PN-ABQ-485

Genetic Improvement of Neem: Strategies for the Future

ceedings of the
ernational Consultation
Neem Improvement
d at Kasetsart Universit
ngkok, Thailand,
- 22 January 1993

Errata

On the back of the title page the International Development Research Centre (IDRC) should be included as one of the contributing agencies.

Page 16, para 4, line 4 the citation Sastry *et al.* should be Sastry and Agrawal.

Pages 79, 80, 84 and 179 the National Academy of Science should be changed to the National Academy of Sciences.

Pages 89, 90 citations for Surendran *et al.* should be 1993 not 1992.

Page 132, line 5 the citation Foster *et al.* should be changed to Cady *et al.*, 1988.

Page 140, para 3, line 2 the citation for Lauridsen should be 1992 not 1991.

Page 185, the reference for Mungkorndin S. should read Mungkorndin, Sompetch. Spotlight on species: *Azadirachta excelsa, Farm Forestry News* 6(1):9-11.

Genetic Improvement of Neem: Strategies for the Future

proceedings of an international consultation
held at Kasetsart University, Bangkok, Thailand
18 - 22 January 1993

editors:
Michael D. Read
James H. French

1993

Winrock International Institute for Agricultural Development
Forestry/Fuelwood Research and Development Project
(funded by USAID)

The Forestry/Fuelwood Research and Development (F/FRED) Project, funded by the U.S. Agency for International Development, is designed to help scientists address the needs of small-scale farmers in the developing world for fuelwood and other tree products. It provides a network through which scientists exchange research plans, methods, and results. Research and development activities center on the production and use of multipurpose trees that meet the several household needs of small farmers.

F/FRED is being carried out by the Winrock International Institute for Agricultural Development. Winrock is a private, nonprofit U.S. organization working in agricultural development around the world. It was established in 1985 through the merging of the Agricultural Development Council (A/D/C), the International Agricultural Development Service (IADS), and the Winrock International Livestock Research and Training Center. Winrock's mission is to improve agriculture for the benefit of people-to help in crease the agricultural productivity, improve the nutrition, and advance the well-being of people throughout the world. Winrock's main areas of emphasis are human resources, renewable resources, food policy, animal agriculture and farming systems, and agricultural research and extension.

Correct citation

Read, Michael D. and James H. French, eds. 1993. *Genetic Improvement of Neem: Strategies for the Future.* Proc. of the International Consultation on Neem Improvement held at Kasetsart University, Bangkok, Thailand, 18 - 22 January, 1993. Bangkok, Thailand: Winrock International. 194 + x pp.

ISBN 0-933595-84-0

Cover: Neem windbreak in Majjia Valley, Niger. A neem decline syndrome in West Africa prompted international attention that lead to the organization of the International Consultation on Neem Improvement. (*photo by Rick Van Den Beldt*)
Insert: Somyos Kijkar, (right) Director of the ASEAN-Canada Forest Tree Seed Centre in Muak Lek, Saraburi Thailand answering questions of Neem Consultation participants regarding propagation of *Azadirachta indica* var *siamensis*. (*photo by James H. French*)

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Contributing Organizations:

Food and Agriculture Organization of the United Nations (FAO)
Centre de coopération internationale en recherche agronomique pour le développement (CIRAD-Forêt)
Comité permanent inter-Etats de lutte contre la sécheresse dans le Sahel (CILSS)
Danida Forest Seed Centre
ASEAN - Canada Forest Tree Seed Centre
Kasetsart University

Printed by: Craftsman Press Ltd.

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Acknowledgements

Preparation of this proceedings was a team effort — drawing upon the energy of friends from Europe, Africa, Asia and the Americas. While core funding was provided by USAID, this event would not have been possible without the support of Hélène Joly (CIRAD-Forêt) who obtained funding from the EEC for African participants. Elmer Lauridsen (Danida - Forest Seed Center), Oudara Souvannavong (FAO - Forest Resources Development Branch) and Cherla Sastry (IDRC) helped sponsor participants and assisted in preparations. The personal interest shown by K. Vivekanandan (FAO - FORTIP), the late Y.S. Rao (FAO - RAPA) was also greatly appreciated. Several Thai organizations were instrumental in making the meeting a success. Suree Bhumibhamon helped organize the conference and served as liaison with Kasetsart University and the National Research Council of Thailand; Somsak Sukwong, Director of RECOFTC, provided meeting and accommodation facilities; Somyos Kijkar, Director of the ASEAN-Canada Forest Tree Seed Centre, organized the study tour. Day-to-day organization of papers, support services and logistics were expertly handled by F/FRED staff. Norma Adams and Rita Butler, from the F/FRED office in Arlington, edited first drafts of several of the papers. Supplemental technical editing was handled by Kamis Awang and Rick Van Den Beldt, while clarification of statistical questions and presentation of charts were provided by P. Venkateswarlu and Atchara Jantrasaengaram. The burden of typesetting and entering of multiple draft corrections was conscientiously done by Leela Wuttikraibundit.

Finally, we are indebted to all of the participants who prepared papers, contributed to the lively discussions and developed a sense of camaraderie during our week-long consultation.

Foreword

The initial stimulus for this consultation on neem improvement arose as a response to the neem dieback syndrome which hit Sahelian Africa in mid-1991. Gerard Vander Berg, a CARE forester in Niger was the first to bring this syndrome to the attention of the world. George Taylor, of the USAID mission in Niger, worked effortlessly to assemble teams of experts to study the dieback and kept colleagues on four continents informed of the status. The idea of an Asian conference to address the issue of international germplasm collection and exchange was considered simultaneously by at least three organizations and eagerly endorsed by national programs throughout South and Southeast Asia, where neem is endemic. F/FRED's involvement was formalized in early 1992, when a Memorandum of Understanding was signed between Winrock International and CIRAD-Forêt to initiate action on this idea.

Planning for the neem consultation was coordinated by Michael Read (F/FRED), Hélène Joly (CIRAD-Forêt), and Oudara Souvannavong (FAO, Rome). The consultation enjoyed a high level of participation. After the meeting, national scientists in Asia were contracted by FAO (Rome) and the F/FRED Project to collect seed from 23 provenances which were exchanged within the region by F/FRED and shipped to Africa and Latin America by DANIDA. This effort had mixed results but, for the most part, seedling nurseries are now in place. These will provide the basis for a series of observational trials in each of the participating countries.

Efforts such as this consultation exemplify the networking approach that has been advocated by F/FRED as an alternative form of technical cooperation. This approach draws upon the combined expertise of scientists from many countries and backgrounds who posses a common mission. Much work remains to be done on neem. Research is needed on seed storage and germination, further seed collections are needed to cover gaps left in the first series of exchanges and multilocation provenance trials must be nurtured and monitored. Some have requested a second international consultation on neem for 1994 to address these and other issues. We certainly hope this will come to fruition. In any case, I believe that the goodwill and cooperation built during this conference will go far to realize completion of these pending tasks.

Rick J. Van Den Beldt
Bangkok, Thailand
October 1993

Preface

As neem begins to receive the international recognition it so richly deserves, farmers and landowners the world over have begun to ask for improved seed and seedlings to plant on their land. Unfortunately, these are not available at the present time. The research community has yet to begin the much needed task of selecting and breeding superior germplasm.

The importance of neem as a multipurpose tree species and lack of progress on its improvement prompted establishment of the International Neem Improvement Network. The network was formed to organize individuals working on neem into a cohesive association to exchange seed and information. To date, there has never been a successful international collection of neem from its naturalized range or a systematic exchange and evaluation of provenances. It was to plan such an activity that the inaugural meeting of the International Neem Improvement Network was organized.

The objectives of this first meeting were 1) to exchange results of research related to genetic improvement, 2) discuss the status of neem research in Asia and Africa, 3) plan a set of international provenance trials and 4) identify priority supplemental studies which are needed. The first two goals were met by presentation and discussion of volunteered and invited papers. The second two were achieved by setting up working groups and asking the participants to develop a plan of work for seed exchange and to propose supplemental studies.

Section one of the proceedings consists of papers presented on neem improvement research in India. Neem is native to India and is highly revered there. Numerous scientists are investigating all aspects of the botany, silviculture, chemistry, and various uses of neem in its homeland, and the four papers presented in this section only touch on some of the fine work being done. They were invited because they focus on issues related to neem improvement.

Section two consists of country reports presented by Asian participants and a summary of papers presented by African delegates. These presentations cover all aspects of neem in their respective countries, not only improvement, because so little improvement work is being carried out except in India.

Section three contains overview papers on the importance of neem to small-scale farmers, pests and diseases of neem, the influence of genotype and environment on chemical compounds in neem, vegetative propagation, and the role that networks can play in supporting national research programs.

Section five is arguably the meat of the conference. It starts out with an invited paper on neem provenance collection and seed handling prepared by specialists from DANIDA and FAO. Using the presentation as a springboard, a working group was asked to make recommendations for neem seed collection, handling and exchange.

They were also asked to develop guidelines for establishing international provenance trials. The results of the working group deliberations are presented as the second paper in this section.

Section six complements section five. This section opens with a discussion of several technical constraints to exchanging neem seed and establishing international provenance trials. Though provenance trials are a necessary first step, complementary research is needed if researchers are to maximize their effectiveness and progress as quickly as possible. The second paper is a list of research priorities for supplemental studies that was prepared by a working group.

The editors of this volume hope that the proceedings will act as a catalyst to stimulate thinking and action among researchers interested in neem improvement. It is encouraging to note that the work plan for seed exchange developed during the conference is already being implemented and that valuable that experience has been gained. To date, seed has been exchanged among five countries in Asia. Seed shipments have been also been sent to Africa and Central America from Thailand and India.

The enthusiasm generated by the First International Consultation on Neem Improvement bodes well for the future. It is hoped that interest will intensify and that a follow-up consultation can be organized to build upon the momentum of the first.

Michael D. Read
Network Specialist and
Neem Coordinator

29 September 1993

Opening Remarks

Banpot Napompeth
Vice-president for Research, Kasetsart University,
Bangkok, Thailand

It is with great pleasure that I open this conference on behalf of Kasetsart University. Neem is growing in importance yearly and it is an honor that Thailand has been selected as the venue to discuss its genetic improvement.

Neem is emerging as one of the most important reforestation and agroforestry species in dry areas around the world. It has been extended well past its native range in South and Southeast Asia and is now found throughout much of Africa, the Caribbean, and Latin America. When one speaks of multi-purpose trees, neem emerges as perhaps the quintessential model. The wood is used for fuelwood, sawn timber and construction while the tree performs a valuable service in shelterbelts and as a source of shade in arid areas.

However, the true potential of neem is made clear only when we look at the traditional uses in its native range. These are many. Neem is used to manufacture soaps, waxes, lubricants, fuels, soil amendments, cosmetics, and pharmaceuticals. The leaves and flowers of selected trees are eaten in Thailand, and used as fodder in many countries.

As a plant protection specialist, I am aware of neem's important role in insect control. There has been a lot of work done on its role as a medium-spectrum insecticide; its effect on over 200 insect pests has already been documented. It may also have an important role to play in future control of nematodes, snails, fungi, and perhaps viruses.

The purpose of this consultation is not to discuss the many uses of neem, however. That will be done during the World Neem Conference which will be held in Bangalore, India next month. You are here to discuss and design an improvement program.

It is my sincere hope that your initiative does not end with mere publication of the minutes of this meeting. That would be an inestimable loss. What we need is action — a concerted effort to follow up on your own recommendations. You must strive to execute the plan of work you will formulate during this meeting. We need to get on with the genetic improvement of *Azadirachta indica*. And we need to coordinate this work internationally for maximum effectiveness.

One of the first tasks before you is to answer the question, "improvement for what purpose?" Tree growth or form? Oil yield? Insecticidal properties? Which of the many uses are you going to address.

There are many other problems with neem improvement work, especially when one speaks of an inter-regional effort. It flowers and sets seed at different times throughout its range. This makes coordinated seed collection difficult. So much seed has been moved around in its range over the last thousand years that its true center of origin is not clear.

After the seed is collected, there is the problem of storage and shipping. Most reports describe the seed as recalcitrant. But this has not been ascertained for certain and requires further study. Ways to harvest, store, transport and handle the seed to ensure its viability are lacking.

Despite the difficulties, improvement work is underway in individual countries. Here in Thailand, I hope you will be able to visit the field planting of the Royal Forest Department and see some of the neem trees in our farmers' fields. I further hope these examples will provide stimulus to your discussions.

On behalf of Kasetsart University, I would like to extend my cordial welcome to all of you and wish you the greatest success in your deliberations over the next week. May you enjoy your stay in Thailand

Thank you.

Neem Improvement in India

Section 1

Neem Research at ICFRE

D.N. Tewari
Indian Council for Forestry Research and Education (ICFRE),
Dehra Dun, India

Neem (*Azadirachta indica* A. Juss.) is an attractive evergreen tree (deciduous in drier areas) native to the Indian subcontinent. It is also cultivated throughout Southeast Asia, Australia, East and sub-Sahelian Africa, Fiji, Mauritius, and many countries in Latin America. A host of products are obtained from the neem tree, many of which have potential use in pest management programs. Neem is a fast growing and sturdy tree which can be established without irrigation in hot and dry regions with low annual rainfall of 500 mm or less. It grows well on poor, shallow, stony or sandy soil where agricultural crops give low yields despite application of fertilizers. The extensive root system of neem has the unique physiological capacity to extract nutrients from highly leached sandy soils. Under plantation conditions the nutrients are subsequently restored to the soil by the litter of fallen leaves and twigs.

Many regard neem a "wonder plant." Enthusiasm about the tree, however, is largely based on anecdotal evidence. Its active principle, a group of about twenty chemicals, needs to be further explored. Research on tree improvement, physiology, entomology, pathology, chemistry and soils are weak. Other studies may include its potential use in fertilizers and animal feed. To optimize production and utilization of neem, a number of investigations have been carried out at ICFRE:

- Tree improvement
    - Germplasm collection/maintenance from within the country and abroad.
    - Identification of plus trees in existing stands and their assemblage in clonal banks.
    - Vegetative propagation and tissue culture.
    - Development of tree ecotypes of the highest quality in terms of azadirachtin content and other desirable compounds.
- Physiological
    - Growth development and yield studies of different provenances under different bioclimatological zones.
    - Studies of photosynthetic and transpiration rates of promising provenances to understand the capacity of neem to growth in shallow soil and under low rainfall conditions.
- Entomological
    - Studies on insect pests of neem, and their control.

  - Bioassay of neem constituents particularly antifeedant (azadirachtin) and oviposition deterrents.
- Pathological
  - Studies on diseases of neem and their control.
- Chemistry of neem constituents
  - Evaluation of various genotypes for their oil, gum, and other pharmaceutical and insecticidal yields.
  - Development of an effective toothpaste.
  - Evaluation of the anti-bacterial and antiviral qualities of neem.
  - Examination of the potential of developing a male contraceptive from the neem leaf.
  - Examination of neem as a prophylactic treatment against Japanese encephalitis.
  - Examination of neem's potential as a hypotensive drug for the treatment of epilepsy.
  - Experimentation with other neem compounds for treating other diseases.
- Silvicultural
  - Study of the effects of neem trees on physical, chemical and biological properties of different soils.
  - Study of the nature and properties of soil humus under neem and the use of neem as a soil ameliorant.
  - Study of the effects of soil moisture stress on neem growth.
- Other
  - Investigating the potential of neem cake as animal fodder.
  - Determining the efficiency of neem enriched urea based fertilizers.

Physical Traits

This paper briefly reviews the results of these studies, which have been previously published in ICFRE reports.

Morphology

Neem grows to a height of 12-15 m (rarely up to 25m) with a short and stout bole of between 3-7.5 m in height and 1.9-2.8 m in girth. The tree generally branches early, forming a broad round crown of bright green foliage. The tree has a moderately thick bark with scattered, small tubercles between numerous longitudinal and oblique wrinkled furrows, dark gray outside and reddish inside. The leaves are imparipinnate, 20-38 cm long, crowded near the branch end; leaflets are 9-13 cm, nearly opposite 2.5-7.5 x 1.2-4.0 cm, oblique, lanceolate, deeply and sharply serrate, glabrous on both surfaces, base inequilateral, acute; petiolules very short. The inflorescence is auxiliary, many flowered, panicle with white, fragrant, shortly pedicelled, pentamerous flowers; both bisexual as well as male occurring on the same tree. Flowers have minute lanceolate and caducous bracts. The calyx is puberulous outside, 5-lobed to the lower half, lobes imbricate, round-ovate, minutely ciliolate. Petals number 5, free, imbricate, 6 mm long, obovate-oblong, faintly puberulous outside ciliolate. Staminal tube are 3-5 mm high, cylindrical, slightly expanded at the mouth, glabrous; terminated by 10 rounded, truncate, emarginate, or bilobed appendages, apiculate, inserted at the base of and opposite to appendages, little shorter than them. Discs are angular, fused with the ovary base. The ovary is glabrous, 3- celled, 2 ovules in each cell, placentation axile; stigma expanded to form a ring with 3- acute, partially fused, papillose stigmatic lobes, included in the staminal tube. The fruit is a drupe, 12-18 mm long, ovoid-oblong, yellowish-green smooth dark yellow and purple when ripe; endocarp thin, cartilageous, with inter-cellular space between the epicarp and the endocarp; walls breaking down to form the mucilaginous mesocarp, seeds 1-2 reticulate. The outer seed-coat has thick-walled epidermis and three layers of loosely arranged cells; the cells of the inner integument elongates tangentiall, to form the inner seed coat.

The seedling

The seeds germinate epigeally. Roots are initially white, later turning pale. The hypocotyl is erect, tapering upwards, smooth and glabrous with 2 epigeous, unequal cotyledons which are fleshy, green on both surfaces and glabrous. Later, as the seedling develops, an erect, greenish brown stem takes shape. Initially leaves are opposite and spiral. The top of the seedling appears glabrous or with minute colorless glands.

The Pollen Grain

The pollen grains number 3 or 4; colporate, prolate-spheroidal or sub-prolate having a smooth exine which is slightly thickened at the apertures.

The Embryo

The embryo proper is found to be derived from both the apical and basal cells of the two-celled embryo. Placentation is partial. The ovary is trilocular at the base becoming unilocular in the ovule-bearing region. Only one ovule develops into a seed. Several cases of twin embryos have been detected in different stages of development, either zygotic in origin or developed from one of the synergids.

Anatomy

Leaf

The adaxial epidermal cells are slightly elongated with straight or slightly undulating walls. The abaxial epidermal cells at intercostal zones are straight to slightly undulating. Costal cells are arranged in rows. Druses are present in the epidermal cells. Stomata are either absent or very few on the adaxial surface. On the abaxial surface, oval shaped stomata occur in very large numbers. These are closely distributed, large, wide open and slightly raised above the surface. Apart from the normal stomata, there are four types of aberrant stomata found. Hairs, when present, are costal, unicellular and multicellular, and sparse to common. Cork warts are occasionally observed on both surfaces of the leaf.

Bark

The bark varies in appearance and thickness according to the age of the part of the tree and, to some extent, to environment and climate. Bark on the younger stem and branches is smooth, soft and greenish slightly rusty in colour and occasionally having alternating green and rusty green longitudinal bands. Its thickness varies from 1.25 to 2.5 cm. The outer rind is rough, woody, very much fissured, often peeling in fairly thick slices. The cambium bark, when exposed has a rose-purple to reddish or purplish-brown color. In a transverse section it appears differentiated into three regions, a narrow purplish peripheral part a whitish middle and, a fairly thick innermost region composed mostly of secondary bast and appearing tangentially lamellated with alternating dull yellowish white and whitish straitions. The bark has a garlic-like odor and a bitter astringent taste. The hard outer rind is absent in the young bark and a distinct phellogen is discernible.

Root

The stout and woody root system consists of a comparatively short taproot and a number of long horizontally lateral roots and their branches. External appearance and internal structure of the bark are usually similar in all roots irrespective of their size, but the relative thickness and degree of hardness of the outer portions of the bark, as well as the texture of the wood, varies in accordance with the age of the root and, to some extent, with the nature of the soil.

The root surface is profusely covered with numerous large narrowly oblong lenticles, arranged closely in regular longitudinal and intermittent transverse or annular rows, imparting a rough appearance to the bark. As in the case of stem bark, the officinal tissue in the root bark is differentiated into a leathery peripheral or rosy part, a somewhat mealy lustrous starchy white, soft middle region and a fibrous tangentially stratified inner portion. The wood is light yellow and in its transverse section, with a large number of pores and fine meduliary rays visible. Highly porous in the younger portions, the wood becomes denser and more compact as it grows older.

Wood

The sapwood is yellow to yellowish-grey, turning pale yellowish-brown on exposure. The heart-wood is reddish to reddish-brown, darkening on exposure. The wood is somewhat lustrous, hard to very hard, usually heavy (air-dried specific gravity 0.72-0.83); interlocked grained, sometimes exhibiting ribbon-grain effect on the longitudinal surfaces; usually medium to somewhat coarse-textured, occasionally a little finer in texture when fast grown; a good pattern is noticeable in plain sawn boards due to parenchyrna bands as well as small knots.

Silvicultural Studies

Neem tolerates fairly heavy shade during early stages of growth, although not without a retarding effect on the seedlings. It is frost tender, and cannot tolerate excessive cold specially when young. It is drought hardy. It does not tolerate excessive moisture and water-logging conditions. Neem is fire-sensitive. It coppices well, withstand pollarding and produces root suckers.

Reproduction

Neem regenerates naturally from seed as well as by coppice and root suckers. The short-lived seed, maturing during rainfall, readily germinates on the ground forming a thick carpet of seedlings. Once established, the seedlings are difficult to eradicate. Neem seeds have a very short viability which drops rapidly after two weeks due to fermentation of the unopened cotyledons inside the inner seed case. Seeds must be sown within 2-3 weeks of their collection. Germination requires 1-2 weeks. The beds need sparse watering. Young plants need protection against frost. Seedlings can also be raised vegetatively through micro-propagation. Air layering of shoots also yields encouraging results.

Plantation

Neem is easily raised through direct sowing, polypot seedlings or root-shoot cuttings. For degraded areas, direct sowing is more successful and economical if adequately protected during the early stages. Polypot seedlings or root-shoot cuttings are more relevant for agroforestry, silvi-pastures and roadside avenue plantations. Successful direct sowing depends on edaphic, climatic, biotic and economic factors prevalent in the area. Strip weeding of young plantations promotes health and survival. Ordinarily, two weedings are sufficient in the first year. One weeding is necessary during the second year. Ordinarily, the first mechanical thinning is required at the age of 3-4 years in the case of direct sowing and 5 years in the case of transplanted seedlings. The rate of growth of neem in plantations varies depending upon the quality of soil. The plants attain a height of 4 m at 5 years and 10 m at 25 years. Neem has a mean annual increment of 2.3-3.0 m^3.

Genetic Studies

The Forest Research Institute at Dehra Dun undertook a survey of different populations of neem as a part of an Indo-Danish Project on tree and seed improvement to undermine the variability associated with place of origin. Seed was collected from 20 sources in 8 states of the country. Large variation was observed in seed weight (105g/1000 to 347g/1000 seed) and leaf length (2.3 cm to 7.2 cm). An experimental plot was also established for future studies. Callus initiation was obtained from the surfaces of sterilized young leaves and petioles of neem. Researches have also succeeded in inducing morphogenesis of the adult embryo of neem. Successful air layering has also been achieved using NAA and IBA. Rooting has been induced in branch cuttings under mist conditions.

Insect Pests And Their Management

Native Pests

In India, 38 species of pests have been recorded. Neem is frequented by species of seed and flower insects (5 species), defoliaters (13 species), root feeders (8 species), many species of sap sucking insects and stem and shoot borers. In addition, two species of mites and one of snail have been reported to attack neem leaves and tender shoots Seed and flower insects include *Araeceurs sutularis, Erythrothrips asiaticus, Otherios fullonica, Pallaloria camalliae* and *Scirtothrips dorsalis*. The stem and shoot borer pests include *Endoclita undulifer*. Defoliators include *Ascotis scelenaria, Boarmia variegata, Cleora cornaria, Cryptocephalus ovulum, Eurema spp., Lasperyesia aurantiana, L. koenigiana, Myllocerus dorsatus, Odites atmopha, Orthacris smylans, Solenopsis* spp, *Crioceris hamsoni*, and *Cryplocephalus aequalis*. The defoliator damage due to larval feeding can be checked by Foliar spraying of Folithion (0.:-0.2%) water emulsion spray.

Most important among sap suckers are *Pulvinaria maxima, Aonidiella (Aspidiotus) orientalis* and helopeliated with disease (*Helopeltis antoni*). Others include *Calipitrimerus azadirachtae, Ceroplastes ceriferus, C. pseudoceriferus, Elaphrithrips* sp. *Heliothrips haemprrhodalis, Kerria lacca, Megaroelum esmedorae, Pulvinaria azadirachtae* and *Taeniothrips longistylus*. Sap sucking arthropods recorded from neem nurseries and plantations include *Dialeurodes armatus, Elaphrothrips* sp., *Lancaniodiaspis azadirachta, Lacanium* sp., *Lepidosaphes meliae* and two species of mites, *Tetranychus fici* and *Tetranychus* sp. Notable among root feeders found are *Holotrichia consanguinea* and *Odonototermes obesus*.

About 15-20% neem seedlings were found to be killed by an unknown species of snail in almost all the neem nurseries at Mehsana and Gandhinagar in Gujarat. These were controlled either by hand picking or sprinkling a salt solution.

Insect Managing Properties

The antifeedant property of the neem seed kernel against a desert locust, *Schistocerca gregaria* was reported in 1962. Since then, much research has been done in India on the chemistry of neem constituents and their effect on insect pests. In agriculture most attention has been focussed on foliage feeding and defoliating insects. In forestry, emphasis has been a control of a poplar defoliator. Neem insecticide has also been found to be very effective against stored-grain pests.

Biological Activity

Neem extracts influence insect activity and behavioral ecology. It acts as a feeding deterrent, growth disrupter, repellent, ovipositional deterrent and as an insecticide. Extracts have been found to impair egg production and hatchability in insects.

Antifeedant Activity

This the most important pesticidal activity of neem seed extracts. Its effectiveness on as many as 100 species of insects has been established.

Insect Growth Regulators

Neem extracts have been found to act as mimics of insect growth regulato.s. Over two dozen insect species ha 'e been found to be affected by neem extracts. Treated insects show a remarkable reduction in their development, growth, and egg production.

Pathological Studies

Fungi causing damage to neem include *Cercospora subsessilis*, *Fusarium* sp., *Colletotrichum gloeosporiodes*, *Alterdnaria alternata*, *Rhizoctonia solani*, *Oidium azadirachtae*, *Corticium salmonicolor*, *Diaporthe* sp. and *Xylria azadirachtae*. Seed in storage is frequently contaminated with *Aspergillus* spp.

Nursery Diseases

Damping off, leaf web blight, leaf spotting and blight are the major reported nursery diseases of neem. Damping off causes particularly great losses in the nursery. *Fusarium oxysporum* has been reported to be associated with the disease. Use of formalin soil treatment and application of Thiram as soil mix and as a seed dressing controls the disease. Leaf web blight is caused by *Rhizoctonia solani*. This disease, which appears at the onset of monsoon, assums serious proportions from mid-July to August. Incidences as high as 100% occur in humid areas and can result in 30-100% defoliation. The disease can be controlled by adopting proper sanitation and cultural practices.

Serious forms of leaf spotting and blight were reported for the first time at the New Forest, Dehradun. It is caused by *Colletotrichum gloeosporioides*. The fungus causes leaf spots which rapidly increase in size. These appear in the nursery at the end of September or first week of October. Disease incidence as high as 100% has been recorded. The fungus, *Alternaria alternata* also caused leaf spotting and bllight during October-November, damaging 80-100% of the leaf area. This is easily controlled by application of Blitox. The fungus, *Pseudospora (Cercospora) subsessilis* also causes leaf spot in neem seedlings, and is effectively controlled with Mancozeb and Brestan. Other diseases include, powdery mildew *(Oidium azadirachtae)*, bacterial leaf bllight *(Xanthomonas azadirachtii and Pseudomonas azadirachtae)*, leaf spot *(Collectotrichum capsici)*, blight and stem rot *(Sclerotium rolfsii)*, and wilt *(Fusarium solani)*.

Plantation Diseases

Important plantation diseases of neem are ganoderma root rot, pink disease and phomopsis twig blight. Root rot is caused by *Ganoderma lucidum* and has been found to occur sporadically in trees when raised without removing the stumps and roots of the original tree crop. It could be controlled by adopting sound silvicultural practices. Pink disease is caused by *Corticium salmonicolor*, becoming serious particularly in warm and humid conditions Twig blight, caused by *Phomopsis* sp., attacks twigs and produces symptoms of dieback.

Utilizational Studies

Fungicidal and antiviral properties of neem

Neem cake has been found to have an inhibitory effect on the growth of phytopathogenic fungi in the cultural medium. Compounds contained in neem oil have also been reported to have inhibitory effect on potato virus.

Wood

Neem wood is moderately heavy, resembling mahogany in appearance. In strength, it resembles teak but is more resistant to shock. It is used for manufacture of furniture carts, axles, yokes and felloes, boards and panels, cabinets drawer bottom packing cases, ornamental ceilings, ships and boats, helms, oars, oil-mills, cigar boxes, carved images, toys, drums, and agricultural implements. Neem is also a good fuelwood.

Bark

Neem bark controls malarial fever and cutaneous diseases. Extracts display antiviral properties. Nimbin and Nimbidin, compounds extracted from neem bark, show anti-protozoal, anti-allergic, anti-dermnatitic and anti-fungal properties. The bark also exudes clear, amber-coloured gum (East-India gum) which acts as a stimulant, demulcent, and tonic useful in catarr and other afflictions. Stem bark contains 12-16% tannin which compares well with avaram. The bark is also useful in the treatment of jaundice.

Leaf

A 7.5-8.0-m-tall tree yields about 350 kg of leaves. Leaves are used as a cattle feed supplement in many parts of India. They possess carminative properties and help in food digestion. They are also useful as mulch and manure. Neem leaves are a good source of protein, calcium, carotene and some minerals. They are particularly useful in alleviating the copper deficiency of most straws and dry fodders. Neem leaves have numerous medicinal uses, e.g. control of intestinal helminthiasis, ulceration of cow-pox, leucoderma, several viral diseases of cattle and birds, cardiovascular diseases of guinea pigs, are diarrhoea, dysentery and tuberculosis in rabbits. Leaves act as an insect repellent and also control nematodes. Dried leaves are used in protecting woolens and books as well as stored grain and potatoes.

Fruit

The fruit-pulp is useful as a tonic, antiperiodic, purgative, emollient and as anthelmintic. It was found beneficial in urinary diseases and in the treatment of piles. Dry fruits are simmered in water and employed to treat cutaneous diseases. The pulp water can be sprayed to protect crops from locusts.

Seeds

A mature tree produces 30-50 kg seed every year. Azadirachtin, an active ingredient of neem is isolated from seed. Powdered seed kernel, when mixed with wheat grain, protects against pests. Kernel suspensions, when sprayed, protect vegetables, cereals and fruit crops from locusts. Aqueous neem seed extract has been found to control the damage caused by the okra mosaic virus.

Kernel oil

The kernel yields a greenish yellow to brown, acrid, bitter fixed oil (40.0-48.9% by weight) known as oil of margosa, having a strong disagreeable garlic-like odor. It is used mainly in soap making and in preparation of toothpaste.

Neem Seed Cake

Neem seed cake is a major byproduct of the neem-oil industry. It contains more sulfur than any other cake. The nitrogen content varies from 2 to 3%. The cake is also used as manure. Neem cake has 6.5% digestible crude protein with 19 amino acids and 62.5% total digestible nutrients. Livestock feeding trials are however not encouraging. Neem seed cake not only acts as an organic nitrogenous manure but also as a nitrification inhibitor. This property helps in efficient use of nitrogenous fertilizers. Urea coated neem cake has been found to regulate the rate of nitrification and increase yields of grain and straw.

Chemical Studies

Leaf

On steam distillation, fresh leaves yield an odorous viscous essential oil which was a mixture of tetrasulphides of C3, C5, C6 and C9 units. Eight saturated hydrocarbons and nine fatty-acids have been isolated. Chemically interesting constituents of neem leaves include limonoids, the most important being nimbin and its derivatives, which include pyronimbic acid, nimbinene, 6-deacetylnimbinene, nimbandiol, nimocinol, nimbocinone, nimocinolide, nimbocinolide, nimbolide, etc.

Seed

Biologically active volatile organic sulfur compounds are liberated by crushing fresh seeds. As many as 25 volatile compounds have been identified with Di-n-propydisulphide being the chief constituent. Seed kernels yield 40-50% oil with a bitter taste and disagreeable odor. The oil has a high tocopherol content. The taste and flavor of crude oil is due to the presence of lipid associates. The purified oil is similar to any other fatty acid. Refined oil is stable and does not become rancid during storage. The oil contains six fatty acids. The most active antifeedant principle present in neem seed is azadirachtin whose presence in the seed varies from 0.2 to 3.5 g/kg. Pure azadirachtin is found as a microcrystalline solid. Other compounds isolated are salanin, 1.3 -

diacetylvilasinin, 3-deacetylsalanin, salanol, derivataives of azadirachtin and a minimum of 30 others which are not as important as azadirachtin.

Bark

Stem and root bark has astringent, tonic, anti-periodic and other medicinal properties. The activity of root bark is greater than stem bark. Stem bark contains 12-16% tannin and 8-11% non-tannin polyphenolics. Three polysaccharides and 24 diturpenoides have been isolated from the bark. Other compounds isolated from the bark include nimbolicin, nimbinene and 6-deacetylnimbinene.

Heartwood and other parts

The heartwood of neem contains esters of B-sitosterol and 24-methylenecycloartenol, B-sitostarol-B-D-glucoside, 24 (28)-diene-3B-ol, 4 a-methyl-5a-ergosta-B, 24(28)-diene 3 B-ol and others. On destructive distillation, it yields charcoal (30%) and pyrolignetic acid (38%). The wood oil contains B-sitosterol, cycloeucalenol and methylenecycloartenol.

Dried flowers are edible and contain B-sistosterol and B-D-glucoside kaempferol, thiomyl alcohol, melicitrin, benzyl alcohol and benzyl acetate. An essential oil has also been isolated from flowers. The pollen contains tyrosine, arginine, methionone, phenylalanine, histidine, glutamic acid and aminocaprylic acid. The older trees exudes a nutritive sap. The most abundant amino acid is aspartic acid. Glucose and arabanose are the major constituent sugars. Neem also contains saponins and other polar components which have not been studied so far. Further studies are needed to get a more complete picture of the yield of various components in different parts of the tree.

Conclusion

Research at ICFRE represent only a small fraction of the entire body of research devoted to neem in India. The potential of neem demonstrated by the results of this research is clear. However, much effort and investment will have to be made before industrial applications are fully realized. Furthermore, in the event that market demand for neem raw materials increases, Indian scientists must be ready with varieties, silvicultural techniques, and planting schemes to meet it.

Neem Research at ICAR

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Neem (*Azadirachta indica*) is a multipurpose tree species (MPTS) with historical, religious, and social uses that have interwoven with the lives of rural and urban people for over 4,000 years. It has gained international recognition only recently, but its utility to the people of India is written in the pages of history. This paper discusses research conducted by ICAR on neem in India. The research has focused on germplasm collection, identification of elites, tree breeding, vegetative propagation, nursery techniques, and seed technology. The studies were conducted by state agricultural universities, national research institutes, and coordinated projects on agroforestry.

