DOMESTICATION OF *NOTHAPODYTES NIMMONIANA* (GRAH.) MABB. :

AN ENDANGERED MEDICINAL TREE FROM WESTERN GHATS OF INDIA

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BY

DR. ANKUR PATWARDHAN

(E-MAIL : ankur_patwardhan@vsnl.net)

(Web site : www.ankurpatwardhan.com)

Research and Action in Natural Wealth Administration (RANWA)

C-26/1 KETAN HEIGHTS, KOTHRUD, PUNE – 411038.

MAHARASHTRA, INDIA.

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Ankur Patwardhan,
M.Sc. Ph.D.

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Executive Summary

Of the 34 global hot-spots of biodiversity, forests along the coastal mountains in the south western parts of India and on the neighboring island of Sri Lanka comprise the *Western Ghats-Sri Lanka* hot-spot (Myers *et al.* 2000). Western Ghats of India occupy the fifth position in terms of their economic potential of its biological resources. India has a rich repository of medicinal plant species (8000 species), which meets the health care needs of 80 % of the population of this country. India ranks 2nd in terms of the volume and value of medicinal plants exported (Ved, 1997). Several tree species from the Western Ghats are gaining international importance due to their newly identified pharmacological and curative properties. This has led to their indiscriminate harvest and hence their very existence is under severe threat. One such medicinal tree is *N. nimmoniana* (Grah.) Mabb. (Syn. *N. foetida, Mappia foetida*), which immediately needs conservation attention and is now confined only to the remnant forest pockets.

Present case study of a RED LIST species documents various steps from recovery to domestication thereby aiding in successful conservation practice (RSG phase 1 and 2 respectively). Recently domestication of plant species that are rich sources of clinically useful drugs has been prioritized. Prominent among them are the anti-cancer agents such as Camptothecins. *N. nimmoniana* is an **endangered** tree that yields Camptothecin (CPT) and is the most convenient source for large-scale production of CPT. CPT is extracted from stem (wood chips) and root bark. Because of destructive harvesting, habitat loss, the population of this species has declined 50-80% (CAMP, 2001). In view of the mounting demand and perceived threat to the native populations, it is highly essential to develop an appropriate strategy (mass cultivation) for its conservation. On this background present study explores various conservation and domestication aspects of *N. nimmoniana* such as;

(a) chemically profiling the species to identify *'elite lines'* (of CPT) from the wild gene pool

(b) clonal multiplication of these *'elite lines'*

(c) transferring the standardized technology to mass propagate the species

(d) developing cultivation protocols for a sustained and managed utilization of the wild pool.

Important findings :

1. Among the fertilizers tested for their effect on growth of *N. nimmoniana*, Di-Ammonium Phosphate (DAP) showed promising results than any other fertilizers studied.
2. Seed weight seems to be affecting seed germination in all localities. Thus, seed weight could form one of the criterion used to determine Quality Planting Material (QPM) standards for mass cultivation of *N. nimmoniana* .
3. CPT content of stem bark increases with the age of the plant.
4. Wild populations from drier localities were found to have higher CPT content.
5. An alternative source (*Ophiorrhiza sp*) was reported to contain 0.2% CPT .

Key achievements :

1. Recognition of *N. nimmoniana* as **'priority species'** for promotion and cultivation by Maharashtra State Horticulture and Medicinal Plant Board **(MSHMPB) and** State Forest Department **(SFD)** *(Annex. 1A & B)*.
2. State forest department with technical guidance from RANWA and Abasaheb Garware College, initiated pilot plantations.
3. A workshop on ***'Nothapodytes nimmoniana : Prospects* of a future crop'** was conducted at the College of Forestry, Sirsi (Box 1). It was attended by almost every stakeholder group of the *Nothapodytes / Mappia* value chain. Major participation was by the farmers. Organic-farming groups, bankers, horticulturists, ayurvedic practioners, nursery men, scientists and industrialists were also represented. This is perhaps the first meet where various issues on *Mappia* cultivation, processing and marketing were discussed.
4. As a promotion strategy, literature regarding cultivation of the species was designed and distributed to the participants *(Annex. 2 & 3)*. Efforts were also made to reach to the grass roots through local print media and by broadcasting on national TV news channel.
5. A network of women self help groups (SHG's) and farmers were established at 5 different locations for field related experiments (Chart 2). They were given training related to propagation of the species (both seed germination and clonal multiplication). Over 15000 saplings were raised and being distributed to farmers from Maharashtra and Karnataka.

6. A Cost-Benefit Analysis (CBA) model has been worked out for mass scale cultivation / commercial plantation (Industrial mode). Efforts are being made to develop cultivation package for small farmers.
7. Experimental plantation plots were established at Maval and Purandar Taluk. Growth response to organic & inorganic manures / fertilizers were studied and discussed in the report.
8. Studies on alternative species yielding CPT, given promising results. The findings were communicated to scientific peer reviewed journal for publication. Poster related to **'Conservation strategies of *N. nimmoniana*'** was presented at **Students Conference in Conservation Science (SCCS), Cambridge, UK.**
9. Several individuals and farmers co-operative showed interest in developing commercial plantations and requested for saplings.

Looking ahead..

1. Scale-up of plantation activities by having multi location trials is required to evaluate the performance of the species in different agro-climatic zones. We intend to do it by increasing capacity to raise saplings (approx. to 100,000) so as to cater the commercial demand and enable multi location field trials. Our CBA model revealed that plantation over 5 ha requires 12,500 saplings.
2. *Nothapodytes* being a tree crop has got 7-8 yrs gestation period. So cultivation package that involves intercropping of annuals so as to optimize gains / benefits need to be worked out.
3. Studies on effect of various growth hormones, stress conditions on growth of *N. nimmoniana* and CPT production can provide meaningful insights for better management of plantations.
4. We intend to apply *N. nimmoniana* model of **'Recovery, conservation and domestication'** to several such other species (RET, endemic) that are under severe threat and are high in demand. E.g. *Saraca asoka* (EN), *Embelia ribes* (EN) etc.

OPENING REMARKS….

India with a rich repository of medicinal plant species (1/4th of the world) meets health care needs of more than 80 % of the population of this country (Ravikumar and Ved, 2000). In past few decades many species of plants all over the world are gaining international importance because of the medicinally or economically important biomolecules they possess. Out of these plants, 25% are found in India, Western Ghats, a mega biodiversity hot-spot (Myers et al., 2000) being an important site of collection. India ranks 2nd in terms of the volume and value of medicinal plants exported (Ved, 1997). Of the 960 traded medicinal plant species from India, 178 species are consumed in volumes exceeding 100 Metric Tonnes per year. Modern medicine is also exploiting some of these resources as major raw material, sometimes leading to over harvests, threatening both, the health care practices and local livelihoods. *Nothapodytes nimmoniana* (*Syn*: *N*. *foetida*, *Mappia foetida)* is one such 'red listed' species that is endangered globally. Camptothecin (CPT) is one such biomolecule, found in *N. nimmoniana*, which has gained attention of many researchers and pharmaceutical industries all over the world because of its potent clinical applications in anticancer therapeutics (Lorence and Nessler, 2004). Because of unique pentacyclic structure of CPT, its artificial synthesis is difficult and one has to depend on the natural sources for raw material. Around 1000-1500 tons of *N. nimmoniana* wood chips are needed to get 1 ton of CPT in terms of raw material. This gives an idea about the scale at which destruction is going on and also sheds light on the extent of cultivation required to protect the wild pool and cater the commercial demand.

Present study looks into the conservation and promotion strategy road map of *N. nimmoniana*. Various aspects of domestication such as mass propagation of species, pilot plantations, effect of fertilizer application, efficient extraction process for active principle i.e. CPT, alternative species yielding CPT are tackled and discussed in the present work.

The present work is divided into following chapters ; (1) Introduction (2) Materials and Methods (3) Domestication studies (4) Outreach and Capacity building (5) Studies on Camptothecin analysis (6) Discussion and looking ahead. Chapter 1 briefly documents current status of *N. nimmoniana* with notes on its trade potential. Current status includes comments on its distribution and population in Western Ghats of Maharashtra. Chapter 2 deals with various protocols for mass propagation and domestication studies besides Camptothecin analysis. Efforts pertaining to domestication such as field trials, awareness generation, training to local 'bare-foot botanists' and SHG's etc. regarding multiplication of the species and nursery development are dealt in Chapter 3 and 4. Chapter 5 comments in detail about CPT analysis and its variation across plant parts and habitats, identification of '*plus* specimens' & alternative source and its relation with ecology. Follow up of the activities and impact / highlights of the current study are dealt in the discussion section (Chapter 6).

CHAPTER - 1

INTRODUCTION

1. INTRODUCTION

1.1 Taxon Data Sheet *

Species : *Nothapodytes nimmoniana*

Synonyms : *N. foetida, Mappia foetida*

Family : Icacinaceae

Vernacular names : Ghanera, Narkya, Amruta

Trade name : Narkya, Amruta

Habit : Tree

Habitat and microhabitat : Semi EVG, Moist deciduous, Fringes of forests, Understorey

Regional distribution : Around Western Ghats crestline of Maharashtra

Population distribution : Highly fragmented

Threats : Trade for medicine (documented - 300-500 tonns/yr), Loss of habitat, fire

Population change (general plus site specific) : 50-80 % reduction to local extinction

IUCN Status : EN-Endangered

Research/Management recommendations : Non destructive harvesting, suitability for different agro-climatic zones for integration into Agro-forestry systems

Planting need : Conservation and production *in and ex-situ*

* Conservation Assessment and Management Plan (CAMP) Data sheet modified based on observations during the present work

1.2 Description of *Nothapodytes nimmoniana*

Figure 1. *Nothapodytes nimmoniana*

Nothapodytes nimmoniana (Grah.) Mabb. is an **endangered** medicinal tree species (Figure 1) from the Western Ghats of India. It is a small, lower canopy tree belonging to family Icacinaceae. The distribution is highly patchy. It is found in different types of forests ranging from secondary forests, moist deciduous forests, semi-evergreen forests to disturbed evergreen forests. It prefers forest edges as microhabitat and commonly referred to as stinking tree because of the foetid smell it emits while flowering. (Other details pertaining to species biology, breeding types, phenology etc. were given in phase-I report).

