowering season, which may cause low auxin levels in females than males after flowering. Male responds more to external auxin application than females. There are no reports on the differential response of sexes to the application of growth regulator.

5.7 Implications of the results to improvement and domestication of *N. nimmoniana*

Understanding biology of a rare species is a prerequisite for its genetic improvement and domestication. Identification of floral variation exhibited in *N. nimmoniana* helps in planning specific crossing programs involving appropriate sexes (Vasudeva *et al.*, 2001). Since this is a self incompatible species crossing can easily be taken up without the risk of self-pollination. Collection of seeds from female individuals would completely ensure cross-pollination. Studying broad phenological patterns and understanding time of anthesis and stigmatic receptivity are crucial in controlled pollination experiments. For the provenance level collection of seed and propagules at seed maturation phase i.e. during October- November is appropriate.

In the present investigation a simple agar based *in-vitro* germination medium was standardized to test the pollen grain viability in *N. nimmoniana*.

Standardized pollen medium was similar in composition to earlier standardized pollen medium for leguminous species by Vasudeva and Yeradoni (1998). This medium can be very reliably and effectively used to monitor the pollen grain viability across time and can also be employed to periodically check the viability in the event of cryopreservation of pollen grains, which is very useful in breeding programs.

The results have indicated that seed viability is retained only for 150 days after maturation. Hence it is necessary to undertake further research on improving seed viability duration. It should be emphasized that a simple pre-sowing treatment of removing the seed coat and soaking with 50 ppm of GA or cold water helps in quicker and uniform germination of seeds. Treatment of stem cuttings with 2000 ppm of Coumarin in dust form resulted in higher rooting and sprouting, which can be effectively employed while making clonal propagation and while establishment of clonal seed orchards.

It is also viable to include *N. nimmoniana* as a component in agroforestry as it show some unique features very well suited for agroforestry systems, which are as follows.

1. *N. nimmoniana* is a shade tolerant species hence it can be included as a component in already existing multistoried systems.
2. Since the species is non-browsable, good coppicer, and seem to withstand pruning / pollarding and recover at a faster rate, it can be a part of hedge or boundary plantations.
3. Since in natural habitats it is found to grow in association with several species it may not have any allelopathic effects making it suitable for agroforestry.

4. Due to fairly good market price and possibility of harvesting with in four to five years, the species ensures early returns.

5.8. Future line of work

- A few more populations of the species should be monitored to assess the change in sex over years and various theories on sex expression be tested.
- Sexes of the individuals need to be characterized using molecular markers.
- Sex-specific alkaloid content, if any, should be studied.
- The extent of genetic diversity in different populations needs to be assessed using isozymes or DNA markers.
- Selection of genotype with higher alkaloid content and its mass multiplication needs to be undertaken.
- Bi-clonal seed orchards can be established to get hybrids using vegetatively propagated plants of male and female gender.
- Method of sustainable harvesting of wood chips should be standardized.
- Suitable agroforestry practices incorporating *N. nimmoniana* should be devised.

Summary

VI. SUMMARY

Nothapodytes nimmoniana is an important source of Camptothecin (CPT), an alkaloid that is mainly used to treat cancer, and to inhibit human immuno-deficiency virus (HIV) as well as infectious anemia virus. It is believed that CPT is the third most important alkaloid sought-after by the pharmaceutical companies around the World. Perhaps this has led to the large-scale exploitation of the species from its wild habitat. CPT is present in wood chips, bark and root of this species, hence whole trees are essentially chopped while harvesting. This has resulted in a sudden decrease in population sizes in wild habitats; hence recently the species has been assigned the threat status of 'Vulnerable/Regionally'.

However there are no detailed studies on its reproductive biology. The present study was conducted on a good population of *N. nimmoniana* found in Joida and Sirsi forest ranges, to understand its reproductive biology and to standardize protocols of vegetative propagation, which are a prerequisite for genetic improvement and domestication of the species. .

Individuals of *N. nimmoniana* bear flowers of all types of sex *viz.* male, female and bisexual. This is the first report on the occurrence of different flower types in this species; all earlier reports mentioned it as bearing only bisexual flowers. Seven sexual systems *viz.* androecious, gynoecious, hermaphrodite, monoecious, andromonoecious, gynomonoecious and trimonoecious were identified at the individual level. Till so for no such report on the occurrence of all seven sexual systems in a single population exists. Larger proportion of individuals of *N. nimmoniana* (44 %) was dioecious, about 12 and 2 per cent of them were hermaphrodite and monoecious, respectively. About 42 per cent individuals showed mixture of sexual systems. Such variation in sex expression suggests that the species may be in the mid stage of evolving from hermaphrodite towards complete dioecy.

Sex ratio of the population was 1:1, indicating a balance in the sex expression at population level. Larger individuals more often tended to be functionally females, while smaller were males. Generally male flowers were larger, produced ample pollen grains and attracted more floral visitors. Female and bisexual flowers were smaller but produced more nectar. Fruit set was higher among female individuals and lowest among andromonoecious sex types. Identification of floral variation exhibited in *N. nimmoniana* helps in planning specific crossing programs involving appropriate sexes. In the present investigation a simple agar based *in vitro* germination medium was standardized to test the viability of pollen which can be routinely adopted in handling pollen grains.

Sixty flowering individuals were monitored for 2 years with respect to phenology. Leaf flushing started during the first week of June and completed during last week of July. The leafless period was very short and occurred during dry season. *N. nimmoniana* initiated flower buds from new shoots after one month of sprouting *i.e.* during monsoon season. This suggests the species behave like a deciduous species.

Fruits of *N. nimmoniana* started maturing from first week of October and ended during last week of November. For complete development of fruits it takes about 65 to 70 days after fertilization. Studying broad phenological patterns and understanding of anthesis and stigmatic receptivity are crucial for controlled pollination. Birds like Red Whiskered Bulbul, Red Vented Bulbul and Pigeon were the major agents of fruit dispersal. *N. nimmoniana* flowers show perfect fly pollination syndrome. About 12 species of Flies, 7 species of Lepidopterans, 6 species of Wasps and 3 species of Hymenopterans and 2 species of Bugs observed during our study. Assisted pollination experiment showed that fruit set in this species is limited by pollination in natural habitats.

Pre-sowing treatments did not result in significant differences in the total per cent seed germination however speed of germination was higher in GA 50 ppm treated seeds. Further, simple removal of seed coat helps in quicker germination of *N. nimmoniana* seeds and resulted in higher seedling growth. Viability studies showed that *N. nimmoniana* seeds belong to sub-orthodox group of seeds. The germination was maximum upto 90 days after seed maturation and gradually decreases across the time, viability will be zero at 165th day.

Vegetative propagation in *N. nimmoniana* through stem cuttings indicated that treatment with Coumarin at 2000ppm induced maximum per cent rooting and sprouting. Application of growth hormone in the form of dust gave better results compared to solution form. Number of sprouts, number of leaves and shoot length showed significant difference in this form of hormonal application. Interestingly, stem cuttings from male tree showed higher per cent rooting, number of root length followed by female and bisexual cuttings, which may be attributed to the differential endogenous hormonal levels.

As an initial step in domestication, *N. nimmoniana* can be adopted as a perennial component of agroforestry systems for multistory and/or bund planting since it is non-browsable, good coppicer without interference. Since the wood chips are still in great demand, growing this species on their marginal lands can benefit the farmers.

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