Ecology

Neem occurs throughout India except in areas above 1,000 m elevation and above 2,000 mm rainfall. It will grow up to 1,800 m elevation if protected. It is found in the Western and Eastern Ghats as well as the Himalayas and forms 1-5% of the Southern and Northern tropical forests, dry evergreen forests, dry deciduous forests, and savannahs. Large trees (30-70 years old) have been observed in forests, agricultural fields, farm bunds, community lands, school yards, roadsides, and in social and religious gathering places.

Neem is a seral species. Successful reforestation of barren lands, such as the ravines of Mahi indicates a role for neem as an important species in succession. It is a subclimax species which is found scattered or in groups in arid and semi-arid tracts. Neem is an integral component of tropical thorn forests, dry deciduous forests, and tropical dry evergreen forests. It establishes well even under severe biotic and edaphic conditions and is a hardy species that can tolerate drought.

Natural regeneration is common due to abundant seed production and seed dispersal by birds, rodents, and humans. Natural regeneration can be utilized to establish neem in forests, on degraded sites, and in farmers' fields (Chinnamani, 1968a). Protection of naturally regenerated seedlings can provide a strong stand of neem even in the denuded ravines of Mahi Gujarat, Chambal, and Yamuna.

Agroforestry and Neem

Agroforestry research on neem began nearly fifty years ago in India. It is now coordinated through the All India Coordinated Research Project on Agroforestry at 31 centers. These include several ICAR central research institutes and state agricultural universities.

Two Indo-U.S. workshops have been conducted by ICAR which focused on neem. The first one was on tree seed technology (1989) and the other on agroforestry (1991). The Tree Seed Technology Workshop was conducted from 22 May - 2 June 1989 at Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu. In this workshop data was presented showing that neem seed can be stored up to 3 months with 26-56% germination, while in the open it can be stored for only 20-30 days.

The Workshop on Agroforestry was conducted from 15-27 April 1991 at Gujarat Agricultural University in Sardar Krushinagar, Gujarat. Papers were presented on tree germplasm and species improvement. Here, traits for selection of mother trees and for progeny evaluation were discussed. It was recommended that mother trees of average or better phenotype and that are more than 20 years old should be chosen based on height, the height to diameter ratio, stem form, relative freedom from forking, crown form, foliage retention in the dry season, and freedom from insect and disease damage. High oil content in the seed and high protein and mineral content in the leaf was considered an added benefit.

It was proposed that progeny trials be carried out and the progeny evaluated for survival at the end of the first growing season for height and foliage retention after 3-5 years and for height, stem diameter, stem form, crown form, and foliage retention after 8-10 years. Initial selections of material for clonal and seedling seed orchards would be made after eight years and verified at age ten. Tree improvement programs for optimizing genetic gain are enhanced by the following activities:

- form working groups among tree breeders
- compile information on breeding efforts in other countries and identify successful technologies
- coordinate exploration of plus trees
- establish clonal and seed orchards from open-pollinated progeny tests
- manage programs on seed collection, storage, and distribution
- begin breeding schemes to produce rapid gain for the next generation

Soil Conservation

Neem is an important tree for optimizing land use in eroded, marginal, saline, and alkaline lands as well as in gullies and ravines throughout India. It establishes successfully in well-drained soils, but does poorly in waterlogged areas. It is found in pure blocks on highly eroded subsoils and exposed areas in Tamil Nadu, Karnataka Andhra Pradesh, and the foothills of the Himilayas. Neem, along with its typically good undergrowth of grass and shrubs, protects the soil from erosion caused by heavy rainfall.

In severely eroded ravines in Mahi Gujarat, neem regeneration has been achieved by closing and protecting areas and by constructing contour trenches and gully plugs. In only 15-20 years it has formed 40-70% of the total tree cover (Table 1).

Table 1. Runoff and soil loss in Mahi ravines, Gujarat, India

Percentage of neem cover	Runoff (%) total rainfall	Soil loss tons per year	Protected (P) or Unprotected (UP)
Neem 0.01 to 2.0%	20-30	30-60	UP
5th year of neem (25%) + grass + scrub	5-10	6-12	P
10th year of neem (30-50%) + grass + scrub	2-7	1-5	P
15th year of neem (50-75%) + grass + scrub	0-1	0	P

In clay soils of Kota, Chambal, neem has replaced *Prosopis juliflora* by 30-60% over a period of 40-50 years. Planted with *Prosopis chilensis* and *Albizzia lebbeck*, neem makes an efficient shelterbelt against wind erosion. Soil loss in the shelter belt area was 3 ton/ha/year compared with 72 ton/ha/year in unsheltered areas.

Regeneration

Extensive work on marginal land closure and eroded sites has been conducted by the Soil Conservation Research Institute, the Arid Zone Research Institute, and other institutions for more than 50 years. Natural regeneration is common in these eroded lands, which occupy 80 million hectares of the total agricultural areas (143 million ha) in India. Neem can regenerate naturally in farmers' fields if the trees are well-protected from any cutting treatments. Planting seedlings in farmers' fields or on community lands is a cheap and effective way to establish neem woodlands (Tables 2 and 3). The species has been used in ravine reclamation in Mahi Gujarat as well as in desert afforestation projects.

Table 2. Closure of denuded ravines with soil conservation in Mahi, Gujarat

	% Tree Composition	
Years	Location A (8 ha)	Location B (84 ha)
0	0.01	0.0
1	1.00	0.5
5	35.00	20.0
10	55.00	30.0
16	70.20	34.2
20	72.00	40.0

Source: Chinnamani, 1968b

Table 3. Composition of tree cover after 20 years in Mahi Gujarat.

	% of Total Tree Cover	
Species (Common Name)	Location A	Location B
Azadirachta indica (Neem)	72	40
Acacia nilotica (Babul)	15	27
Prospis cineraria (Khejri)	4	27
Bombax ceiba (Semal)	4	2
Holoptelia integrifolia	2	1
Hardwickia binata	2	1
Acacia senegal	1	2

Source: Chinnamani, 1968b.

Environment

Neem is commonly grown to shelter people and cattle from the burning sun in arid areas. Farmers indicate that neem is one of the preferred species for shade, along with *Ficus religiosa* and *F. bengalensis* because it has a leafless period of not more than 1-1.5 months.

Neem is considered to have a medicinal effect on its immediate environment. Trees are planted at schools, playgrounds and along roadsides so men, women and children can relax under the tree in the afternoon for this purpose.

Leaf Fodder and Twigs

Neem provides good fodder for goats and camels. Much work has been done by ICAR and by Indian veterinarians and animal scientists. In Kota, Rajasthan, a diet of 10-25% neem leaf fodder was fed to goats and it resulted in good economic gains. In arid Gujarat and Rajasthan, leaves are lopped for fodder 1-2 times a year, yielding 20-350 kg/tree/lopping, depending on the tree's age, diameter, and size. Silvipastural studies with neem and four grasses (*Panicum antidotale, Dichanthium annulatum, Cenchrus celliaris*, and *C. setigrus*) proved as efficient as the grasses grown with *Acacia tortilis, Albizzia lebbeck*, and *Holoptelia integrifolia* for dry-matter production.

Neem leaf fodder contains 16-18% crude protein, 16-22% crude fiber 7-10% total ash, 1-2% Calcium, 0.2-0.4% Phosphorus, 7-9% DCP, and 50-55% TDN (Patel *et al.*, 1962; Jayal, 1963; and Ketkar, 1976). Twigs are harvested and used extensively as toothbrushes. Only fresh twigs 1-1.5 cm in diameter and 10-15 cm in length are used. In Gujarat and other areas near cities, farmers cultivate neem and babul (*Acacia nilotica*) at 2 x 2 m spacing and harvest them for toothbrush twigs. These are bundled in lots of 10, 20, and 50 and sent to markets. Farmers earn approximately 60,000-120,000 rupees/ha/yr (US$ 2,000-4,000/ha/yr).

Social Aspects

Neem holds an important place in Indian life. Trees 40-50 years old are social gathering places; they have religious significance and are worshipped. Elders, women, children, and hermits congregate around neem trees to talk, work, or play.

The seeds are collected exclusively by women and children and provide a source of income (Table 4). Women also engage in seed processing (drying and threshing the pods and winnowing) to earn extra income. A woman can earn 10-50 rupees/day as a laborer, depending on the location of the nursery. Women can earn more money if they operate their own nurseries as entrepreneurs.

Table 4. Quantities of tree seeds collected and income generated per day by women.

Species	Collection (kgs)	Income (rupees)
Neem	20-30	30-45
Jamun	30-40	30-40
Mango	25-30	40-60
Tamarind	20-30	60-80
Babul	15-25	20-30
Subabul	25-30	10-15

Source: Chinnamani and Chandra, 1991

Fuel and Timber

Farmers use 15-25 year old neem trees for construction timber. Gujarat farmers raise neem on farm boundaries for timber and harvest after 15-20 years. Usually neem is not preferred as a source of fuelwood. It is, however, used along with other fuelwood when necessary.

Oil, Medicine and Other Uses

Neem oil and cake from the seed are used for various purposes. Neem oil is in high demand throughout India. In Gujarat labor costs are high and seeds are rarely collected for oil from scattered trees. Instead, collection is done from small plantations.

Extensive research is being conducted on neem as a source of medicine and insecticide. Neem sap is rarely used but has beneficial medicinal properties.

Water-washed seed kernel cake is fed to piglets at a level of 10%. It has 11.2-25.4% crude protein, 8.4% DCP and 57.8% TDN. A low mixture of 10-25% neem cake can be used as a replacement for groundnut cake. It contains such anti-nutritive factors as nimbin, salamin, azadirachtin, and tannin (Ranjha 1990; Sastry *et al.*, 1992).

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National Level Neem Provenance Trials at Jodhpur

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Neem (*Azadirachta indica* A. Juss) is a valuable multipurpose tree species for both rural and urban people. Almost all parts of the tree have one or several uses.

Neem is widely distributed in several parts of the world. It is found in dry areas of India, Pakistan, Sri Lanka, Malaysia, Indonesia, Thailand and Myanmar. It has been planted successfully on plantations in the Sudan and Sahelian zones of Africa as well as in Sierra Leone, Malawi, Zimbabwe, Tanzania, Zanzibar and the non-Sahelian areas of Guinea, Nigeria and Ghana. In India, the tree is distributed in tropical dry areas ascending up to 1,200 m altitude in the hills. It is largely found in the states of Uttar Pradesh, Haryana, Punjab, Himachal Pradesh, Rajasthan, Delhi, Gujarat, Madhya Pradesh, Bihar, Maharashtra, Andhra Pradesh, Karnataka, Tamil Nadu, Kerala, Orissa, West Bengal and Assam. The tree generally occurs naturally but is also planted by villagers in fields or homestead plantations. The species has recently been taken up for large scale plantation in village woodlots and road/rail side plantations in many states of the country, particularly in Madhya Pradesh, Maharsashtra, Gujarat and Rajasthan.

Neem can grow in a wide variety of soil conditions. It performs better than most other tree species on dry, stony, clayey, sandy and shallow soils. It can grow in all kinds of black cotton soils. It tolerates seasonal water logging of short duration only. It thrives in a wide climatic range but it is very susceptible to frost. The absolute maximum shade temperature and the absolute minimum temperature found in its zone of distribution ranges from 40-47°C and 0-15°C respectively. Its best growth is found in areas having rainfall of about 750-1,000 mm, but it can survive in areas receiving only 150 mm of annual rainfall. It can tolerate long dry seasons. Neem is a light demanding tree but tolerates fairly heavy shade during early years, especially in dry areas. It does not tolerate excessive cold especially in the seedling and sapling stages. It coppices well, produces root suckers, and withstands pollarding. Tree growth is fast for the first five years and then slows down gradually.

Planning of Provenance Trials

Neem is native to India and is widely distributed. Variation in natural populations is quite possible. There is an urgent need to survey and delineate different provenances to exploit natural variation that should be evaluated in multilocation trials. Such trials will help in formulating recommendations for planting a particular provenance for a particular use. Provenance testing will also help in breeding programs, since only the best races will be utilised for breeding.

One of the earliest provenance trials of neem in India was made by the Forest Research Institute (FRI), Dehra Dun under the Indo-Danish project. In order to understand the variability and nature of the species based on place of origin, a survey of provenances was conducted in Uttar Pradesh, Haryana, Rajasthan, Madhya Pradesh, Orissa, Maharashtra, Tamil Nadu and Andhra Pradesh. Seeds were collected from 20 sources and were subjected to various laboratory studies. Because Dehra Dun is a frost affected area and the seedlings are frost tender, the provenance trial was established in Bijnor Plantation Division. Provenance study of neem was carried out in the Arid Forest Research Institute (AFRI) at Jodhpur in 1992.

Planning of the provenance trial was done in January-February, 1992. The entire zone of natural distribution of neem was marked on the map and the area was divided into 2' latitude x 2' longitude grids, each representing an area of approximately 44,000 sq. km. Climatological information was superimposed on these geographical units and more uniform areas were combined while dissimilar areas were further sub-divided into more homogeneous units. A total of 50 such unit areas were identified for collection of data and seeds.

The areas so selected were far from the Institute so assistance was sought from local forest officers and Directors of forest research institutes located in these areas. These officers were contacted to give details on naturally occurring neem stands, phenological information and help in seed collection. They were also requested to identify the optimum period of seed collection. Out of 60 persons contacted in the area, only 41 responded and helped in collection of phenological information, identification of proper neem stands and collection of seeds.

Phenological Information

Neem is essentially an evergreen tree. In very dry localities, the tree is leafless for a short period during February-March. New leaves appear in the month of March-April before old leaves have completely shed. Flowering takes place during February-April. The period of flowering depends upon latitude and climatic factors. In south India, flowering takes place during early February to early April. In the southeastern parts of Tamil Nadu, which receives winter rainfall from the retreating monsoon, neem flowers by the end of March or beginning of April. Between latitude 20° N and 30° N, flowering takes place between mid-March to the end of April with 4.5 days increase for each 1° increase in latitude.

In some areas of northern and northwest India, restricted flowering and fruiting in some trees have been observed during September-November (Mitre, 1963). A number of neem trees at Jodhpur were fruiting during November-December (Dwivedi, 1992). Detailed observation reveals that fruiting during November-December is rather restricted. Only small branches bear fruits. The total yield of fruits was approximately 1 kg/tree in six trees at Jodhpur. The seeds have been collected for detailed studies. Flowering and fruiting in neem in some parts of West Bengal is reported to take place throughout the year (Guhabakshi, 1984).

Generally, mature fruits are obtained 10-12 weeks after flowering (Maithani *et al.*, 1989). Fruits ripen between early May to late July. In sub-Himalayan tracts, fruits generally mature during August.

The fruit of neem is an ellipsoidal drupe, about 1.25 cm long. Its color is green and on ripening turns yellow. The fruit is generally one seeded (rarely two). The tree starts flowering and fruiting at 5 years but good yield of fruits is obtained after 10-12 years. A medium sized tree produces 37-55 kg seeds (Anon., 1978).

Seed Collection

For germination purposes, the proper time for seed collection is reported to be when fruits attain maximum green weight. At this stage, the embryo is fully developed and major change in the color is yet to take place. Generally, this period is 10-12 weeks after the onset of flowering (Maithani *et al.*, 1989). Since neem seeds have short viability, teams were formed to carry out seed collection consisting of scientists and staff of the institute. They were sent to the identified areas after receiving information from the contact persons. Details of the dates and places where these parties collected seeds are given in Table 1.

The seed collection teams which went to Coimbatore and Dharwar collected the seeds in the last week of June, when the seeds were over-ripe and were present only on few trees. The result was that germination was very low, particular for seeds from Dharwar. The seed collection team at Pinjore (Haryana), which is situated on the foothills of the Himalayas, reached the site ahead of the scheduled period of fruit maturity. Seed embryos were not fully developed and did not produce any seedlings. Seeds from Maharashtra, Andhra Pradesh, Madhya Pradesh, Orissa, Bihar, West Bengal, Rajasthan, Gujarat, Haryana and Punjab were collected during the 1st to 3rd week of July which was an optimum period of seed collection.

Seeds collected by different teams were received in the Institute in three forms: 1) as fruit, 2) depulped and properly air dried and 3) depulped and partially dried. No work was carried out on the method of seed storage but experience indicates that depulped and air dried fruits are better for storage and transportation purposes The seeds obtained were sown within a period of 2-3 days in germination trays. Some seeds from all samples were separated for undertaking seed studies.

Seed Studies

The collected seeds were depulped, cleaned and air-dried. Morphological features of the seed such as seed weight and size from each source were studied (Table 2). The seed weight of 100 seeds varied from 12.0 g to 29.1 g. Seeds with less weight were mostly those from dry areas of the country such as Jaisalmer, Bikaner, Mathura, Jodhpur and Sawai Madhopur. Seeds collected from Dharwar and Coimbatore were also light in weight. Presumably these seeds were collected late and therefore, were not of good quality.

The heaviest seeds were from Solapur, Nagpur (Maharashtra), Sikar (Rajasthan), and Jhansi (Uttar Pradesh). The weight of 100 seeds was more than 25 g. Seed obtained from other sources weighed between 18 to 25 g. Seed size was found to be directly proportional to seed weight. Kernel length varied from 1.00 cm to 1.65 cm. Kernel width ranged from 0.50 cm to 0.70 cm.

The germination percentage of seeds from different sources was also studied (Table 3). Germination depended mostly on the condition of the seed and the time interval between seed collection and seed sowing. Seed collected from Dharwar (Karnataka) and Pinjore (Haryana) showed very low germination rates because collections were improperly timed.

The highest germination was obtained when freshly collected seeds were used. For example seeds from Jodhpur were collected on 7th July and were sown in germination trays on 8th of July, with 93.3% germination. Similarly, seeds collected from Indore, Jaisalmer, Bikaner and Solapur also gave good germination because the time between seed collection and sowing was short. Seeds obtained from Bilashpur and Raipur (Madhya Pradesh), Amravati and Nagpur (Maharashtra) gave higher germination percentages even when the interval between seed collection and seed sowing was more than two weeks. These seeds were immediately depulped, air-dried and sown in polythene containers upon receipt in Jodhpur.

Neem seeds do not retain their viability for more than a few months and viability falls rapidly after two weeks (Dent, 1948). Loss in viability is due primarily to fermentation of the unopened cotyledons inside the inner seed case. Seeds with green cotyledons germinate. If they have turned yellow or brown they will not germinate. Seeds stored in well aerated containers at room temperature have been reported to retain viability up to 6 months but germination fell from an initial 60% to 15% (Maithani *et al.*, 1989; Chaisurisri *et al.*, 1986).

Studies conducted at AFRI, Jodhpur showed that seeds from different sources have different viability and germinative capacity. The viability and germination capacity starts decreasing after the second week of seed collection. The seeds from 12 seed sources stored in ordinary containers retained their viability up to 4 months while, in some cases, the viability dropped considerably after 2-3 weeks.

Seedling Height

After germination, the seedlings were transferred to polythene bags. The polythene bags contained the usual soil mixture 1:1:1 ratio of soil, sand and farmyard manure. The polythene bags were well watered. About 2 months after sowing, the seedlings showed variation in height. Details of average seedling height calculated 2 months after sowing is given in Table 4 for different seed sources.

The reason for variation in seedling height after two months cannot be determined. It is assumed to be due to factors such as seed weight, condition of the seed and several other internal factors. Their performance in the nursery will be further observed before they are transferred to the field. In the field, a randomised block design is proposed, each treatment having 25 plants with tree replications.

Difficulties Encountered in Provenance Trials

- A large number of officers did not respond to the request for assistance and, therefore, there was difficulty in selecting proper stands for collecting seeds. Some of officers who responded did not provide enough support in seed collection.
- Information gained regarding optimum period of seed collection from different areas was only tentative. Local officers were requested to suggest the most appropriate period for seed collection, but many of them did not report the precise period. In some cases, the period indicated by them was either too early or too late, which hindered seed collection. Ideally, scientists from the Institute should have visited the area themselves to access the optimum period of seed collection.
- Seeds collected from different sources were not uniform. Some were overripe, while others were raw. Therefore, the germination and viability studies were confounded by these initial differences.
- In many cases, seeds were collected in the form of fruits while in some cases they were depulped and air dried. During seed collection, in some locations, there was continuous rain and humidity was very high. This makes air drying seeds difficult.
- There was considerable time lag between seed collection and seed sowing between locations. The places which were close to the Institute had seeds sown within 3-4 days of collection. However, seeds obtained from distant places involved a time lag of 10-15 days which led to differences in germination. Seeds collected at inappropriate times coupled with differences in time lag resulted in different germination percentages from different seed sources.
- All efforts were made to collect seeds from naturally growing trees. However, in three cases, seeds were collected from plantations which may not be truly representative of the area. Large natural stands are difficult to find. Neem occurs naturally in traditional agroforestry systems.
- Some seed sources gave very poor germination rates, requiring sowing large quantities of seed to obtain a sufficient number of seedlings. The seed collection teams collected 1-2 kg of seed from each source which was not sufficient for

conducting other seed-related studies. It is now realised that each party should have collected a larger quantity of seeds, particularly when seed is overripe on the time lag between collection and sowing is anticipated to be long.

- Seedlings were raised in polypots, taking care to prevent insect and fungal attacks. However, seedlings obtained from some sources were severely affected by an unknown disease. Care should be taken to discard infested seeds.

Proposed Studies

- The present trial should lead to identification of better provenances for producing timber, fodder, oil and azadirachtin. We propose to repeat collections and field plantings to ensure sufficient representation from some areas.
- Germplasm from other countries is proposed to be collected and tested. The AFRI provenance trial is proposed to be expanded to a few other locations.
- Based upon differences in morphology and behaviour, there is a distinct possibility that *Azadirachta indica* has more than one subspecies or variety. Therefore, taxonomic studies are proposed for this species.
- Neem trees flower generally during February-April. However, restricted flowering has been observed during October-November. It is proposed to study the nature and extent of such flowering and fruiting as well as the quality of seeds.
- Plus trees are proposed to be identified in the existing seed stands for future progeny trials and clonal banks.
- Neem seeds have short viability. Seeds from some sources showed remarkably short viability. Studies relating to increasing the viability period are proposed.
- Vegetative propagation and tissue culture research are proposed to avoid the problem of low viability and variability of seeds.

Table 1. Date of seed collection and the form in which the seeds were collected.

Code No.	Source of Provenance	Date of collection	Condition of the seed
1	Dharwar (Karnataka)	29.6.92	depulped and slightly moist
2	Kota (Rajasthan)	4.7.92	depulped, dried, without treatment
3	Indore (Madhya Pradesh)	4.7.92	depulped, dried, without treatment
4	Ujjain (Madhya Pradesh)	5.7.92	depulped, dried, without treatment
5	Jaisalmer (Rajasthan)	4.7.92	dried drupes
6	Bikaner (Rajasthan)	4.7.92	dried drupes
7	Jodhpur (Rajasthan)	7.7.92	ripe drupes
8	Pune (Maharashtra)	2.7.92	depulped, dried, treated with ash
9	Satara (Maharashtra)	30.6.92	depulped, dried, treated with ash
10	Solapur (Maharashtra)	30.6.92	depulped, dried, treated with ash
11	Sawai Madhopur (Rajasthan)	4.7.92	depulped, dried, without treatment
12	Nagpur (Maharashtra)	3.7.92	depulped, dried, without treatment
13	Jhansi (Uttar Pradesh)	6.7.92	depulped, dried, without treatment
14	Kanpur (Uttar Pradesh)	4.7.92	depulped, dried, without treatment
15	Rajkot (Gujarat)	6.7.92	depulped, dried, without treatment
16	Palanpur (Gujarat)	8.7.92	depulped, dried, without treatment
17	Gandhinagar (Gujarat)	7.7.92	depulped, dried, without treatment
18	Pali (Rajasthan)	9.7.92	ripe drupes
19	New Delhi	10.7.92	depulped and slightly moist
20	Coimbatore (Tamil Nadu)	2.7.92	depulped, dried, treated with saw-dust
21	Sikar (Rajasthan)	7.7.92	depulped, dried, treated with ash
22	Mathura (Uttar Pradesh)	9.7.92	depulped, dried, without treatment
23	Pinjore (Haryana)	15.7.92	unripe drupes
24	Gurgaon (Haryana)	14.7.92	ripe drupes
25	Amrawati (Maharashtra)	4.7.92	depulped, dried, without treatment
26	Ravinagar (Maharashtra)	3.7.92	depulped, dried, without treatment
27	Ranchi (Bihar)	9.7.92	dried drupes
28	Muzaffarnagar (Uttar Pradesh)	15.7.92	depulped, dried, without treatment
29	Bankura (West Bengal)	10.7.92	dried drupes
30	Mulag (Andhra Pradesh)	10.7.92	not completely depulped, dried, treated with ash
31	Raipur (Madhya Pradesh)	13.7.92	depulped, dried, without treatment
32	Angul (Orissa)	14.7.92	depulped, dried, without treatment
33	Jabalpur (Madhya Pradesh)	15.7.92	Dry drupes
34	Hoshangabad (Madhya Pradesh)	14.7.92	Dry drupes
35	Shivpuri (Madhya Pradesh)	13.7.92	Dry drupes
36	Rewa (Madhya Pradesh)	17.7.92	Dry drupes
37	Maihar (Madhya Pradesh)	16.7.92	Dry drupes

Table 2. Morphological parameters of neem seeds (kernel)

Code No.	Source of Provenance	Weight of 100 seeds (gm)	Average kernel length (cm)	Average kernel width (cm)
1	Dharwar (Karnatak)	12.3	1.2	0.5
2	Kota (Rajasthan)	16.4	1.3	0.6
3	Indore (Madhya Pradesh)	21.3	1.5	0.6
4	Ujjain (Madhya Pradesh)	18.5	1.3	0.6
5	Jaisalmer (Rajasthan)	13.8	1.3	0.6
6	Bikaner (Rajasthan)	14.6	1.2	0.6
7	Jodhpur (Rajasthan)	15.3	1.2	0.6
8	Pune (Maharashtra)	18.1	1.5	0.6
9	Satara (Maharashtra)	19.0	1.4	0.6
10	Solapur (Maharashtra)	25.0	1.4	0.6
11	Sawai Madhopur (Rajasthan)	18.4	1.3	0.6
12	Nagpur (Mahrashtra)	19.7	1.2	0.7
13	Jhansi (Uttar Pradesh)	25.0	1.4	0.6
14	Kanpur (Uttar Pradesh)	21.1	1.5	0.6
15	Rajkot (Gujarat)	19.9	1.4	0.6
16	Palanpur (Gujarat)	19.6	1.5	0.6
17	Gandhinagar (Gujarat)	22.1	1.5	0.6
18	Sumerpur, Pali (Rajasthan)	17.3	1.1	0.6
19	New Delhi	20.2	1.4	0.6
20	Coimbatore (Tamil Nadu)	13.4	1.2	0.6
21	Sikar (Rajasthan)	24.6	1.3	0.6
22	Mathura (Uttar Pradesh)	16.8	1.4	0.5
23	Gurgaon (Haryana)	19.3	1.3	0.6
24	Amrawati region (Maharashtra)	21.2	1.4	0.6
25	Ravinagar (Maharashtra)	29.1	1.6	0.7
26	Ranchi (Madhya Pradesh)	13.0	1.3	0.5
27	Muzaffar Nagar (Sikandarpur)	18.1	1.2	0.6
28	Bankura (West Bengal)	22.0	1.3	0.7
29	Mulag (Andhra Pradesh)	22.1	1.4	0.7
30	Raipur East (Kharora)	21.3	1.6	0.6
31	Angul (Orissa)	20.5	1.3	0.6
32	Jabalpur (Madhya Pradesh)	20.4	1.3	0.6
33	Hoshangabad (Madhya Pradesh)	20.3	1.4	0.6
34	Shivpuri (Madhya Pradesh)	21.3	1.4	0.6
35	Rewa (Madhya Pradesh)	18.2	1.4	0.6
36	Maihar (Madhya Pradesh)	21.4	1.5	0.7

Table 3. Seed collection and germination from different areas.

Code No.	Source of Provenance	Date received at AFRI	Date of sowing in nursery	Interval between seed collection & sowing (in days)	Germination %
1	Dharwar (Karnatak)	2.7.92	4.7.92	5	0
2	Kota (Rajasthan)	6.7.92	7.7.92	3	40.0
3	Indore (Madhya Pradesh)	8.7.92	8.7.92	4	85.0
4	Ujjain (Madhya Pradesh)	8.7.92	8.7.92	3	57.6
5	Jaisalmer (Rajasthan)	8.7.92	8.7.92	4	75.0
6	Bikaner (Rajasthan)	8.7.92	8.7.92	4	73.3
7	Jodhpur (Rajasthan)	8.7.92	8.7.92	1	93.3
8	Pune (Maharashtra)	9.7.92	10.7.92	8	31.6
9	Satara (Maharashtra)	9.7.92	10.7.92	10	30.0
10	Solapur (Maharashtra)	9.7.92	10.7.92	10	80.0
11	Sawai Madhopur (Rajasthan)	10.7.92	10.7.92	6	40.0
12	Nagpur (Mahrashtra)	10.7.92	10.7.92	7	5.0
13	Jhansi (Uttar Pradesh)	10.7.92	10.7.92	4	53.3
14	Kanpur (Uttar Pradesh)	8.7.92	9.7.92	5	53.3
15	Rajkot (Gujarat)	10.7.92	13.7.92	7	38.3
16	Palanpur (Gujarat)	10.7.92	13.7.92	5	5.0
17	Gandhinagar (Gujarat)	10.7.92	13.7.92	6	23.3
18	Pali (Rajasthan)	10.7.92	13.7.92	5	55.0
19	New Delhi	13.7.92	13.7.92	3	40.0
20	Coimbatore (Tamil Nadu)	12.7.92	13.7.92	11	3.3
21	Sikar (Rajasthan)	12.7.92	14.7.92	7	46.6
22	Mathura (Uttar Pradesh)	13.7.92	15.7.92	6	30.0
23	Pinjore (Haryana)	18.7.92	24.7.92	9	0
24	Gurgaon (Haryana)	16.7.92	18.7.92	4	6.6
25	Amrawati (Maharashtra)	15.7.92	16.7.92	12	20.0
26	Ravinagar (Maharashtra)	15.7.92	16.7.92	13	56.6
27	Ranchi (Bihar)	16.7.92	16.7.92	7	25.3
28	Muzaffarnagar (Uttar Pradesh)	16.7.92	16.7.92	1	68.3
29	Bankura (West Bengal)	20.7.92	21.7.92	11	1.6
30	Mulag (Andhra Pradesh)	21.7.92	21.7.92	11	20.0
31	Raipur (Madhya Pradesh)	22.7.92	25.7.92	12	55.0
32	Angul (Orissa)	22.7.92	27.7.92	13	73.3
33	Jabalpur (Madhya Pradesh)	22.7.92	27.7.92	12	1.6
34	Hoshangabad (Madhya Pradesh)	22.7.92	27.7.92	13	41.6
35	Shivpuri (Madhya Pradesh)	22.7.92	25.7.92	12	53.3
36	Rewa (Madhya Pradesh)	22.7.92	25.7.92	18	63.3
37	Maihar (Madhya Pradesh)	22.7.92	27.7.92	11	78.3

Table 4. Height of seedlings from different areas.

Code No.	Source of Provenance	Seedling height (cm)
1.	Dharwar (Karnatak)	-
2.	Kota (Rajasthan)	10.9
3.	Indore (Madhya Pradesh)	12.3
4.	Ujjain (Madhya Pradesh)	15.7
5.	Jaisalmer (Rajasthan)	6.5
6.	Bikaner (Rajasthan)	7.5
7.	Jodhpur (Rajasthan)	7.1
8.	Pune (Maharashtra)	11.5
9.	Satara (Maharashtra)	20.6
10.	Solapur (Maharashtra)	10.8
11.	Sawai Madhopur (Rajasthan)	9.3
12.	Nagpur (Mahrashtra)	8.6
13.	Jhansi (Uttar Pradesh)	9.2
14.	Kanpur (Uttar Pradesh)	13.7
15.	Rajkot (Gujarat)	14.5
16.	Palanpur (Gujarat)	12.9
17.	Gandhinagar (Gujarat)	12.8
18.	Pali (Rajasthan)	12.8
19.	New Delhi	18.3
20.	Coimbatore (Tamil Nadu)	16.3
21.	Sikar (Rajasthan)	10.2
22.	Mathura (Uttar Pradesh)	9.7
23.	Pinjore (Haryana)	-
24.	Gurgaon (Haryana)	8.0
25.	Amrawati (Maharashtra)	7.5
26.	Ravinagar (Maharashtra)	9.9
27.	Ranchi (Bihar)	6.0
28.	Muzaffarnagar (Uttar Pradesh)	10.2
29.	Bankura (West Bengal)	8.4
30.	Mulag (Andhra Pradesh)	9.0
31.	Raipur (Madhya Pradesh)	16.7
32.	Angul (Orissa)	25.1
33.	Jabalpur (Madhya Pradesh)	22.3
34.	Hoshangabad (Madhya Pradesh)	18.3
35.	Shivpuri (Madhya Pradesh)	19.3
36.	Rewa (Madhya Pradesh)	14.7
37.	Maihar (Madhya Pradesh)	18.1

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Tree Improvement and Seed Management in Indian Neem

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Chaney and Knudson (1988) claim that neem (*Azadirachta indica*) is native to the Indo-Pakistan sub-continent. Neem is a versatile tree with a variety of uses in industry, medicine, energy, and timber (Ketkar, 1976; Radwanski, 1977 and 1981; Radwanski and Winckens, 1981; Michel-Kim and Brandt, 1981; and Fagoonee, 1982). Neem cake, the residue left after extraction of oil from the seed, makes an excellent fertilizer and soil conditioner (Ahmed and Grainge, 1985 and 1986; Munavu, 1984). It is also fed to animals in Andhra Pradesh (Christopher, 1970). Oil extracted from the kernels is used extensively in soap manufacture and in oil lamps in some villages. Neem bark contains 12-14% tannin. Neem is widely recommended for rural development projects and has been successful in arid, tropical and sub-tropical regions worldwide (Goor and Barney, 1976). Uses that have most interested scientists, especially entomologists, are the pesticidal properties of the entire plant (Atwal and Panji, 1964; Girish and Jain, 1978; Attri and Prasad, 1980; Ali *et al.*, 1983; and Pruthi, 1937). Many researchers have emphasized the need for developing countries to minimize farmers' dependence on imported pesticides by utilizing indigenous resources (Fagoonee, 1980; Jacobson, 1981 and 1986).

Tree Improvement

Though neem constitutes an important component in both social forestry and agroforestry programs, genetic improvement efforts have been modest. Seeds for forest renewal programs are still collected from wild, unimproved stands with poor survival and sub-optimal growth. In July-August 1990, a program with the following objectives was started in 18 neem-growing districts of Tamil Nadu: identifying superior phenotypes; establishing genotypic values for selected trees through progeny testing; and establishing seed orchards for mass-production and supply of improved seed materials. By using straightness of bole, intensity of branching, crown spread, fruit-bearing efficiency, and fruit yield as selection criteria, 149 plus trees were identified. Seeds from the trees were evaluated for lenght, width, 100 seed wt. germination percent and oil content (Table 1). One-hundred seed weight ranged from 17.4 to 29.5 g. Variation in oil content, however, was narrow (40-44%). No correlation between seed parameters and oil content was noted.