1.3 Current status of *N. nimmoniana*

Since there is no convenient synthetic source for CPT, one has to depend on raw material from natural populations. *Camptotheca acuminata* (tree of Chinese origin) and *N. nimmoniana* are the only convenient sources for large scale extraction and purification of CPT (Padmanabha *et al.*, 2006). As CPT accumulates in stem and root bark of *N. nimmoniana*, whole tree is cut to generate biomass for extraction. In Indian market, the current demand for its biomass is 500-700 metric tons. In Maharashtra, overexploitation and habitat destruction for raw material has led to population decline by 50-80 % in last decade (CAMP, 2001). Total loss has been recorded from certain areas. Currently the species population density is as low as 1-2 individuals /ha in some areas. However it extends up to 30-40 individuals/ha at some localities such as forest of Mahabaleshwar where populations of *N. nimmoniana* are surviving against the severe threat of destruction.

1.4 Trade of *N. nimmoniana* for Camptothecin

In India, research on clinical trials of CPT is only at laboratory scale. Countries like Japan, USA and Spain are into the commercialization of CPT as drug. These countries import dried raw material from India and is one of the leading exporters worldwide. According to SFD records, the annual demand from Japan for dried stem of *N. nimmoniana* was 200-300 tonnes in 1994 (Dr. Maslekar. *Pers. Comm*.,). Since then there has been increasing trend. The trade volume increased to 1600 tons in the year 2002. In 2006-08, the reported trade is in the volumes exceeding 1000 tonnes, whereas unreported trade is thought to be at least twice the reported one. The ever-increasing worldwide market of Irinotecan and Topotecan (semi synthetic CPT analogues) has currently reached **1000 million US dollars,** which represents approximately 1 ton of CPT in the terms of raw material (Watase *et al*., 2004). To meet this ever increasing demand for CPT related drugs from world wide, more and more plants are cut, dried and are exported. This export business is completely managed by private sector. The collectors have trained the local tribal and rural laborers in cutting and drying processes. They are paid Rs. 10-15/ kg of dried stems and exported at the price of 1500 USD per kg (meaning Rs. 60,000 i.e. 1000 times higher price).

Figure 2. Making wood chips of *N. nimmoniana ready for sale*

Images courtesy : Dr. Vasudeva

Key facts:-

*Around **1000-1500 tones** of N. nimmoniana wood chips are equivalent to ~ 1 ton of CPT in terms of raw material exerting tremendous pressure on natural population. Decline in natural populations has thus necessitated that efforts needs to be taken to cater commercial demand as well as reduce threats to natural population. This can be achieved by adopting strategies such as domestication and raising commercial plantations.*

1.5 Objectives

Present study addresses various aspects of domestication by adopting two pronged strategy;

1. **Mass propagation with the help of farmers.** This involves,
    - Networking with farmers and women SHG's across northern Western Ghats
    - Conducting propagation trials at varied locations
    - Studying relation between seed size, seed weight and germination
    - Effect of bag size on growth of saplings
    - Checking response to fertilizers/ manures such as Di-ammonium Phosphate (DAP), Urea
    - Experimental plantation at various localities

2. **Screening and CPT profiling**
    - Chemical profiling of *N. nimmoniana* populations to identify high yielding elite lines
    - Screening alternative species for CPT content (e.g. *Ophiorrhiza sp.*)

CHAPTER - 2

MATERIALS AND METHODS

2. MATERIALS AND METHODS

I. Domestication studies :

Experimentation regarding pilot plantations was conducted at Maval and Purandar taluk. Whereas 4 locations along northern Western Ghats were chosen for CPT profiling studies of *N. nimmoniana* and 2 for *Ophiorrhiza* resp. (details given below).

2.1. Study area :

(i) Maval taluk : Study area is situated towards north west of Pune in Maval taluk (18° 36'N, 73° 26' E) and is on the eastern slope of the Western Ghats at an elevation of about 700m a.MSL (Fig. 3). The average annual rainfall is approximately 4000 mm with 8-9 months of dry period length. The mean maximum temperature is 35°C and mean minimum temperature is 12°C. The forest type is mainly secondary moist deciduous with scrub patches in between.

(ii) Purandar taluk :. Study site is located on the eastern side of Pune in Purandar taluk (18^0 25' N, 74^0 08' E) (Fig. 4) and the conditions are drier. Area mainly dominated by xerophytic plant species. The average annual rainfall is around 700mm with 9-10 months of dry period length.

2.2. Relation between seed weight, seed size and germination:

Seeds of *N. nimmoniana* are oval in shape. They were collected from 5 different localities. Seed coat was removed and parameters *viz.* length, breadth and seed weight were measured before sowing seeds (n = 50 for each locality). Percent germination was monitored at regular intervals.

2.3. Effect of bag size on growth of saplings :

Saplings were planted in nursery bags of different sizes: 5" X 7" and 12" X14" and monitored for collar diameter and shoot length. Set of 30 saplings was monitored for both the bag sizes. One set was kept as control without application of any fertilizer. Control reading was taken for every set before application of fertilizers. For checking the effect of fertilizers and bag size on growth, the difference between the control reading (before application) and present month reading was taken. Data were subjected to statistical analysis.

2.4. Response to fertilizers:

Growth of saplings to fertilizers such as Diammonium phosphate (DAP) and Urea was seen. The parameters measured were,

1) Collar diameter
2) Shoot length

The measurements were taken every one month for first 6 months and then at every 3 months interval. Fertilizers were applied to saplings in nursery bags and saplings planted on field in Maval taluk (n=83) (moist deciduous region) and Purandar taluk (n=80) (arid region).

Dose: Control: No fertilizer

DAP: 0.5g per sapling

Urea: 0.5g per sapling

Sets of 30 saplings were monitored for every treatment. The fertilizers were applied once in a year.

II. Camptothecin (CPT) profiling :

2.5. Protocol for Extraction of CPT:

2.5.1. Sample processing : Plant parts were separated into roots, stem and leaves.

- Samples were dried at 60^0C for 48 hrs
- Powdered samples were extracted in 70% ethanol so as to get final concentration 100mg dry wt/ 10ml ethanol.
- Extraction was done at 55^0C for three hrs in water bath.
- Extracts were kept overnight for cooling
- Centrifugation: 13,000 rpm for 20 min.
- Filtration: Double filtration with same 0.2 µm pore size filter.

2.5.2. Preparation of Standard:

- CPT dissolved in DMSO : Methanol (1:50)

2.5.3. Detection of CPT:

- High Performance Liquid Chromatography (HPLC) analysis (Perkin Elmer)

2.6. Sites of collection of *N. nimmoniana* :

(i) Raigad taluk : It is the northernmost of the sites selected. It is situated north west to Pune and is on the western slope of the Western Ghats. The area is dry-deciduous with

some open scrubs scattered in between. Average annual rainfall is 1000-1400 mm; dry period length is 8-9 months; elevation is 1000 m a. MSL.

(ii) Purandar taluk : same as above.

(iii) Sawantwadi taluk 1 : It is the southern most site selected for the sample collection and is also the southernmost site of distribution of *N. nimmoniana* in Maharashtra. Average annual rainfall is >6500 mm; dry period length is 8 months; elevation is 750-900 m a. MSL. The area mainly comprises of semi-evergreen and some opened up areas.

(iv) Sawantwadi taluk 2: The area harbours open disturbed semi-evergreen type of forest.

2.7. Sites of collection of *Ophiorrhiza sp* :

(i) Radhanagri : Study site is located in Kolhapur district in Radhanagri taluk (Fig. 5) (16^0 13' 35.5"N, 73^0 57' 22.3" E; elevation:922 m a.MSL). Dry period length is about 7 months. The area harbours moist deciduous type of forest.

(ii) Koyna – The area supports semi-evergreen type of forest. It is located between 17^0 41' N, 73^0 42' E ; Elevation : 769 m a.MSL, with dry period length of about 8 months (Fig. 6).

CHAPTER - 3

DOMESTICATION STUDIES OF N. NIMMONIANA

3. DOMESTICATION STUDIES OF *N. NIMMONIANA*

3.1. Relation between seed weight, seed size and germination:

Seeds across different localities show variation in weight and size (Table 1).

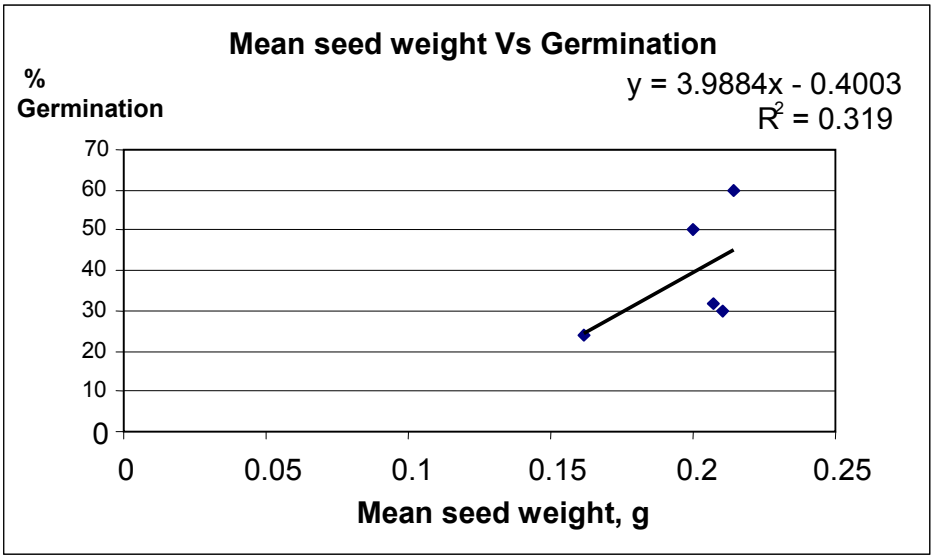
Table 1 : Mean seed weight and % Germination across localities

Locality	Mean seed weight * (g)	% Germination
Maval	0.1614 ± 0.033	24
Mahabaleshwar	0.2003 ± 0.060	50
Satara	0.207 ± 0.039	32
Radhanagari	0.2106 ± 0.040	30
Sindhudurg	0.214 ± 0.062	60

* n= 50 for each locality

Seed weight seems to be affecting germination of seeds. There is positive correlation between mean seed weight and % germination (Fig. 7).