Table 1. Geographic variation in seed attributes and oil content in neem

District	Seed length (cm)	Seed width (cm)	100 seed weight (g)	Germination (%)	Oil content (%)
Chengai M.G.R.	1.40	0.65	20.5	72.4	42.2
Chidambaranar	1.40	0.69	21.7	78.0	41.6
Coimbatore	1.56	0.60	25.3	74.4	43.1
Dharmapuri	1.48	0.62	17.4	73.0	43.4
Dindigul-Anna	1.54	0.57	21.5	73.5	40.2
Kamarajar	1.27	0.55	18.0	79.4	42.9
Kanyakumari	1.50	0.68	24.0	81.9	41.6
Madurai	1.52	0.56	21.7	80.3	41.4
North-Arcot	1.46	0.63	19.3	80.8	42.0
Pasumpon	1.48	0.58	19.3	80.4	43.1
Periyar	1.56	0.60	25.3	77.3	43.1
Pudukkottai	1.48	0.58	18.6	80.2	43.9
Ramnad	1.58	0.64	29.5	78.0	41.6
Salem	1.46	0.56	20.4	78.0	40.1
South Arcot	1.43	0.53	22.7	83.0	41.9
Thanjavur	1.56	0.58	19.6	72.0	40.8
Trichy	1.52	0.71	22.3	89.2	41.9
Tirunelveli	1.52	0.63	20.9	84.2	44.3
LSD (0.05)	0.20	0.11	2.8	10.2	2.1

In a separate study, seeds collected from 28 phenotypically superior trees, representing 12 districts, were evaluated for the same characters (Table 2). Significant variations among the families were manifest in all the seed attributes investigated. Oil content ranged from 41 to 52%, indicating some scope for individual tree selection for high oil content. Variation in oil content due to geographic location was not apparant. Seed size or weight and oil content were not correlated.

Variability in Azadirachtin

The yield for ethanol extractions of azadirachtin from 100 g of seeds varied from 0.2 g (using the same sources of commercial Indian seed) to 3.5 g (from Ghana), and up to 6.2 g (from Togo) (Anon, 1987). Seeds of Kenya, Nigeria, and Ghana reported greater azadirachtin content (1.0-3.5 g/kg) than commercial Indian neem (0.2-0.75 g/kg) (Morgan, 1982). Seeds from 32 phenotypically superior trees from several Indian locations were estimated for azadirachtin. Large variation in azadirachtin, ranging from 0.29 to 8.83 g/kg seed, was evident. This suggests an opportunity to select for higher azadirachtin content (Table 3). However, no correlation between oil and azadirachtin content was observed.

Table 2. Inter-one-parent family variation for seed parameters

One-parent family district	Seed length	Seed breadth	Oil content	100 seed weight
Dindigul Anna	1.57**	0.78**	48.18**	29.0**
	1.56**	0.86**	46.19	28.0**
	1.33	0.64	47.79**	20.0
	1.40	0.68	48.12**	19.0
Dharmapuri	1.45	0.70*	45.08	25.9*
Pudukkottai	1.32	0.63	44.42	20.0
	1.61**	0.68	49.73**	21.0
	1.21	0.66	46.68	20.0
Trichy	1.43	0.65	41.49	23.0
Ramnad	1.24	0.63	50.98**	19.5
Chengai MGR	1.57**	0.59	50.89**	21.0
	1.47	0.76**	43.46	25.5**
South Arcot	1.46	0.66	49.23**	21.0
	1.45	0.64	48.23**	23.0
	1.63**	0.66	51.00**	26.0**
Salem	1.90**	0.81**	50.85**	25.0**
	1.64**	0.65	47.40	24.0
Periyar	1.21	0.68	47.24	20.0
	1.44	0.63	46.52	21.0
	1.31	0.62	52.25**	20.0
Coimbatore	1.23	0.64	50.76**	18.0
	1.76**	0.71**	41.60	25.0**
	1.36	0.55	52.66*	24.0
Dharmapuri	1.44	0.76**	46.23	17.0
	1.50**	0.70*	43.87	20.0
	1.63**	0.63	44.22	21.0
Tanjore	1.22	0.56	46.38	17.0
	1.46	0.67	44.82	26.0

* Higher than general mean plus one S.E.
** Higher than general mean plus two S.E.

Table 3. Oil content and azadirachtin content of neem seeds in various locations of Tamil Nadu

Location	Oil content: Seed basis	Oil content: Kernel basis	Azadirachtin content (mg/kg kernel)
South Arcot (red soil)	24.87	36.87	558.9
Tanjore (black soil)	24.72	36.72	1,179.0
Kanyakumari	26.43	38.43	951.8
Coimbatore district	24.60	36.60	5,945.0
Kanyakumari	28.59	40.59	1,130.1
Kovilpatti	25.99	37.99	2,991.5
Paramakudi	26.20	38.20	348.7
Sankarankoil	27.91	39.91	3,667.0
South Arcot district	23.97	35.97	3,830.0
Chidambaranar	28.39	40.39	3,248.0
Sathyamangalam	26.43	38.43	5,487.0
Dindigul district	28.37	40.37	3,199.0
Bannari (2)	23.51	35.51	4,824.0
Pudukkottai (thin red loam loose)	24.67	36.67	2,119.0
Alangulam	27.99	39.99	1,593.0
Trichy (deep red loam)	24.07	36.07	5,007.0
Periyar district	27.00	39.00	3,011.0
Kanyakumari	29.07	41.07	3,300.0
Tuticorin	26.51	38.51	8,832.0
Kovilpatti	27.20	39.20	293.4
Kanyakumari	28.20	40.20	5,211.0
Tanjore (black soil)	26.32	38.32	4,181.5
Paramakudi	27.43	39.43	3,155.4
Kanyakumari	28.06	40.06	2,232.0
Vridhachalam (1)	25.03	37.03	3,536.0
Tanjore (coastal river alluvial)	25.84	37.84	488.0
Trichy (deltaic alluvial loamy)	28.11	40.11	3,308.0
Tanjore (black soil)	28.71	40.71	1,522.0
Panrutti (1)	28.10	40.10	1,414.0
Vasudevanallur	26.22	38.22	707.9
Tirunelveli	28.25	40.25	3,628.0
Alangulam	31.10	43.10	1,464.0

Stable Genotypes

A phenomenon of crucial importance in tree improvement is the genotype x environment interaction. This underscores the need for conducting genetic tests in a variety of environments and selecting genotypes that perform well under all environments or that show little interaction over differing environments. A stable genotype shows high mean yield, regression coefficient (b) around unity, and mean squared deviation (s^{-2} d) around zero. Based on b and s^{-2} d, good performers (mean + SE) can be resolved again into four groups (Singh and Singh, 1980).

Genotypes falling into Group I are considered highly stable; those in Group II will have above- or below-average response and will be suitable for stress or favorable growth phases; those in Groups III and IV will have unpredictable performance. (Table 4) Applying these distinctions, 28 one-parent families were evaluated for phenotypic stability 45, 90, and 180 days after planting. Five characteristics (height, stem diameter, root length, sturdiness quotient, and volume index) were analyzed. The 28 families resolved into just two groups (I and IV). From a holistic perspective, families 27 and 28 are considered highly stable (Table 4). The study indicates the possibility of identifying stable genotypes that would perform consistently under diverse habitats.

Table 4. Group of families based on stability analysis for specific characteristics

Group	Height	Diameter	Root length	Sturdiness quotient	Volume index
I	2,9,19,24, 27,28	7,19,20,22, 23,25,27,28	15,16,18,19, 22,23,24,27	8,17,21	7,20,22, 23,27,28
II	-	-	-	-	-
III	-	-	-	-	-
IV	7,16,22,23	-	21,26,28	2,9,10,14, 16,24	19

Vegetative Propagation

Poor storability of neem seeds underscores the need for devising suitable clonal propagation methods (Chaney and Knudson, 1988). One ton of seeds procured from Burma failed to germinate in the Philippines because they lost viability during transport (Ahmed and Grainge, 1986). Clonal propagation is advocated as an alternative to conventional breeding. Studies were carried out to delineate a suitable method for producing vegetative propagules.

Hardwood cuttings (20 cm long and 0.5 cm wide) were collected from the lower half of the crown in an 8-year old woodlot following the method of Girouard (1970) spruce. The basal end of the cuttings was cut obliquely to increase the rooting surface area, quickly dipped for 60 seconds in growth regulators (IAA, IBA, or GA), and planted to 10 cm depth in 20 x 10 cm polypots. The potting mixture was 750 g of field soil, sand and FYM in the proportion 4:1:1. After planting, the polypots were transferred to a mist chamber maintained at 80% relative humidity and approximately 30° C. After planting (135 days), the following parameters were assessed:

- percentage rooting
- number of primary roots
- root length
- number of sprouts
- number of leaves
- leaf length

The percentage rooting was highest with under 1,000 ppm of IAA and IBA (Table 5). Using IBA, the number of sprouts and leaves and leaf length were distinctly superior. IBA at 1,000 ppm is, therefore, advocated as a promising treatment for promoting rooting of cuttings.

Effect of Grading Depulped Fruits on Seed Viability and Vigor

Ripe fruits collected from a 20-year-old tree were depulped, washed thoroughly, air-dried for 8 hours, and resolved into sinkers and floaters by the liquid flotation technique. The two components were germinated separately on sterilized quartz sand in enamelled trays. Twenty-one days after sowing, the number of germinants was counted and the percentage computed. The dry weight of 10 random seedlings was obtained and vigor index calculated as the product of percentage germination and seedling dry weight.

In the total depulped seedlot, floaters accounted for 18%. These were characterized not only by lesser weight but also by a higher proportion of endocarp (Table 6). This may be attributed to poor filling of kernels. In addition, their germinability (55%) and vigor index (3,850) were low. The sinkers, in contrast, recorded 35% higher values for viability measured as maximum germinability 17% higher vigor measured in terms of seedling dry weight, and 92% higher vigor index. It is, therefore, advocated that depulped drupes be density-graded and only the sinkers used for seedling production.

Table 5. Effect of growth regulators on propagation of *Azadirachta indica*

Growth regulator	Levels (ppm)	Percentage rooting	No. of sprouts	No. of leaf	No. of primary roots	Root length (cm)
IAA	500	54.6	1.3	9.0	4.6	9.4
	1,000	72.6	2.0	13.3	9.3	13.4
	1,500	62.3	1.6	12.6	3.6	11.2
	2,000	65.3	1.6	16.6	4.6	12.6
Mean		63.7	1.6	12.8	5.5	11.6
IBA	500	48.3	1.6	13.7	6.6	7.7
	1,000	66.6	3.3	17.6	8.0	8.5
	1,500	60.6	2.3	16.0	4.3	12.1
	2,000	63.6	3.0	16.0	5.0	17.9
Mean		59.7	2.6	15.8	5.9	11.5
GA	500	53.0	1.6	11.0	6.8	12.8
	1,000	58.6	2.0	17.0	7.0	17.0
	1,500	53.0	1.6	16.0	4.3	16.3
	2,000	60.0	2.3	11.0	4.0	12.5
Mean		56.1	1.8	13.7	5.5	14.6
Control		52.8	1.3	8.0	5.1	7.8
LSD (0.05)						
Growth regulators (G)		3.1	0.07	1.2	0.4	1.3
Concentration (C)		3.6	0.09	1.4	0.6	1.5
G x C		6.0	0.15	2.5	1.1	2.6

Table 6. Effect of fruit density on seed viability and vigor in neem*

Fruit density grade	Percentage to total depulped fruitlot	100 fruits weight(g) depulped	Proportion to total depulped fruit: Kernel	Proportion to total depulped fruit: Endocarp	Percentage germination	Dry weight (mg/seedling)	Vigor index
Sinker	82.0 (64.9)	24.8	64.0 (53.1)	36.0 (36.8)	90.0 (71.5)	82.0	7,380.0
Floater	18.0	21.5	57.0 (49.0)	42.0 (47.8)	55.0 (47.8)	70.0	3,850.0
LSD (0.05)	0.75	0.21	1.00	0.72	1.47	1.5	3.7

*Numbers in parentheses represent arc-sin transformation

Seed Storage

Neem is generally considered recalcitrant because its seeds are shed at relatively high moisture content and are prone to dehydration and chilling injuries (Emah, 1986; Maithani *et al.*, 1989). It has been reported that neem seeds, under proper management, show a germination of 42% after 5 years of storage at 4° C and 60-65% after 9 months of cold storage (Roederer and Bellefontaine, 1989; Souvannavong, 1992). Under Indian conditions, viability is lost 2-3 weeks after collection. If drupes are collected when the color of the epicarp is yellowish-green and are still on the trees, viability is reported to be conserved. This has not yet been established for Indian neem (Souvannavong, 1992). However, studies carried out to devise a low-cost method for prolonging the storability of neem seeds yielded promising results (Ponnusamy *et al.*, 1991).

Sinkers, separated by the flotation method and air-dried, were transferred (after treatment with Dithane M-45) to 12 earthen pots (50 seeds per pot); the open pots were buried up to neck level in 20-25% moist sand bed under a thatched shed. The sand moisture content was maintained by replenishing water every three days. A similar number of seedlots stored in ambient material served as the control. The treatment was set up in quadruplicate in a completely randomized block design. At monthly intervals for three months, replications were drawn from the two storage media, and 25 seeds from each pot were tested for germination. Ten seeds were assayed for moisture content using the technique tested by Willan (1985). Initial moisture content and germinability were determined prior to storage. While the mean temperature during the storage period was 33.8° C in the ambient material, it was 25.6° C inside the pots buried in moist sand.

The initial moisture content of the air-dried seeds was 30.8%, and the initial germinability was 90% (Table 7). For seeds stored in ambient conditions, there was a progressive decline in moisture content over time. The cumulative loss over the 3 months was 15.3% or half the initial moisture content. Concomitant with this drop in moisture, viability of seeds, measured as a ratio of maximum germination, progressively diminished to 8% at the end of three month. In contrast, the seeds stored in earthen pots recorded a germination of 62% at the end of three months. The moisture content of these seeds remained almost constant (30%).

Table 7. Effect of wet versus ambiant storage on seed viability and moisture content in neem.

Storage method	Seed moisture content (%) Storage period (months) 0	1	2	3	Seed germination (%) Storage period (months) 0	1	2	3
Earthern pot (wet)	30.8 (33.7)	30.5 (33.5)	30.4 (83.4)	30.1 (33.2)	90.0 (71.5)	86.0 (68.3)	78.0 (61.7)	62.0 (51.1)
Ambient	30.8 (33.7)	22.6 (28.3)	17.4 (24.5)	15.5 (23.1)	90.0 (71.5)	74.0 (58.6)	26.0 (30.4)	8.0 (16.3)
LSD (0.05) for: Method (M)				0.10		1.11		
Period (P)				0.15		1.58		
M x P				0.21		2.23		

Numbers in parentheses are arc-sin transformations

The poor storability of seeds in ambient conditions may be attributed to their rapid dehydration. This has been demonstrated in *Mangifera indica*, *Shorea roxburghii*, *Hopea odorata*, and *Symphonia globulifera* (Corbineau and Come, 1988). Seed of *S. roxburghii* and *H. odorata* died when moisture content reached 17%. The threshold was 30% for *M. indica*, and 37% for *S. globulifera*. It has been suggested that recalcitrant seeds lack the mechanism to allow drying to low moisture contents (Berjak *et al.*, 1984). Neem seeds contain high percentages of both saturated and unsaturated fatty acids. Free fatty acids within seeds could be responsible for this loss of viability (Chaney and Knudson, 1988). Long-chain, unsaturated fatty acids cause swelling of isolated mitochondria and impairment of their normal function (Bewley and Black, 1982). Absence of appreciable senescence for seeds in earthen pots may be ascribed to maintenance of moisture content and the possibly of non-formation of free fatty acids.

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Neem Research in Other Countries

Section 2

Studies on Neem in Thailand

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Neem (*Azadirachta indica* var. *siamensis* Valeton) is native to Thailand and found throughout the country. Some common names for the species are Sadao Thai, Sadao Ban (central), Dao (south), and Siliam (north). Neem grows in various types of soil, varied rainfall conditions, and at altitudes of 50 - 500 m above sea level. Preferable pH is between 6.2 - 6.5, but neem can adapt to a lower pH (5). The species grows poorly in waterlogged or salt affected areas, but grows well in dry areas with average rainfall 450 - 1,150 mm/year (Puriyakorn *et al.*, 1993).

Recent studies in Thailand identified three varieties of neem: *Azadirachta indica* A. Juss, *A. indica* A. Juss var. *siamensis* Valeton, and *A. excelsa* (Jack) Jacobs (Boonsermsuk and Chittachamnonk, 1989). According to discussions with prominent botany experts in Thailand, it can be concluded that *A. indica* A. Juss and *A. excelsa* (Jack) Jacobs were introduced to Thailand (C. Pengklai and T. Santisuk, personal communication, 1992). *A. indica* is seldom found in Thailand. This variety is known as *Sadao India*, or India type; *A. excelsa* is known as *Sadao Chang*, or giant type. A common method for identifying the species that occur naturally in Thailand is to observe the leaves. The natural distribution of neem has been described by many authors (Jacobs, 1961; National Academy of Sciences, 1980; Bhumibhamon, 1987; Chailertpongsa and Boonsermsuk, 1989; Boonsermsuk and Chittachamnonk, 1989; Lauridsen *et al.*, 1991; and Puriyakorn *et al.*, 1993).

Neem has multiple uses, including construction timber, tool manufacturing, firewood, charcoal, pest repellent, and, to a certain extent, food (Huttacharoen, 1987). Neem is widely grown in Thailand, particularly var. *siamensis*. The first plantation was established by the Royal Forest Department (RFD) at Prachuap Kiri Khan more than 20 years ago. Neem is a multipurpose tree species (MPTS) promoted by the RFD. More than 3 million seedlings are distributed to local people each year.

Tree Improvement Research

Intensive studies have been conducted, beginning in 1983, when neem was first considered as a wood for energy. Demonstration plantations of (var. *siamensis*) were established at the Ratchaburi Forest Research Station, Ratchaburi Province. A number of research projects were begun in 1980; reports and publications soon followed. Topics included planting techniques and growth trials, yield analysis, biomass studies, and seed research (Puriyakorn, 1983; Thoranisorn, 1991; Boontawee *et al.*, 1987; P. Wasuwanich and S. Kijkar, personal communication, 1992). Neem seed is classified as recalcitrant but it has been demonstrated that the seed can be stored for longer periods if the moisture content is reduced and storage temperature is lowered (-20° C) (Lauridsen *et al.*, 1991; and P. Wasuwanich and S. Kijkar, personal communication, 1992).

No information on genetic variation has been published or reported in Thailand. Due to its economic importance, rapid growth, and range of site adaptability, the Research Section of the RFD has established research projects on neem improvement. Activities include plus tree selection, grafting studies, progeny testing, and establishing gene conservation. To date, 32 plus trees in the Kanchanaburi, Ratchaburi, and Suphanburi regions have been selected; 30 clones of these trees were produced and are kept at the Silvicultural Research Center No. 3, Kanchanaburi Province. (Planting materials were derived by means of grafting).

Gene banks will be established in 1993 at six new locations under RFD administration. It is anticipated that neem research, particularly improvement programs and propagation techniques, will be seriously developed. A summary of research completed over the past 10 years is included in Tables 1-3.

Recommendations for Future Research

It is generally accepted that neem is a multipurpose tree species. More research should focus on provenance trials, progeny tests, improvement studies, vegetative propagation techniques, seed storage, and studies on oil and chemical properties.

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Table 1. Survival percentage and growth of *A. indica* var. *siamensis* planted in different parts of Thailand at age 5 years.

Location	Spacing (m)	Survival (%)	Growth	
			Ht (m)	Dbh (cm)
Chiangmai (North)	1 x 0.5	12.9	1.7±0.4	1.1±0.4
	1 x 1	11.7	2.0±1.1	1.3±1.2
	1 x 1.5	22.5	2.8±1.1	2.9±1.9
	1 x 2	25.0	3.1±1.4	2.7±1.8
	2 x 2	25.0	3.1±1.8	2.3±1.7
Mean		19.4		
Kon Kaen (Northeast)	1 x 0.5	62.0	3.1±1.2	2.6±1.6
	1 x 1	60.3	3.5±0.9	3.6±1.1
	1 x 1.5	55.6	2.7±0.9	2.8±1.3
	1 x 2	78.6	3.3±0.9	3.3±1.3
	2 x 2	71.4	3.2±0.9	3.2±1.5
Mean		65.6		
Ratchaburi (Central)	1 x 0.5	73.5	4.7±1.6	3.5±1.5
	1 x 1	79.7	5.5±1.8	4.6±1.7
	1 x 1.5	83.3	5.9±1.7	5.1±1.8
	1 x 2	85.7	5.6±1.6	5.4±2.1
	2 x 2	78.6	5.9±1.7	6.4±2.4
Mean		80.2		
Chantaburi (East)	1 x 0.5	14.8	3.0±1.1	3.5±2.1
	1 x 1	17.3	2.3±0.5	2.4±1.5
	1 x 1.5	28.3	2.5±0.7	2.8±1.5
	1 x 2	20.7	2.7±0.9	3.6±2.1
	2 x 2	19.6	3.3±1.1	4.5±2.3
Mean		20.0		

Boontawee, B. *et al.* (1987)

Table 2. Survival percentage of *A. indica* var. *siamensis* at 7 and 8 years at Ratchaburi

Spacing (m)	7 yrs	8 yrs
0.5 x 0.5 (40,000)	89.4	87.9
0.75 x 0.75 (17,778)	94.0	93.3
1.0 x 1.0 (10,000)	97.1	97.1
1.5 x 1.5 (4,445)	98.4	98.4
2 x 2 (2,500)	98.0	98.0
4 x 4 (625)	98.4	98.4
6 x 6 (278)	92.0	92.0
8 x 8 (157)	100.0	100.0

After Vicharn Preuksakorn (1992).

Table 3. Total stem volume (1000 m^3h^{-1}) of *A. indica* var. *siamensis* plantation at different ages (Ratchaburi)

Population/ha	Spacing (m)	Age (yr)*			
		2	4	6	8
40,000	(0.5 x 0.5)	15.5	32.8	48.7	59.4
17,778	(0.75 x 0.75)	19.0	39.4	62.9	76.6
10,000	(1 x 1)	21.4	58.0	83.2	102.1
4,445	(1.5 x 1.5)	13.2	39.5	74.2	86.5
2,500	(2 x 2)	5.9	22.3	39.0	44.6
625	(4 x 4)	3.5	20.3	40.0	52.4
278	(6 x 6)	0..8	7.5	20.9	29.1
157	(8 x 8)	0.6	4.8	16.7	26.7

*After Somchai Thoranisorn (1991) for 1-6 yrs
After Vicharn Preuksakorn (1993) for 7-8 yrs

Neem in Myanmar

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Neem (*Azadirachta indica* A. Juss) originated in South and Southeast Asia and is widely distributed in Bangladesh, India, Myanmar (Burma), Pakistan, and Thailand (Hla Tin Oo, 1987).

Although the species is found throughout most of Myanmar, records from old working plans and an inventory carried out by the National Forest Management and Inventory Project from 1981 to 1988 show that neem was found growing in the natural forests of the country's central dry zone. The species, therefore, can be considered native to Myanmar. Evidence of neem introduction cannot be found.

The species is rarely used as timber or fuelwood in Myanmar. It has been used mainly as a shade tree and for roadside plantings. Extracts from neem seed make effective insecticides and the oil can be used to manufacture soap. The byproduct (neem cake) is used as fertilizer.

Tender, young leaves are popularly used in pickled salads. Also, they are used in medicinal preparations as remedies for skin diseases and ulcers. A traditional practice is to use neem leaves to protect books, clothes, stored food, and grains against damage caused by storage pests (Hla Tin Oo, 1987).

Current Distribution

Neem is widely planted as a shade and roadside tree. It can be found in areas where rainfall is as low as 460 mm and as high as 5,080 mm (Hla Tin Oo, 1987). However, according to the old working plans and the inventory data, the tree can be found in the natural forest only in Sagaing, Magway, Mandalay, and Bago Divisions (Table 1). Of the 482 species inventoried in these forest divisions, neem made up 0.02% of the total.

According to Troup (1921), neem can be found in abundance in areas where the rainfall ranges from 460 to 1,140 mm and where the maximum shade temperature reaches 49° C. However, it cannot tolerate frost or waterlogging. Although the species can be planted in high rainfall areas in Myanmar, it has been found that the fruit yield from these areas is as low as 4 kg per tree and heavily infested with fungus due to high moisture content (Hla Tin Oo, 1987).

Table 1. Growing stock of neem in Myanmar

Sr. No.	State/division	Township	Inventory area (in acres)	No. of trees 2' 0" +	No. of trees per 1,000 acres
1	Bago	Bago	531,630	898	1.69
2		Padaung	381,630	2,632	6.90
3	Mandalay	Tatkon	324,860	3,499	10.77
4		Thabeikkyin	428,590	898	2.10
5		Yamethin	148,500	2,664	17.94
6	Magway	Gangaw	445,080	1,765	3.97
7		Pauk	259,410	8,827	34.03
8		Saw	370,660	11,490	31.00
9		Salin	183,490	897	4.89
10		Setoktara	537,920	11,398	21.19
11		Pwintphyu	92,000	2,694	29.28
12		Myothit	133,530	4461	33.41
13		Minbu	122,260	8,025	65.64
14		Taungdwingyi	180,640	2,774	15.36
15		Ngaphe	242,690	19,263	79.37
16	Sagaing	Mawlaik	787,700	10,593	13.45
17		Paungbyin	827,700	21,189	25.60
18		Pinlebu	540,450	6,967	12.89
19		Indaw	331,230	2,632	7.95
20		Katha	468,080	11,429	24.42
21		Wuntho	230,220	1,734	7.53
22		Kawlin	312,570	1,765	5.65
23		Banmauk	756,190	7,960	10.53
24		Mingin	820,540	5,265	6.42
25		Kani	544,600	15,306	28.11
26		Yinmabin	12,540	897	71.53
27		Pale	219,000	2,632	12.02
28		Kantbalu	575,210	12,264	21.32

Source: National Forest Management and Inventory Project (1991)

Ongoing Tree Improvement Research

Research on seed processing and storage is being conducted at the Forest Research Institute, Yezin, Myanmar. Seeds were collected directly from the trees and depulped. They were then subjected to different methods of storage.

Storage

Seeds with different initial moisture contents (41%, 30%, and 18%) were stored in open bamboo trays at room temperature (30-33° C) and in an air-conditioned room (22-25°C). Germination tests were then conducted at regular intervals. These same parameters were tested storing the seeds in closed containers and then testing germinability.

According to Figures 1 and 2, the initial moisture content does not seem to have much effect on storage. However, the seeds in the air-conditioned room (23° C) can be stored longer than those at room temperature (31° C).

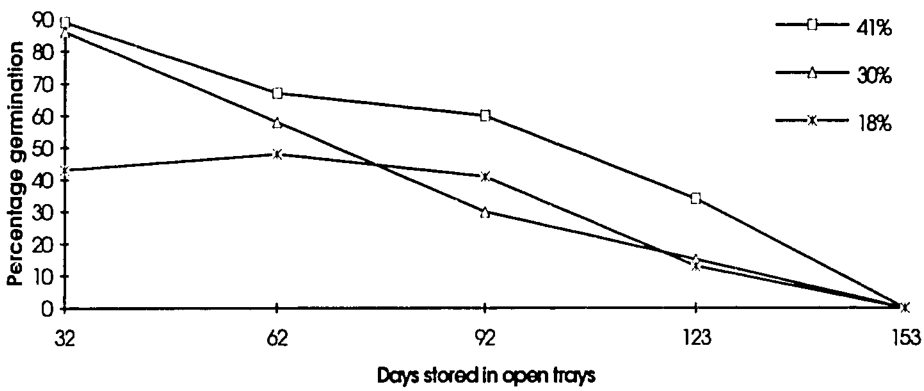

Figure.1 Germination percentage of seed stored at 31° C

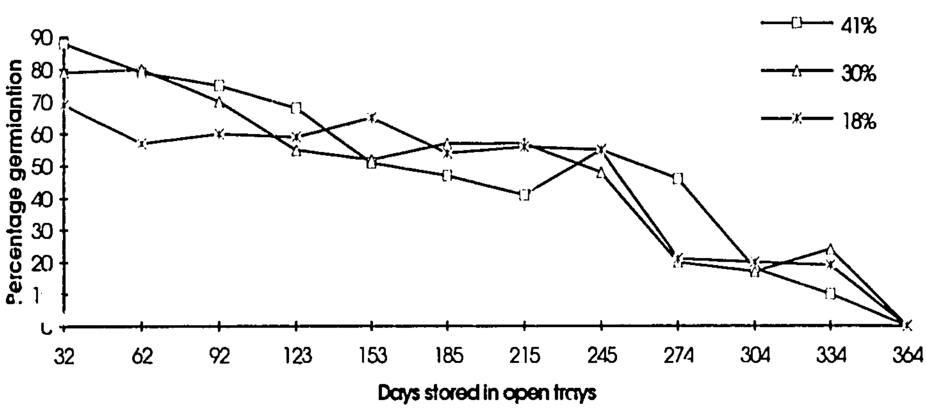

Figure. 2 Germination percentage of seed stored at 23° C

Storage at Room Temperature

Storage at room temperature affects moisture content and germination. These variables were studied by conducting moisture content measurements and germination tests until no germination occurred.

According to Figure 3, the moisture content dropped from 41% to 6% by day 116. Germination dropped gradually to 11% on day 116, at which time the seed moisture content was 6%

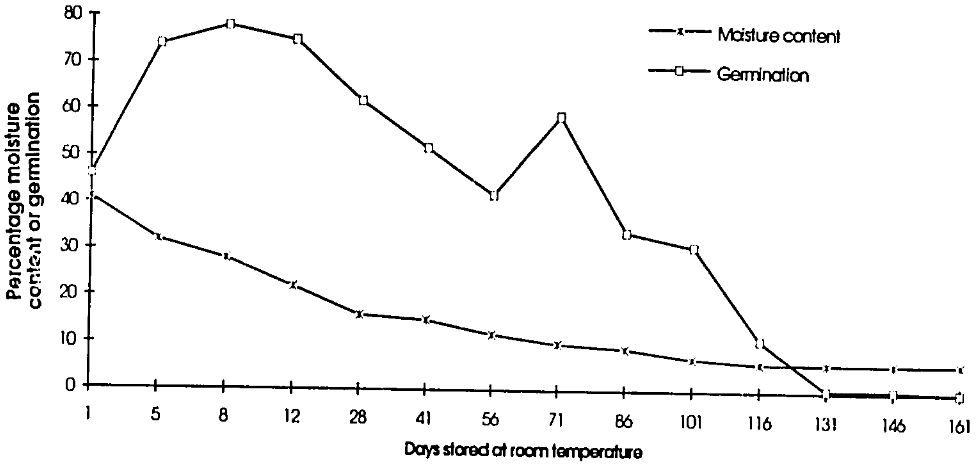

Figure 3. Drop in moisture content and germination % with increase in no. of days stored at room temperature

According to Figures 4 and 5, 30% initial moisture content produced the best results. Fungus was found at both 41% and 30% moisture content; no fungus was observed at 18% moisture content. For each germination test, 100 seeds x 4 replicates was used.

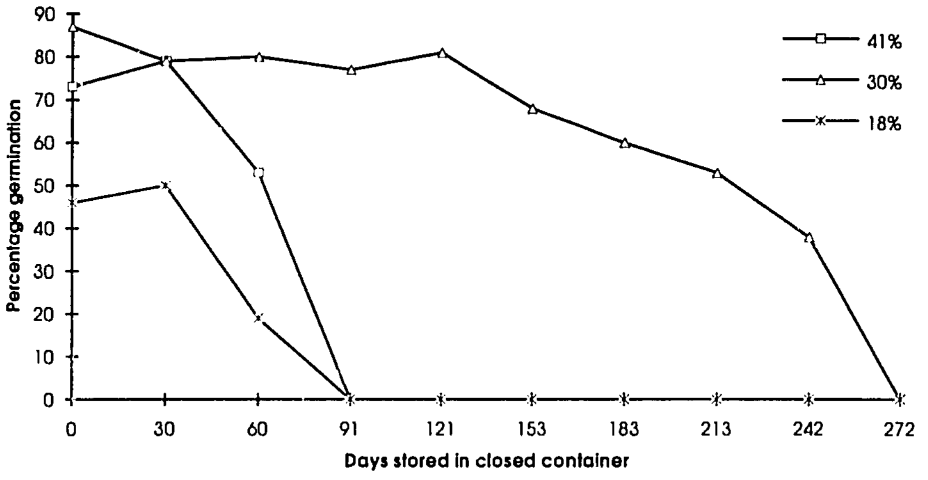

Figure 4. Germination percentage of seed stored at 31° C

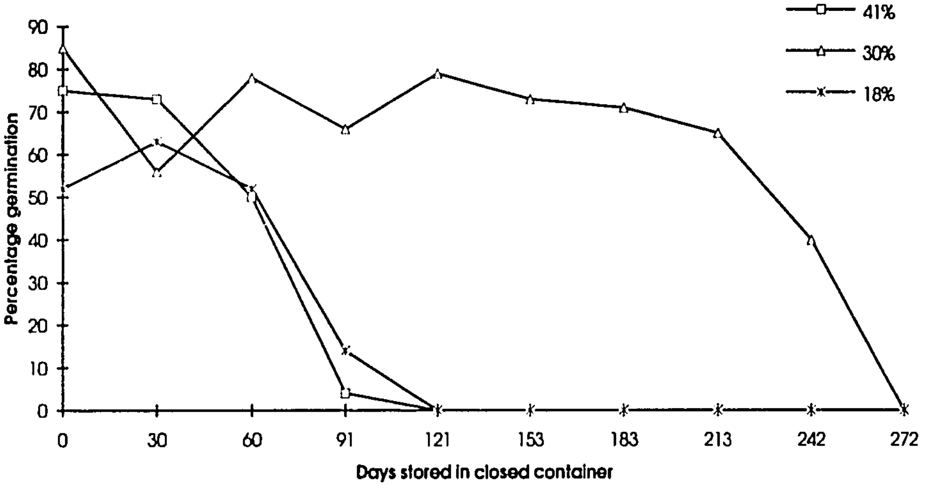

Figure 5 Germination percentage of seed stored at 23° C

Summary

- Seed stored better in an air conditioned room than at room temperature in one study; there was no difference in another.
- Initial seed moisture content had no effect on the long term storability of neem seed in one study. An initial seed moisture content of 30% was significantly better than 41% or 18% in another study.
- Seed moisture content dropped to 6% after 116 days of storage at room temperature.

Recommendations for Future Research

Further research is needed on methods of seed storage and transportation to facilitate seed exchange and germplasm conservation. Provenance trials and selection for high-yield seed trees (specifically for Myanmar) are needed.

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Distribution, Performance, and Utilization of Neem in Nepal

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Neem (*Azadirachta indica*) is a multipurpose tree species of great promise for resource-poor, developing countries. It has been grown by farmers in Nepal's warmer, southern regions for at least a century. Most trees are found along avenues, in orchards, and on homesteads. To date, large-scale, commercial neem plantations have not been reported in Nepal.

Although neem is found both in the wild and on cultivated land, its indigenous habitat in Nepal has not yet been fully established. Most researchers believe it is an exotic species with characteristics widely adaptive to Nepal's eco-physical conditions (Stainton, 1972; Pandey, 1982; Jackson, 1987; and Kayastha, 1985). However, some older farmers from the Dang and Surkhet valleys in western Nepal claim to have spotted neem in the natural forests as long as 20 years ago. According to them, the species is no longer found in the wild due to excessive cutting for fuelwood, medicine, and fodder. Assuming that neem is exotic, it is believed that the tree was introduced to Nepal by traders and farmers bringing the seeds from India after discovering the multiple benefits of neem and by birds and other agents of seed propagation transferring neem seeds from India into Nepal's Tarai region. Based on Brandis (1921), Duthie (1903), Troup (1981), and Kanjilal (1928), as quoted in Tewari (1992), the natural habitat of neem could be the Siwalik forests in Uttar Pradesh.

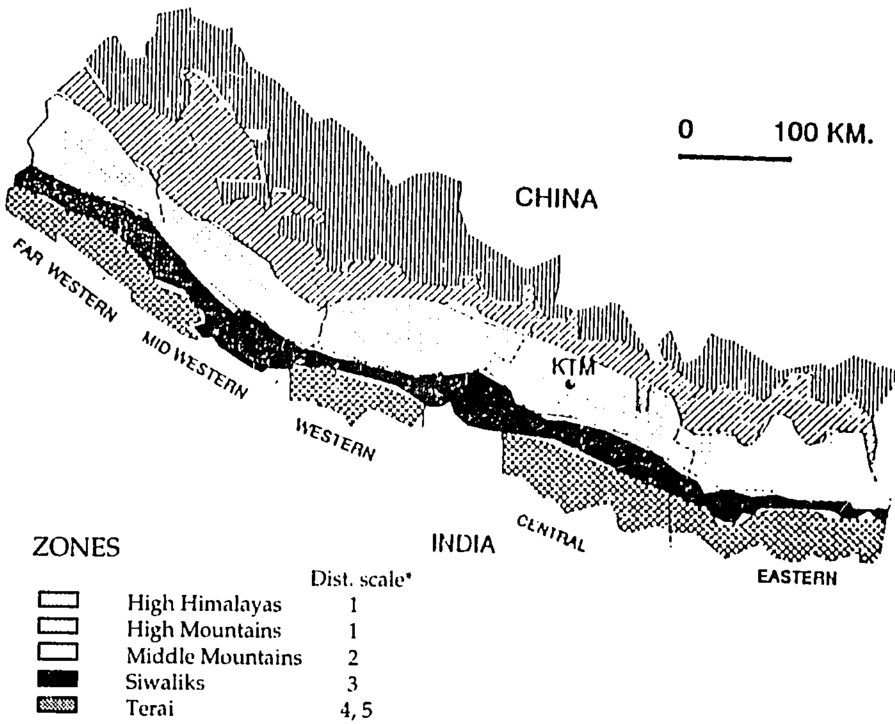
The major uses of neem in Nepal are shade, toothbrush sticks, medicinal herbs, storage pest repellents, fodder, firewood, timber, fruit, and animal bedding materials. Neem is truly a multipurpose tree in Nepal.

Current Distribution and Cultivation

Distribution

Neem is a medium- to large-sized evergreen/deciduous tree found both in the wild and in homestead gardens of the Tarai and Inner Tarai regions, up to an elevation of 900 m. It is more common in the dry western area of the Tarai than in the wet eastern area (Figure 1). It has a large, dense, and circular-shaped, spreading crown. It is a hardy, light-demanding tree species. It can be found in almost every village in the Tarai regions of Nepal (Kayastha, 1985). The species prefers a moderately dry climate (Singh, 1982). To date, it has not been found in elevations above 1,000 m. It can grow on degraded and dry sites. It is a common roadside tree in western Nepal and is also commonly found on farms and homesteads throughout the plains (Tarai and Inner Tarai regions).

Figure 1. Map showing distribution of neem cultivation in Nepal.



* Scale notation: 1 = not found, 2 = few isolated trees, 3 = many scattered trees, 4 = often planted, 5 = common.

Source: Department of Survey.

Climate and Soil

Neem can grow on sites with a maximum temperature above 40° C and a minimum of 2-15° C. It cannot withstand frost or freezing temperatures. The optimum rainfall range is 450-1,125 mm (Singh, 1982; Kayastha, 1985; Howell, 1989). Relative humidity in its natural areas during summer is 60-90% and in winter is 40-70%. Neem does best on well-drained soils with a fairly high subsoil water level. It can, however, persist under adverse soil conditions where few other tree species can grow (Howell, 1989). Neem tolerates most soil types except heavy clays. Growth is better on deep permeable soils, even almost pure sand, and it succeeds on dry, shallow, stony soils with a deep water table. It does not tolerate waterlogging and should only be planted on well-drained sites. It prefers soils with a neutral pH.

Leaf Shedding, Flowering, and Fruiting

Neem leaf shedding and flowering are controlled by the climatic conditions of the locality. The tree becomes leafless for only a short period. Based on observations, the trees are deciduous in the dry, western Tarai but remain nearly evergreen in the wet, eastern and the Inner Tarai zones. In most of the Tarai, neem flowers the first week of May. The fruit ripens from June to July (Howell, 1989).

Silvicultural Characteristics

Neem cannot survive under intense shade, weed competition, or frost conditions. The seedlings are intolerant of waterlogging and tap roots rot when soil moisture is excessive. It coppices well and produces root suckers in dry localities. It withstands pollarding well. It is browsed by goats and can be destroyed by fire (Singh, 1982).

Ongoing Improvement Research

Neem improvement research in Nepal is still in its infancy. Most of the tree introduction, nursery techniques, and utilization work have been carried out by voluntary researchers and farmers. In fact, farmers are more knowledgeable about neem improvement than many researchers. Nevertheless, the following research activities have been carried out by various agencies, farmers, and researchers:

Nursery Techniques

The seeds must contain green cotyledons at the time of planting. To achieve this, the seed should be sown as soon as possible after collection. Depulping and cleaning the seed improves germination. Fresh seeds give about 60-70% germination. Neem can be raised successfully through direct sowing, outplanting of entire plants, or stump planting. Direct sowing is preferred over the other two methods. The soil is worked to a depth of about 15 cm, and the seeds are sown at a depth of 1.5 cm.

Germination and Seedling Development

Neem propagation is commonly done through direct seeding and nursery-raised seedlings. Seeds do not show any dormancy; once sown on well-worked seed beds, they germinate quickly. Germination is epigeous. The growing period ceases from November to March. Important factors affecting seedling growth are moisture availability and weed competition (Kayastha, 1985; Howell, 1989).

Growth and Development

Neem grows rapidly once seedlings are established. Weeding is more important for survival and rapid growth than irrigation. Growth from the sapling stage onward is fairly rapid.

It can be assumed that the allometric relationships in neem do not differ markedly from other commonly grown, on-farm trees in Nepal. Therefore height- and Dbh-based growth models can be developed for this tree species. An annual girth increment of about 2-3 cm is usually obtained. In well-managed plantations, an annual girth increment of about 8 cm has been found (Table 1). Based on the observed growth patterns, the following allometric relationships have been found:

- $\log H = 0.56 + 0.56 \log(d)$ s.e. = 0.194 $r^2 = 0.83$
- $\log H = 1.56 + 0.43 \log(A)$ s.e. = 0.30 $r^2 = 0.59$
- $\log d = 1.76 + 0.78 \log(A)$ s.e. = 0.39 $r^2 = 0.74$

where H = height in m
d = dbh in cm
A = age in years

Utilization

Fodder

Farmers in Nepal commonly lop and feed neem foliage as green fodder to their animals. They report that the leaves are quite palatable and can meet maintenance requirements of bullocks and male buffaloes. The leaves are reported to have a crude protein content of 12-18% with a satisfactory digestibility coefficient. Ketkar (1977) reported the possibility of using neem cakes as part of the famine feed along with bagasse, urea, molasses, cottonseed meal, minerals, mixture, salt, and vitamins A and D. Neem cake has high fiber; thus, it is not a true concentrate.

Firewood

Neem provides good firewood. The wood weighs 560-850 kg/m, and has a high calorific value. It is also a durable and decorative timber. In Nepal, neem trees from the wild may have vanished due to excessive use as firewood.

Table 1. Performance of individual neem trees in the Tarai and Inner Tarai regions

Location	Dbh (cm)	Height (m)	Age (yr)	Remarks
Butwal Plywood Nursery, Butwal	12.7	6.3	3.5	Bund Plantations
	11.7	6.2	3.5	
	9.7	5.8	3.0	
Butwal-Bhairahawa Highway	15.6	7.2	4.0	Roadside Plantations
	22.5	10.8	6.0	
	35.0	15.6	7.0	
East West Highway Tamnagar, Butwal	35.2	13.6	3.0	Homestead Plantations
	51.6	13.2	21.0	
	9.0	5.0	2.0	
	3.0	4.2	2.0	
	37.5	8.2	17.5	
	46.5	8.6	20.0	
Lipni village, Chikani VDC	34.8	12.0	8.0	Homestead Plantations
Amalekhganj, Bara	58.5	16.0	6.0	Roadside/ Homestead Plantations
	101.1	22.3	65.0	
	37.0	12.5	10.0	
	22.0	10.5	8.0	
	32.5	13.5	11.0	
	87.3	21.5	19.0	
	51.5	20.5	15.0	
Nijgarh, Bara	32.5	14.2	9.0	Roadside/ Homestead Plantations
	14.2	9.0	3.0	
	21.4	15.4	4.0	
	27.5	13.5	7.0	
	35.4	12.8	8.0	
	8.9	6.5	2.0	
	30.0	11.5	5.0	
	27.5	10.0	4.0	
	29.0	14.0	5.0	
	18.0	7.5	3.0	

Storage Pest Repellents

Neem leaves, kernel, oil, seed cake, fresh fruit, and other products provide effective control against such larvae pests as lacorn weevil, locust, fall army worm, shield bug, Mexican beetle, diamond moth, beet leaf caterpillar, termite, aphid, and cabbage butterfly.

Other Common Uses

Farmers interviewed reported the following common uses of neem:

- Leaves are mixed with warm water and used for bathing to cure skin irritants and diseases.
- Leaves are rubbed on bruises, cuts, and rashes to cure or relieve these problems.
- Oil is used in homeopathic medicines to treat such common ailments as stomach ulcers, worm infections, and rheumatism. Also, neem leaves are placed on sleeping beds to suppress small pox and other skin infections.
- Farmers commonly mix neem leaves with major food grains to repel such insect pests as moths, rice weevils, and red flour weevils. Some farmers (especially upper caste Brahmins and Chettris) will spread a layer of green neem leaves in food storage bins. Other castes reported simply mixing the leaves and twigs with the grain before storing. Around 5-6 kg of green neem leaves are mixed with about 150 kg of rice and wheat seed grains and then sun-dried.
- One farmer in Nijgarh Village, in central Nepal reported that he used neem bark and seeds to suppress malarial symptoms in his children.
- Young neem leaves, shoots, and twigs are used as green vegetables, chatani, and other delicacies, especially when tender and succulent. They are eaten during hot and dry seasons to prevent heat stress.
- Fruits are edible; children and birds commonly eat them. Although bitter when ripe, young fruits are reported to be sweet.
- Leaves and twigs are used as bedding materials by most farmers in the Inner Tarai region of Nepal. Besides repelling bugs and other insects, the mulch mixed with animal dung makes a good organic fertilizer. Tewari (1992) noted that neem leaves and cakes increase soil fertility and water-holding capacity and reduce soil acidity. Although neem is not a leguminous tree and does not fix nitrogen neem-based organic manures are believed to facilitate the growth of nitrogen-fixing bacteria.
- The only reported or observed disadvantage of neem is the attractiveness of ripe fruits for the housefly and other disease-carrying insects. Villagers claimed these pests sometimes cause stomach ailments and other health problems. This is one reason why farmers may lop neem branches before fruiting. This obviously affects seed production and subsequent uses of seeds for other potential uses.

Recommendations for Future Research

Based on interest shown and problems identified through discussions with neem growers in the Tarai and the Inner Tarai regions, the following research areas are recommended for implementation as part of neem improvement research in Nepal:

- Collection of native germplasm. Although only one provenance is believed to be growing in Nepal, in an east-west stretch more than 1,000 km in length and 50 km in width, some morphological variations have been detected. The major differences are in leaf shedding, fruiting season, and tree form. This task will capture all the genetic and phenotypic diversity in Nepal.
- Establishment of seed orchards by identifying plus trees. A commonly stated reason for not planting neem trees is a lack of adequate seeds and seedlings. Therefore, from the collection of national and international provenances, plus trees will be selected and seed orchards established.
- Development of more-appropriate and cost-effective nursery techniques. Although preliminary nursery techniques have been developed for neem seedling production, better seedling preparation methods, appropriate soil mixtures, and proper size of polybags are yet to be determined.
- Provenance evaluation under different ecological and climatic conditions. Promising provenances from India, Burma, and Nepal should be screened for best growth and biomass on a short-rotation basis. Only the selected provenances should be used for on-farm trials, including agroforestry trials.
- Utilization of leaves, bark, seeds, and roots in medicine, storage pest repellents, and fertilizer additives. To refine traditional use practices adopted by farmers for leaves, barks, seeds, and roots in storage pest repellents, trials should be conducted to discover the most effective neem products and methods for controlling storage losses of food grains.
- Investigation of organic manure quality. Animal bedding is the third most important MPTS product in Nepal. Some farmers have already reported using neem leaves and twigs as bedding and composting materials. Investigations should be conducted to learn the magnitude of fertility improvement in neem-based organic manures as compared to conventional ones.
- Devise extension programs. At present, farmers are either ignorant of neem's potentials or are incapable of obtaining seeds and seedlings for large-quantity planting. Appropriate extension programs are needed to promote widespread planting and management of neem trees.

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Neem in Sri Lanka

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Azadirachta indica (Neem) goes by many names. These include *Kohomba* in Sinhala, *Vempu* in Tamil, *Neem* in Hindi and margosa in English. Neem grows abundantly in the drier parts of Sri Lanka, concentrated in the Northern and Southern Provinces. It commonly grows to a height of 13-15 m with dbh of 0.5-1.0 m and bearing a dense crown. Flowering is typically between March-May but this may extend until December or even later. Fruiting is abundant from October-December. Seed viability is poor. It is cultivated extensively in home gardens in dry and intermediate zones and, to a lesser extent, in the wet areas. It has become very popular as avenue planting in urban areas for environmental protection.

Uses of Neem

Nearly every part of the neem tree can be used for some purpose. It is said that the value of its by-products is greater than that of the timber in its 50-55 year rotation. Traditionally the tree in used by rural people in ayurvedic (herbal based) medicine as it is reputed to have both therapeutic and antiseptic properties.

Timber

Neem timber is used for making special furniture such as wardrobes, book cases, closets, etc. where neem's insect repelling properties are useful. Although it has satisfactory strength, furniture dealers do not prefer neem due to it's rough and interlocked grain which does not take a good polish. Normally a good polish is required for Sri Lankan furniture. However, in recent years blackened neem furniture has become fashionable among city dwellers. Supply of timber is another problem, as there are very few planted forests of neem. The present distribution of the species only covers 2,600 ha in the Eastern, Northwestern and Southern areas of the country (Jayasekera, 1991).

Fresh Leaves

Fresh leaves display antiseptic, antibacterial, and pesticidal properties. They also destroy intestinal helminths when taken with garlic or bee honey. Leaf extract can be sprayed for controlling insects. Leaves contain the alkaloid paraisine (Jayaweera, 1981). Young leaves are used as fodder for cattle which increases milk secretion and helps in digestion. They also make good green manure and mulch.

Dried Leaves

Dry leaves produce essential oils which are antifungal and pesticidal. They are used in grain storage, particularly paddy.

Flowers

The flowers are cooked as a curry or made into soups.

Fruit

The pulp is edible and can be used as a tonic to cure urinary diseases. The fruit contains the alkaloid margosine and the bitter principle nimbidin (De Siiva, 1991).

Kernel

Kernel oil help cure chronic skin diseases, ulcers, rheumatism, leprosy, sprains, malarial fever, asthma and ear aches. It can be used as an antihelminthic in cattle and elephants. The oil can also be used as a feed for cattle as well as for treating manure and as an insecticide and nematocide. In Sri Lanka it is used in manufacture of margosa soap and toothpaste. Extracts are reported to be antidiabetic, antibacterial and antiviral.

Bark

The bark is said to control malarial fever, skin diseases, catarrh, and typhoid fever. It also contains margosine.

Gum

This can be used as a stimulant as well as an adhesive in silk dyeing and as a medicine for catarrh.

Commercial Availability

Neem and its products are widely used in ayurvedic medicine and are an integral part of rural culture in Sri Lanka. The principle users are low income families. The 13,132 registered ayurvedic physicians in the country are the largest users of neem product, although low availability is a problem due to the limited planting in home gardens.

The main producer of margosa based medicines in Sri Lanka is the Ayurvedic Drug Corporation which comes under the Ministry of Ayurvedic Medicine Consumption of margosa products by the corporation in recent years is as follows:

Table 1. Consumption of margosa products by the Ayurvedic Drug Corporation.

Components	1987	1988	1989
Bark (kg)	1,900	1,950	1,850
Leaves (kg)	4,000	6,000	1,550
Oil (kg)	1,500	1,600	1,490

The volumes given above represent a very small portion of the total as most medicinal preparations are based on prescriptions given by village level ayurvedic physicians. The potential for use of neem in medicine is not fully exploited due to lack of incentives and facilities for refining the products.

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Neem Improvement in Bangladesh

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In Bangladesh, *Azadirachta indica* (neem) is cultivated in highlands (on homesteads) throughout the country. Neem is a common household tree in northern Bangladesh, including Rajshahi. It is of considerable interest to farmers and plays an important role in agroforestry systems in Bangladesh. Common uses are described below:

- Trees on homesteads are believed to purify the air around the house and keep the homestead free of diseases.
- Some people make a daily drink from leaves soaked in water overnight. It is often taken in the morning to purify the body and maintain health.
- Leaf litter provides nutrients to shallow-rooted crop plants.
- Neem seed cake is used alone or with urea as fertilizer for rice cultivation; it is mixed with soil (1.5 t/ha) to prepare fields for transplanting and to reduce attack of rice stem borers (Katz, 1991).
- Neem is used to prevent soil erosion and help in soil conservation.
- Neem oil is rubbed on farm animals to protect against skin parasites; the oil is used on people to treat skin diseases and lice.
- Neem oil is used to lubricate cartwheels and machines.

Product of Neem

Wood

The wood is hard and resistant to termites and wood borers. The timber is ideal for furniture and construction. Sattar *et al.* (1992) observed that neem, in general, possesses higher strength values than teak. It is classified as a heavy wood with specific gravity of 0.74 in air-dry condition. Its shrinkage co-efficient is 22.02. In light of shrinkage and shrinkage co-efficient values, it may be grouped as a medium-class wood. It is easy to dry and cures without defects. It takes 7.5 days to kiln-dry 2.5 cm planks from a green state to 12% moisture content.

Chowdhury and Akhtaruzzaman (1992) observed that the fiber length of the species is lower than the average value of paper-making hardwoods. The paper-making properties of the pulp are inferior. Hannan *et al.* (1992) observed that the wood is hard and heavy and that sawing is difficult. Neem shows excellent machining properties except for planing. In handtool tests, boring trials, resulted in 100% defect-free samples while mortising tests produced only fair products. Turning tests gave excellent results, yielding 80% defect-free samples. Neem finishes to an excellent high gloss. Akhter *et al.* (1992) observed that neem is hard to treat with preservatives like CCB (2:2:1). Penetration with CCB and creosote is 0.1 cm for the side and 0.2 cm for the end.

Oil and Seed Cake

Oil is extracted by crushing and pressing the seeds in an oil mill. A traditional "ghani" is generally used in Bangladesh.

The remains of seeds after oil extraction is known as neem seed cake. It is used as a fertilizer for soil improvement either alone or with urea. It enhances the effectiveness of urea by reducing the rate of nitrogen release. It has a suppressive effect on various soil-borne diseases caused by fungus or bacteria and on stem borers.

Current Distribution

The density of neem trees is not high in Bangladesh, except in some parts of the country (Rajshahi, Sirajganj, Bogra and Dinajpur districts in northern Bangladesh). Islam (1983) estimated the density of neem in North Bengal at about 50 trees/square mile. In the 1960s, its density was estimated at about 200 trees/square mile. However, many trees were cut during the early 1970s. Katz (1991) estimated a maximum neem tree density of 10 trees/square mile for the greater Rajoir area of Madaripur District. Reza *et al.* (1992) estimate the total number at about 3,404,000, covering 0.84 million hectares. Figure 1 presents a rough picture of this distribution. Of this total, 383,000 trees are on small plantations and under 10 years old. The estimated total of scattered trees in the country is 2,831,000, of which 2,125,000 are less than 10 years old. Neem is a major species in the Tista meander flood plain and in the high Barind Tract, where it is found mostly on homesteads (Abedin and Quddus, 1990).

Neem grows in the greater Rajoir area of Madaripur District, where Gano Unnayan Prochesta (GUP), a non-government organization (NGO), is studying the insecticidal actions of neem trees in. Kar (1992) stated that the species grows abundantly in the High Barind Tracts, and it can be grown in highland and homestead areas (31% of the country). A survey conducted in 1990 for the High Barind Tract showed that 42% of the farmers own neem trees. The average number of neem trees per farm was 5.11 (Kar, 1992). He observed that only 25% of the neem trees growing in the Barind area were more than 10 years old. This suggests a high demand for construction timber and fuelwood.

Hussain *et al.* (1991) recommended growing neem as an upper canopy tree in the Barind Tracts after a Diagnosis and Design (D&D) exercise conducted by the Bangladesh Agricultural Research Council, the Bangladesh Agricultural Research Institute, and the International Council for Research in Agroforestry (ICRAF). The species is recommended for the medicinal values of its leaves; income generation for rural people through the sale of products; pesticides; fuelwood; and neem seed cake production. According to the Bangladesh National Herbarium, neem distribution in northern Bangladesh is higher than in other areas of the country. It is cultivated at higher elevations, especially on homesteads. Few neem trees grow in the hilly areas of Bangladesh, in the low-lying areas of Sylhet, or in coastal and mangrove areas.

Some NGOs, the Forest Department, and the Bangladesh Tobacco Company Ltd. (BTC) have planted neem as part of their plantation programs. The Swiss Development Cooperation (SDC), under its agroforestry action-research program, has supported 10 NGOs in planting neem. The NGOs involved are the Community Development Association, Samaj Unnayan Proshikkan Kendra, Shahid Smrity Sangha, Come to Work, Tengamara Mahila Sabuj Sangha, Rajshahi District Unemployed and Landless Farmers Accessory Organization, Mohila Shilpa Pratstan, Association for Community Development, and the United Development Initiatives for Programmed Actions.

These NGOs have organized farmers to plant different tree species in their crop fields and homesteads to meet fuelwood and timber requirements of the rural poor. So far, 27 species have been tested; of these, 11 are suitable for northern Bangladesh. Under this agroforestry program, nearly 3,800 neem trees have been planted in crop lands and homesteads of the participating farmers. The survival percentage of the trees in the crop fields was 47%; on homesteads, it was over 80%. The main causes of neem mortality were identified as human interference and livestock grazing.

The BTC has planted neem on roadsides; these are reportedly performing well. The Forest Department has planted 400 neem trees on embankments surrounding the Dhaka Airport, to control flooding. The species is planted with the view that it would not be browsed by cattle and other livestock. Previously the area was planted with *Acacia* and *Albizia* spp.

Gono Unnayan Prochesta (GUP) raised 900 neem saplings in 1990, which it planted in Madaripur (Katz, 1991). In general, seedlings are sold in village markets during tree planting seasons. Rural people buy the seedlings for their homesteads from rural markets.

Tree Improvement Research

The Bangladesh Forest Research Institute (BFRI) is mandated for tree improvement research in Bangladesh. To date, BFRI has not undertaken any neem improvement research. GUP and SDC have funded 10 NGOs to study neem seed viability, seed storage techniques, and nursery techniques.

Seed Collection

In Bangladesh, neem flowers and fruits annually. Flowering occurs in March-April, and fruiting occurs in July-August. Young trees produce fruits after 4-5 years. A mature tree yields 20-40 kg of fruits/year.

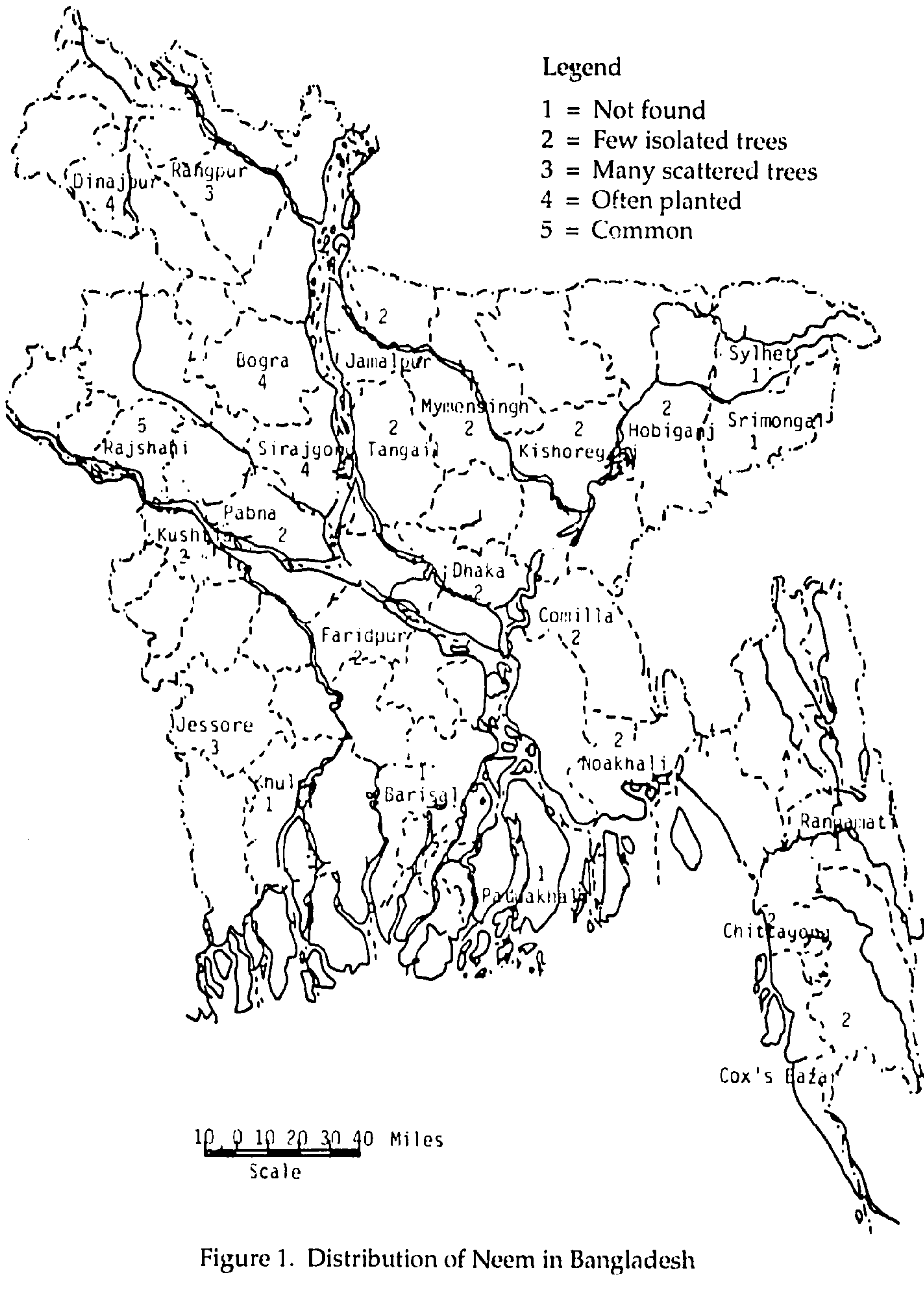

Figure 1. Distribution of Neem in Bangladesh

Seeds are collected when the fruits are ripe. When mature, the fruits become yellow and fall to the ground. Collecting green, unripened fruits results in cleaning and drying difficulties. It is also reported that ripe seeds possess more insecticidal properties. Since not all seeds on a given tree ripen at the same time, collecting ripe seeds is spread over a three-week period. To ensure that ripened seeds are collected, one should collect the seeds that have fallen to the ground every day or every other day and not pick them from the tree. Daily seed collection takes only about 10 minutes and can be done easily by the children of a homestead. Depulped seeds can be found because birds may eat away at the soft and sweet pulp. Such depulped seeds can be collected as well as seeds with pulp. Lone trees, exposed to maximum sun, produce high quantities of seed.

Seed Processing and Storage

Collected seeds should be processed immediately to avoid rotting. The process is as follows:

Remove pulp and clean seeds

The pulp is removed easily by rubbing seeds in a traditional bamboo sieve and washing away pulp particles repeatedly until the seed shell is completely free of soft fruit pulp particles. Cleaning unripened seeds causes two problems; 1) pulp cannot be removed easily, and 2) the seed shell is too soft and breaks easily.

Dry seeds in the shade

Drying in sunlight may reduce the insecticidal efficacy of the seeds. It is important to dry the seeds thoroughly; otherwise, fungus growth occurs quickly.

Store dry seeds in dark, well-ventilated conditions

Baskets or jute bags are more suitable containers than polybags. It is recommended to use small containers because air flow in the middle of large containers tends to be weak. During the rainy season, it is recommended to re-dry stored seeds every 2-3 weeks.

Protect stored seeds from fungus attack

Two types of fungus attack the seeds. One produces a black dust, covering the seeds, and can be seen first outside the seed shell. The other destroys the whole seed kernel before it becomes visible as a greenish-yellow dust from the outside. Thus, when checking for fungus attack, open some seeds and look at the kernels. Seeds attacked by fungus should be discarded to avoid possible spreading. There is no available information on whether fungus attack reduces insecticidal properties.

Nursery Practices

Germination is epigeal, and sown seeds should be covered with only 1 cm of soil. Neem seedlings/saplings can be grown in polyethylene bags. Germination of freshly collected, depulped seeds is high (100%). Thus, if fresh seeds are used, one seed per polyethylene bag is sown. Seed viability lasts only 2-3 weeks. Subsequently, the germination rate declines. Dried seeds are soaked in water for 3 days before sowing; germination rate is lower than that of fresh seeds.

For most tree species in Bangladesh, seeds are sown in February-March and transplanted after 5-6 months (July-August). For neem, however, this pattern is not possible. Neem seeds ripen in July-August and, as mentioned above, are viable for only a short time. Thus, the seeds are sown in July-August. The saplings must be transplanted in a young stage in September or early October. This is only possible if proper nursery practices are maintained. The other option is to keep the saplings in the nursery for 10 months until the beginning of the rainy season. In this case, it is important to use larger polyethylene bags and to watch that roots do not penetrate the bags and grow into the outside soil.

Seedlings are also raised in seed beds and the roots of the 1-to-2-year-old seedlings are pruned two weeks prior to selling. First, 50% of the roots are pruned, and a polyethylene sheet is laid out so that newly developed roots cannot penetrate the outside soil. After one week, the remaining roots are pruned, and the seedlings are removed from the seed bed with a ball of soil. Such seedlings are kept under shade for 3 days for hardening and then for marketing (Ahmed and Kalam, 1991). Neem saplings grow slowly; the root system, however, is well-developed. After the first year, the trees grow quickly and can reach 10 m in height after only 5 years.

Research Recommendations

As agroforestry and participatory forestry activities gain momentum in Bangladesh, NGOs and governmental organizations become increasingly involved in tree plantation. On homesteads, the most preferred tree species are jackfruit and mango; these are used as cash crops for fruit and other wood and non-wood products. In some areas of northern Bangladesh, *Melia azaderach* (known locally as *ghora* neem) is the third most preferred species on homesteads. In crop lands, the growth of *ghora* neem was found to be as good as that of eucalypts (Ahmed *et al.*, 1989). Rural people give equal preference to both neem and *ghora* neem for their insecticidal properties and other uses. On almost all homesteads, neem trees are grown because of local beliefs that it keeps people in good health. Because the leaves are compound and small in size, sunlight penetrates to the ground, allowing other plants or crops on the homestead to prosper.

Farmers grow trees that yield fruit or grow fast. Neem found in Bangladesh, however, is fast-growing. Therefore, it is necessary to find trees with such characteristics as faster growth and higher yield. Research on neem should focus on the following to meet the needs of rural people:

- Screening and testing fast growing provenances;
- Developing seed orchards to meet high demands from NGOs for plantation programs;
- Studying vegetative and sexual periodicity and pollination behavior;

- Studying variability among populations found on homesteads and along roadsides;
- Selecting provisional plus trees and establishing progeny trials;
- Developing improved seed sources and establishing demonstration plots using genetically superior seed;
- Developing seed collection, storage, and handling procedures;
- Studying the nature of seed dormancy; determining proper methods to break seed dormancy and enhancing germination percentage;
- Developing a seed grading system on the basis of size and weight to achieve higher germination percentages and better initial height;
- Studying soil dynamics under agroforestry systems with different crop combinations;
- Developing vegetative propagation techniques;
- Establishing spacing trials for optimum production/unit area;
- Introducing provenances with adaptability for degraded and denuded hills;
- Developing plantation techniques;
- Studying growth and yield under different conditions; and
- Studying neem pests and diseases.

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Neem Distribution and Research in Indonesia

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Neem is an introduced species in Indonesia. It has several common names: *imba* or *mimba* (Java), *intaran* (Bali), and meupleuh (Madura). Neem typically grows in monsoon forests.

Originating in India and Myanmar (Burma), neem has been naturalized in other tropical zones with dry climates: Cuba, Fiji, Guinea, Haiti, Malawi, Malaysia, Mauritius, Nicaragua, Nigeria, Pakistan, Saudi Arabia, Sierra Leone, Sri Lanka, Suriname, and Zanzibar (National Academy of Sciences, 1980; Ahmed and Grainge, 1988). Neem tree products have multiple uses: termite-resistant timber, fodder for cattle, gum exudates for medicinal uses, extracted seed oil for soap manufacture and pesticides, and fuelwood (National Academy of Sciences, 1980).

Few neem trees are found in Indonesia, however, the species is expected to be successful in the dry, tropical areas of eastern Indonesia. Because neem has high potential for various uses, it is suggested that the species be developed in various social forestry programs. To benefit communities living in close proximity to forested areas, this species might also be promoted in agroforestry systems. In Indonesia, neem is used primarily for construction. Its many wood uses should be promoted as it has good mechanical, physical, and chemical properties.

Distribution

Neem is thought to have arrived in Indonesia from Burma and is common throughout open scrub forests in dry zones. Neem trees are found in the dry lowlands of East Java, Bali, Madura, West Nusa Tenggara, and East Nusa Tenggara (Heyne, 1987). In Java, neem grows in areas 1-300 m above sea level. In West Nusa Tenggara, the trees are grown in areas 400 m above sea level with a periodic dry season (Baker and Van Den Brink, 1965). Martono (1928), a former botanist of the Forest Research and Development Centre in Bogor, mentioned that neem has never been found growing naturally in Sumatra, Kalimantan, Sulawesi, or Irian Jaya.

To date, plantations are grown primarily in dry areas of eastern Indonesia. In East Java and West Bali, the trees are grown for shade along roads; these trees are managed by local government, and, in some areas of West Nusa Tenggara and East Java, neem is used for fuelwood. In Besuki, Panarukan, and Situbondo (East Java), neem trees are tapped to extract gum exudates used to make paper glue. Leaves are used as medicine to treat malaria. Neem's strong wood is popular for construction.

In Java, neem has been planted as a substitute for teak as the primary fuel for a sugar factory (Harreveld, 1915). It was reported that the Medicine and Spices Research Centre established neem plantations in Indramayu (West Java) to study medicinal and pest-repellent properties.

Research Status and Neem Development

In Asia much neem research has been conducted particularly on silviculture, plantation, production, and utilization. Nevertheless, research results might not be applied thoroughly in Indonesia due to ecological or socioeconomic conditions.

Neem is now included as one of eight priority multipurpose tree species (MPTS) in Indonesia. This was approved during the 1991 National Workshop on Multipurpose Tree Species Research and Development (Tampubolon and Hidayat, 1991). Neem is considered a suitable species for development. Further research is needed in selecting appropriate models for neem cultivation by smallholders.