Figure 7 : Relation between seed weight and Germination



3.2. Effect of bag size on growth of saplings:

Saplings grown in bag size of 12”X14” show higher growth rate as compared to control with bag size of 5”X7” (Fig. 8A). t values show that there is significant difference in shoot length ($t_{critical}$ = 2.00 , $t_{calculated}$ = 5.8, $p<0.05$, $n=28$). This is because, saplings in small sized polybags show tendency of root coiling (Fig. 8B).

Therefore to achieve better performance in the field when saplings are out planted, care should be taken to maintain saplings in large polybags.

Figure 8A and 8B

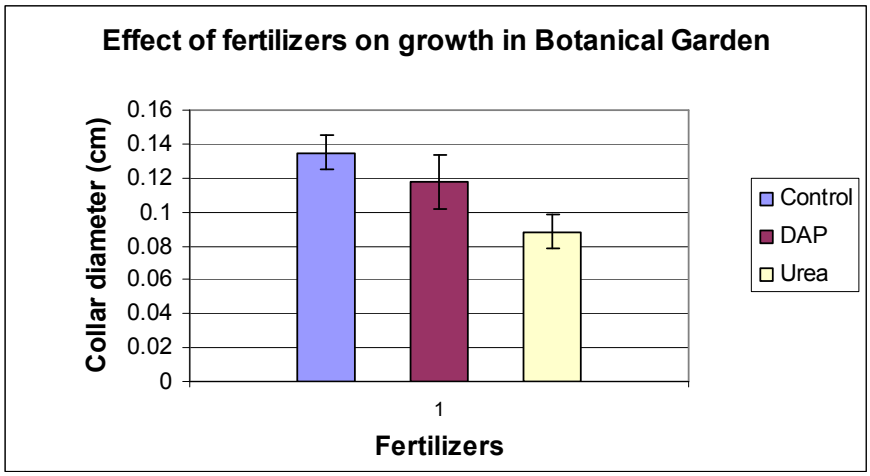
3.3. Response to fertilizers:

Figure 8C indicates design and monitoring of various fertilizer treatments given *N. nimmoniana.*

Figure 8C. Monitoring growth of *N. nimmoniana*

Comparison between collar diameters of saplings grown in polybags is given in Fig. 9

Figure 9 : Effect of fertilizers on growth of saplings



Though control saplings are showing more growth, DAP shows pronounced effect on growth of saplings in the long run than Urea (nursery raised saplings of 3 yrs was found responding better to DAP than Urea and control). Hence in further experiments comparison across DAP and Urea is considered.

N. nimmoniana was planted as a hedge crop in Maval (Fig. 10), while as a regular crop in Purandar (Fig. 11).

Figure 10 : Experimental plantation of *N. nimmoniana* at Maval

Figure 11 : Experimental plantation of *N. nimmoniana* at Purandar

Chart 1. Field layout of *Nothapodytes nimmoniana* plantation

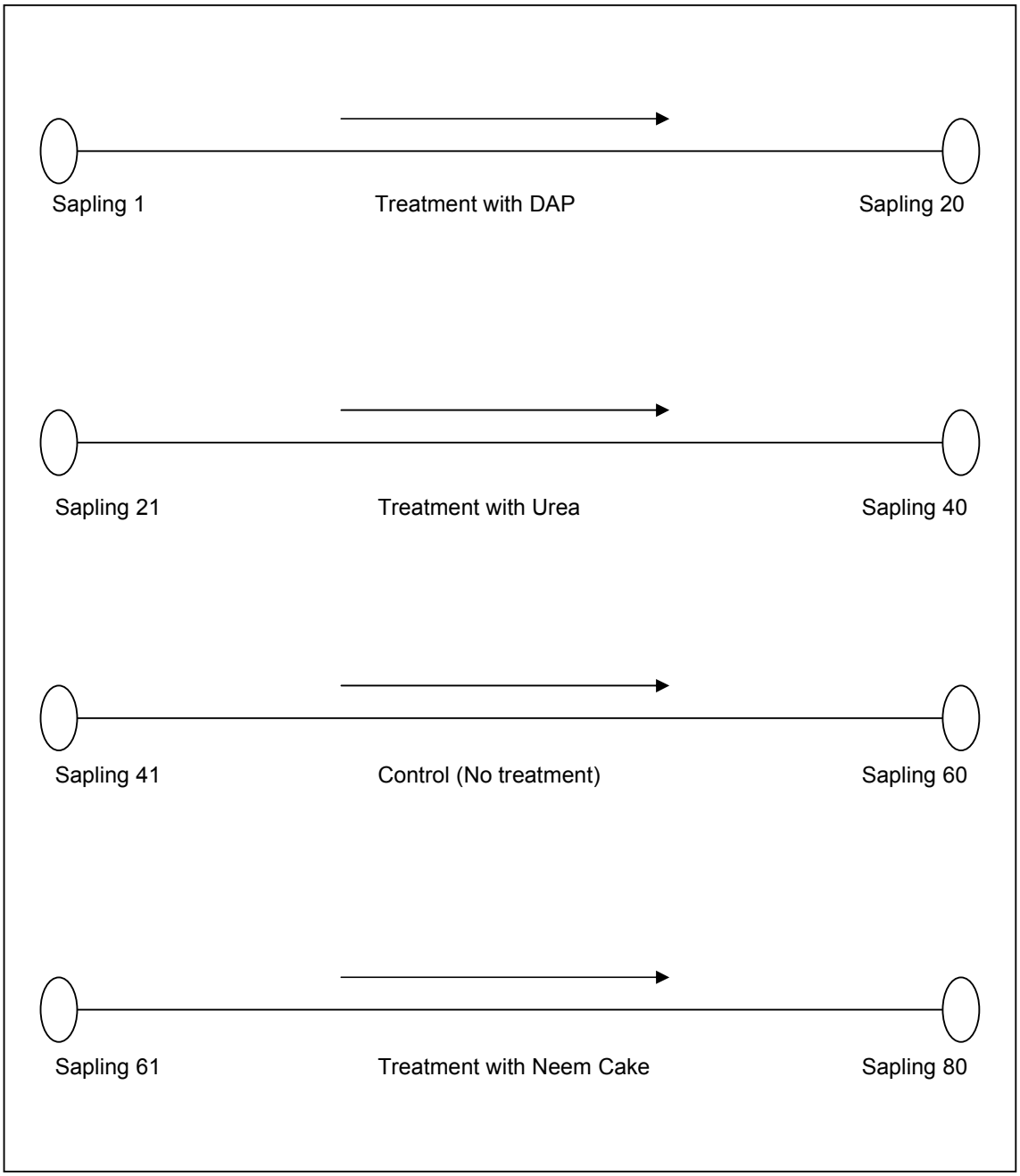
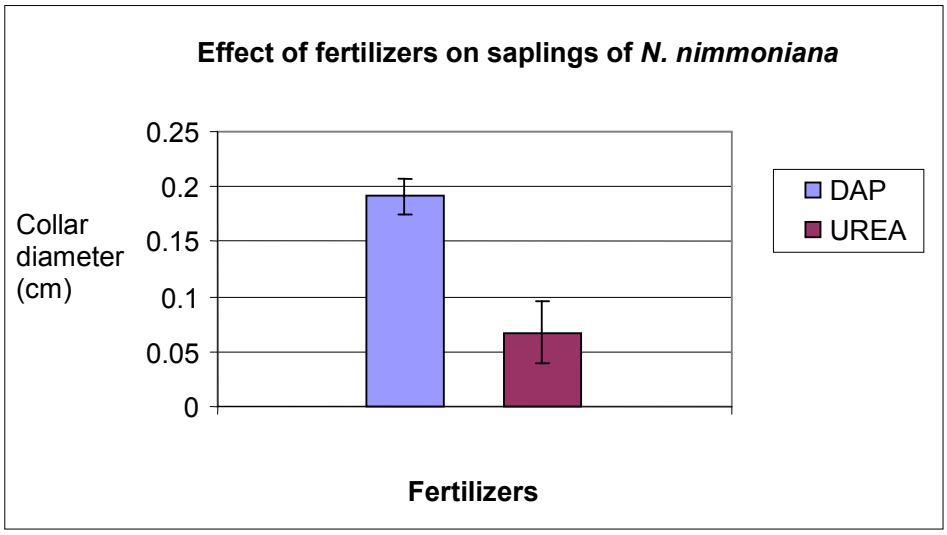


Figure 12 and 13 gives comparative account of effect of fertilizer on collar diameters of saplings planted in field in Maval and Purandar resp.