In Indonesia, the development of neem improvement is limited. The status of botanical research seems sufficient for tree improvement. However, in terms of silviculture (especially in relation to performance and production of neem), more information and research are needed.

A study on neem tree physiology was conducted in Indonesia in 1943 by Verhoef concerning the ability of roots to grow in oxygen-deficient soil (Tampubolon and Hidayat, 1991). Several field trials on seed processing and storage were conducted by Syafei (1959) and Syamsuwida (1991).

The Indonesian government has considered developing neem utilization techniques involving small farmers. Neem's various uses could help meet farmers' needs for fuelwood, timber, medicine, fertilizer, and fodder. It can also improve the productivity of their lands through agroforestry systems. According to Tampubolon and Alrasyid (1989), neem development should be promoted in the rainfed zones of the northern areas of East Java, as well as in North and South Bali, West Nusa Tenggara, Central Mollucas, the Palu Valley, and Merauke.

Recommendations for Future Research

Further research is needed in the following areas:

Tree improvement

- collecting germplasm in Indonesia and elsewhere
- establishing seed orchards and prevenance trials
- developing vegetative propagation and tissue culture methods

Ecology and silviculture

- relationship between habitat and performance
- phenology of flowering and fruiting
- seed collection, processing, and storage
- nursery techniques
- plantation methods
- silvicultural practices
- pest and disease management

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Neem in Malaysia

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Azadarichta indica is not an indigenous species to Malaysia. It was initially introduced from Burma (Mabberley and Pannell, 1989). It is also believed that Indians who migrated to Malaya in the 1940's brought neem with them.

Neem, especially the leaves and seeds, is mainly used for medicinal purposes such as treatments for small pox and ringworm (Azizol, per. comms.), malaria and ulcer (Burkil', 1966).

Current distribution of neem in the country

Neem trees in Peninsular Malaysia are mainly planted in gardens and around homes. In a current study (Azizol, pers. comm.) neem trees are found mainly in the northern states of Peninsular Malaysia such as in Penang Island, Langkawi Island, Perlis, Kedah and Perak.

Ongoing neem improvement research

There is no neem improvement research, except for small planting trials on sandy tin tailings with the objective of determining moisture availability. Survival was recorded as high as 100% (Ang, pers. comm.)

There was also a study on seed storage and viability. Seeds of neem are recalcitriant and storage by desication is only viable for about seven days (Baskaran, pers. comm.).

Recommendations for future research

Provenance research with the objective of determining adaptability to adverse sites such as tin-tailing areas is recommended. There are no references available related to neem improvement research in Malaysia. There is, therefore, an opportunity for useful research in this area.

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Neem in Pakistan

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Neem is a large, deciduous tree attaining a height of 12 to 15 meters. It is native to Pakistan but more often cultivated in homesteads or stripsides. In Pakistan it is cultivated in Larkana, Thatta, Bahawalpur, Faisalabad, D.I. Khan, Dera Ghazi Khan, Muzafar Garh, Rahimyar Khan, Hyderabad and Karachi

Biology

Flowering and Leaves

In Pakistan neem flowers during April-May and the color of the flower is whitish. Leaves are compound, simply imparipinnate, 20 to 38 cm long and crowded at the end of the branches.

Seed

Seed ripens in June-July. The number of fruits per kilogram varies from 2,500 - 3,000. Seed viability is short, usually only 2 months. Germination varies from 30-70%.

Silviculture

Seed Collection and Sowing

Seed should be collected from the tree at maturity, not from the ground. Seed is sown immediately after collection and depulping. No special treatment of the seed is necessary.

Propagation

Direct sowing into seedbeds or into pots is commonly practiced. Sowing is done in lines 25 cm apart in drills at 15 cm spacing. Sowing in July gives good germination. Pricking out is done in August. In the hot weather watering with partial shade gives very good results.

Nursery plants are lifted at 12-13 months and their roots and shoots pruned for planting out in the field. Two weedings in the first season are necessary.

Plant spacing

Generally 2 x 2 m spacing is followed for in block plantations; when grown with agriculture crops, the distance between two rows are kept wide. Cotton and sorghum are grown between the rows in different parts of the country.

Cultural Practices

It is reported that regular weeding in neem stimulates growth of the trees (Sheikh, 1988).

Soil and Water Requirements

Neem can easily be grown on dry soils. The tree is in' lerant of excess moisture in the soil. Proper irrigation increases biomass production.

Types of Plantings

It is suitable for agroforestry, wind breaks, shelterbelts, block plantations as well as linear plantations. In Karachi and D.I. Khan neem is successfully planted on road sides.

Rotation

Rotation depends upon the plantation objectives. For building poles and firewood, it is normally grown on a coppice rotation of 8 years.

Uses of Neem Components

Pesticides

Pakistan is spending Rs. 3.26 billion on import of approximately 5,000 mt of pesticides annually. Failure of pesticides is due to resistance, resurgence and secondary pest outbreaks. Environmental degradation due to excessive use of pesticides has led to a renewed interest in natural pesticides for ecological sustainability. Dr. Ghulam Jilani and his colleagues at GSRL have emphasized developing pesticides from neem as a potential source of biologically active chemicals. Neem seed extract controlled more than 80% of major stored grain insect pests and reduced grain damage up to 6 months and remained quite effective up to 13 months in a farm level trial in Sindh.

Techniques have been developed to prepare a ready-to-use pest control material known as neem seed bitters. Applications of neem seed bitters in field trials on rice in Larkana and Thatta and in cotton in Bahawalpur and Faisalabad have successfully controlled pests. This biopesticide is environmentally safe and is available at low cost. The neem seed bitters are produced by soaking powdered neem seed kernel in water for 12-16 hours at room temperature. The filtrate is then freeze-dried which yields a yellowish brown crystalline material which is instantly soluble in water. Standardized and stable formulations of neem seed bitters are being developed by the Research Institute of Chemistry, Karachi University. (PARC, 1991).

Gum

The bark exudes a clean, bright amber-colored gum which is collected in small tears and fragments. It is said to form a portion of the East India gum. Medicinally, it is a stimulant.

Fibre

The bark yields fibre which is of little economic value but is commonly employed in manufacturing rope.

Oil

A fixed, acrid, bitter oil, deep yellow in color, and strongly disagreeable in flavour, is extracted from the seed by boiling or applying pressure. It is employed medicinally as an anthelmintic and antiseptic, and is also used by poorer people for burning in lamps. The smoke is said to be offensive.

Medicine

The bark, leaves, and fruit are used as medicine. The bark is a bitter, tonic astringent, which has traditionally been used to treat fever, thirst, nausea, vomiting, and skin diseases. The root-bark is more effective in action than the bole bark and young fruit.

The leaves are used as a pot-herb, being made into soup and curry with other vegetables. The slightly aromatic and bitter taste which they impart to curries is relished by some. The leaves are an old and popular remedy for skin diseases. The fresh juice of the leaves is administered with salt to treat intestinal worms and with honey for skin diseases and jaundice. As an external application for skin diseases, neem leaves are used in a variety of forms such as a poultice, ointment and liniment. A strong decoction of fresh leaves produces an antiseptic which may be used in place of a weak solution of carbolic acid. A hot infusion of the leaves is used for fomenting swollen glands, bruises, and sprains, and appears to be an anodyne.

The fruits are described as a purgative and emollient, which are useful in the control of intestinal worms, urinary diseases and piles. The oil obtained from the seeds is employed in skin diseases and ulcers. The dry seeds posses almost the same properties as the oil when brushed and mixed with water or some other liquid.

The oil is antiseptic and has proved to be a useful in treating skin diseases, ulcers, rheumatism and sprains. Its antiseptic property is being adopted for the manufacture of a medicated soap, since the oil saponifies readily. This soap is very serviceable for the purpose of washing sores and for general uses similar to those of carbolic soap.

The flowers are useful in some cases of atonic dyspepsia and general debility. The gum has medicinal properties which are better than other remedies such as gum arabic and feronia gum. The *toddy* or fermented sap of the tree appears to be of great service to some chronic and long standing cases of leprosy and other skin diseases including consumption, atonic dyspepsia, and general debility (Watt, 1891).

The presence of a neem tree is generally presumed to improve the health of people in its neighborhood. It is believed to be a prophylactic against malarial fever and even against cholera.

Food and Fodder

The leaves are cooked with other vegetables in the form of a curry or are simply parched and eaten. The leaves impart a bitter taste to the food, but this seems to be liked by some people.

Wood

Neem sapwood is gray while the heart-wood is red. It is very hard weighing from 45 to 53 lb per cubic foot. It is commonly used for construction of carts and making agricultural implements. Owing to its durability, it is largely employed by the Hindus to make idols. Neem is recommended for carpentry work, including door panels, rails, sash frames, and furniture. It has also been highly recommended for making trunks and chests, which can withstand attacks of white-ants and protect the contents from other insects.

Other uses

Twigs are used for cleaning teeth and the oil is employed for burnir [illegible])il obtained from neem is used as a hair tonic by the women of Sindh because of its fragrance and also to kill vermin. The leaves are largely used to protect clothes, books and papers from insects. Neem is extensively planted as an avenue tree for which it is excellently adapted. The leaves make a good fodder for animals.

Recommended Research

- Provisional Plus Trees (PPT) must be selected and seed orchards established for better quality seed production.
- Seed collection and storage studies should be initiated as the viability of seeds is very low.
- Provenance trials should be established to identify fast growing and high yielding varieties from indigenous and exotic sources.

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Neem in Africa:
A Summary of Country Reports

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This report is a compilation of data collected from oral presentations and working papers presented by the African participants. Scientists who participated included Boudary Ourman Diallo of Burkina Faso, Abibou Gaye of Senegal, Souley Habi of Niger, Sidibe Modibo of Mali, Tal Moulnang of Chad and Ebenezer Olapade of Nigeria. Without their contributions this paper could not have been written.

History of Neem in Africa

Neem (*Azadirachta indica* Juss.) is naturalized for cultivation over most of tropical Africa. It is reported that Indian immigrants introduced neem in Mauritius and may also have taken it to continental Africa. It was also introduced by French and British migrants to West African (National Academy of Science, 1992). Neem is now widely planted in Mauritania, Senegal, Gambia, Guinea, Ivory Coast, Ghana, Burkina Faso, Mali, Benin, Niger, Nigeria, Togo, Cameroun, Chad, Ethiopia, Sudan, Somalia, Kenya, Tanzania and Mozambique. In these countries, the tree is particularly adapted to dry and low lying areas.

Ghana

The first introduction of neem in West Africa appears to have been in Ghana between 1919 to 1927 by Brigadier General Sir Frederich G. Guggisberg (National Academy of Science, 1992). It is reported that the first neem trees were planted in the northern territories and progressively spread throughout Ghana. They later reached the Sahelian region in what is now Burkina Faso, Mali and Niger.

Nigeria

Neem was first introduced to Nigeria in 1928 from India and probably Ghana. It was successfully established in Born province and then replanted in Sokoto, Katsina and Kans provinces in the 1930's. It has adapted well to the ecogeographical variations of Nigeria being widely distributed from the arid north to the humid south. (Oni, 1992).

The tree is fast-growing, can attain 28 m in height, and has a dense crown. Its thick leaves and stem epicuticles reduce water loss making it drought resistant (Larcher, 1975). The deep taproot system and profuse lateral root system also make it suitable for windbreaks and shelterbelts to protect against the southward encroachment of the Sahara Desert (Fasehun and Abolarin, 1980).

Neem may begin flowering and fruiting after five years. Flowering is usually profuse beginning in February; fruiting is common from April to June. Mature fruits are collected either from the tree crowns or picked from the ground under trees. Seeds have been reported to loose viability rapidly after two weeks but can be stored for 12 months under dry, cool conditions (Tela, 1983).

Freshly collected, viable seeds germinate within seven days of sowing in either sterilized or garden soil. It is better to transplant seedlings using the "ball" planting method rather than the "bareroot" or "stumping" method (Oni, 1992). It was observed that the Majiya Forest Reserve in Sokoto failed in 1951 because the plantation was established by direct sowing. The Gabo forest reserve, however, succeeded because transplants were used (Tela, 1983).

Niger and Burkina Faso

It is probable that neem reached Niger and Burkina Faso from Nigeria, mainly by colonial foresters. The following experiments were conducted in Burkina Faso:

- productivity of a neem stand in Ouagadoufo and Gouse in 1966
- plant spacing
- comparative species trials
- provenance trials
- phytosanitary prospectus
- utilization of neem in agroforestry
- live fences
- alley cropping
- utilization of neem seed products

Senegal

The neem tree was introduced in 1944 to the forest reserve of Mann from India and Ceylon, probably through a seed collection mission of the Senegalese agronomist Djibril Sene in the 1960's. After ten years the species began to be widely spread by developmental forestry projects. Many of the neem trees now in Senegal probably also came from neighboring countries (Mauritania, Mali) as a result of the far-sighted efforts of Djibril Sene (National Academy of Science, 1992).

Uses

Neem is a multipurpose tree widely used in Africa. Many parts of the tree are used by local people including the trunk, branches, leaves, seeds and bark. Some common uses of neem are discussed below.

Environmental Protection

In most West African countries neem is cultivated within villages and along roadsides for shade, as an ornamental, and for windbreak. It provides good shade and the surface root system stabilizes the soil. The tree grows in dry areas with 200 mm/year rainfall or more. It has good drought resistance and grows on very poor soils, including those which are dry, light, stony, sandy and clayey. However, it does not tolerate inundated conditions.

Energy, timber, and furniture

In the dry areas of West Africa, neem is used as fuelwood. However, the scent of the smoke makes it unattractive for utilization in the house. The branches are excellent for fencing and roofing because of their straightness and resistance to termites. The wood is also well adapted to making tools and, in Nigeria, the timber is used to produce high-quality lumber for house construction and furniture making.

Agroforestry

Neem trees are used as windbreaks and shelterbelts in arid, northern parts of Niger and Nigeria. The leaves are used for improving the soils as mulch and green manure. In Niger many farmers have found this manure efficient. However, it was reported that, in Senegal, neem trees are not used widely in agroforestry systems except as windbreaks. The reason is that the tree has a shallow and invasive root system which hinders development of vegetable crops.

In Nigeria neem was reported to coppice from felled stands. It can regenerate from the roots which makes it amenable to vegetative propagation using different auxin treatments. It is possible to produce clonal materials for plantation establishment.

Fodder

A recent survey in Niger indicated that 69% of the farmers reported that their animals occasionally consume neem fodder. The animals mentioned were sheep, goats, cows, camels, and (rarely) donkeys or chickens.

Medicine

Neem trees are well known for their medicinal properties. Different parts of the tree are used in the treatment of infections and diseases.

- **Leaves:** Leaf decoction was reported to be used for the treatment of skin diseases in Senegal and leprosy in Chad. In Mali, the tree is called *Saijiri*, which means "the tree against hepatitis." In Nigeria it was reported that the scent of the leaves repels insects—particularly mosquitoes. Leaf decoctions are also said to be used for preparation of antimalarial remedies and stomach medicines. In Nigeria the leaves are commonly used to prepare anti-malarial remedies in many homes, and, in some cases, they are used as pesticides. Some people believe that the scent from the leaves has insect-repellent properties, particularly against mosquitoes.

- **Seeds**: Investigations are needed to determine the efficiency of neem oil, crushed berries, and powdered seed in protecting stored produce, especially potato and bean. Seed cakes are commonly used in Chad as an insecticide. Seed cakes are reportedly used for termite and nematode control in Burkina Faso. Nigeria reported that 5-10 seeds kept in a 25 kg bag of beans will protect the beans from damage by weevils or bruchids.
- **Bark**: The tree bark is used in preparation of herbal therapeutics for diabetes and malaria.

Other Potential Uses of Neem

Recent studies focused on the potential for neem exploitation in Niger. The following potential uses were reported:

- **Pesticides**: Insects cause important problems for all types of crops in West Africa. Based on research results obtained in Niger, Nigeria, Ghana and Togo on maize and sorghum, neem-based pesticides are promising.
- **Manure**: Ongoing research in collaboration with ICRISAT suggests the use of neem in preparation of organic fertilizers.
- **Soap**: Production of soap is feasible at commercial and local levels.

Technical Bottlenecks and Problems

Very little research has been done on neem in Africa. Some efforts have been made by CTFT (now CIRAD-Forêt) in some West African countries. The results obtained show enormous potential for neem in West Africa. The reasons are good productivity for fuelwood and timber, good adaptation to Sahelian ecological conditions, and satisfactory growth. However, some bottlenecks remain to be solved by future research:

- the genetic base of the species in Africa is narrow
- seed storage is difficult
- control of pests and diseases is not adequately understood
- appropriate technologies for utilization of neem products are undeveloped

Recommendations

International cooperation

There is a great need to promote international and regional cooperation to develop sound programs for neem in Asia and Africa. International organizations (FAO, CIFOR, IBPGR, and ICRAF), regional networks (F/FRED and the CICSC Tree Improvement Program), and national programs should contribute to developing strategies for cooperation between countries.

- **Collaboration:** Efforts should be made to stimulate and support neem programs and networks.
- **Exploration:** International organizations should strengthen existing initiatives and networks on neem regarding seed collection and tree breeding.
- **Genetic engineering:** Genetic engineering techniques may have a significant impact on the use of neem. International organizations may play a role in strengthening national programs to use these techniques.
- **Mycosymbionts:** Research on mycosymbionts may increase the adaptation of neem to specific conditions.

Research

- ***In situ* germplasm conservation:** There is a need for knowledge on *in situ* conservation methodologies. In particular, methods of measuring population size, locations, and diversity are needed. Also the impact of different silvicultural treatments on the genetic diversity of neem will help future development efforts. This should not be to the neglect of ex situ conservation strategies such as germplasm banks.
- **Seed technology:** Important areas for neem research include:
  - seed characteristics (recalcitrant/intermediate)
  - monitoring of seed development
  - relation between moisture content, temperature and longevity
  - storage damage (genetic)
  - storage preparation techniques
  - cryopreservation techniques
- **Population genetics:** There is a need for better knowledge of population limits, phenology, pollination mechanisms and ecological information in order to make wise decisions for seed collection and breeding work. Morphological variability and isoenzymes should provide information on genetic architecture and serve as guides for sampling and assessment in planning collecting activities and field trials.
- **Genetic improvement:** Utilization of neem forest genetic resources through species and provenance selection:
  - There is a great need to support the establishment of a breeding population with a large genetic base in Africa.
  - Breeding tools should be available for application such as vegetative propagation by cuttings and grafting.
  - Methods for detection of pathogens should be developed. Improved quarantine procedure are required to avoid the transfer of pests and diseases through movement of plant materials.

Genetic Resources Information

There is a need for more information on neem genetic resources. Research is needed on genetic conservation, identifying local producers and establishment of provenance and progeny trials for tree improvement. This information should be available and accessible to interested breeders. A software system should be available to maintain and manage the information.

Training and Extension

International and regional workshops on neem genetic resources will be required in the future on specific aspects and to evaluate achievements of by the networks. Specific publications on neem will also be required.

Strengthening National and Regional Programs

National programs and regional networks should be strengthened with appropriate human, material and financial resources to contribute to better conservation and improvement of neem resources in Africa, Asia and India.

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Invited Papers

Section 3

Neem Production and the Small Farmer

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Neem (*Azadirachta indica*) is a versatile tropical tree with immense potential to protect the environment while developing sustainable agriculture. Apart from its drought and saline tolerance, neem seed production can contribute to the income of rural people. However, neem cultivation is not popular because many people are unaware of its benefits and economic advantages.

To improve the productivity of neem and to popularize its cultivation, particularly among small farmers, it is necessary to formulate a strategy that will include the standardization of propagation techniques, selection of superior germplasm, development of low-cost silvicultural techniques, orientation of farmers to proper collection and processing of seeds, and establishment of a strong extension network to highlight the benefits of neem for improving the economy and protecting the environment.

Environmental Protection

Neem has great potential to help small farmers and the environment in the tropics, particularly in India. This tree can help solve environmental problems such as how to rehabilitate degraded ecosystems and wastelands, reduce the use of agro-chemicals (fertilizers and pesticides), and generate income for small farmers. Little attention has been given to understanding the economics of neem and to popularizing its cultivation; impressive work has been carried out worldwide on its use as a pesticide and germicide, with significant potential for commercialization. Use of environment-friendly neem products for plant protection, particularly fruit and vegetable crops, not only can avert a major environmental crisis but can also help small farmers engage in agricultural production with low external inputs.

To popularize neem as a safe bio-pesticide in technologically advanced products, it is necessary to improve the productivity of the species. However, the present level of knowledge about germplasm variation and silvicultural requirements is superficial. There is an urgent need to explore the possibilities for improving the productivity and quality of neem and to develop a technology package for small farmers.

Distribution

A native species of the Indo-Burma region, neem is distributed throughout India, Pakistan, Bangladesh, Sri Lanka, Burma (Myanmar), Thailand, Malaysia and Indonesia. It is also planted extensively in the Middle East and tropical regions of Africa. Neem has been held in great esteem in India, and references to the diverse uses of this plant are found in ancient religious literature. According to a survey conducted in 1959 (Ketkar, 1976), about 13.9 million neem trees are distributed throughout India, with about 55.7% in Uttar Pradesh. It is often planted along roadsides in that country.

Neem is a large evergreen tree, reaching 15-20 m in height, with a semi-straight to straight trunk 30-80 cm in diameter and spreading branches that form a broad crown. A hardy species, neem performs better than many fast-growing trees in arid regions, surviving more than 100 years. Neem has adapted to a wide range of climates. It thrives well in hot weather, where the maximum shade temperature is as high as 49° C and can tolerate temperatures as low as 0° C, in altitudes up to 1,500 m.

The tree grows in most types of soils including, clayey, saline, and alkaline, with a pH of up to 8.5, but does particularly well on black cotton soils and deep, well-drained soils with good sub-soil water. Unlike most other multipurpose tree species (MPTS), it thrives well on dry, stony, shallow soils, and even on soils having hard calcareous or clay pan, at a shallow depth. The tree improves soil fertility and water-holding capacity; it has a unique property of calcium mining, neutralizing acidic soils. Neem grows naturally in areas where the rainfall is 450 to 1,200 mm. However, it has been introduced successfully in areas where the rainfall is as low as 250 mm. It cannot withstand water-logged areas and poorly drained soils.

Production and Uses

Neem has several economic advantages over other MPTS grown in the tropics. The tree can be harvested for timber after 35 - 40 years. The sapwood is grayish white and the heartwood is red to reddish brown, resembling mahogany.

The wood is aromatic, moderately heavy (specific gravity 0.72-0.83), narrowly interlocked, with uneven grains, and medium-to-coarse texture, durable and not easily attacked by insects. The timber is medium refractory and seasons well even when sawn wet. The wood is easy to work by hand or machine, but does not polish well. The wood properties presented in Table 1 clearly indicate that neem timber can be closely compared with teak in many respects.

Table 1. Comparison of neem and teak timber properties

Properties	Quality Index	
	Teak	Neem
Weight	100	124
Strength (as beam)	100	87
Stiffness (as beam)	100	81
Suitability (as post)	100	82
Shock resistance	100	105
Shape retention	100	77
Shear	100	129
Surface hardness	100	131
Nail holding ability	100	144

Source: CSIR (1985)

The wood is used for constructing as posts, beams, door and window frames, for furniture, carts, axles, yokes, naves, and ship and boat building, helms and oars, oil mills, cigar boxes, carvings, toys, drums, and agricultural implements (CSIR, 1985; Troup, 1986).

Establishment and Growth

Neem grows slowly during the first year. Young neem plants cannot tolerate intensive shade, frost, or excessively cold conditions. After establishment, the saplings start growing faster and compare well with other MPTS.

Under a multi-locational silvicultural study carried out in western India, the growth data indicated that neem can adapt well to high pH, saline soils, as well as to other shallow and sandy soils. During the first five years of this trial, neem ranked third, next to Eucalyptus and Leucaena, in both height and diameter at breast height (dbh), which can be attributed to a high calcium content and adequate moisture supply. However, the study indicated that the fertility of the soil is more critical than moisture supply for growth.

The annual biomass increment of neem plantations has been reported at 3-10 m^3/ha, which is considered medium-fast growth, next to such fast-growing tree species as Eucalyptus, Casuarina, Leucaena, and Acacia (of humid regions). The wood makes good fuel, although it emits more smoke than acacia and casuarina wood (Hegde, 1991).

Non-wood Products

Apart from its favorable characteristics as a timber tree, neem can contribute significantly to the income of rural people through annual seed production. Neem seeds are not only rich in oil but also contain compounds that have medicinal and pesticidal properties. Neem leaves, known for their bitterness, also contain useful compounds like nimbin, nimbinene, nimbandiol, nimbolide, desacetylnimbinene, and quercetin, which are used in making ayurvedic medicines and pesticides. The leaves, mixed with other fodder, are fed to livestock. In certain parts of Andhra Pradesh, farmers feed neem leaves to cattle and goats immediately after parturition to increase milk secretion (CSIR, 1985). The leaves contain 12.40-18.27% crude protein, 11.40-23.08% crude fiber, 43.32-66.60% nitrogen-free extract, 2.27-6.24% ether extract, 7.73-18.37% total ash, 0.89-3.96% calcium, and 0.10-0.30% phosphorus. These chemical compositions vary with the season and location. The foliage of neem is considered a good fodder, although it is not possible to lop the trees regularly. Generally, farmers in North India lop the trees in winter, while in South India the trees located in agricultural fields are lopped at the time of sowing kharif or rabi crops to reduce shade.

Neem starts producing seeds after 5-6 years and a mature tree yields an average of 25 kg of seeds annually. With an average price of 2 rupees per kg of seed, a tree generates a gross income of 50 rupees per year. With about 150-200 trees per hectare, the annual income is 10,000 rupees (US$ 350), higher than returns from many food crops.

Although neem seeds contain azadirachtin and many other compounds useful in medicines, pesticides, bactericides, and fungicides, they are crushed for oil, and about 95% are used in soap production. This is, in fact, an inferior use of the product because the same material, in a different form, can be used as a pesticide of high value. Processing neem as value-added products can command higher prices for seeds.

Neem cake is used as livestock feed, manure, insecticide, and nematicide. It not only provides organic nitrogen, but also inhibits the nitrification process when mixed with urea, before applying in the field. Such use of neem-coated urea (90:10) can save about 30% of the total chemical nitrogen requirement of crops, which otherwise would be wasted, and can help reduce the cost of agricultural production (Hegde, 1991; Ketkar, 1976).

Environmental Safety

Neem-based pesticides are safe, non-toxic, and leave no residues on agricultural produce. As the pesticidal preparations are simple, farmers can make direct use of these products locally, without any additional cost.

Use of neem products for plant protection can help reduce air pollution and prevent food poisoning. It can also reduce the demand for chemical pesticides, which will subsequently reduce industrial production of agro-chemicals.

Despite the benefits of neem, there is no significant increase in area under neem cultivation. Neem products are not yet popular among Indian farmers. Therefore, it is necessary to solve the problems related to productivity of neem trees and develop suitable strategies to popularize its cultivation, particularly on wastelands.

Problems of Neem Production and Utilization

The problems of neem production are related to its establishment; as well as improvement in growth, yield, seed quality, and profitability. Adoption of neem production and use in farming systems is equally challenging and needs attention from promoters.

Propagation

Neem is propagated primarily through seed, which has a short viability of 3-4 weeks. Enclosed in a fleshy drupe, the seed contains 35-45% oil and about 30% moisture and needs proper handling, not only to extend its viabilitv but also to prevent rancidity. Although the usual practice is to collect fresh seeds naturally dropped on the ground for sowing, new techniques have been developed to extend the seed viability by improved handling and storage practices. Roederer and Bellefontaine (1989) have reported 42% germination of neem seeds stored 5 years at 4° C. According to Van Den Beldt and Bhumibhamon (1992), collection of newly ripened fruits, depulping, quick washing, shade drying, and storage of seeds in cloth bags at low temperatures of approximately 4° C can improve seed viability. Surendran *et al.* (1992) have reported that the storage of depulped and shade-dried seeds in earthen pots containing wet sand (30% moisture) can retain the viability up to 62% after 3 months.

Because the seeds contain certain chemicals that inhibit germination, it is recommended to soak the seeds in warm water at 65-70° C for 30 minutes to improve germination.

As the flowering pattern of neem is influenced by altitude and temperature, fruiting is delayed in areas where winter is severe or prolonged. The fruits start ripening in early April in Tamil Nadu and Karnataka, while peak fruiting season in central and northern India is from June to August. The nursery activity to raise neem seedlings in most parts of the country should begin in June or July; the recommended practice is to establish the nursery in February or March and to plant seedlings of 3-4 months at the onset of rains in June or July. Such practices will ensure better establishment, and the saplings can withstand the dry summer better. Any delay in raising the nursery will result in either using small seedlings or delay planting. In both cases, survival will be poor. The other option of direct sowing of seeds is not ideal because of slow growth of neem seedlings, particularly during the first year. For successful promotion of neem cultivation, it is essential to develop low-cost technology for extending the seed viability.

Another option for successful establishment of neem plantations is through stump planting, which is considered better than direct seeding or planting seedlings. Stumps are generally prepared from 2-year-old seedlings raised in seedbeds by trimming the plant to retain 22 cm root and 5 cm shoot portions. The stumps are prepared under shade and transported in wet gunny bags (Singh, 1982).

Although the most popular and economic method of propagation is through seeds, propagules can also be produced through root suckers and stem cuttings. It has been reported by Surendran *et al.* (1992) that application of IBA and IAA (1,000 ppm) induced rooting in neem stem cuttings. Air-layering can also be produced with the help of IBA or NAA. As vegetative propagation can induce early fruiting without losing any economic traits of the mother trees, perfection of this technique for large-scale production is necessary to popularize neem cultivation.

Variation in Germplasm

Because neem is an open-pollinated species, a wide variation in the germplasm has been reported from neem-growing regions. The neem seeds produced in Niger and other African countries are rich in azadirachtin, although neem was introduced to these countries from India. A few neem provenances in Thailand grow fast with a clear bole, larger leaves, dark pink to light brown bark, and a long drupe, almost twice the size of the fruits found in India, but with low azadirachtin content.

Despite its many pesticidal properties, neem is susceptible to a few diseases and pests. The fungi that attack neem are *Cerospora leucosticta, C. subsessilis, Fomes sensex, Polyporus gilvus*, and *Xylaria azadirachta*. The wilting of neem trees is a serious disease, which has been killing mature trees in Africa during the past few years. It has no serious pests, except for a few leaf-eating insects.

The likelihood is good for improving the productivity of neem through selection of superior germplasm for higher yield, better quality, and stronger resistance to pests and diseases.

Fruiting

The agro–climatic conditions of the site also affect the yield and quality of neem seeds. A good rainy season helps the trees maintain a good crown in order to synthesize large amounts of carbohydrates necessary for seed development. A few good rain showers after the fruit sets will also influence the fruit size, in turn, increasing the yield (Hegde, 1991). Systematic studies are needed to understand the flowering behavior and tendency of alternate bearing.

Silvicultural Practices to Improve Productivity

Although neem has an edge over many MPTS grown in arid regions and saline soils, it is necessary to develop ideal silvicultural practices to improve its productivity.

Establishment

Suitable site preparation practices ensure better establishment of neem seedlings. Such soil- and water-conservation measures as contour bunding and trenching, plugging of gullies, and formation of basins also ensure better establishment and growth.

Neem seedlings may fail to establish due to water-logging in some areas. In such cases, it is necessary to establish plantations on raised mounds or on ridges. Nutritional deficiency in infertile soils may affect the growth. On such sites, application of organic matter and chemical fertilizers can help.

Mulching the plant basin helps conserve moisture. It has been observed that even young trees are affected by moisture stress, resulting in wilting of terminal buds. In extreme cases of drought, the plants shed their foliage and become dormant until the rainy season begins. In the absence of terminal buds, the auxiliary buds sprout after the rains, and the plants remain bushy, stunted, and fruiting is delayed (Hegde, 1991). Such moisture stress can be avoided by mulching the plant basins during the initial 2-3 years.

To improve the productivity of the plantation, depending on the agro-climatic conditions of the site, a spacing of 6 x 6 m or 8 x 8 m is recommended. As it will take at least 8-10 years for the trees to attain good size, mixed plantations of neem can be established with leguminous trees of shorter rotations.

Seed Collection and Processing

The traditional practice of neem seed collection is to hand-pick or broom the fruits fallen on the ground. The seed is coated with sweet pulp in a thin skinny epicarp and needs to be separated, sun-dried, and stored until crushed for oil extraction. Any delay in separating the seed and drying will affect the quality with respect to oil and azadirachtin content. Properly dried seeds can be stored up to 1 year; however, the recommended storage is a minimum of 3 months after collection for maximum oil recovery (CSIR, 1985). The seed contains 44.7% kernel and 55.3% shell. The kernels constitute only 35% of the fresh fruits.

The seeds are decorticated before extracting the oil. Decorticated seeds can be stored for 8-12 months without any deterioration in quality. In India, the commercial oil-extraction process has been well-developed, but the real problem lies in collecting and drying seeds at the village level.

Despite a high demand and an attractive price, only about 25-30% of the neem seed is collected in India, and the annual value of the uncollected seeds is estimated at more than 10 billion rupees (US$ 3.5 million).

The most important reasons for disinterest among farmers is their lack of knowledge about the handling of seeds and inadequate local marketing facilities. The price realized by seed collectors is 500-2,500 rupees per ton of seed; mill owners are willing to pay up to 4,000 rupees per ton. An important reason for poor price realization is poor quality seeds. Some farmers tend to keep the fruits a few day after collection without separating the skin. In the absence of bright sunlight, drying is slow; the seeds have a high moisture content, and the oil gets rancid and turns black. Due to poor recovery of oil, the price paid for such seeds is low. Thus, the seed collectors need to learn better handling and drying techniques.

The other major problem is collection of clean seeds. Many collectors sweep the ground under the trees to collect the seeds. In the process, gravel and dirt get mixed in with the seed, which results in lower priced seed. This can be avoided by cleaning the ground before fruits ripen. Temporary flooring and spreading gunny or plastic sheets under the tree can help in collecting clean seeds, without any wasted labor. Organizing seed collectors' cooperatives for marketing and oil extraction can help farmers realize better prices.

Neem and Small Farmers

Small farmers throughout the tropics can benefit from neem trees in the following ways:

- cultivating neem trees on unproductive sites;
- generating employment and additional income;
- improving the profitability of agriculture;
- meeting domestic needs for fodder, fuel, and timber; and
- ensuring environmental and health safety.

Planting of Neem Trees

The performance of neem is superior to most indigenous and exotic species in dry areas, in almost all types of soils, except in poor sandy areas. The cost-benefit analysis indicates that the cultivation of neem trees on wastelands is more profitable than cultivating food crops in non-irrigated areas. As most small farmers own unproductive and marginal plantation areas, which are not ideal for crop production, neem can be cultivated either as a sole crop or on field bunds to improve farmers' livelihood. However, to avoid the problem of long gestation periods, other species can be introduced on the plantation.

Generation of Employment and Additional Income

Neem contributes to the income of small farmers through the sale of seeds. However, picking, cleaning, and drying seeds must be carried out regularly at an appropriate time, which is a problem for small farmers.

Improving the Profitability of Agriculture

Neem can also increase crop production and improve the profit margin of small farmers. The trees on field bunds or on wastelands help improve the micro-climate and ensure soil and water conservation, thereby improving agricultural productivity. Neem, being resistant to most pests and diseases, does not serve as an alternative host in agricultural fields. The use of neem seeds for pesticide preparations can reduce the cost of chemical pesticides significantly and improve crop yields. Neem contains more than 25 different organic compounds; there is little chance for pests to develop resistance; hence, crop protection is further ensured over a longer period of time. The blending of neem cake with urea can reduce fertilizer requirements, thereby improving profitability.