Figure 12. Effect of fertilizers on growth at Maval

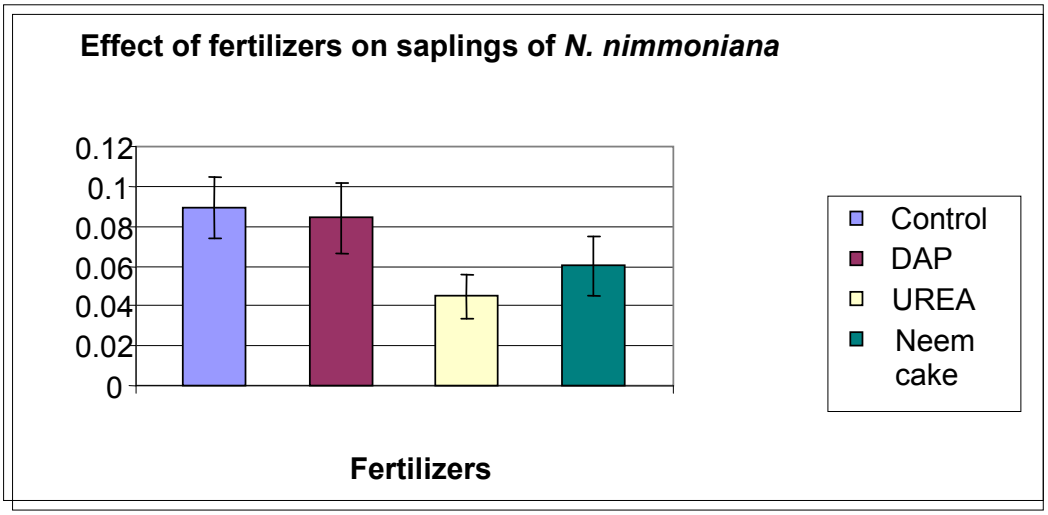


Though artificial fertilizers support growth, it is seen pronounced in DAP treated saplings than in Urea ($t_{critical}$ = 1.4432, $t_{calculated}$ = 1.9896, $p < 0.05$).

There is not significant difference in shoot length of saplings treated with DAP and Urea.

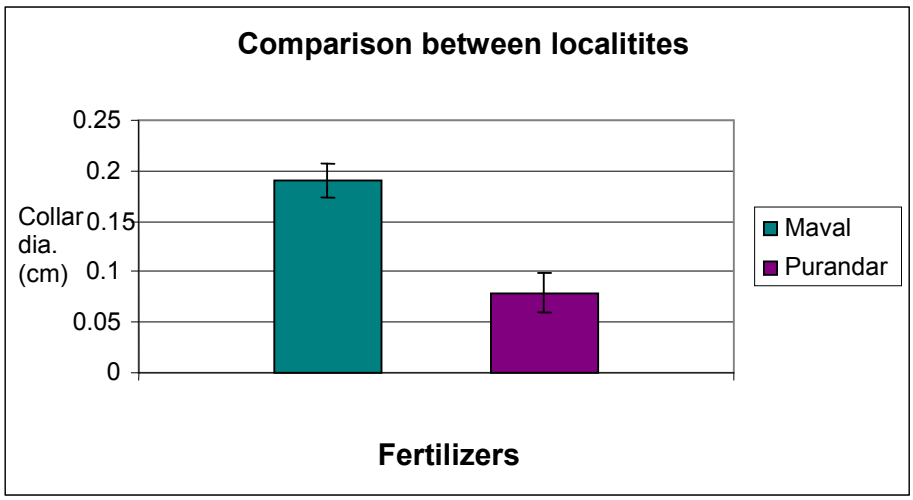
For Purandar region, ANOVA was performed to see the difference in performance of different fertilizers. It was found that DAP shows significantly pronounced growth followed by Neem cake (organic) and then followed by urea. In case of shoot length, there is significant difference among three fertilizers. DAP showed pronounced effect than other fertilizers.

Figure 13. Effect of fertilizers on growth at Purandar



Comparison across localities : As DAP was found to have pronounced effect than other fertilizers, effect of DAP on collar diameter was seen across localities and depicted in Fig. 14.

Figure 14. Effect of DAP across localities



t – test results show significant difference in growth of saplings in Maval and Purandar, Maval showing higher growth than Purandar. $t_{critical} = 1.99$, $t_{calculated} = 3.76$, $p < 0.05$.

CHAPTER - 4

OUT REACH AND COMMUNITY PARTICIPATION

4. OUT REACH AND CAPACITY BUILDING

Conscious and directed efforts were taken to reach various stakeholders *(Annex. 4)* of the *Nothapodytes* value chain. The activities conducted were at different scales, ranging from grass root (farmers, Self Help Groups) to policy level.

1) A workshop on ***'Nothapodytes nimmoniana : Prospects* of a future crop'** was organized (Box No. 1) for creating awareness amongst stakeholders. Literature regarding propagation and cultivation of the species was designed and distributed to the participants

2) Series of focused and targeted interactions with State Forest Department and State Medicinal and Horticultural Plant board were conducted.

3) As a promotion strategy, efforts were also made to reach to the grass roots, farmers and cultivators through local print media and by broadcasting on national TV news channel. (*Annex*. *6A-6C*).

4) Training to women self help groups (SHGs) and nursery-men regarding propagation and cultivation of the species.

Box No. 1 : *Nothapodytes* promotion workshop

A workshop on ***'Nothapodytes nimmoniana : Prospects* of a future crop'** was held at the College of Forestry, Sirsi, University of Agricultural Sciences, Dharwad. It was attended by almost every stakeholder group of the *Nothapodytes* value chain. Major participation was by the farmers. Organic- farming groups, bankers, horticulturists, ayurvedic practioners, nursery men, scientists and industrialists were also represented. This was perhaps the first meet where various issues on *Mappia* cultivation, processing and marketing were discussed.

Box No. 1 *contd..*

Key Decisions of the Plenary Session:

1. To fix an achievable target for planting stock and recognize nursery persons who can take up this activity all along the Western Ghats.
2. To approach the various agencies to include *Nothapodytes* as a profitable medicinal plant.
3. To create *Nothapodytes* forum, to facilitate information exchange on cultivation, processing and marketing of *Nothapodytes*.
4. To encourage conservation of *Nothapodytes* germplasm in academic institutes.
5. To identify gaps in the understanding of biology and response to cultivation practices of *Nothapodytes* .
6. To encourage organic cultivation of *Nothapodytes*.
7. To screen the other important chemical properties of *Nothapodytes*.

Honorable Forest Minister felicitating Mr. Sharad Shinde, a farmer from Maval

Training to women Self Help Group members regarding mass propagation

Chart 2. Nursery of *Nothapodytes* established at various locations in northern Western Ghats

Locality : Maval

Information dissemination through various agencies ;

Source : National Television Interview (DD News)	Source : Print media

Discussion with academicians and forest officers

CHAPTER - 5

CAMPTOTHECIN STUDIES

5. CAMPTOTHECIN STUDIES

5.1. Introduction

Camptothecin (CPT) is 3rd most promising alkaloid of the 21st centuary (Lorence and Nessler, 2004). CPT is a monoterpenoid indole alkaloid, originally isolated from *Camptotheca acuminata*, a tree native to south China. It was first isolated in 1966 by Wall and Wani from *C. acuminata*. It is water insoluble molecule, having unique pentacyclic structure. ***It has gained attention of many researchers and pharmaceutical industries all over the world because of its potent clinical applications in anticancer therapeutics.*** Two water soluble derivatives of CPT, Irinotecan and Topotecan, have been approved by Food and Drug Administration (FDA) of USA for their clinical applications in colorectal and ovarian cancer (Lorence and Nessler, 2004). Apart from anticancer activity, CPT has also been shown to have anti HIV-I activity (Priel *et al.,* 1991). It is also known to be effective against trypanosomes and *Leishmania sp.* (Bodley and Shapiro, 1995).

Apart from *N. nimmoniana* and *C. acuminata* (family: Nyssaceae and order: Cornales) following plants have been detected to synthesize CPT.

Table 2: Species of plants synthesizing CPT

	Species	Family
1	*Ervatamia heyneana*	Apocynaceae
2	*Ohpiorrhiza pumila*	Rubiaceae
3	*O. mungos*	
4	*Mostuea brunonis*	Gelsemiaceae
5	*Merrilliodendron megacarpum*	Icacinaceae
6	*Pyrenacantha klaineana*	

Because of an unique structure of CPT, its artificial synthesis is difficult and one has to depend on the natural sources for raw material (Suhas et al., 2007). ***Detailed studies on alternative species are lacking and one needs to look into it critically as they can also be proved as potential sources of CPT drug.***

Significance of CPT and its relation with decline in population of N. nimmoniana has already discussed in previous section. CPT content varies among species and varieties but even more significantly within the plant parts (with different tissues, tissue age and seasons) (Li et al., 2002). This implies that analyzing various tissues, at different ages and in different seasons for CPT content may come out with better options for sustainable extraction of CPT. Present study was especially designed to;

(a) identify 'elite lines' / '*plus specimens'* of *N. nimmoniana* in terms of high CPT content.

(b) see variation of CPT across age of the plant and seasons

(c) analyze alternative species yielding CPT

5.2. Detection of 'Plus Specimens' :

As stated earlier, '*plus*' specimen is the one which has some additional property or some enhanced property. In our study we recognize plus specimen as the one which has stem bark CPT content higher than the normal range which is encountered in *N. nimmoniana*, 0.14 to 0.24 % (Aiyama *et al.*, 1988).

While analyzing CPT content of stem bark samples from different localities, we have identified a **'*plus*'** individual which showed CPT of 1.15% from *Raigad* area. CPT of this individual was analyzed for 3 times and the CPT content was found to be consistent (1.15±0.06%). CPT content of >1% has also been found in some individuals from southern Western Ghats. (Suhas *et al.,* 2007). These individuals were 'marked' for further studies in clonal propagation.

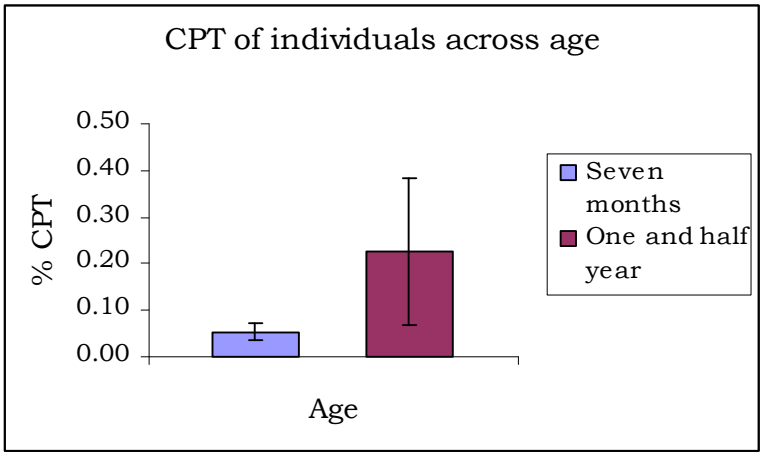
Figure 15: Marking populations in the wild

5.3. Variation in CPT with age of the plant :

According to the biosynthesis pathway, CPT is synthesized in leaves and is mainly accumulated in the stem and root bark of the plant. **As the plants mature, CPT accumulation in their bark will go on increasing till some concentration after which concentration will remain in more or less in proportion with the bark thickness. This pattern of accumulation studied in detail, would help to find out the appropriate age of harvest of stem from the plant so as to get maximum possible concentration.**

Two age groups were available for the analysis; six months old plants from experimental field plot and one and half year's old plants. Stem bark samples of 5 individuals of each age group were collected and their CPT content was analyzed.

Figure 16 : CPT across age of the plants



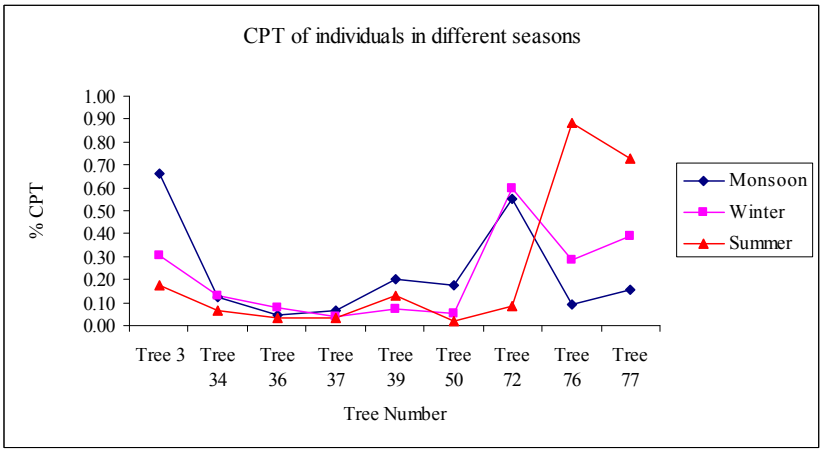
CPT content of one and half year's plants was found to be significantly higher than that of seven months plants. $t_{calculated} = 4.056$ and $t_{critical} = 2.306$ at $p<0.05$.

5.4. Variation in CPT of individual plants across seasons and sex :

5.4.1. CPT across the season :

18 bark samples, from mature trees from the wild (*Maval* area), were collected in following seasons, August 2006 (monsoon), January 2007 (winter) and April 2007 (summer). The results were represented in fig 17.

Figure 17: CPT variation across seasons

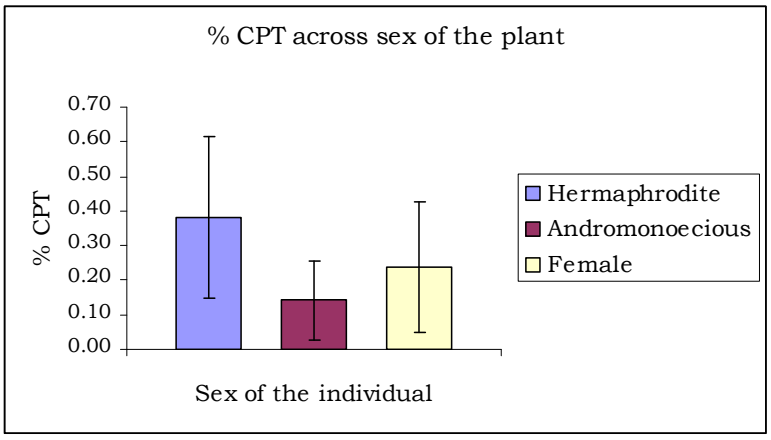


ANOVA was performed on the results obtained, and it was found that variation in the CPT content of plants was not significantly different for different seasons. When CPT of an individual was observed over the seasons, it was observed to vary but no trend was seen in such variation when observed collectively for all individuals.

5.4.2. CPT across the sex of the individual :

There are different sexes present in *N. nimmoniana* and there is possibility that plants of different sex produce and accumulate different levels of CPT. Attempts were made to analyze if there is any significant difference in the CPT of the individuals of different sexes from the samples collected. The results were depicted in fig 18. The samples were collected in monsoon which is the flowering season of *N. nimmoniana*, so that sex of the flowering plants is confirmed at the time of the collection. Samples collected were from Hermephrodite, Female and Andromonoecious individuals.

Figure 18 : CPT content in different sexes of plant



ANOVA test was performed to analyze the difference between sexes of the plant for its CPT content. No significant difference was found in the CPT content across sex of the plant. In general Hermaphrodite plants showed highest CPT content.

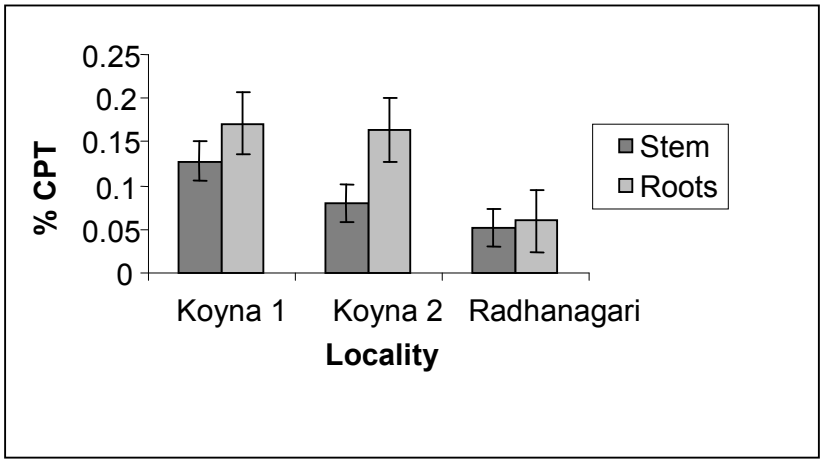
Soil fertility, coppicing are found to be affecting CPT levels in C. acuminata seedlings (Liu et al., 1998). Since CPT is produced by the plant as a defense strategy against herbivory and CPT being a secondary metabolite which is generally synthesized under stress conditions, it will be important and interesting to study what kind of stress conditions are responsible for high levels of CPT in the plant. For this, multi-location field trials at different agroclimatic zones are a prerequisite. So studies on effect of various fertilizers (both inorganic and

organic) on CPT levels, impact of stresses like water stress, difference in CPT levels across plantations in rainy Vs. dry areas could be future course of action. By employing effective coppicing strategies one can get 2-3 kg dry biomass per plant in N. nimmoniana after 7 yrs of plantation. Especially strategies like coppicing can be used as a successful harvest practice (as it is responsible for higher surface area to bark ratio and bark being the main source of CPT) when it comes to commercial plantation.

5.5. Studies on *Ophiorrhiza sp.,* an alternative species yielding CPT :

Ophiorrhiza is a rare, understorey herb found in areas of high rainfall. CPT content of plant parts of 15 individuals was analyzed. Fig. 19 shows mean CPT of plant parts of these individuals. We checked for the variation across stem and root tissues of *Ophiorrhiza sp.* Leaves though screened for CPT were not considered for further analysis owing to their very less content (0.03 ± 0.02 %). CPT content from root (mean value) was found to be highest among all plant parts analyzed in all localities.

Figure 19 : Percent CPT across plant parts and localities



Per cent CPT from roots was found to be significantly higher than stem for each locality. t- test results: Koyna 1 : t critical =2.09, t- calculated = 2.54; Koyna 2 : t critical = 2.08, t calculated = 3.57

5.6. Soil analyses :

Surface soil samples from Koyna 1 and Radhanagari were subjected to analysis of various physicochemical parameters elaborated in Table 3.

Table 3: Results of soil analyses

No.	Physicochemical parameters	Koyna 1	Radhanagari
1	pH (1 % solution)	5.93	5.85
2	Soil Texture	Medium Loam	Clay Loam
3	Total Nitrogen (%)	0.7392	0.2304
4	Total Phosphorus (%)	0.099	0.07037
5	Total Potassium (%)	0.2905	0.2322
6	Cation Exchange Capacity (milliequi/100g sample)	34.8452	27.4745
7	Total Organic Carbon (%)	8.6624	1.487
8	Total Organic matter (%)	14.9339	2.563
9	Total Alkalinity (mg $CaCO_3$/L)	228	153
10	Total Hardness (mg $CaCO_3$/ L)	0.34	0.18
11	Calcium Hardness (mg/$CaCO_3$/L)	0.18	0. 14
12	Iron (%)	5.6948	7.1037

CHAPTER - 6

DISCUSSION AND LOOKING AHEAD….

DISCUSSION….

N. nimmoniana is a red listed species. There is a difficult task ahead of all conservation biologists, as they have to find solutions such that harvest pressure on wild population is reduced and at the same time commercial demand is also fulfilled. One way to address this is to promote commercial cultivations, as they help in reducing the harvest pressure on the wild population (Ravikumar and Ved, 2000). Studies presented here have given promising results in achieving that goal.

- Among the fertilizers tested, DAP showed promising results than any other fertilizers studied. In both the localities, DAP (source of nitrogen and phosphorus) showed pronounced effect than Urea (only nitrogen source). Preliminary findings presented in the report on growth of *N. nimmoniana* will help to determine the conditions to be incorporated when *N. nimmoniana* will be taken as a commercial crop.