Small farmers who require fodder, fuel, and timber do not have the capacity to procure these products from markets. Therefore, neem trees can provide them fodder and fuel occasionally. Although neem timber takes 30-50 years before it can be harvested, it is excellent material for building houses and furniture.

With the use of neem products as pesticides, small farmers can avoid using poisonous chemicals as pesticides and avoid health hazards that frequently occur in developing countries. Promotion of botanical pesticides also prevents residues on foods and protects the environment.

Promotion of Neem Production

Considering the present scope for improving the productivity of neem trees and involving small farmers in neem cultivation, the following activities can be initiated at the grassroots level in India and other developing countries:

Packaging of Silvicultural Practices

It is necessary to develop a standardized package of silvicultural practices, including superior provenances, seed storage, economic production of propagules, techniques of soil and water conservation, and establishment of plantation. These silvicultural practices should ensure better growth, earlier fruiting, higher yield, and better recovery of oil and other components.

Popularizing the Use of Neem Products

Neem products, particularly seeds, are not considered dependable for plant protection. Hence, use of neem products should be tested and popularized among farmers through field demonstrations. Villagers should be taught how to prepare pesticides using neem seeds instead of depending on commercial products. This requires a strong extension network of both government and non-government organizations to disseminate information about the technology, costs and benefits of neem cultivation.

Marketing Produce

It is necessary to develop grassroots farmer organizations or NGOs to help villagers collect, clean, dry, and sell neem seeds without any deterioration in quality. Small-scale industrial units for oil extraction and pesticide preparation can be developed through such organizations. They can also promote neem products that must compete with those of well-organized, multinational pesticide firms.

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Pests and Diseases of Neem

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Neem (*Azadirachta indica*), a multipurpose tree native to the Indian subcontinent, has been widely planted in the tropics. The fruits, seeds, and foliage contain several compounds that repel or kill insects, inhibit the growth and development of fungi, and limit infectivity of plant virus (National Research Council, 1992). It would, therefore, seem logical to expect a tree that produces these compounds to be relatively free of pests and diseases. However, neem is subject to a number of pests capable of causing serious damage. These must be considered when recommending neem for tree-planting programs.

Pests

Insects

Eight orders and 32 families of insects have been recorded feeding on neem (Tables 1-8) (Browne, 1968; Schmutterer, 1990; Tewari, 1992). As is true for most trees, every part of neem, including flowers, fruits, foliage, stems, bark, wood, and roots, is subject to injury. However, relatively few pest species cause serious damage. Those reported as damaging are described below.

Foliage Feeding Insects

A number of foliage feeding insects have been reported; extensive defoliation of neem does not occur frequently.

- Fire ants (*Solenopsis* spp.) caused severe defoliation of 3-6-year-old neem trees in Andhra Pradesh, India. Neem was the only plant fed upon, even though other plants were available (Raghunath *et al.*, 1982). In Central and South America, leaf cutting ants (*Acromyrex* spp.) are common neem defoliators (Schmutterer, 1990; and National Research Council, 1992).
- Loopers (*Lepidoptera: Geometridae*) have been occasionally reported as pests of neem. *Ascotis selenaria*, a widely distributed species, defoliates neem in India. *Bourmia variegata* defoliates neem nurseries in India, and *Cleoria cornaria* is found both in nurseries and on young seedlings (Tewari, 1992). Caterpillars of the genus *Eucema* (*Lepidoptera: Pieridae*) also have been reported as neem defoliators (Table 4).

Stem and Wood Borers

The powder post beetles *Apate monachus* and *A. terebrans* (*Coleoptera: Bostrychidae*) are native to most of Africa, having a wide range of host plants, including neem. The larvae feed in the sapwood of dead trees and logs and reduce it to a fine powder. Adults attack young trees and bore into small stems and branches. This either kills trees directly or makes them susceptible to mechanical injury. Both species have been introduced to the Caribbean Islands and Brazil. *Bostrychopsis jestiva* larvae cause damage to the wood of several tree species in New South Wales and Queensland, Australia, including neem (Browne, 1968; Schmutterer, 1990; Tewari, 1992).

- *Cydia* (=*Laspyresia*) *aurantiana* and *C. koenigian* (*Lepidoptera: Olethreutidae*) are both shoot borers and foliage feeders of neem in India. Boring can kill buds and cause excessive branching. Up to 55% of neem shoots have been reported infested by *C. aurantiana* (Madhavan Pillai and Gopi, 1990).
- Several species of termites (order *Isoptera*) have been reported (Table 5). They are sometimes locally damaging but do not kill living trees (Browne, 1968; El Amin *et al.*, 1984; Tewari, 1992).

Sucking Insects

Sucking insects (orders *Hemiptera* and *Homoptera*) constitute the largest group of insects that utilize neem as a host plant. At least 20 species, representing nine families, have been recorded (Tables 2 and 3). They feed on the foliage, branches, and stems of host plants. This causes desiccation of plant tissue and results in drying of the foliage, defoliation, stem and branch dieback, and occasional tree mortality. Some species have salivary toxins that accelerate damage and tree death.

- An insect known as the tea mosquito (*Helopeltis antonii*) (*Hemiptera: Miridae*), attacks terminal shoots of neem in India. Feeding causes a drying of foliage and terminal shoots. This is believed to be due to a phytotoxic reaction to the saliva of the feeding insects. While large trees tend to recover, heavy infestations have been shown to cause seedling mortality. Widespread neem damage from this insect has been reported in southern India. *H. antonii* is also a pest of cashew (*Anacardium occidentale*) plantations. It is believed that large numbers of insects are carried on northeast monsoons from cashew to neem plantings from October to December (Madhavan Pillai and Gopi, 1990).
- The Oriental yellow scale or cochineal (*Aonidiella orientalis*) (*Homoptera: Diaspididae*), has caused widespread damage to neem in parts of Africa and India. This insect attacks the foliage and young stems. Heavy feeding gives trees a burnt appearance (Boa, 1992). *A. orientalis* has a wide distribution and has been reported from China, Southeast Asia, Australia, the Indian subcontinent, Iran, the Arabian Peninsula, the United States (Florida), and the Caribbean. It is found primarily on citrus and tropical fruit trees (International Institute of Biological Control, 1987). *A. orientalis* has been found on neem in India and Africa (Browne, 1968; Singh, 1990; Tewari, 1992). There is a report of its 1935 occurrence on neem

in the Sudan (El Amin, 1984). In 1972, an infestation was recorded in Cameroon. During the mid 1980s, the insect spread over much of central and northern Cameroon to Chad, Mali, Niger, and Nigeria (Boa, 1992). In Nigeria, infestations have been treated with chemicals and infested limbs have been removed (Akanbi *et al.*, 1990). An outbreak of *A. orientalis*, which occurred in the Lake Chad Basin in 1987, is believed to be related to stress associated with the lowering of the water table (Lake Chad Basin, 1988; Schmutterer, 1990).

- The neem scale (*Pulvinaria maxima*) (*Homoptera: Coccididae*) is a pest of neem in India. This large, scale insect occurs throughout the Indian subcontinent and Malaysia and has been recorded causing epidemics in central and southern India. Both adults and nymphs feed on the sap of tender shoots and leaves. A heavily infested tree in an advanced stage of attack is conspicuously coated with thick, sticky white patches. Heavy infestations lower tree vigor and promote premature leaf fall and dieback of infested shoots. Repeated attacks can kill trees (Tewari, 1992). The feeding scales produce honeydew, a sweet substance that serves as a medium for the growth of sooty molds (Singh, 1990).
- The lesser snow scale (*Pinnaspis strachani*) has a wide distribution and host range. Colonies of this woolly scale attack shoots and foliage of neem in Africa, Asia, and Latin America (Schmutterer, 1990).

Mites

An eriophyid mite (*Calipitrimerus azadirachtae*) has been recorded on neem foliage in India. It has been found infesting both tender shoots and foliage. Feeding causes yellowing of foliage, deformity, and desiccation of foliage and shoots. At times, damage can be severe (Madhavan Pillai and Gopi, 1990; Tewari, 1992).

Mollusks

An unidentified mollusk has caused 15-20% seedling mortality in neem nurseries in Mahsana and Gandhinagar, India. Feeding occurs on the tender stem above the root collar and results in girdling (Tewari, 1992).

Mammals

In Nigeria, damage to neem by several species of mammals has been reported. Browsing by domestic goats (*Capra hircus*) damages neem plantings. The red flanked duiker (*Cephalophus rufilatus*) occasionally causes slight bark damage. The Nigerian hare (*Lepus crawshayi*) is suspected of eating the tops of neem seedlings in forest nurseries (Browne, 1968).

Diseases

A number of biotic and abiotic factors are known to cause disease of neem. These are reviewed by Browne (1968), Tewari (1992), and the National Research Council (1992).

Nursery Diseases

Damping off, a disease that affects germinating seedlings of many plant species under conditions of excessive moisture, also affects germinating neem seedlings. In India, up to 20% seedling mortality has been reported in nurseries at New Forest, Dehra Dun. The fungus *Fusarium Oxysporium* is generally associated with diseased seedlings.

Several fungi that attack neem foliage can cause damage in nurseries. Rhyzoctonia leaf web blight, caused by *Rhyzoctonia solani*, forms grey-brown spots on neem foliage. Infected leaves are joined together by fungus hyphae as if caught in a spider's web. This disease is common in nurseries after the regular monsoon rains begin. Infection by colletotrichium leaf spot and blight, caused by *Colletotrichium gloeosporide*, also results in leaf spots that rapidly increase in size and cover large areas of foliage surface. Other fungi that damage neem foliage in nurseries are *Alternaria alternata* and *Pseudocercospora (=Cercospora) subsesslis*.

Foliage Diseases

The fungus *Odium azadirachtae* causes a powdery mildew of neem foliage and several bacteria, including *Pseudomonas viticola*, *P. azadirachtae*, and *Xanthomonas azadirachtii*, cause leaf spot diseases.

Root Disease

In India, root rot is caused by the fungus *Ganoderma lucidum*. This fungus has a worldwide distribution and a broad host range. Sporadic infections occur in young neem plantings when stumps and roots of the previous tree crop are not removed from the site. The fungus attacks the sapwood and causes a white, spongy rot. Symptoms of infection are pale, thin foliage and branch dieback. Fruiting bodies often occur at the base of the stem.

Stem Diseases

In India, neem is one of many plants affected by pink disease. Caused by the fungus *Corticium salmonicolor*, this disease can cause serious losses, especially under warm, humid conditions. The earliest sign of infection is the presence of white or pink pustules on dead bark. A conspicuous pink layer of fungus mycelium spreads over the bark. In time, the bark may be entirely destroyed and the outer layers of wood killed. Branches are killed, quickly causing the foliage to wilt and turn black (Browne, 1968; Tewari, 1992).

Phomopsis twig blight, caused by the fungus *Phomopsis* spp., has been reported on neem from New Forest, Dehra Dun, India. This fungus attacks twigs and causes branch dieback. Dark-colored, pinhead size pycnidia form on infected twigs in large numbers (Tewari, 1992).

Parasitic Plants

Mistletoes (family *Loranthaceae*) are epiphytic, semi-parasitic plants that occur in the crowns of many tree species. They depend on their host plants for water and soil nutrients. Neem is one of many hosts of the mistletoe *Dendropthoe falcata* (= *Loranthus longiflorus*) that is widely distributed on the Indian subcontinent and the Solomon Islands. A mistletoe of the genus *Tapinanthus* infests neem and other trees in Nigeria. This mistletoe is capable of killing branches and causing deformity (Browne, 1968).

Neem Decline

A decline or disorder, due to unknown causes, has been reported from several countries in West Africa (Brata, 1991). The most conspicuous symptoms associated with this condition is loss of older foliage. This is often preceded by yellowing of older leaves. Foliage loss gives tree crowns an open appearance with clumps of leaves concentrated at the branch tips. This symptom has been termed a "giraffe's neck" (Boa, 1992). Other reported symptoms include distorted branches, branch dieback, stunted and dry foliage, red discoloration of the cambium layer, and exudation of sap from the branch tips.

Neem decline was first reported from Niger and has since been reported from Burkina Faso, Cameroon, Chad, Mali, and Nigeria. Experts who have examined declining trees and plantings believe that the decline is due to such site-related stresses as low soil moisture, competition, intercropping, and animal traffic (Boa, 1992; Ciesla, in press; Hodges and Beatty, 1992). In addition, trees planted in West Africa are believed to have originated from a limited seed source and may have low genetic variability. Consequently, the ability of the neem population to cope with environmental stress may be limited. Investigations are continuing, however, to determine if a fungus, plant virus, or mycoplasma-like organism (MLO) might be involved.

Discussion

A wide range of insects, fungi, bacteria, parasitic plants, mammals, and other organisms can affect the health of neem. Fortunately, most of these organisms only cause minor damage. However, there are several species that can cause widespread and severe damage. These include the oriental yellow scale (*A. orientalis*) in India and the Sahel and the tea mosquito (*H. antonii*) in India. Several fungi are pests of neem, especially in nurseries. A widespread decline of neem in the Sahel, possibly resulting from the interaction of several abiotic and biotic factors, is currently of concern in Niger and neighboring countries.

Pest management is an essential part of tree planting programs, including those in which neem is a component. A range of pest management tools are available. Chemicals are effective tools for management of neem pests in special situations (nurseries or seed orchards). They are not recommended for large-scale use, however, because the costs are high, and they may present environmental and human health hazards. Biological control may be an effective tool, especially if introduced pests threaten a neem resource. Cultural techniques, matching tree species to site, maintenance of optimum stocking levels, or elimination of alternate hosts can be effective techniques for preventing losses due to pests. Broadening the genetic base of the neem populations through introduction of new germplasm may increase the resilience of future neem plantings to pest damage or to the stresses that predispose trees to a buildup of pest populations.

A point to consider in tree planting programs is to avoid reliance on a single species, regardless of how promising that tree might be. Using two or more species, preferably of different genera, will provide greater biodiversity and resilience of the overall program to the damaging effects of pests, even if the individual species are planted in pure stands.

Table 1. Coleoptera (beetles) reported from *Azadirachta indica*

Family	Species	Portion of Tree Attacked	Region/ Country	Economic importance	Reference
Anthribidae	*Araecerus sutularis*	Seeds	India Pakistan	Locally damaging	Browne (1968) Tewari (1992)
Bostrichidae	*Apte monachus* *A. terebrans*	Wood of dead trees (larvae) Shoots and branches of live trees (adults)	Africa Dominican Republic	Locally damaging	Browne (1968) Schmutterer (1990) Tewari (1992)
	Bostrychosis jesvita	Wood	Australia	Occasional pest	Browne (1968) Tewari (1992)
Buprestidae	*Sternocera interrupta*	Foliage	Nigeria	Minor	Browne (1968)
Cerambycidae	*Anoplophora chinensis*	Stems	China Japan Korea Myanmar Taiwan	Minor	Tewari (1992)
	Phryneta spinator	Stems	S. Africa	Minor	Tewari (1992)
Chrysomelidae	*Crioceris hamsonii*	Foliage	India	Minor	Pillai & Gopi (1990)
	Cryptocephalus aequalis	Foliage	India	Minor	Pillai & Gopi (1990)
	C. ovulum	Foliage	India	Minor	Pillai & Gopi (1990)
Curculionidae	*Apirocalus mus*	Foliage	Papua New Guinea	Minor	Schmutterer (1990)
	Myllocerus dorsalis	Foliage	India Sri Lanka	Minor	Browne (1968) Pillai & Gopi (1990)
	Siderodactylus sagittarius	Shoots	Nigeria	Minor	Browne (1968)
Platypodidae	*Doliopygus unispinosus*	Wood	Ghana Nigeria Zambia	Degrades wood	Browne (1968) Tewari (1992)
Scarabaeidae	*Holotrichia consanguinea*	Roots (larvae)	Asia	Nursery pest	Tewari (1992)

Table 2. Hemiptera (bugs) reported from *Azadirachta indica*

Family	Species	Portion of tree attacked	Region/ Country	Economic importance	Reference
Miridae	*Helopetis antonii*	Foliage Shoots	India Pakistan	Damaging	Browne(1968) Pillai&Gopi(1990)
	Megacoelum	Foliage	India	Minor	Tewari(1992)
Pentatomidae	*Agonoscelis versicolor*	Foliage Shoots	Nigeria	Millet pest	Browne(1968)

Table 3. Homoptera (aphids, scales, etc.) reported from *Azadirachta indica*

Family	Species	Portion of tree attacked	Region/ Country	Economic importance	Reference
Aphididae	*Aphis gossypii*	Foliage Shoots	Philippines Dominican Republic	Minor	Schmutterer(1990)
Aleyrodidae	*Dialeurodes armatus*	Foliage	India	Minor	Pillai&Gopi(1990)
	D. citri	Foliage	USA	Minor	Tewari (1992)
Coccididae	*Ceroplastes ceriferus*	Foliage Shoots Stems	India Pakistan W. Australia	Minor	Browne(1968) Tewari(1992)
	Ceroplastes pseudoceriferus	Foliage Shoots Stems	E. Asia	Minor	Browne(1968) Tewari(1992)
	Ceroplastes spp.	Foliage Shoots Stems	India Myanmar	Minor	Pillai&Gopi(1990) Schmutterer(1990)
	Macropulvinaria jacksonii	Foliage Shoots	Niger Nigeria Togo	Damages street trees	Schmutterer(1990)
	Pulvinaria azardichtae	Foliage Shoots Stems	India	Minor	Browne(1968) Tewari(1992)
	P. maxima	Foliage Shoots Stems	India Malaysia Pakistan	Pest	Browne(1968) Pillai&Gopi(1990) Singh(1990) Tewari(1992)

Table 3 (continued)

Family	Species	Portion of tree attacked	Region/ Country	Economic importance	Reference
Coccididae (continued)	*Vinsonia stellifera*	Foliage	Benin Dominican Republic Philippines	Minor	Schmutterer (1990)
Diaspididae	*Aonidiella orientalis*	Foliage Stems	Africa Asia Near East N. America	Pest	Akanabi *et al.* (1990) Boa (1992) Browne (1968) El Amin *et al.* (1984) Schmutterer (1990) Tewari (1992)
	Aspidiotus hederae	Foliage	Uruguay	Minor	Tewari (1992)
	Parlatora crypta	Foliage Stems	Niger	Minor	Schmutterer (1990)
	Pinnaspis strachani	Shoots Stems	Africa Asia Latin America	Common	NRC (1992) Schmutterer (1990)
Lacciferidae	*Kerria lacca*	Stems	Asia	Culivated for laquer Minor pest	Browne (1968) Tewari (1992)
Margarodidae	*Aspidoproctus bifurcatus*	Stems	Ghana Nigeria	Minor	Browne (1968) LCBC (1988)
Pseudococcidae	*Pseudococcus maritimus*	Fruits Shoots	Egypt	Minor	Tewari (1992)

Table 4. Hymenoptera (bees, wasps, ants) reported from *Azadirachta indica*

Family	Species	Portion of tree attacked	Region/ Country	Economic importance	Reference
Formicidae	*Acromyrmex* spp.	Foliage	C. America S. America	Common defoliator	NRC(1992) Schmutterer(1990)
	Solenopsis spp.	Foliage	India	Minor	Raghunathetal(1982) Tewari (1992)

Table 5. Isoptera (termites) reported from *Azadirachta indica*

Family	Species	Portion of tree attacked	Region/ Country	Economic importance	Reference
Macrotermitidae	*Macrotermes bellicosus*	Roots Stems	Nigeria	Locally damaging	Browne(1968)
	Macroterme natalensis	Roots Stems	Nigeria	Locally damaging	Browne (1968)
	Odontotermes obesus	Roots	India	Locally damaging	Tewari(1992)
	Odontotermes nilensis	Stems	Sudan	Minor	El Amin *et al.*(1984)

Table 6. Lepidoptera (butterflies and moths) reported from *Azadirachta indica*

Family	Species	Portion of tree attacked	Region/ Country	Economic importance	Reference
Diaspidae	*Parlatoria camalliae*	Flowers	India	Minor	Tewari(1992)
Geometridae	*Ascotis selenaria*	Foliage	E. Hemishpere	Minor	Tewari(1992)
	Boarmia variegata	Foliage	India	Nursery pest	Tewari(1992)
	Cleora cornaria	Foliage	India	Minor	Tewari(1992)
Hepialidae	*Endoclita undulifer*	Branches Stems	Bangladesh India Myanmar Taiwan	Minor	Tewari(1992)
Lymantridae	*Leptocenerin reducta*	Foliage	Australia	Can cause heavy deoliation	Tewari (1992)
Noctuidae	*Othries fullonica*	Fruit (adults) Flowers (larvae)	E. Hemisphere	Minor	Tewari (1992)
Olethreutidae	*Cydia (=Laspeyresia aurantiana*	Foliage Shoots	India	Kills shoots	Pillai & Gopi(1990) Tewari (1992)
	Cydia (=Laspeyresia koenigiana	Foliage Shoots	Asia Australia	Kills shoots	Browne(1968) Tewari(1992)

Table 6. (Continued)

Family	Species	Portion of tree attacked	Region/ Country	Economic importance	Reference
Pieridae	*Eurema* spp.	Foliage	India	Outbreaks reported	Tewari (1992)
Pyralidae	*Hypsipyla* spp.	Shoots	S. Australia	Unknown	NRC (1992)
Tortricidae	*Adoxophyxes aurata*	Foliage	Asia Papua New Guinea	Minor	NRC (1992) Schmutterer (1990)
Xyloryctridae	*Odites atmorpha*	Foliage	India Sri Lanka	Minor	Browne (1968) Tewari (1992)

Table 7. Orthoptera (crickets, locusts, etc.) reported from *Azadirachta indica*

Family	Species	Portion of tree attacked	Region/ Country	Economic importance	Reference
Acrididae	*Orthacris simulans*	Foliage	India	Minor	Tewari (1992)

Table 8. Thysanoptera (thrips) reported from *Azadirachta indica*

Family	Species	Portion of tree attacked	Region/ Country	Economic importance	Reference
Phlaeothripidae					
	Erythrothrips asiaticus	Flowers	India	Minor	Tewari (1992)
Thripidae	*Elaphrothrips* spp.	Fungus infested Bark, Twigs and Foliage	India	Minor	Tewari (1992)
	Heliothrips haemorrhoidalis	Foliage Shoots	Global	Minor	Tewari (1992)
	Scirtothrips dorsalis	Flowers	India	Minor	Tewari (1992)
	Taeniothrips longistylus	Foliage	India	Minor	Tewari (1992)

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Geographical Variation in the Chemical Composition of Neem

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Much has been said and written about almost every aspect of neem. While some of the medicinal attributes claimed cannot be substantiated, its insect control properties are well documented and world attention is mainly due to these.

Scientists have been eagerly looking for an effective, safe and viable pest control agent that can replace toxic synthetic pesticides which cause enormous damage to the environment and public health. Synthetic pesticides have also been a drain on third world economies as they require foreign exchange. Also, much of the underdeveloped world is in the tropics, an ideal breeding ground for insects. Neem, therefore, assumes added importance in the tropical world where it grows. It is often recommended as a species for afforestation programs in view of its frugal nutritional requirements and tolerance of a wide range in climatic conditions. Neem is also used in some countries for timber, firewood, and wind-breaks. Thus, even without its insect control and medicinal properties, it has value for greening deforested areas. This use will continue irrespective of the practicability of using neem for its other potential uses.

It is not necessary in this forum to review all the existing information on neem's potential in pest control, medicine and afforestation. The paper will, rather, discuss geographical variation and surmise why neem is grossly under-utilized at the present time. It will also identify what technology and information gaps prevent its optimum utilization.

The last count of neem trees in India, taken over four decades ago, placed the number at 14 million. This count excluded certain important areas of the country and the current number is now estimated at around 20 million. Assuming the 20 million figure, the trees should yield about one million tons of neem seeds or half-a-million tons of kernels, the main source of pest-control agents. The seeds contain about 45% oil. The oil, being non-edible, is used primarily for soap-making. Given current estimates, only about 100,000 tons of neem seed (10% of total production) are actually collected and processed for oil (Tewari, 1992).

Over 40 commercial products based on neem are currently available on the Indian market. These include cosmetics, medicines, fertilizers and insecticides. Use of neem for pest control does not even constitute 1% of its total utilization in spite of world attention to pest-control problems. This is probably because of several uncertainties regarding the extent of neem production and its accessibility. Also, questions remain regarding its chemical composition, efficacy, technology and safety.

More than 300 compounds have been isolated and characterized from neem. These include over 70 limonoids (or tetranortriterpenoids), 30 other terpenoids, sulfur compounds, glycerides, flavanoids and carbohydrates. Among these, the limonoids azadirachtin, salannin, meliantriol, nimbin and nimbidin have been well characterized. These compounds are important for their confirmed antifeedant and ecdysis inhibition properties on several pests. No single compound is credited with all of the physiological properties attributed to neem. Among the antifeedants, azadirachtin remains the best studied. It has been demonstrated that there is a close correlation between the azadirachtin content of extracts and their bioactivity. Azadirachtin is the most abundant among the known bioactive compounds.

As the focus of this meeting is genetic improvement of neem, one must look at geographical variation of its chemical composition. How much azadirachtin is present in the kernels? Reported figures vary from neem seeds of different geographical origins. It is well known that the chemical composition of plant materials is subject to variation with climate (altitude, temperature, rainfall, soil nutrients, etc.), as well as the age of the tree and fruit storage conditions (temperature, humidity and duration). Though neem is native to South Asia, its propagation in other tropical countries has resulted in the existence of distinct variants. Systematic identification of the varieties and their chemical composition needs to be undertaken. Variation in azadirachtin content is reported from different trees of the same region by different groups (Table 1). The discrepancies could arise due to nonuniformity in methods for determination of azadirachtin content. Sometimes it is reported in terms of the yield of isolated azadirachtin by weight, but with undefined purity; at other times it is by HPLC. Some methods are solely based on bioactivity.

Ermel *et al.* (1987) analysed 66 neem seed samples by HPLC. The samples came from India, Togo, Nicaragua, Sudan, Mauritius, Niger, Indonesia and Burma (Table 2). They drew the following conclusions from their analyses:

- All neem seed samples contained azadirachtin.
- Azadirachtin content varied from 0.5 to 6.8% of the extract.
- High azadirachtin content is not restricted to any specific country.
- Compounds other than azadirachtin also vary markedly in concentration

It is interesting to note the wide differences in the figures given in the two tables. Table 1 is based on the total azadirachtin content in the kernel while Table 2 gives the percentage of azadirachtin in the kernel extract concentrate. The numbers in Table 1 are based on data published in peer-reviewed scientific journals while those in Table 2 are from conference proceedings. The methods used for sampling and storing the seeds, the number of samples, laboratory methods and statistical validity of the figures need to be established. HPLC seems to be the most convenient, rapid and reliable method for determining azadirachtin content.

Table 1 Concentration of azadirachtin in neem seed kernels as reported in scientific journals

Country	Concentration (%)	Reference
India	0.250	Schroeder & Nakanishi. (1987)
India	0.024	Lee & Kocke. (1987)
India	0.006	Brayanyamasaki, *et al.*(1986)
India	0.075	Butterworth & Morgan. (1971)
India	0.018	Ubel, Warthen, Jacobson. (1979)
Haiti	0.003	Kubo, *et al.* (1986)
Togo	0.009	Kraus *et al.* (1987)

Table 2. Variation of azadirachtin in different seed samples from neem trees in a given region (Ermel *et al.*, 1987)

Country	No. of trees	Range (%)	Mean value (%)
Nicaragua	15	2.8 - 6.8	4.79
Togo	17	2.2 - 6.4	3.87
India	7	1.5 - 5.5	3.50
Sudan	15	1.0 - 2.8	1.90
Mauritius	4	2.7 - 3.7	3.29
Niger	3	0.5 - 2.0	1.53
Indonesia	2	4.7 - 4.8	4.75
Burma	5	2.5 - 5.5	3.92

Visual presentation of data from the 66 samples above (Figure 1) gives a clear picture of the range among samples. Samples from 4 countries (Mauritius, Niger, Indonesia and Burma) are too few to be of statistical significance. Of the 5 samples from Burma, 2 had 6% azadirachtin and 3 had 3%. Among the countries from which a larger number of samples were analysed, Nicaragua consistently showed a high concentration (5%) and Sudan low (2%). Meanwhile, Togo samples showed that there could be considerable variation in the azadirachtin content within a region. There were surprisingly few samples from India, and there was no consistency even among them.

A detailed analysis of Indian neem, including correlation of environmental factors with bioactivity, was undertaken by R. P. Singh's group at the Indian Agricultural Research Institute (1987). They selected 9 ecotypes spread throughout the country. Half of these were fruiting in April and the other half in March. The results are summarised in Figure 2. The annual average temperature throughout the country, as well as the temperature during the fruiting season, was fairly constant (except for Pune). The same was true for the average relative humidity. The average rainfall was low both in Hissar and Delhi as well as in Pune. The bioactivity of neem seeds from

Hissar and Delhi was highest, while those from Pune were the lowest. The bioactivity, percentage of extractives, and the oil content are also shown in the bar diagram (Figure 2). No correlation was found between ecological factors and bioactivity.

There has been very little investigation on variation in content of other neem compounds. There have also been very few reports of the stability of these compounds to ambient conditions of light, temperature and humidity. On exposure to ultraviolet light (254 nm; 10 cm distance), azadirachtin content in neem kernel extract was found to decrease by 65% in 14 hr. Salannin decreased more rapidly, by about 20% in 10 min and 95% in 14 hr. Azadirachtin declined by 20% following four hours exposure to sunlight, and by 50% after 1 week (Figure 3) (Ermel *et al.*, 1987).

Azadirachtin is quite stable at room temperatures (even up to 40 degrees C) and fairly stable up to 65 degrees. However, a combination of high temperature and humidity appears to have a devastating effect on azadirachtin. While the concentration of azadirachtin remained at about 85% of the initial level after storage at 60 degrees (and 7% relative humidity for 21 days, it reduced to about 30% of the value after 3 days at 100% r.h. Storage of the seed in the shell at an ambient temperature below 40 degrees C and at low humidity is strongly recommended for accurate content analysis and for extraction of pest control agents. In any case, extraction soon after harvesting seems desirable in view of the apparent instability of azadirachtin under various storage conditions. As already noted, both azadirachtin content and its storability in neem seeds of different genotypes and ecotypes needs further systematic investigation.

In conclusion, I suggest the following approaches to the topic under discussion.

- Use of a rapid and reliable method for determination of azadirachtin content in neem seeds. HPLC is best suited for the purpose. The protocol for sample preparation and azadirachtin quantification is available from the author.
- To produce acceptable results, the number of samples, the method of sampling and storage, sample preparation techniques, and the protocol for analysis must be agreed upon in accordance with requirements for statistical validation. Inter-laboratory correlation and networking for sample exchange is highly desirable.
- Precise geographical location, agroclimatic data, age of the tree, time of seed collection and conditions of storage must be recorded. These data must be published with the results in order to be easily accessible to other researchers, preferably in peer-reviewed journals.
- We must address ourselves to the technology and information gaps that need to be bridged before the promise of neem can be realized.

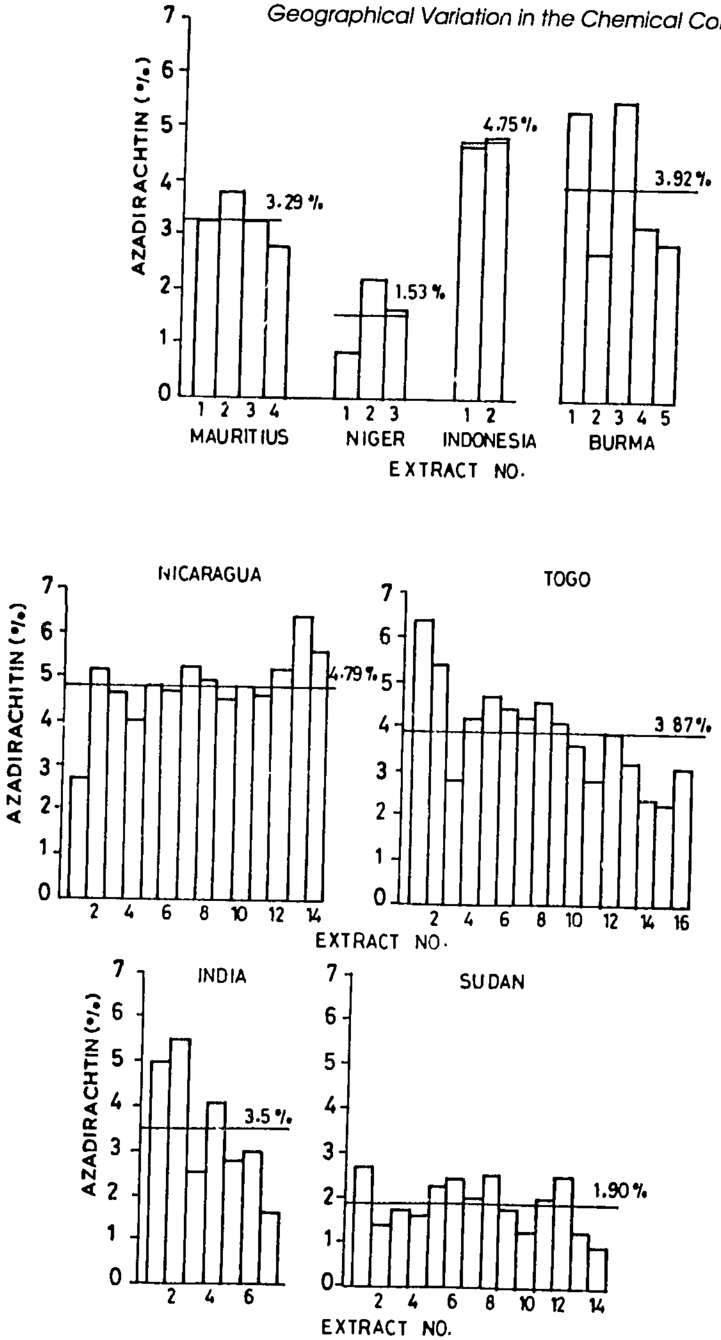


Figure 1. Concentration (%) of Azadirachtin in extracts of neem seed (Ermel *et al.*, 1987)

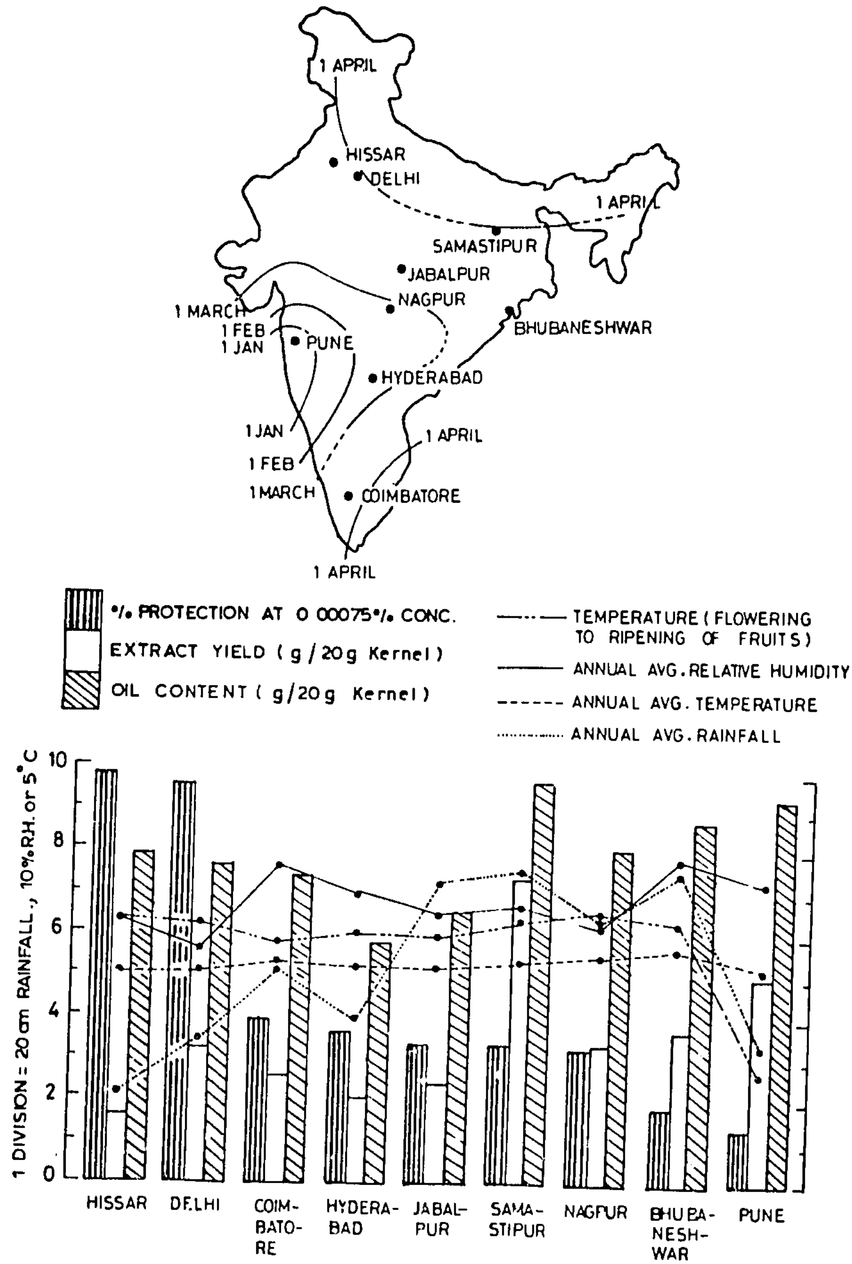
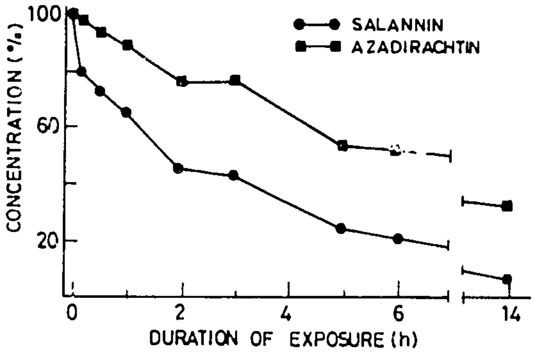
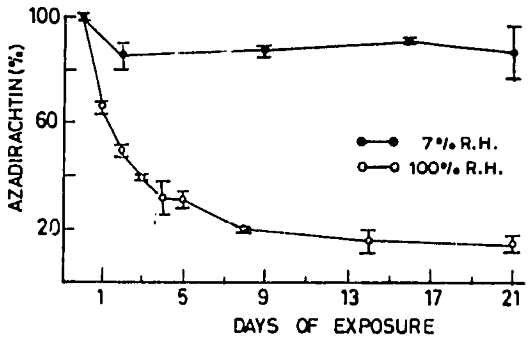


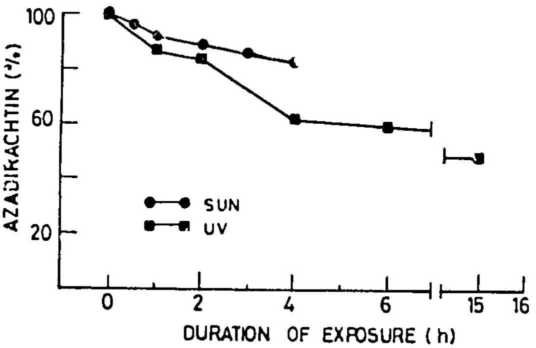
Figure 2. Variations in antifeedant efficacy (as expressed in percent protection), extract yield, and oil content of nine neem seed kernel ecotypes (Singh, 1987)



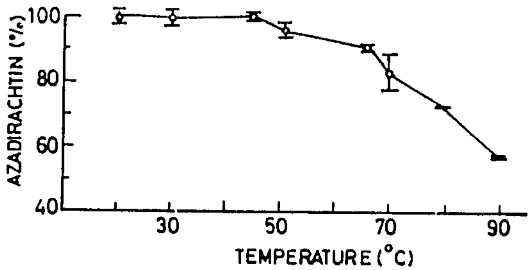
(a) UV_{254} nm DEGRADATION OF AZADIRACHTIN AND SALANNIN



(c) AZADIRACHTIN CONTENTS OF EXTRACTS FROM NEEM KERNELS STORED AT 60°C UNDER DRY AND HUMID CONDITIONS



(b) PHOTODEGRADATION BY SUNLIGHT or UV_{254} nm RADIATION OF AZADIRACHTIN



(d) EFFECT OF 24-h INCUBATION OF A NEEM SEED KERNEL EXTRACT AT INCREASING TEMPERATURES, ON THE AZADIRACHTIN CONTENT

Figure 3. Ermel *et al.*, 1987

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Grafting of *Azadirachta indica* var. *siamensis* in Thailand

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Introduction

As a multi-purpose tree, Thai neem (*Azadirachta indica* var *seamensis*) is commonly used as a plantation and wood farming species. Its wood is suitable for general construction and furniture manufacturing. In Thailand, neem flowers and young shoots are consumed as green salad and in curries. This reduces seed production and limits the availability of genetic materials for expansion.

There is still some doubt whether Thai neem seed is orthodox or recalcitrant. Its viability and germination percentage drop rapidly after collection. Through special treatment it is reported to be storable up to 12 years, but this has not yet been verified in Thailand.

An alternative to planting seed is vegetative propagation, through root cuttings, tissue culture, grafting, marcotting, or air-layering. This paper summarized work at the ASEAN-Canada Forest Tree Seed Centre on grafting of neem.

Grafting Techniques

Grafting is a technique used for joining two parts of different plants together in such a way that they will unite and continue their growth as one plant (Kajornsrichon, 1991)

Grafting can be done using apical grafting (or top-working), side grafting, and approach grafting. For *A. indica* var. *siamensis*, only apical grafting (or top-working) methods were found to be successful. Three methods of apical grafting were tried—splice grafting, cleft grafting, and whip or tongue grafting.

Success or failure of grafting depends on the following factors.

Experience

Workers with adequate training and experience produced better results. At the ACFTSC there was great variation in grafting success even among well-trained workers (Pong-anant *et al.*, 1989).

Species

The species of scion and stock plays an important role. Plants of the same species may graft perfectly, however, grafting between species, even those in the same genus, may or may not be successful. Grafting *A. excelsa* on *A. indica* var. *siamensis* at ACFTSC is acceptable while *A. indica* var. *siamensis* on *A. excelsa* is incompatible.

Size and stage of scion and root-stock

Scion and root-stock size affects the success of grafting. Generally, scion and stock should be of the same or similar sizes so that their cambial tissues can be in intimate contact.

Stage of development

Grafting scions of less than one year to root-stock of not more than two years produced the best results. Grafting of mature material on the juvenile stock may hamper development of the grafted plant and prolong the time to seed production. Also, there could be incompatibility problems.

Surrounding conditions

According to Pong-anant (1987), the time of year had no significant effect on grafting. He observed, however, that locations with optimum temperature (20 - 30°C) and high relative humidity (above 80%) yield better results.

Contamination and pests

High temperature with high relative humidity may induce black mold at the union of the grafting materials and result in scion mortality.

Stock

Stock could be either seedlings, rooted cuttings, or marcots. However, materials produced from vegetative propagation have no taproot and thus are more susceptible to drought or storm. Healthy seedlings with strong root systems are the best material.

The best rootstock for grafting is 1.0-1.5 cm diameter at 10 cm above the root collar. Seedlings of this diameter should be 0.75-1.0 m. tall. This requires 1-1.5 years in the container. To avoid root curling, stock should be root-pruned at least 3 months before grafting. Root pruning does not affect grafting, but improves the vigor of the grafting material after outplanting.

Scion

The scion is a short piece of detached shoot containing several dormant buds. When united with the stock it comprises the upper portion of the graft and becomes the main stem and/or branches of the new grafted plant. The scion should be a twig or branch of a selected mother tree. It should be less than 1 year old (current-season) with healthy buds and of a similar size to the root stock. It should be free of pests and diseases.

A. indica var. siamensis can be grafted all year round, but success varies with season. Summer scions (early April) are twigs or branches with green-colored bark at the tip. These are highly susceptible to pest infestation. The lower part of the scion is brown with shorter internodes. Only the lower part should be used. Wet-season scions (early August) have elongating internodes. Fresh, green-color tissue at the tip should be discarded, and only the brown, lower part of the twig used. Winter scions (early December) are leafless and dormant. The twig or branch can be used wholly as a scion.

Because summer and wet-season scions may be subject to pest or disease infestation after cutting its fresh tip, it is suggested that grafting be done in the winter since no special treatment of the scion is needed. Dormant buds of winter scions emerge sooner than those of summer or wet-season scions, possibly because their vigor is enhanced by some period of dormancy.

After collection, leaves of the scion should be removed to minimize evapotranspiration. Defoliated scions are then wrapped in moistened cloth and put in a tightly sealed plastic bag. Bags should be sprayed with water and kept in a cool and shaded place. Transportation to the grafting site should be as soon as possible after collection.

Grafting Methods

The apical grafting methods used at ACFTSC are as follows:

Splice grafting

- A 4-5 cm long sloping cut at 10-15 cm above the root collar of the stock.
- A long sloping cut is also made at the base of the scion the same length as the cut on the stock.
- The scion and stock are slipped together, ensuring good contact between the two cambial layers.
- The graft is tied with grafting tape. Vaseline wax (mixed with Benlate pesticide) is applied to reduce water loss and to protect the union from pest infestation.

In case of different sizes of stock and scion, the scion is placed on the stock so that at least one side of the cambium of each part is in contact. If the scion is much bigger than the stock, one side of the scion should be shaped to match the size of the stock, keeping one side of the cambial layer intact and in contact with the stock.

Cleft grafting

- The top of the stock is cut at about 10 cm above the root collar. The cut should be sharp and perpendicular to the root-stock.
- The stock is cut vertically about 4-5 cm deep at the middle of the top cut.

- The base of the scion is cut on both sides to make a tapering wedge-shaped shoot similar in length to the depth of the cut of the stock.
- The grafting knife is used to open the cut on the stock. The wedge-shaped scion is inserted into the cut and to ensure that cambial layers of both scion and stock are in contact.
- The graft is then tied and waxed.

Whip (or Tongue) grafting

- A long sloping cut 4-5 cm long is made 10-15 cm above the root-collar of the stock.
- A second downward cut is made starting 1/3 of the distance from the tip to the base of the first cut.
- A long sloping cut is made at the base of the scion the same length as the cut on the stock.
- A second cut on the scion is made, also at 1/3 distance of the first cut.
- The stock and scion are then slipped together, with the tongues interlocked.
- The graft is tied and waxed.

Table 1. Percentage grafting success by two worker using three techniques in three seasons at Muak Lek, Thailand.

	Summer (Apr)			Wet season (Aug)			Winter (Dec)		
Grafting Methods	Splice	Cleft	Tongue	Splice	Cleft	Tongue	Splice	Cleft	Tongue
Worker 1	70	60	80	60	60	80	100	70	90
Worker 2	70	40	90	90	100	100	70	90	90
Average	70	50	85	75	80	90	85	80	90

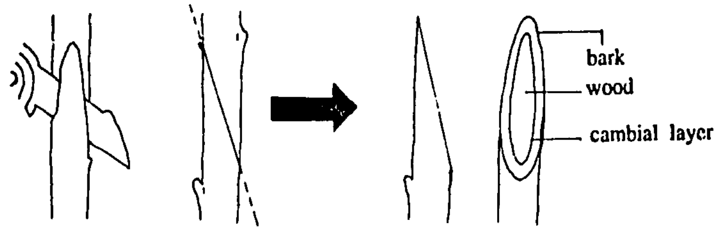
Source: Pong-anant, 1989.

Treatments After Grafting

To assure the stability of the grafted plant, the union should be tightly tied with grafting tape and waxed with vaseline and pesticide to prevent loss or penetration of water and to prevent contamination. Grafting tape is normally bound from the lower part upwards.

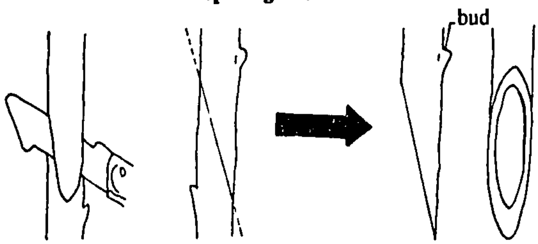
To prevent or minimize evapotranspiration of the grafted plant, the materials should be kept under shade (50 - 70% shade) and covered with a plastic tent. A large plastic bag may be used to cover the grafted material. The plastic bag may be partially

Preparing the stock



A long, sloping cut 1 to 2 inches long
is made at the top of the stock.

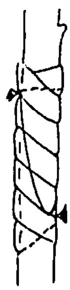
Preparing the scion



A long sloping cut is made at the base of the
scion the same length as the cut on the stock.

Grafting

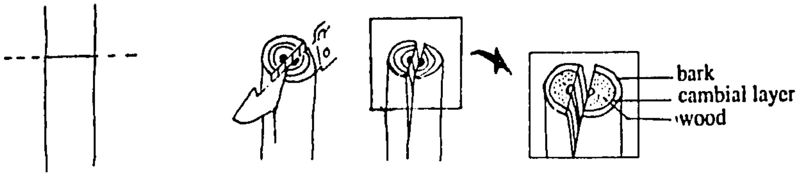
The stock and scion are slipped together, ensuring that there is good contact between the two cambial layers.



The graft is then tied with polyethylene tape and waxed with vaseline to reduce water loss.

Figure 1. Splice graft

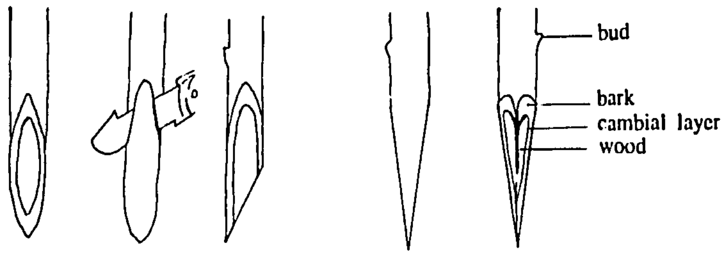
Preparing the stock



The top of the stock is cut horizontally.

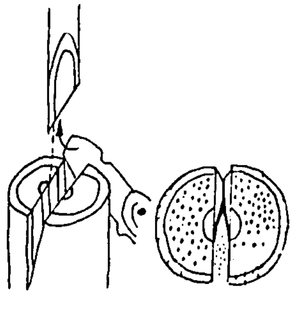
A vertical cut 1 inch long is made in the middle of the stock.

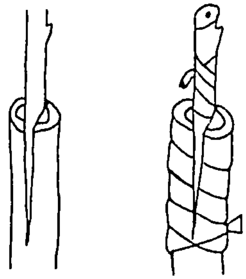
Preparing the scion



The scion is made with a long, gradually tapering cut.

Grafting



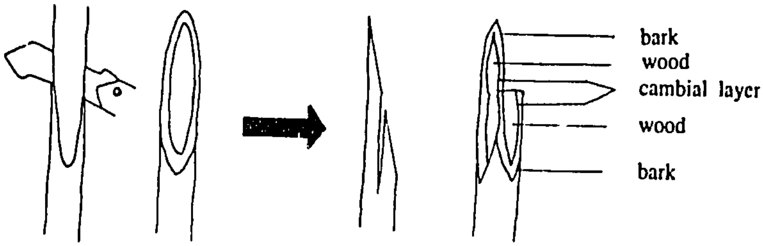


The scion is pushed into the stock cut leaving a part of the scion cut surface exposed.

The graft is then tied and waxed.

Figure 2. Cleft graft

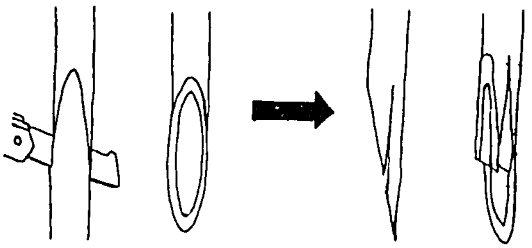
Preparing the stock



A long, sloping cut 1 to 2 inches long is made at the top of the stock.

A second downward cut is made starting 1/3 of the distance from the tip to the base of the first cut.

Preparing the scion

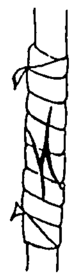


A long sloping cut is made at the base of the scion the same length as the cut on the stock.

A second cut under the first is made the same as for the stock.

Grafting

The stock and scion are slipped together, the tongues interlocking.



The graft is then tied and waxed.

Figure 3. Whip or tongue graft

filled with water to keep relative humidity high and to supply water to the grafted plant. The bag is opened after 25 days to check the percentage of success. Successful grafts will have fresh buds or new leaves. Failures will have dried tips or shoots, or brown, dry buds. However, even plants with dried top shoots may have dormant buds that can emerge later. To test for this, pruning shears are used to cut the scion bit by bit from the top downwards. A successful graft will reveal fresh tissue.

A mixture of vaseline and pesticide is applied to the graft. Treated plants are then kept under shade for a few more days. Grafting tape is removed 3 months after grafting, or when the union becomes swollen. Shoots emerging from the stock are plucked off and the adventitious bud destroyed. This ensures that the root stock will provide enough water and nutrients to the graft material. Plucking is best done manually, as tools may leave adventitious buds on the stock that could produce new shoots later. Plucking shoots from the stock should be done regularly until outplanting.

Grafted materials should be kept under shade for the first 3 months. After that, they may gradually be exposed to open sunlight for hardening until ready for outplanting in the wet season.

Outplanting

Outplanting of the grafted materials should be done after there is an assured supply of water or rainfall. Planting holes are filled with a mixture of topsoil and compost or other organic fertilizers, then topsoil is added again to prevent direct root contact with fertilizer. At planting, the union of the grafted plant should be slightly above ground to prevent termite damage and infestation by fungi, diseases and pests. Also, with the union above ground, it is easier to observe and remove shoots growing from the stock.

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Opportunities For Regional Research Networking: The F/FRED Experience

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Sharing of learning experiences is becoming increasingly more critical for scientific researchers. Although more institutions and scientists are pursuing research, resources available to scientists are limited. Recent advances in communication systems allow sharing of information and experiences, and there are mechanisms available through which such sharing can be promoted and encouraged.

Networking is a concept most recently accepted by development agencies and developing countries as being a cost-effective mechanism for strengthening institutional capability and causing rapid change in application of research findings. Burley (1985) defines network as 'an informal or formal arrangement of cooperation between institutions with similar conditions and problems but without the immediate resources to find answers to those problems individually'.

Mechanisms

Operationally, networks may take many different forms. Several of the existing forestry and agriculture networks in Asia constitute the following (MacDicken, 1989):

- Loosely grouped associations of researchers who divide research problems into network assignments, either formally or informally. An example of informal division of topics is the Northern Thailand Natural Resources Management Network (NORMNET), an advisory group of agency officers and researchers, whose members jointly identify and help allocate funds for research projects of relevance to the participating development agencies. The formal division of topics is found in the approach adopted by the Forestry Research Support Program for Asia and the Pacific (FORSPA), whereby research fund is disbursed according to identified research themes based on submitted proposals which have been positively evaluated by expert teams.
- Groups of participants who conduct a set of centrally coordinated experiments to solve specific research problems. This approach concentrates greater resources on a well-defined problem, and is often used by international agricultural research centers to test food crop varieties (Plucknett and Smith, 1984). Rice variety trials coordinated by the International Rice Research Institute and

network experiments on multipurpose tree species supported by the Forestry/Fuelwood Research and Development (F/FRED) Project are examples of this type of network.

- Other networks are primarily communications-oriented. They comprise of groups of people who communicate regularly in areas of common interest. Organizations which produce research publications and newsletters such as the Nitrogen Fixing Tree Association (NFTA) and the ASEAN Timber Technology Centre (ATTC) exemplify this type of network.
- Some networks provide a wide range of services and support among network participants. This type of "integrated network" combines aspects of all three of the above concepts. It facilitates the training and development of network participants and includes publications and research components. Examples of this type of network are the Multipurpose Tree Species (MPTS) Research Network which is supported by the F/FRED Project, and the recently launched FAO/UNDP-sponsored Tree Breeding and Propagation Regional Project based at Los Banos, Philippines.

Strengths of the Network Approach

A network approach generally reduces costs, minimises duplication, boosts efficiency and leads towards national self-sufficiency in research capability or management. It provides for a speedy technology transfer, research material and guidelines not easily available to all potential beneficiaries, and yield research results that are greater in extent and value than the sum of the individual activities that are components of the network. The benefits of a network are therefore most valuable in countries with limited funds and scientific manpower (Burley, 1985).

However, success may not come as easy as it appears. In discussing networking in international agricultural and forestry research, Plucknett and Smith (1984) and Burley (1985) identified the following factors for success:

- The problem should be clearly defined and a realistic research agenda proposed.
- The problem must be widely shared.
- There must be strong self-interest.
- The participants are willing to commit resources, such as personnel and facilities.
- Outside funding is available to develop the network and keep it running for at least the first few years.
- The participants must be sufficiently trained and experienced to make a contribution.
- The network must be guided by strong and efficient leaders who have the confidence of the participants.

- Participants should be prepared to share the results of national activities related to the network's research through formal publications, informal reports, newsletters and conference papers.
- Participants should be prepared to establish mechanisms for extension of technological findings to the 'user'.

Problems and Opportunities

While the concept of networking and international collaboration appears straightforward, the path is full of hazards, especially when the linkage is between countries of different regions with people of different cultural and religious backgrounds, attitudes, and training. In addition, the linking institutions might have widely contrasting backgrounds with varying resources. The problem could further be heightened if the institutions are inflexible with their own programs (Salleh and Chan, 1986).

Burley (1985) identified nine main groups of problems that have been experienced in the existing forestry research networks. These are outlined below, and many of them have also been shared by F/FRED.

Choosing Species or Topics

Deciding on appropriate species and subjects for research has to take into account of the cost-effectiveness of limited available resources and the widest potential socio-economic benefit. For a network that covers a wide geographical region and traversing different ecological zones, this can pose a problem. The MPTS Research Network adopts the approach of continual consultations with its cooperators, and the division of research activities according to the ecological zones. New species have been added to the initial priority list. Neem and Jackfruit are two examples. Similarly, priority research areas, especially for its small research grant program, have been revised through the course of the project to reflect changing needs.

Identifying the Leading Center

Successful networks need strong and efficient leaders that can provide firm leadership, sympathetic guidance, relevant information, experimental designs, and where necessary, data analyses. The MPTS Research Network has circumvented this problem by establishing a Network Secretariat based at Kasetsart University. The Network Secretariat enjoys strong support from the Network Research Committee who advises on the research directions, as well as from various national networks with their own leading institutions.

Identifying Network Participants

For networks to be effective, membership should not be too exclusive, except where there are legal or other constraints. One of the great advantages of networking is that a greater number of field sites provides increased information on the effects of interaction between site and genotype or management.

Encouraging Active Participation and Providing Resources

Even when potential participants can be identified they may not see the importance of joining a network, or they may not have sufficient resources to follow the agreed research agenda. Collaboration among the participants and Secretariat staff is one of the key strategies adopted by the MPTS Research Network. Potential benefits must be clarified and agreements made regarding mutual commitment of resources. Training courses or workshops are also organized to enhance the capability of staff in participating institutions.

Communications and Feedback

To be effective the results of all the participants' research should be combined, circulated to all collaborators, and published for the benefit of others. Feedback from the field to the Secretariat is sometimes sporadic because of shortages of staff and skills. The F/FRED Project has developed MPTSys, an integrated information and decision support system which can be used to store, manage, analyse and exchange data. This is made available to cooperators and for some needy participating institutions, computers are also provided. Where necessary, training is also offered on how to use the program. Regular visits to the collaborators' sites have helped to alleviate the data exchange problem. In addition, Farm Forestry News, the Project newsletter keeps the participants regularly informed of network activities.

Plant Material

Most of the existing forestry research networks require exchange of plant material between countries. This is usually in the form of seed which should be accompanied by certificate of origin and quality. Before seeds are exchanged, it is mandatory that at least two requirements are met. Plant health requirements of the importing countries must be known in advance and steps taken to satisfy these so that there is no unnecessary delay at the customs which can jeopardize the seed viability. Longevity of seed viability and how it is affected by storage and transport conditions must be fully understood, so that necessary precautions can be taken to maintain viability. Recalcitrant seeds such as Inga and neem demand special attention in their handling. The possibility of exchanging tissue cultured or micropropagated material of improved germplasm should not be discounted.

Amount of Material for Comparative Testing

When a network is involved in germplasm evaluation, it has several aspects that are in mutual conflict. For example, to improve the precision of estimating genotype-site interaction effects, the number of field sites, with consequent increase in environmental variation, should be increased. But the amount of seed required also increases. For some species with wide geographical distribution, it may be difficult to make seed collection covering the whole range. On the other hand, where numerous seedlots could be easily collected, not all are likely to have future applications in all sites. There must be a balance between the need to test everything and trying to identify specific seedlots for certain sites.

Experimental Design

The experimental designs for field trials are commonly expected to be produced either by the network leader or through planning meeting of some cooperators. This is particularly important for some scientists who are not too familiar with the basic principles of design and analysis. Depending on the material and sites, designs may be simple (e.g. randomized complete blocks) or more complex (e.g. incomplete block designs, etc.). Similarly, how the trial should be managed must be agreed upon well in advance. Manuals should be provided giving guidelines which allow certain flexibilities for individual participants to make their own decisions and to develop future programs.

Comparability of Assessment

For intersite analysis to be meaningful, data from different collaborators should be strictly comparable in terms of method of assessment, precision and freedom of bias. Standard variables and methods can be formalized at the planning meeting and clearly be written up in the network trials manual.

F/FRED'S Experience: A Case Illustration

The MPTS Research Network

The 10-year F/FRED project began 1985. It was designed to help scientists cooperatively address the needs of small farmers for fuelwood and other tree products. The MPTS Research Network has been established under this project. Presently, the MPTS Network has over 20 participating institutions in 11 countries in the Pacific and South and Southeast Asia. Mechanisms for network development include scientific and organizational meetings, cooperative research projects, long and short-term training, travel grants, publications and small research grants. A number of other donor organizations have collaborated in supporting network activities.

The MPTS Research Network is built upon national-level networks formalized by MPTS research meetings held annually in participating countries. These meetings provide opportunities for discussion of MPTS research issues; provide national level links to the regional research network; monitor and evaluate on-going F/FRED-funded research projects; develop a prioritized list of MPTS-related research topics; and select representatives (a social scientist and a biological scientist) to serve on the MPTS Research Network Research Committee. A number of countries have initiated national level research programs on MPTS.

The MPTS Research Network is guided by a Research Committee and a Steering Committee. The Research Committee consists of two representatives from each participating country who are selected at the national MPTS research meeting. The committee recommends network research direction, priorities, and standard methodologies. The Steering Committee advises on policies and strategies, promotes programs and activities, and reviews Research Committee recommendations. The Steering Committee is made up of three members elected by the Research Committee and four permanent positions. The permanent positions include the Chair of the Research Committee, the ranking Asian member of IUFRO, the FAO Regional Forestry Officer for Asia and the Pacific, and the Dean of the Faculty of Forestry at Kasetsart University, the institution that hosts the Network Secretariat.

In the establishment and management of the MPTS Research Network, the following factors have contributed to its success (MacDicken, 1989):

- an active core of network participants
- adequate management of conflicts of interest
- flexibility of project support
- a supportive and neutral institutional base
- sustained, long term financial support
- strong scientific and professional interest
- cooperative research activities
- optimization of compromise
- appropriate research funding levels
- effective coordinating staff
- extensive donor cooperation and coordination

Network Experiments

F/FRED has supported a series of multilocation network trials in the humid/subhumid and arid/semi-arid zones of South and Southeast Asia. Several MPTS and their genotypes of potential for small farm planting have been chosen. The trials incorporate the following features:

- common design and standard methodology,
- common minimum data set,
- common germplasm,
- thoroughly described soils and climate at each site,
- data exchange and professional interaction among participants,
- intersite combined analysis of data.

The approach adopted in carrying out these trials consists of the following aspects, coordinated by the Network Secretariat:

Planning Meeting

The meeting, attended by potential cooperators, plans for the experiment. Agreements are sought on matters of experiment objectives, experimental design, standard methodology, minimum data set to be collected, germplasm to be used and their sources of supplies, sites selection, the commencement time, how data should be exchanged and analyzed, and the propriety of research results and authorship of publications. The protocols for the establishment, management, assessment and data exchange are written up into a manual distributed to all cooperators.

Trial Establishment

It is important to get a clear idea of the number of sites and participating institutions or individuals to be involved in the trial early. This will help in determining the amount of seed required, so that seed ordering or collection can be done sufficiently. Preliminary information on the climate of the sites is helpful to the coordinator in determining the growing season, so that seed can be dispatched in time. Awareness of the quarantine requirement, and the need to handle the seed appropriately by the participant is critical in getting good establishment. Guidance is provided by the network coordinator, where necessary in areas such as seedling production and trial layout.

Trial Monitoring and Data Exchange

Despite the agreement reached at the planning meeting, and the procedure prescribed in the manual, some data are not collected or assessment is not done at the stipulated times.

Continual communication between the network coordinator and the collaborators is important in minimizing this problem. In addition, this will also help in facilitating data exchange. Visits by the network coordinator to trial sites at the time of data collection can also assist the participant in measurement decision such as tree form classification. Free exchange of data is not only encouraged between the Network Secretariat and the collaborators but also among the cooperators themselves.

Data Analysis and Publication

The network cooperators usually analyse and publish the data from their own sites. Sometimes, several of them combine their data and come out with a joint publication. But for a more complete intersite analysis, the task is usually undertaken by the Network Secretariat, and the authorship of the resultant publication is shared by all cooperators. The MPTSys provided to the cooperators can assist in this task.

Nature of Cooperation and Support

All network experiments have been carried on the basis of cooperation between the F/FRED Project and the participants. The F/FRED Project only provides partial support in terms of finance, germplasm and expert advice. The cooperating institutions because of their strong interest, usually commit some of their resources to ensure success. F/FRED also collaborates with other donor agencies such as FINNIDA, ACIAR, CSIRO and ODA in supporting the trials. In such cases, it is important to clearly define the respective roles.

Conclusions

Networking offers a means of increasing the efficiency and effectiveness of forestry research in this region. The MPTS Research Network provides a basis and a model for expanded collaboration beyond this region. Options to consolidate network programs should be carefully considered to avoid confusion, duplication of effort and the wastage of time and money.

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MPTSys and Its Role in Networking

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Networking has been recognized as a useful, cost effective means of enhancing research on multipurpose tree species. Networks can increase the efficiency and effectiveness of forestry research. Their development is a time consuming and expensive process, one that demands extensive coordination and communication among interested institutions and individuals. Yet, networks can provide sustainable, long term capacity to produce and communicate research results (MacDicken *et al.*, 1990).

Burley (1987) defines agriculture and forestry research networks as "informal or formal arrangements of cooperation between institutions with similar conditions and problems but without the immediate resources for finding solutions to these problems individually". The concept of networking is not new. Indeed, it dates back to the beginnings of institutionalised science. Communication between researchers, the sharing of hypotheses, discussion of research methods, professional society meetings and cooperative research projects have existed in some form for centuries.

In 1986, the multipurpose tree species research network was formed with support from the Forestry/Fuelwood Research and Development Project (F/FRED), funded by the United States Agency for International Development (USAID). The F/FRED project provides a network through which scientists exchange research plans, methods and results. Research and Development activities center on the production and use of trees that meet several household needs of small farmers in Asia. This project is implemented by the Winrock International Institute for Agricultural Development, a private, non-profit US organization working in agricultural development around the world.

F/FRED network experiments are designed and conducted by groups of Asian MPTS researchers. Ready access and exchange of network data, using standardized data collection techniques, is a major contribution of the F/FRED network to its cooperators.

A network experiment is a multilocational set of trials designed and conducted by a group of MPTS research cooperators. Critical elements include experiment design, common germplasm, a mutually agreed upon minimum data set and standardized methodology. Network experiments are a cost effective way to evaluate improved management practices under a spectrum of different agroecological and socio-economic environments. Cooperators contribute to the joint effort and, in turn, each scientist derives benefits from the combined network data. The network also provides a forum for strengthening scientists and institutions through improved MPTS research methodology. The F/FRED organizes experiments based on environment zones i.e., humid, sub-humid and arid, semi-arid zones (Foster *et. al.*, 1988).

Database Development and Management

Information exchange is one of the most important ways to ensure the success of any regional network. This process involves scientists, administrators, extension workers, entrepreneurs and farmers.

The F/FRED database system was developed through 1) joint planning with collaborating Asian scientists in the F/FRED research network, 2) coordination with global efforts of MPTS database program specialists, and 3) study of existing databases from key forestry and agricultural organizations. Several regional and global databases are supported in the system. The system addresses one of the F/FRED's core objectives, that of providing better information management to enhance analysis and publication of research results related to multipurpose tree species. To provide a common, easily accessible system for information management, the F/FRED project of Winrock International has designed, developed and implemented the information and decision support system, known as "MPTSys".

MPTSys is an integrated, user-friendly microcomputer software package designed to help scientists, administrators, and extension workers organize, manage, and share research information on multipurpose tree species. This system is based on a modular approach to system design. The components are database management and application programs. Stand alone components give the system flexibility which allows users to install selected components to meet their individual needs and resources. The system is decentralized and does not require users to have previous training in computers or database management. Standardized screens, options and menus along with help windows, reduce the time required for learning this application.

The database management programs have standard options to add, search, modify, delete, browse and output records. MPTSys can provide improved information management to enhance research analysis and publication of results as well as assist in decision making about multipurpose trees.

Subsystems, in the context of MPTSys, determine the logical and structural linkages between MPTSys components. By focusing on subsystems, it is not necessary to link each component with every other component. Instead, the components are linked in predefined ways to accomplish predefined tasks. These linkages can be classified as data or control. Control linkages allow users to move from one component to the other; data linkages transfer data between components.

MPTSys consists of five research databases, three reference databases and three decision support systems. The research databases are-- experiment, summary, soil, climate and farm and village forestry databases. The reference databases are-- species digest, abstracts and MPTS specialist databases. The decision support systems are-- Data analysis and modeling, growth simulation and species x environment modeling.

Experiment database

The experiment database, the flagship of the system, has supported the multilocational trials being carried out by the network since 1987. Known as MPTDATA, the experiment database not only allows easy data exchange of network experiments among researchers but can also be customized to include data from non-network trials to meet independent research needs. Researchers can enter information directly into the system. Data can be selectively retrieved, viewed and printed to disk file or printer.

The experiment database is the prime residence for experiment data sets generated by the F/FRED research network. It is ideally suited for comparative analysis from a series of trials with common treatments, experimental designs and methodologies. The experiment database is furthermore linked with a data analysis and graphics package. Database files on experimental design, treatment to plot assignments and user selected plot measurement data are joined within MPTSys and automatically inputted into MPTStat for an intra-site analysis of the data. Frustrations associated with preparing and analysis of data for input to an external data analysis package are eliminated.

Summary database

After performing appropriate statistical, economic and other analyses, the summarised information from experiments can be entered into another database that contains highly condensed, key information on MPTS which is suited for practical management decisions. This is called the summary database.