- Seed weight seems to be affecting seed germination in all localities. Thus, seed weight could form one of the criterion used to determine Quality Planting Material (QPM) standards for mass cultivation of the species.

- Individuals having very high CPT content (*plus* specimens) were encountered from wild populations while screening. Some individuals showed the CPT content which is about 350% more than the CPT range (0.14-0.24 %) recorded by Aiyama *et al.*, (1988). These individuals belong to deciduous areas with lower rainfall. Thus scale-up of existing nursery facilities so as to facilitate multi-location field trials across different agroclimatic zones will prove to be an important step towards raising plantations. This will give an insight into suitability of the region where *N. nimmoniana* can be taken as a commercial crop.

- CPT content of stem bark increases with the age of the plant. Our previous studies and studies by Padmanabha *et al.* (2006) showed that CPT content does not vary with the GBH of the plants above 16 cm. The samples which we had collected were young samples of GBH less than 15 cm. CPT content of these age groups increased significantly with age of the plant. Doing further studies on CPT content of the plantations from varied locations with increasing age (1 yr, 2 yr, 3 yr etc.) would yield

better results and would lead to determination of optimum age of harvesting of the stem after cultivation. Such studies incorporating growth and CPT content will help achieving higher yield in terms of CPT content per unit biomass.

- CPT content from *Ophiorrhiza sp.* is found comparable to that of *N. nimmoniana. N. nimmoniana* is found to produce CPT in the range of as low as 0.03% to as high as 1% or even 2.5% with the mean of 0.3% (Suhas *et al.,* 2007). In comparison to that, *Ophiorrhiza sp.* contains 0.2% CPT.

FUTURE PLANS….

- Owing to its inclusion in the **'priority species list'** for cultivation and promotion (*Annex. 1A,B*), there is a strong user interest by various stakeholders. With the publication of encouraging results in the local news media (*Annex. 5A-C*) many farmers, self help groups and private cultivators have approached us to find our supply extent (*Annex. 4*). We intend to scale up the nursery facilities and ultimately intend to delist this species from RET category.

- State Forest Department and State Medicinal Plant Board has expressed keen interest in replicating the *Nothapodytes* model of conservation to similar such species.

- *N. nimmoniana* is being proposed as a candidate in agroforestry programme. However, being a slow growing species, it takes 7-8 yrs for first harvest. In such cases, *Ophiorrhiza sp*. and other medicinal annuals can be considered as potential candidate as an intercrop with *N. nimmoniana.* In such case standardization of propagation techniques and developing cultivation strategy could be the focus of future studies.

CHAPTER - 7

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BIBLIOGRAPHY

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ANNEXURE

Annexure 1A

Maharashtra State Horticulture & Medicinal Plants Board
Sakhar Sankul, Shivajinagar, Pune – 411 005.
Phone: 020-25534860 Fax: 020-25511302 E-mail: mshmpb_pune@rediffmail.com

No.MSHMPB/Tech-3/2209/08
Pune 411005, date- 11-06-08.

To,
Dr. Ankur Patwardhan,
Lecturer,
Biodiversity Section,
Dept. of Microbiology,
Garware college, Karve Road,
Pune – 411004.

Sub: Finalization of list of priotised medicinal plant species for the State of Maharashtra.

Sir,

The list of Medicinal Plant Species priotised for the State of Maharashtra according to their conservation and economic value have already been sent to you vide this office letter no. MSHMPB/Tech-3/MPS/08-09/1627/08, dated 29/04/08.

Encl-As above

(NHN SHAIKH)
Consultant
MSHMPB, Pune

Annexure 1B

Medicinal plant species prioritized for the State of Maharashtra according to their conservation and economic values

TREE SPECIES

Sr. No.	*Botanical Name*	Local Name
1.	*Aegle marmelos* (L.) Corr.	Bel
2.	*Alstonia scholaris* (L.) R. Br.	Saptparna
3.	*Callophyllum inophyllum* L.	Undi
4.	*Cassia fistula L.*	Bahava
5.	*Emblica officinalis Gaerin.*	Amla
6.	*Garcinia indica* (Du Petit. thou) Choisy.	Kokam
7.	*Gmelina arborea Roxb.*	Shivan
8.	*Mesua ferrea* L.	Nagakesar
9.	***Nothapodyfes nimmoniana* (Grah.)Mabb.**	**Narkya, Amruta**
10.	*Oroxylum indicum* (L.) V en t.	Tetu
11.	*Pongamia pinnata* (L.) Pierre.	Karanj
12.	*Premna obtusifolia R.Br.*	Agnimanthah
13.	*Pterocarpus marsupium* Roxb.	Bibla, Bij a
14.	*Santalum album* L.	Sandal
15.	*Sapindus laurifolia* Vahl.	Ritha
16.	*Saraca asoca* (Roxb.) de. Wilde.	Sita Ashok
17.	*Strychnos nux-vomica* L.	Kaj ra
18.	*Symplocos laurina* (Retz) Wall.	Lodhrah, Mirioli
19.	*Syzygium cumini* (L.) Skeel	Jambhul
20.	*Terminalia ovata (Roth)*	Arjun
21. /	*Terminalia bellirica* (Gaertn.) Roxb.	Beheda
22.	*Terminalia chebula* Retz.	Hirda

Contacts:

Dr. Ankur Patwardhan
Research and Action in Natural Wealth Administration (RANWA),
c/o Mr. Bhushan Sathe
16, Swastishree Society, Ganeshnagar, Pune- 411 052.
E-mail: ankur_patwardhan@vsnl.net

Dr. R. Vasudeva
Associate Professor, Department of Forest Biology,
College of Forestry, Sirsi-581 401.
University of Agricultural Sciences, Dharwad, Karnataka.
E-mail: vasukoppa@gmail.com

A Workshop on

***Mappia foetida:* Prospects of a future crop**

Organized by
College of Forestry, Sirsi,
University of Agricultural Sciences, Dharwad

RANWA, Pune

Venue : College of Forestry, Sirsi
Date : 6th October 2007
Time : 9:30 am to 4:30 pm

RANWA

Rufford

Cultivation Techniques for *Mappia foetida*

Fruit collection and seed storage:
1) Ripen seeds are black in colour.
2) Fruits should be collected in the period of November - January
3) Fruits should be depulped and should be dried in shade.
4) Seeds should be stored in airtight containers under ordinary room temperature if not used immediately.
5) For nearly 100% viability, storage should not exceed 45 days.

Pre-sowing treatments:
1) Treatment with cow-dung slurry :
-Soak seeds with seed coat intact in fresh cow dung slurry for 24 hrs
-Remove seeds from slurry and sow them

2)Cold water treatment:
-Remove seed coat with pointed forceps without harming the seeds inside.
-Soak the seeds in cold water for minimum 12 hrs.

Sowing methodology:
Seed bed preparation:
1) Seeds should be sown immediately after pretreatment in a raised nursery bed of size 1.2 X 7.0 X 0.3 m^3.
2) Seed bed should be drenched with 0.1 % bavistin to protect against fungal attack.
3) Seeds should be sown at the depth of 2 cm in flat position.
4) Moist conditions should be maintained.

Seed bed protection:
Spray the seedlings and drench the soil with 0.2% solution of Carbendazim (Trade name- bavistin) once in 10 days to control fungal attack.

Seedling transplantation:
Seedling should be of height 8-10 cm before they are transferred to poly-bag (size 6"X9"), filled with soil: sand: farm yard manure (FYM) in 2:1:1 proportion along with 10g of finely powdered swine manure.

Fertiliser application:
1)After two months of growth, 1g DAP supplied to seedlings as a top dress Direct contact of fertilizer with root zone should be avoided.
Spraying insecticides such as 0.2% Dimethoate(trade name- Rogar) is recommended.

Vegetative propagation:

1)season for cutting- February to March
2)Preparation of cuttings- Branches of pencil thickness girth are given a slant cut at the basal end.
3)Application of growth hormones- IBA (Indole butyric acid) dose-2000ppm in dust form.
4)Rooting media- Sand : coir in 1:1 proportion.
5)Propagation Chamber- Chamber with temperature range 28°-33°C and Relative humidity-90%
6)Planting the rooted cuttings- Cuttings should be hardened for about 30 days before they are out planted.

Plantation of seedlings on field:

1) The seedlings should be grown in nursery for about 1 year before they are outplanted.
2) The pits of size 1ft X 1ft X 1ft should be used for plantation of the seedlings.
4) The pits should be filled with soil & cow-dung manure in 1:1 proportion to maintain porosity & hence prevent water logging.
5) Seedlings can be planted at 2m X 2m interval.
6) Preferably give a dose of fertilizers like DAP(0.5-1g) for about two times in first year after plantation for good growth.

A workshop on
Mappia foetida: Prospects of a future crop


About *Mappia foetida*

1. *Mappia foetida* is an endangered tree species from western ghats of India. It is commonly found along the crestline.
2. It occupies varied types of habitats such as moist deciduous,semi evergreen and secondary forest formations. However it prefers forest edges as microhabitat.
3. An alkaloid known as Camptothecin (CPT) is extracted from the stem and root bark of the species. This alkaloid is widely used in the treatment of cancer.
4. The plant is a good coppicer and is not preferred by cattle for fodder (non-browsable).
5. It is a good candidate to be incorporated in agro-forestry systems. It grows well under shade and prefers soil with good moisture content.
6. The species usually flowers during August and the fruits ripe till December. Thus December onwards one month is a good time for collection of seeds.

Cultivation Techniques for *Mappia foetida*


Seed Propagation

Pre-sowing treatments:
1. Seeds should be shade dried for a week before sowing.
2. Depulped seeds need to be kept in air-tight containers and should be sown within 45 days for maximum efficiency of germination.
3. Removal of seed coat and treatment with cow dung slurry enhances germination.

Sowing methodology:
1. Seeds should be sown immediately after pre-treatment in a raised nursery bed drenched with 0.1% bavistin for protection against fungal attack.
2. Seeds sown at the depth of 2cm in flat position.
3. Keep seed bed in moist condition.



Plantation on field

1. Seedlings should be grown in nursery for 1 year before they are outplanted.
2. The pits of size 1ft X 1ft X 1ft should be used for plantation of the seedlings.
3. The pits should be filled with soil and cow-dung manure in 1;1 proportion to maintain porosity and hence prevent water-logging.
4. Recommended spacing of about 2m X 2m for plantation.
5. Preferably give a dose of fertilizers like DAP(0.5-1g) for about two times in first year after plantation for good growth.

Vegetative propagation

1. Best season for stem cuttings starts from February to March
2. Branches with pencil thickness girth are cut with a slant cut at the lower end.
3. Treat the basal cut ends with IBA- 2000 ppm in dust form
4. Sand: coir (1:1) can be used as a rooting medium

Propagation chamber:
1. Chamber with temperature maintained between 28-33°C and relative humidity of 90% should be used.
2. Cuttings should be hardened for 1 month before plantation on field
3. Green house temperature should be maintained between 18-30°C with 60-70% relative humidity.


Dr. A. Patwardhan
RANWA and Garware College, Pune

Dr. R. Vasudeva
College of Forestry, Sirsi

Acknowledgements: Rufford Maurice Laing Foundation, UK

Annexure 4

Some key stakeholders interested in *N. nimmoniana* promotion

Name	Category	Activity
Mr. Vitthal Shinde	Farmer	Nursery / cultivation
Mr. Sharad Shinde	Farmer	Nursery / cultivation
Mr. Madhukar Desai	Farmer	Cultivation
Mr. Salunke	Farmer	Cultivation
Mr. Dilip Bhosale	Farmer	Cultivation
Mr. G.N. Gaikwad	Farmer	Cultivation
Mr. Michael Lewis	Farmer	Cultivation
Mr. Nitin Gudge	Forest Dept.	Cultivation
Mr. Puranik	Farmer	Cultivation
Mrs. Nalini Mahale	Individual	Cultivation
Mr. Dilip Yedve	Farmer	Cultivation
Mr. Jalinder Pawar	Farmer	Cultivation
Mr. Mansingh Shinde	Farmer	Cultivation
Mr. Suresh Kunjir	Farmer	Cultivation
Mr. Shivram Gavade	Forest Dept.	Nursery
Mr. Santosh Taware	Individual	Nursery
Mr. Ganesh Dhumal	Individual	Nursery
Mr. Babu Pawar	Individual	Nursery
Mrs. Anita Sawant	Self Help Group	Nursery
Mr. Atmaram Raul	Self Help Group	Nursery
Mr. Ashok Khadse	Forest Dept.	Promotion
Mr. B. H. Gaikwad	Forest Dept.	Promotion
Mr. Bhurke	Forest Dept.	Promotion
Mr. Sunil Limaye	Tribal Dept.	Promotion
Dr. Ajay Namedo	Academician	Research
Dr. Arun Chandrashekhar	Academician	Research
Mr. V. B. Avad	Academician	Research
Mr. Agavekar	Industry	Promotion
Mr. Menon	Industry	Promotion
Mr. Arvind	Industry	Promotion

RANWA comes to the rescue of Narkya plant

BY A STAFF REPORTER
reporters@sakalherald.com

KOTHRUD: With the development of nursery techniques, there is a ray of hope for the endangered medicinal plant Narkya or Amruta (Nothapodytes Nimmoniana) which is exploited for its anti-cancer properties.

The technique, which is developed by an NGO - Research and Action in Natural Wealth Administration, Kothrud, Pune (RANWA), if accepted can satisfy industrial demand of raw material without exerting pressure on the plant which has dwindled considerably. The detailed booklet was released by State Forest Minister Baban Pachpute at Garware College on Saturday.

NEW NURSERY TECHNIQUES

The plant found in the Western Ghats has great demand from the pharmaceutical industry the world over as it yields an anti-cancer alkaloid called Camptothecin (CPT). The species was cut illegally and supplied to Indian pharma companies and also smuggled abroad. RANWA was conducting a project on urban ecosystem with special reference to Pune-Mumbai urban belt when it was noticed that there was no continuous distribution of Narkya in the Western Ghat.

Earlier, an independent survey by RANWA from Mulshi to Amboli revealed that about 50 to 80 per cent of the Narkya population was already reduced, said Dr Ankur Patwardhan, Biodiversity lecturer at Garware College and honorary secretary of RANWA.

"We felt there was a need to explore the ways of plantation of Narkya so that it could be conserved", said Patwardhan adding that, the idea was also to provide some incentive to the local farmer population.

Thus, the project for standardising Narkya propagation techniques was taken up by RANWA in 2003-04 in association with Biodiversity Department students of Garware College.

Radhika Kanade, a Biodiversity student in Garware College and a researcher with RANWA said, "The first nursery adopting the standardised techniques was set up by Sharad Shinde, a farmer in Shilim village in Maval taluka. About 2,000 seedlings were produced and supplied to institutions and individuals at the rate of Rs 15 per plant."

"In Maharashtra, no big plantation has come up yet. But, in Karnataka efforts by Dr R Vasudeva Associate Professor of Department of Forest Biology, College of Forestry, Sirsi, are yielding good results." But the post-plantation activities like harvesting and transportation need to be streamlined which are time consuming, added Kanade.

'नरक्या'चे संवर्धन केल्यास स्वतंत्र बाजारपेठेची हमी

वनमंत्री पाचपुते यांचा विश्वास

पुणे, ता. १२ : "आजवर 'नरक्या' (अमृता) वनस्पतीची पन्नास टक्के तोड झाली आहे. त्याची नव्याने लागवड आणि संवर्धन करणे आवश्यक असून, तसे झाल्यास उत्तम हमी देणारी स्वतंत्र बाजारपेठ निर्माण होऊ शकते," असा विश्वास वनमंत्री बबनराव पाचपुते यांनी व्यक्त केला.

आबासाहेब गरवारे महाविद्यालयातील 'जैवविविधता विभाग' व 'रानवा' यांच्या वतीने राबविण्यात आलेल्या 'नरक्यासंवर्धन' प्रकल्पांतर्गत डॉ. अंकुर पटवर्धनलिखित 'नरक्या : लागवड आणि संवर्धन' पुस्तकाचे प्रकाशन श्री. पाचपुते यांच्या हस्ते झाले. त्या वेळी ते बोलत होते. महाविद्यालयाचे प्राचार्य सुभाषचंद्र भिडे, माजी वनसंरक्षक अधिकारी माधव गोगटे, ज्येष्ठ पर्यावरणतज्ज्ञ माधव गाडगीळ, 'रानवा'चे अध्यक्ष अजय दीक्षित, श्री. पटवर्धन या वेळी उपस्थित होते. "महाविद्यालयातील जैवविविधता विभागांतर्गत राबविण्यात येणाऱ्या संशोधन प्रकल्पासाठी वन खात्याच्या वतीने एक हेक्टर जमीन उपलब्ध करून देऊ," असेही त्यांनी सांगितले. श्री. दीक्षित यांनी प्रास्ताविक केले. राधिका कानडे यांनी सूत्रसंचालन केले. डॉ. मिलिंद वाटवे यांनी आभार मानले.

आबासाहेब गरवारे महाविद्यालय : डॉ. अंकुर पटवर्धनलिखित 'नरक्या : लागवड आणि संवर्धन' पुस्तकाचे प्रकाशन शनिवारी वनमंत्री बबनराव पाचपुते यांच्या हस्ते झाले. त्या प्रसंगी (डावीकडून) माधव गाडगीळ, श्री. पाचपुते, सुभाषचंद्र भिडे, डॉ. पटवर्धन.

Nursery technique of cultivating 'Mappia Foetida', widely used in the preparation of cancer drugs, has been researched and chronicled for the first time by RANWA. This is a welcome move for the conservation of wild medicinal plants in the country

Shot In The Arm

By Richa Bansal

Providing the much-needed research back up to the dwindling gene pool of wild medicinal plants, RANWA — a city-based environmental NGO — has for the first time researched and documented the nursery propagation of *Mappia Foetida* (commonly known as Amruta or Narkya), a plant used widely in the preparation of cancer medicine.

Found only in the wild, RANWA took up the research in October 2002, when a study by the Pune-based Medicinal Plants Conservation Centre (MPCC) revealed that the plant was an endangered species, with almost 80 per cent of its population lost.

"Because of the medicinal properties of its roots and stem, Mappia was being smuggled to other Indian states, Western European countries, the UK and Japan," says Dr Ankur Patwardhan, a scientist at RANWA involved in the research. "As a result its population dwindled. When there was a sudden realisation that the supply was not meeting even half of the demand, conservation came into focus," he adds.

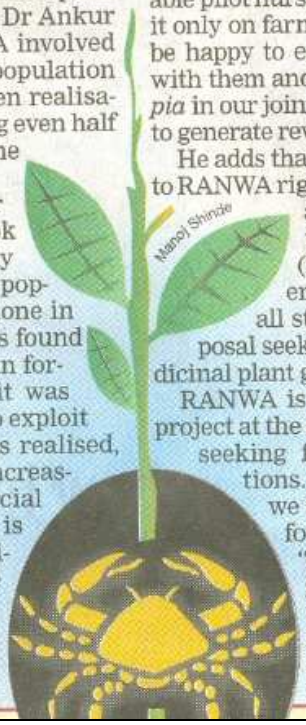
According to deputy conservator of forest (DCF), Pune, Ashok Khadse, smuggling was not the only cause for the decline in the plant's population. "Smuggling can only be done in reserve forest areas. But *Mappia* is found growing more in farmlands than in forest areas," says Khadse. "Since it was available in ample, farmers used to exploit it. However, once its shortage was realised, the only way of meeting the ever increasing demand was through commercial plantations. RANWA's research is thus commendable, as they have finally made it possible to cultivate and harvest *Mappia*," he adds.

Mappia Foetida is endemic only to the Indian subcontinent and was found abundantly in the districts of Satara, Kolhapur, Sangli and Sindhudurg in the Western Ghats of Maharashtra.

RANWA began its research by studying the ecology of the plant for the first two years. This included studying its flowering season, seed collection, understanding the right time for harvesting, the conditions under which it flourishes etc. "Once we had studied its ecology, we established a pilot nursery on farmland at Shilim village in Pune district," says Patwardhan. "We researched how its germination techniques can be best achieved by using seeds as well as cuttings," he adds. The NGO recently released a booklet detailing the process.

The forest department has shown a keen interest in the nursery cultivation of Mappia post RANWA's research. Says Khadse, "We are willing to give RANWA forest land near NDA, where they will have to carry out a commercially viable pilot nursery project. So far they have done it only on farmland. Once they do this, we will be happy to enter into a commercial venture with them and include the cultivation of *Mappia* in our joint forest management programme to generate revenue for the committees."

He adds that this land can be made available to RANWA right-away. Besides this, Khadse has also sought funds under the National Medicinal Plant Board's (NPMB) scheme to set up nurseries for cultivation of the plants in all states. "We have submitted a proposal seeking nearly Rs 2 crore for our medicinal plant garden at Kothrud" says Khadse.



RANWA is also starting a pilot plantation project at the same farmland at Shilim village, seeking funds from different organisations. "Through this pilot plantation, we will be able to take our research forward," says Patwardhan. "Moreover, not only will farmers gain by selling the harvest, *Mappia* has the added advantage of being a candidate species for agro forestry," he says.

विशेष

कर्करोग, एड्स या रोगांवरील उपचारासाठी अत्यंत प्रभावी असलेल्या नरक्या वनस्पतीला परदेशात प्रचंड मागणी आहे. अस्तित्व धोक्यात आलेल्या या वनस्पतीची रोपवाटिका उभारून पुन्हा तिचे उत्पादन सुरू करण्यासाठी पुण्याच्या तरुण शास्त्रज्ञाने प्रकल्प सुरू केला आहे, त्या विषयी...

नरक्या जगवा, पैसे कमवा!

नरक्या ही भारतीय मातीतील आयुर्वेदिक वनस्पती. काही वर्षांपूर्वी फारशी माहिती नव्हती. जैविक विविधतेसाठी प्रसिद्ध असलेल्या पश्चिम घाटात ती मुबलक होती. काही व्यक्ती या वनस्पतीचे महत्त्व जाणून होत्या. कर्करोग, एड्स या रोगांवर उपचारासाठी ती अत्यंत प्रभावी असून, परदेशात तिला प्रचंड मागणी आहे. या वनस्पतीच्या विक्रीतून लाखो रुपये मिळविता येतात, हे काही जण जाणून होते. त्यांनीच या वनस्पतीची बेसुमार तोड करून तस्करी सुरू केली. हा व्यवसाय चांगलाच फोफावला. गेल्या दहा वर्षांत ८० टक्के वनस्पती भारतातून नाहीशा झाल्या. तस्करीची पाळेमुळे खोदेपर्यंत नरक्याची मौल्यवान मुळे मात्र नावालाच उरली होती.

पुढे तस्करीवर नियंत्रण आणण्यास वनखात्याला यश आले. आता मुख्य काम होते नरक्याच्या बचावलेल्या झाडांचे संवर्धन करणे. एवढेच नाही तर रोपवाटिका उभारून पुन्हा तिचे उत्पादन सुरू करणे आणि झाडांची संख्या वाढवणे.

पुण्याच्या आबासाहेब गरवारे महाविद्यालयात सूक्ष्म जीवशास्त्र विभागात प्रपाठक असलेले डॉ. अंकुर पटवर्धन यांनी या कामाला हात घातला आहे. डॉ. पटवर्धन गेल्या काही वर्षांपासून मावळ (जि. पुणे) तालुक्यात स्थानिक शेतकऱ्यांच्या मदतीने 'नरक्या'वर प्रकल्प राबवत आहेत. रानवा या पुण्यातील स्वयंसेवी संस्थेने या प्रकल्पासाठी पुढाकार घेतलाय. त्यातून रोपे तयार करणे, ती वाढवण्याचे तंत्रज्ञान डॉ. पटवर्धन यांनी विकसित केले आहे. याविषयीची पुस्तिकाही नुकतीच त्यांनी प्रकाशित केली आहे.

डॉ. पटवर्धन प्रकल्पाविषयी म्हणाले, "नरक्या वनस्पतीला अमृता असे म्हणणे योग्य ठरेल. या वनस्पतीच्या मुळात व खोडात कँप्टोथेसिन नावाचे अल्कलाईड असते. त्याची पावडर करून ती विकली जाते. कर्करोगावरील उपचारासाठी त्याला जगात महत्त्व येत आहे. ही पावडर १६०० रुपये किलो आहे. शुद्ध उत्पादनाला आंतरराष्ट्रीय बाजारात तब्बल एक लाख रुपये किलो भाव आहे. नरक्याची आंतरराष्ट्रीय बाजारपेठेत मागणी ४०० ते ५०० टन आहे. पुरेशा उत्पादनाअभावी पुरवठा त्याच्या ५० टक्केही नाही. जपान, इंग्लंड, अन्य युरोपीय देशांना तिची निर्यात होते."

मावळ, मुळशी प्रकल्पात रोपे वाढवण्याबाबत विविध प्रयोग केल्याचे पटवर्धन यांनी सांगितले. बी पेरणीपूर्वी विविध बीजप्रक्रिया करून पाहिल्या. त्यातून ७० ते ७५ टक्के उगवणक्षमता मिळवण्यास आम्हाला यश आले. छाटे पद्धतही वापरली, तीही यशस्वी होते असे आढळले. छाटे पद्धतीचा फायदा असा, की ज्या झाडांमध्ये औषधी द्रव्याचे प्रमाण चांगले आहे, त्यांची निवड करून अधिक अर्क देणाऱ्या नव्या जाती विकसित करणे शक्य होते. बी पेरण्याचा कालावधी, वाफा कसा घ्यायचा, रोपे उगवून आल्यानंतर घ्यावयाची काळजी, पुनर्लागवड करण्याची पद्धत या प्रत्येक

डॉ. पटवर्धन

टप्प्याचा सविस्तर अभ्यास केला. त्यातून रोपे तयार करण्याची पद्धत विकसित केली.

बांधावर लागवड करण्यासाठी दोन बाय दोन मीटर अंतरावर लागवड केली जाते, अशा तऱ्हेने हेक्टरी दोनशे झाडे बसतात. शेतात लागवड करायची असेल तर एकरी एक हजार झाडे बसतात. प्रत्येक झाडामागे एक किलो जैविक वस्तुमान मिळते. धारवाड कृषी विद्यापीठांतर्गत शिरसी येथील वन्य महाविद्यालयातील सहायक प्राध्यापक डॉ. आर. वासुदेव यांचाही महत्त्वाचा सहभाग होता. त्यांच्या पुढाकाराने कर्नाटकातील शेतकऱ्यांनी शंभर एकरवर नरक्याची लागवड सुरूही केलेली आहे.

पंधरा रुपये अशी एका रोपाची किंमत आहे. शेतकऱ्याने एकदा प्रशिक्षण घेतल्यानंतर तो स्वतः रोपे तयार करू शकतो, वाढवू शकतो व संवर्धनही करू शकतो. संबंधित प्रकल्प पुढे सुरू राहणार आहे. वनस्पतीतील औषधी द्रव्य मुळे व खोडांसोबत बिया व पानांमध्येही आढळते. मात्र पानांमधून ते अधिकाधिक कसे मिळेल यावर आम्ही अधिक संशोधन करणार आहोत असेही पटवर्धन म्हणाले. नरक्यासाठी बाजारपेठ कोठे उपलब्ध आहे याविषयी विचारले असता ते म्हणाले, की भारतात या वनस्पतीची गरज असणाऱ्या संस्था किंवा खोत उपलब्ध आहेत. त्यांच्याविषयीची माहिती शेतकऱ्यांना दिली जाईल. आमचे मुख्य काम वनस्पतीवर शैक्षणिकदृष्ट्या संशोधन करणे आहे. बाजारपेठेत आपली फसवणूक होणार नाही याची जबाबदारी मात्र शेतकऱ्यांनाच घ्यावी लागणार आहे.

नरक्याच्या विकासासाठी वनमंत्रीही प्रयत्नशील

वनस्पतीतील औषधी गुणधर्म व आंतरराष्ट्रीय बाजारपेठेत असलेली त्याची किंमत लक्षात घेऊन अधिकाधिक अर्क देणाऱ्या जातींवर संशोधन करणे गरजेचे असल्याचे मत राज्याचे वनमंत्री बबनराव पाचपुते यांनी नरक्या वनस्पतीच्या पुस्तिका प्रकाशनावेळी व्यक्त केले आहे. नरक्याच्या विक्रीतून शेतकऱ्यांचा फायदा होण्यासाठी संयुक्त वनव्यवस्थापन समितीच्या माध्यमातून नरक्यासंबंधीचे धोरण आखले जाणार असल्याचेही त्यांनी म्हटले आहे. तेंदू पत्त्यात अशा प्रकारचे धोरण राबवून आदिवासी लोकांचा फायदा झाला आहे. नरक्या संशोधनासाठी गरवारे महाविद्यालयाला एक हेक्टर जागा उपलब्ध करून दिली जाईल, असे आश्वासनही वनमंत्र्यांनी दिले आहे.

अधिक माहितीसाठी :
डॉ. अंकुर पटवर्धन (९८२३८१२६५३)

- मंदार मुंडले