Soil Database

The soil database stores chemical and physical characteristics of tropical soils at the horizon level. Two major data files comprise the soil database. One contains information associated with a single soil profile and does not vary according to soil layer. It uses a soil pedon number as a primary key to distinguish among pedons. The other contains information associated with a single layer of a soil profile.

Climate Database

This database contains long term climate data obtained from the Plant Protection and Production Division of the Food and Agriculture Organization of the United Nations. Primary files contain station information and climate data. Climate variables are mean rainfall, minimum and maximum temperatures, vapor pressure, wind speed, sunshine, radiation, daytime and night time temperatures, etc.

Farm and Village Forestry Database

This database stores and manages socioeconomic information at household, village, district, and national levels, which helps scientists understand how these factors affect small farmers.

Abstract Database

Literature citations and abstracts relevant to MPTS are contained in this reference database. There are about 500 abstracts available now.

Species Digest

This database stores species characteristics and environmental requirements. About 175 species included.

MPTS Specialist Database

This database provides quick access to the names, addresses, and expertise of researchers active in MPTS research worldwide.

Data Analysis and Modeling

This is a general purpose statistical analysis and graphics package which has been integrated into the F/FRED MPTSys. The primary objective of MPTStat is to offer researchers an easy to learn yet flexible data analysis package to handle a range of research design situations.

Growth Simulation

This is a computer model designed to predict yield and biomass of tropical MPTS under various management conditions, soil types, and climate conditions. For example, users can quantify trade-offs between species and planting density in terms of wood and foliage biomass production.

Species x Environment Modeling

MPTModel, is an interactive decision support package, which examines environment by genotype interactions and provides performance prediction analyses. Tailored to support network multilocational trails, MPTModel allows a series of experiments with standardized treatment designs to be combined and analysed in an intersite analysis. This helps in evaluating the consistency of species and management performance across environments. Both MPTStat and MPTModel help Network cooperators eliminate the time consuming steps of organizing and entering a data set into a decision support package.

For the full benefit of network trials to be realized, the plot and site data from each trial must be combined in an inter-site analysis following the intra-site analysis of each trial. Interactions between experimental treatments and sites have to be evaluated, and if important, be incorporated in modeling efforts for prediction of wood and foliage biomass. Graphics capability is an important tool in inter-site analysis for interpreting the variability of treatment effects from site to site. A preliminary evaluation of important site characteristics for understanding treatment-site interactions is enhanced with graphics as well as a summary presentation of the data. MPTSys is designed to perform these steps of analysis.

The hardware requirements for running MPTSys include an IBM compatible PC/XT (AT is preferred), one hard disk (20Mb recommended, 2 Mb free), 512K bytes of RAM memory installed (300 free), one 360K Floppy disk drive (for installation and backup), an enhanced graphics adapter (EGA card), a monitor with at least 640x400 resolution, and a printer. The only software requirement is DOS: PC-DOS v3.1 or higher, or MS-DOS v3.2 or higher.

MPTSys utilizes dBASE III Plus, a widely-used commercial relational database application for organization, maintenance, and manipulation of data. However, dBASE III Plus is not required on the user's computer and no previous knowledge is needed. Screens for data entry, help windows, and other aids have been programmed for easy use of the system by the user. The F/FRED system was compiled using Quicksilver, one of several excellent compilers available for dBASE. Compilation allows the MPTSys to execute faster, handle windows, interact directly with the operating system, and manage memory more efficiently.

Training

A series of training courses were organized for cooperators and technicians of the F/FRED humid and sub-humid zone experiment network. The user-friendliness of the system, especially for data entry, allowed rapid comprehension with a minimum of frustration for new users. Much valuable feedback and recommendations for enhancements of the system were generated which will help make MPTSys more useful to cooperators both within and outside the F/FRED network.

End Users

Researchers will benefit from timely analyses of experiment results through the data analysis package of MPTSys, the use of an integrated and personalized reference source including citations and abstracts, and the access to a database of other specialists in MPTS research. Technicians can use the system to quickly and conveniently store experiment results, retrieve experiment data for verification, modify data stored in the database, and generate hardcopy records for reports. Managers can utilize the system for decision making through the environment and simulation modeling information of MPTSys, and for the MPTS specialist database and network directory which gives possible contacts with individuals specializing in selected areas.

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Provenance Trials

Section 4

Neem Provenance Collection and Seed Handling

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Before attempts are made to study population structures and to genetically improve a species, its pattern of distribution, and possible extent of variation must be explored; otherwise, proper sampling schemes cannot be devised.

To plan the collection and distribution of propagative material properly ,knowledge must be obtained on seed ripening periods, seed physiology and collection, handling, storage, and transport techniques. Planning needs should also account for matching possible dispatch dates with actual optimal sowing periods.

To ensure that proper performance evaluation is obtained, provenances or families must be tested over varying conditions of ecology and management.

Despite the extensive use of neem, few studies have been undertaken regarding its genetic diversity. Attempts to widen its narrow genetic base in areas of introduction have failed; e.g., in Africa. This is largely due to problems with the seeds of the species.

Occurrence and Distribution Pattern

Genetic structures of natural, wild populations of a species would usually be expected to differ from those of newly established plantations. Landrace structures may differ depending on how long the respective populations have been exposed to conditions in an area.

Where a natural population may have segregated into sub-populations of more or less closely related individuals, e.g., *Terminalia superba* (Vigneron, 1985), or have complicated patterns of distribution of genotypes, a plantation may be expected to be a random mix of several genotypes; however, this may not always hold true. Landraces may have distribution patterns of genotypes similar to natural populations if the species has spread or regenerated naturally. If the species has been propagated under forest or other management systems through artificial sowing or planting, the population structures may continue a random mix of genotypes; i.e., if one is not talking about seed or fruit orchards with special designs for the location of genotypes.

Natural Occurrence

The natural distribution of *Azadirachta indica* has been described by several authors, but the descriptions are mostly general. The best are old, considering the changes that have occurred to tree vegetation in most countries.

According to Troup (1921), "It is common in the open shrub forests of the dry zone of Burma, and it is found apparently wild on the Siwalik Hills; ... if wild anywhere in India, it is probably so in the forests of the Carnatic and parts of the Deccan. In the Saharanphur Siwaliks I have found it in some quantity on the inner ridges in places where it is difficult to believe it could have spread from cultivated trees. It is cultivated all over India and Burma, more especially in the drier parts of the country, and has evidently become wild in many localities as an escape from cultivation...."

Jacobs (1961) states neem as natural to upper Burma (but cultivated throughout India), Ceylon, Indo-Chinese Peninsula (not in Malaya), and East Java to Sumbawa. According to National Academy of Sciences (1980), neem is native to the dry forest areas of Pakistan, India, Sri Lanka, Burma, Thailand, Malaysia, and Indonesia. Bhumibhamon (1987) cites that *Azadirachta indica* is indigenous to India, Burma, Java, and the Lesser Sunda Islands.

It is unclear whether the occurrences mentioned by the National Academy of Sciences in Malaysia and Indonesia, by Jacobs in Java and Sumbawa, and by Bhumibhamon in Java and Sunda Islands are in fact, *A. indica*, *A. excelsa*, or both. Jacobs (1961) does state that *A. excelsa* is generally confused with *A. indica* in those areas.

Natural Occurrence of Varieties

Two varieties of the species are found in Thailand. One, *Azadirachta indica* siamensis Val., has larger leaves and appears more robust and vigorous than the other, which belongs to what one may call the holotype found first of all in India. The latter exists in Thailand only as planted trees.

The two varieties of *Azadirachta indica* have been differentiated with respect to pollen morphology and isozyme systems (Boonsermsuk and Jitjamnong, 1989). They can be distinguished from each other based primarily on their patterns of variation in the isozyme system. The pollen differed in the thickness of the pollen membrane, but not in other pollen characteristics.

In Thailand, distribution of the variety *A. indica* siamensis has clearly been influenced by human beings. In general, the spread of neem seems to have been a slow, gradual spread of existing populations, and can be considered a form similar to a truly natural process of extension. People's contribution to the spread of neem in Thailand seems to have been to pave the way through forest clearing and land preparation and to leave the tree once established. Thus, in Thailand, neem may be considered as naturally occurring in all areas, except where it has recently been planted (often along with the holotype) in roadside plantations or where a special "sweet" type has been obtained from the Petchabun area to other parts.

The variety is distributed throughout the southern, central, northern, and eastern parts of Thailand, with the densest populations occurring on termite mounds and ridges in paddy fields, and more scattered in some of the dry dipterocarp forests.

In one locality, near the provincial town of Tak, a dense population of neem trees is located deep in a moist, mixed deciduous teak-bearing forest. There is no other neem in the vicinity of this stand.

The variety has two types: one with white flushing inflorescences and the other with red ones. The former is often collected and used in preparation of food. The wood of the latter is said to be liked less by insects and is preferred in the making of furniture, especially beds. This information rests solely on farmer statements. It has not been verified whether these types are caused by genetic or local effects.

It is unclear from the literature whether the occurrence in Myanmar is the holotype or the variety siamensis, but Troup (1921) does not distinguish the types in India and Burma. There is no obvious way of connecting the occurrences in Myanmar and Thailand or in India and Myanmar.

Regarding the situation in Laos, Cambodia, and Vietnam, it is not evident to what extent both the holotype and the variety occur. Obvious migration routes exist between Laos and Cambodia with Thailand and between Cambodia and Vietnam.

Plantations and Landraces

The holotype of *A. indica* Juss (called "Sadao india" or "Quinine" in Thailand) is found sporadically, mostly in temple compounds where it has been introduced primarily because of its medicinal properties. These are said to be more pronounced than those of *A. indica* siamensis. One monk stated that he had not seen this type until the naval base at Sattahip was established in the 1920s. He suggested that the Thai Navy introduced the type. This was also suggested by an 83-year-old farmer, who was in the navy before the second world war. Near Prachuab Kirikan, in the Peninsula, the two varieties hybridized, to produce progeny of intermediate characteristics. A little north of this area, hybridization does not occur because of asynchronous flowering of the varieties.

Troup (1921) states that in India and Burma neem "... is cultivated all over ... more especially in the drier parts of the country, and has evidently become wild in many localities as an escape from cultivation ...." These countries may thus present a more complicated picture than Thailand. One would have to distinguish between truly wild populations, landraces, and newly established plantings.

In Africa, neem has been introduced to many countries earlier this century and exists now in populations that would be termed *landraces*. An account of period and mode of introductions to Africa is provided under Country and Region Reports in the symposium proceedings. Introductions of neem to America are fairly recent, and true landraces may not yet exist.

Seed Collection Sampling Strategy for Provenance Tests

Sampling Among Populations (Provenances)

Before attempting seed collection for provenance/progeny studies, it is essential to make a proper exploration in which the occurrences of neem have been defined and in which the origin of populations have been examined (wild populations, landraces, or newly-established plantations). For the two latter categories, the origin should be established, and attempts should be made to verify the representation of the original stand in the plantations; i.e., how many seed mother trees were collected from, how were they distributed over the area, and how large the area.

As a further help in devising proper sampling schemes, variation patterns may be studied through observing such characteristics as leaf morphology. Such studies would add to the time needed for proper preparation. It should be kept in mind that different characteristics may have different patterns of variation.

In Thailand, the occurrence of neem was examined during the period 1988-1989 (Lauridsen *et al.*, 1991). In this work, the nature of a number of populations was examined, and provenances were defined and described, including a statement of seed-production capacity. It is possible to refer a number of provenances to certain ecological situations, using either the seed (or ecological) zoning system devised by Eis (1986) or by Kaosa-Ard (1983) for teak. However, no attempt was made to study phenotypical variation. Work of this nature has not been reported from other countries, but it would facilitate the planning of possible provenance collections if exploration work were carried out.

Information on population variation has been presented by Ermel *et al.* (1983). They studied the concentration of azadirachtin in seed samples from African and Asian provenances and found a range in content from 1.5% to 4.8%. Possibly, the effect of the genetic population variation is confounded with the direct environmental effects.

Singh (1988) reported on the insect antifeedant properties of neem seed from nine localities. He found large differences between seed from different localities. However, the effects are confounded with the direct effect of environment.

For provenance studies, one may use both plantations and natural populations. Plantations located adjacent to the natural populations where they originate could be excellent for collection of seeds because collection may be easier than in the natural populations and because any effect of inbreeding that may have occurred in the natural populations would be alleviated through crossings in the plantation. However, before using plantations, their history must be thoroughly explored.

Landraces are also important to include because they represent occurrences and adaptations of the species in special environments, but they should have been through several generations in the concerned environment. Possibly, landraces should only be tested locally because they may represent a limited genetic variation, where one should generally aim at a wider genetic base.

Each situation would require specific design of sampling for obtaining good representation of available genotypes. One cannot devise a proper sampling scheme without some prior idea of the nature of the populations as discussed above.

Ideally, provenance testing should be carried out in two or more stages (IUFRO, 1965). In the first stage, the objective would be to "Outline general patterns of inherent variation. The number of provenances to be tested will depend upon the variability of the species and environments over its natural range. The second or later stage trials should find sub-regions and finally provenances having greatest productivity." However, the costs involved in provenance testing are high, and we may try, as much as possible, to minimize the number of stages required, or to aim at the later stage.

For the important study of genotype-site interaction, we should have the same set of provenances represented on all sites. This would, however, in the case of neem with many potentially different provenances, lead to impractically large experiments. One may well do with less.

With many provenances representing different environments, a practical alternative would be for each trial environment to select a set of provenances, where most of the environments they represent matches the trial environment, and to select a few provenances representing extreme environments to be included. One would, in this way, still have a sufficiently large number of the same sets of provenances represented on several sites to permit inferences of interactions and could keep trials at practical sizes.

Sampling Within Populations (Provenances)

How large is a provenance? One may recall the practical objective of provenance research (IUFRO, 1965), which is "to locate populations of trees, or provenances, whose seed will produce well-adapted, productive forests in a given region."

It is suggested that one should aim at provenances sufficiently large to potentially produce enough seeds for the establishment of 5-50 hectares of plantation, seed production stand, or breeding population.

When choosing trees in a provenance for collection, the principles differ for natural populations and plantations. In the former, seed trees should be selected so far from each other that there is little chance of collecting from closely related trees. A rule of thumb is to select trees at intervals exceeding normal seed-fall distance. One should also aim at covering the whole, not only part, of the provenance.

The number of seed trees should, in principle, reflect the variability of the population (i.e., the larger the variation, the larger the sample, and vice-versa), but one should always aim at a minimum of 20-25 trees, preferably more.

How much seed is to be collected? Naturally, this will depend on the number of willing participants.

Number of Provenance Trials

Seed requests are often received in which the stated objective is provenance testing, and enough seed for one or two trials should be supplied. This would be appropriate if the trial were to be located on the specific site where plantations of the species were to be established.

One may consider, however, that a test may be performed over a relatively short period under the prevailing climatic conditions. Thus, provenances would be tested only under the particular conditions of climate and soil-climate interactions. Climate shows long-term changes, and in semi-arid and arid areas, conditions may sometimes be extremely adverse. The risk is that provenances may be tested in a period with better-than-average climatic conditions.

If trials are located in many different countries (hence, different continents), one may have a better chance of different climatic situations, and provenance interactions with site conditions would have a higher possibility of being exposed. Or, one may have a better possibility of identifying, with high certainty, provenances that perform stably under different conditions, with especially good tolerance to adverse conditions. This is the virtue of planned and coordinated international provenance trials.

The amount of seed to collect from any provenance can be determined only when we know the participants and which site conditions will be tested.

Seed Problems

Poor storability of neem fruits has been a major obstacle to proper provenance testing. A wide variation in germination has been reported. This is generally due to seed source, but much of the reported variation may be due to methods of collection and seed handling (Chaney and Knudson, 1988).

Until recently, it was generally acknowledged that neem seed looses viability shortly after collection, and neem was considered a species with recalcitrant seed characteristics. Recent work in France and England, however, has demonstrated that neem seeds may, in fact, be true orthodox; possibly for only some occurrences of the species. Two cases show typical germination levels and viability losses, and they reflect possible differences in seed physiology between populations from different continents.

Ezumah (1986) found, for seed from Nigerian provenances, germination of freshly collected seeds, dried at different regimes for 8 days and of different moisture contents to be: fresh-undried MC 39%, G 83%, air-dried MC 16%, G 83%, sun-dried MC 11%, G 83% (test method not indicated). Viability dropped by 50% after 8 weeks of storage in airtight tins, and was completely lost after 12-16 weeks. Cold storage at 6-7° C gave poorer results than storage at 26-28° C. He found optimal germination at 25° C.

Chaisurisri *et al.* (1986) found, for seed collected in Thailand, that the drying regime would significantly affect seed viability during storage. For up to 8 weeks storage, 2-3 days drying in ambient conditions in the laboratory (30° C, 75% RH), in a dehumidified air-conditioned room (25° C, RH), and in the sun in a glass house (T and RH), viability was held above 90%. For more than 8 weeks storage, sun-drying had a significant advantage over the other treatments. Storage in gunny bags at 15° C was clearly better than other combinations of storage conditions, including airtight bags and storage at 2° C. Up to 62% germination (range 20-62) was still found after 16 weeks of storage, and a complete loss of viability was found at 24 weeks of storage under all conditions. Moisture contents were, after the optimal drying periods of 2-3 days, still reported at a level of 46-50%; after 11 days in a dehumidified room and 7 days in sunlight, they were 38% and 36%, respectively. Moisture content continued to drop, but was still 26% after 16 weeks of storage in gunny bags. Tests used the oven method, drying at 105° C for 6 hours.

Bellefontaine and Audinet (In press) reviewed recent research on neem seeds in India, Pakistan, Haiti, and France (in collaboration with Senegal, Cameroon, and Burkina Faso). They note the characteristic difference between India, Pakistan, and Thailand on the water contents of seeds. Seed from the former countries has higher moisture contents than the latter after various drying regimes. They speculate that the introductions of neem in Africa and Central America have adapted to the particular conditions there, to become a type with orthodox seeds.

Chaney and Knudson (1988) found the endocarp of the neem fruit a physical barrier to water and gas exchange, and suggest that the germination is lost as the endocarp dries out. They also found that the endocarp does not contain water-soluble germination inhibitors. The authors further point to the role of unsaturated fatty acids and ethylene, and suggest that the effect of removal of the endocarp of neem fruits on germination could be a change in ethylene concentration.

Bellefontaine and Audinet (In press) continued research similar to that of Chaney and Knudson. They studied germination after seed storage of different provenances and maturity states. They tried treatment combinations of storage of stones and seeds, with sowings as stones or seeds stone as the depulped, clean fruit; i.e., seed, seed coat, and endocarp; seed is the seed and seed coat. In addition, they tested the effect of the following storage conditions:

- When stones were stored, germination was better when the endocarp was removed before sowing. After eight years of storage, sowing as stones stored for eight years resulted in 20% germination, whereas sowing as seeds gave 70% germination. For eight-month storage, the figures were 59% and 97%, respectively. Moisture contents of stones or fruits significantly affected germination; 11% for stones and 9% for seeds resulted in 0% and 19% germination, respectively.
- Storage as seeds for up to 12 months gave germination similar to that of stones stored (and then sown as seeds); i.e., 71% versus 75%.
- Storage of stones at 4° C for more than four months showed significantly better germination than storage at a constant ambient temperature.

The above, together with a review of available relevant literature, shows the following:

- Neem seeds of certain origins can be stored for many years (eight) with only about 30% loss of initial viability.
- Cold storage affects seed viability differently, depending on seed origin, but the effects may be confounded with the effects of different moisture contents.
- It is tentatively concluded that moisture content can, for some African and Central American provenances, be lowered to 5-7% for seeds or stones, whereas it cannot be lowered to safe storage levels for provenances from the original occurrences of neem.
- Fruit endocarp appears to prevent moisture and gas exchange between seed and environment.
- It is often not possible to see which test for moisture content has been used; i.e., results may differ merely because of differences in test method or reporting.
- Fruit-drying conditions are not always provided when research results are reported.

For initial discussion of the possible differences in storage behavior between Asian and other provenances and the possibility of adaptation, one may observe the conditions under which the species occurs. In Thailand, neem may often occur under widely different conditions with respect to water. Seeds ripen in some areas during long intervals between rain showers and where evaporation considerably exceeds rainfall. In other areas, water conditions would generally be favorable for seed germination. The same would possibly be the case in India, where neem is found under a wider range of environmental conditions, including very dry ones. It is possible that neem has adapted its seed physiology to the prevailing environmental conditions; thus, before concluding that there are continental differences in seed physiology, one should examine fruit and seed physiology, germination, moisture content, and storability from a wider range of conditions in the respective countries.

One should work from the hypothesis that seeds from the moister provenances may be recalcitrant, those from the drier provenances may be orthodox, and that there may be intermediates.

Regarding drying regimes, temperature and humidity levels during drying should be presented. The reason is that too high temperatures may negatively affect seed viability and vigor, or too rapid a drying (low initial relative-humidity level) may stop the drying early in the process. Also, one may consider providing a different system for monitoring seed drying than is usual. Vertucci and Roos (1990) discuss recommendations provided for storage of seeds, and suggest that the optimal level of seed moisture content for seed storage can be identified more readily by the relative humidity than by the seed moisture content. One estimates that, for orthodox seeds, a safe level for storage or transport is below 6-7%. Vertucci and Roos (1990) found the optimal level of moisture content to vary considerably depending on lipid (fat or oil) content and that the relative humidity kept at around 18% would ensure maximum vigor after storage at 35° C (Table 1). Suggesting that oil content in neem seeds may vary with provenance and that a temperature of 35° C would easily be encountered during seed transport, one may make use of the suggestion of Vertucci and Roos, where seed drying may be of relevance to ensure the best results after transport and storage; i.e., one should try to maintain the final humidity at an optimal level. It should be examined how the humidity level should vary throughout the process; a gradual decrease ending with the optimal level. The influence of the endocarp on the drying process should also be taken into account in research on drying operations.

Table 1. Critical moisture levels for various physiological activities (%)

		Onset of respiration		Moisture level for maximum vigor after storage at 35° C	
	Lipid content*	MC**	RH	MC**	RH
Sunflower	58	15	92	3.01	18
Peanut	45	16	92	3.29	16
Lettuce	37	17	91	4.49	21
Soybean	20	19	91	5.57	20
Pea	2	21	90	6.63	19

* Lipid content as percentage of dry weight
** MC as g H_2O/g fresh weight
Source: Modified from Vertucci and Roos (1990)

Test methods and reporting forms for moisture content must be clearly presented with research results; i.e., oven method, temperature and length of drying period, and wet or dry weight.

Research work is needed regarding the influence of the endocarp on imbibition, considering also that in very dry fruits the seed may have retracted from the endocarp, leaving only a limited pathway for water. Even though the endocarp may permit water passage, the rate of uptake may be too slow, leaving ideal conditions for fungal activity to negatively affect viability.

Neem fruit, being a juicy drupe, is prone to rapid deterioration due to fermentation. The seed quality is dependant on the maturity of the fruit at time of collection; the treatment after collection will greatly affect quality. Also, seed handling is a matter of applying well-known techniques.

Seed Collection, Handling, Storage and Transport

Collection Period

Neem fruits develop and ripen during a period of 1-2 months. Because of the nature of the fruits, these have to be collected during certain periods of their development or ripening. The optimum period is when the color of the drupe turns from full green to yellowish-green. However, it is also possible to collect earlier, when the fruit is still green. This depends on whether the fruit has attained its full size and the seed has all structures well-developed. The collection of still-green fruits along with fully ripe, yellow fruits would mean a more effective use of resources for collection.

Fruits should be collected directly from the trees because fruits that have already fallen to the ground may have lost quality. It is implicitly understood that the ground should be cleared of fallen fruits, with a tarpaulin or something similar spread under the tree. Whether to pick the fruits or to beat or shake branches depends on whether only fully ripe fruits or green fruits are to be collected and the amount of seed to be collected from each stand. The process of beating or shaking branches causes undeveloped, immature fruits and seeds to fall and potential for further harvest is less.

Fruit Processing

For maximum seed quality, fruits should initially be graded. Undeveloped, immature, and damaged fruits should be discarded; fully developed fruits should be separated into color groups (e.g., fully green, greenish-yellowish, and yellow fruits). The latter group must be depulped on the same or the following day because fermentation starts rapidly. The other groups can be left in a shaded place until they have ripened and can be depulped. However, the still-green fruits may be depulped immediately, without significant loss of quality, but with more difficulty. Whenever fruits are kept in bulk, they should be kept in a thin layer to avoid damage due to suppression or heating. If fermentation, for some reason, has started in a bulk, spraying the lot with water several times a day to wash out fermentation products will prevent loss of seed quality.

Depulping is usually done by hand, which is easy for fully ripe fruits and labor intensive for green fruits. The depulped stones are washed in running water, avoiding soaking. Amata-Archachai and Wasuwanich (1986) discuss the use of a Dybvig seed cleaner that can depulp around 700 liters of fruits per day.

Further research on drying is needed before general guidelines can be established for different provenances. We cannot yet define the boundaries between possible recalcitrant (if they do exist) and orthodox neem. Packing materials and storage conditions can also be identified in general once we have a better idea of possible types of neem seed.

Documentation

Detailed information on a particular provenance and seedlot is a necessity for a good trial scheme. Participants should always be provided with full information of the materials they have received. Too often, inadequate information is provided with seedlots.

A provenance may be independently described, since it may be the base for a number of seed collections. Such information may need updating, since tree populations change due to management or to uncontrollable factors.

A seedlot collected in a particular provenance must be adequately labeled and documented. Most importantly, the lot must be adequately labeled--one label inside the bag or container and one fixed solidly on the outside. Labels must be moisture-proof and tear resistant. Such basal information as species name, provenance name or reference to provenance, date of collection, container number, and weight should be on the label of each container. The labels should follow the seedlot through transport and processing.

In addition to the label, detailed information on the collection must follow a seedlot to the participant in the trial scheme. Besides species name, provenance reference, and collection period, information on genetic representation must be provided (e.g., number of seed mother trees, their spacing, area covered, possibly shown on a map, and possible selection criteria; ideally, provenance collections should include the variation of genotypes in a provenance). A general account of the abundance of fruiting and frequency of fruiting trees would tell something about genotypic representation. Seed test results for the processed seedlot, germination percentage and moisture content, together with recommendations on storability, imbibition method, sowing method, and seedbed condition, would provide a good background for best plant production.

Seed Distribution for Trial Establishment

In implementation of a provenance/progeny trial scheme, it is evident that one cannot consider handling seed from all provenances as orthodox seeds; hence, a number of unusual obstacles will be encountered when dealing with orthodox seeds.

If one cannot wait for the relevant research to be completed or if the research results confirm that some provenances may have recalcitrant seed characteristics, one should spend more effort and resources on the timely collection, dispatch, and receipt of seeds.

The problem is less serious in the case of Thailand, where fruits mature within two months. The above research shows that fruits are easily stored for up to three months without significant loss of viability. This means that all provenances can be collected, stored, and dispatched to participants in complete sets. The biggest problem is that the optimal sowing periods in the recipient countries may not fit the dispatch time.

In India, where fruits mature over a much longer period (from May to August), one may reach the storage period limit for some provenances. Also, the same problem exists as in Thailand for matching dispatch and sowing dates.

As an alternative to seeds, one may consider producing stumps in the countries of material supply. Seeds may be sown immediately after collection, and stumps can be lifted and dispatched any time during the following year as appropriate for the recipient countries. Any difference in plant size will last in the trials for some years but will, under good management conditions, possibly disappear. Stumps are robust and can tolerate some adverse conditions in transport. Stump production will add to the costs of trial work, and there is the added risk of transferring disease. These factors may prevent this method from being applied.

It will be difficult to provide a strategy for collecting, handling, and dispatching material until we better understand the properties of Indian and Thai neem seeds and until it is firmly established who will participate and under what climatic conditions they will establish trials.

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Guidelines for Managing International Neem Provenance Trials

Report of Working Group One

One of the primary reasons for convening the First International Consultation on Neem was to plan a research strategy for the genetic improvement of neem. Two working groups were formed for this purpose. One was asked to develop a strategy for seed collection and exchange and to plan a set of uniform international provenance trials. The second group was asked to recommend supplemental studies which should be carried out in support of the provenance trials and future neem improvement work. This paper summarizes the results of the first group's deliberations.

It was affirmed that a set of international provenance trials should be established to evaluate seed lots collected from as wide a range as possible, with particular emphasis on seed collected from neem's native range. The trials would incorporate the following features:

- common design and standardized methodology
- common minimum data set to be collected
- common germplasm
- complete soil and climatic data at each site
- data exchange and professional interaction among participants
- intersite combined analysis of data

Participants divided into two sub-groups. One was asked to make recommendations regarding seed handling and storage while the other concentrated on trial layout and management procedures. This document is a synthesis of the two sub-group reports. It provides guidelines for research collaborators who will participate in the network trials and includes the agreed-upon standard procedures to follow for seed collection, storage and exchange. It also described a methodology for establishing international provenance trials as well as recommended nursery and field management practices.

It was acknowledged by all those in attendance at the meeting that the work plan drawn up is both tentative and ambitious. This is because there is a lack of basic knowledge about some of the technical issues, for example, the optimum procedures for neem seed drying and storage. Also, many of those in attendance were not empowered to make a firm commitment on behalf of their country to participate in the program. Finally, many of the representatives indicated that, although they were extremely interested in conducting collaborative research on neem, they would require at least some level of external funding in order to participate.

In spite of this, it was agreed by all present that the program should begin without delay using currently available funding. It was felt that experience gained by beginning immediately would prove invaluable in subsequent years. The participants suggested that another meeting be organized the next year to revise the work plan in light of new research data on neem seed handling and review of the experience of participants who exchanged seed during the first year. The organizing committee offered to help by coordinating implementation of the agreed upon work plan and by seeking funding for a follow-up meeting.

Defining and Sampling Provenances

Provenance Delineation and Description

Natural populations will be given priority for sampling. Plantations originating from the same environments as natural populations and planted in the same location will also be considered. Old man-made plantings and populations derived from such plantations may be impossible to distinguish from natural populations and will, therefore, also be included.

The delineation and identification of provenances will be based upon ecoclimatic zones and latitudes. Provenances which represent characteristic ecoclimatic zones and a range in latitudes will be selected. A certain limited number of these will be made available to the international scheme, and a further limited number will be designated as a core-group of provenances. This core group will be represented on all trial sites. In addition to the core group provenances, cooperators will be able to select another 15 provenances of their choice from the list given in Attachment 1.

A careful description of each identified provenance must be made. The purpose is primarily to ensure that any repeated collection can be made in the same area where the original provenance was collected, but it is also to provide information helpful when selecting provenances for trials. Attachment 2 is an example of a provenance description which includes all the data which should be collected. Additional information may be required to meet local needs.

Selection of seed trees within a provenance

In principle, random sampling should be used to capture as much genetic variation in the provenance as possible. **Plus trees should not to be selected.** The trees for collection should be uniformly distributed over the provenance and represent the whole area.

Spacing of seed trees may vary but trees should be a minimum of 50 meters apart. The number of trees collected per provenance must be at least 25; preferably more.

Seed Collection*

Throughout this paper, the term "fruit" is used to refer to fresh neem fruit which includes the fleshy pulp. Stone and seed are used interchangeably to refer to fruit without the pulp. Stones (seeds) consist of an outer endocarp and an inner kernel.

Time to Collect

The optimum time for collection is when the color of the fruit begins to turn yellowish-green. Green and yellow fruits can be collected at the same time in order to make more effective use of resources, but this should only be attempted if those collecting the fruit have good knowledge of fruit development. Immature fruit may not germinate well and could result in seedlings of poor vigor. Furthermore, it is extremely difficult to depulp green fruit. Yellow fruit, on the other hand, is over-mature and has a short shelf life.

Amount to Collect

- Each trial will contain four replicates of 25 trees (100 seeds). Additional seed is needed to compensate for poor seed viability and seedlings which die in the nursery. Extra seedlings must also be raised as replacements for trees which do not survive outplanting.
- It is therefore recommended that 1,000 seeds be collected per provenance trial, or **1/2 kg of dry clean seed per provenance for each cooperator who will receive a shipment**.
- Twenty sites are tentatively planned for the international provenance trials. Hence, **the total seed requirement is 10 kg per provenance.** This should be adequate to supply seed for additional studies as well.
- Note that stone weight may vary. The above calculations assume a weight of 0.2 grams. If the seed you collect weighs significantly more or less than this, adjust the weight of seed collected accordingly.

Collection Methodology

- Fruits should be collected directly from the tree. **Do not collect fruits that have already fallen to the ground** as they will yield seed of poor quality.
- The ground should be cleared of fallen fruits, or a tarpaulin should be spread under the tree to separate picked from fallen fruits.
- Shake branches with poles or pick the seeds directly. Climbing may be necessary for tall trees. In the process of beating or shaking branches, immature fruits and seeds may also fall.
- Ideally, only physiologically mature, green-yellow fruit should be collected. If fruit on the tree is at different stages of maturity, the same tree should be re-

collected later so that only fruit of the correct maturity is harvested on any given day.

- In practice, it may not be possible to harvest the same tree several times. In this case, mature green fruit can also be harvested along with green-yellow fruit. The procedure is the same, but depulping the fruit is difficult.
- Fruits should be collected in baskets, gunny bags, or another type of container which allows the fruit to be exposed to air. Do not use plastic bags or plastic buckets to collect and store the fruits. Lack of aeration causes fermentation.
- Fruits from each tree should be kept separate during collection and processing (collect seed on a single tree basis).
- Each basket or bag should be tagged separately with a solid tag, using waterproof writing. (see example in Attachment 3).

Seed Processing and Storage

Grading and Depulping of Fruits

- Fruits must be processed (graded, depulped and dried) immediately after harvest. The amount of time between harvest and processing should not exceed 48 hours.
- During transport, fruits with pulp should be protected against pressure and excessive shaking. Fermentation of green-yellow fruits must not be allowed to occur. Should fermentation take place, fruits must be washed in running water and spread in a thin layer to cool and dry them.
- Do not store fruits in the refrigerator
- Grade the fruits as follows:
  - The undeveloped, immature and damaged fruits should be discarded;
  - Fully developed fruits should be separated into three color groups:
    - fully green
    - green-yellow
    - yellow
- The fully yellow fruits must be depulped the same day if possible, as fermentation proceeds rapidly.
- Fully mature green and yellow-green fruits can be left for ripening in a shaded place until they soften and can be depulped easily. They may also be depulped immediately without any loss in quality, but this can be difficult when the fruits are hard.
- Although soaking green fruit in water or allowing partial fermentation to occur facilitates depulping, there is some evidence that these practices may negatively affect seed germination. As there is not enough information available yet to

make firm recommendations, it is important that you **carefully document the procedures used in seed collection and processing.**

- After depulping, carefully wash the stones in clean water. Take care to remove all the flesh, but do not soak the stones.

Stone Drying

- Recent research suggests that neem seed can be dried to 8% moisture with full retention of viability (i.e., the seed is orthodox). Seed dried to this extent can be stored and transported safely in moisture-proof containers such as plastic bags.
- Other researchers argue that neem seed does not tolerate drying (the seed is recalcitrant), and that it must be kept at higher moisture content in a well-aerated container.
- There is also evidence that neem may be neither orthodox or recalcitrant, but intermediate in its characteristics. Several researchers have reported that seed can be dried successfully and retain its viability, but that germination drops drastically after several months in storage.
- In the absence of definitive information about the nature of neem seed, the following tentative recommendations are made:
  - Dry seed in the shade for two days. Spread the depulped, cleaned seed in a monolayer on top of a tarpaulin, newspapers, or jute bags in a shaded area or inside a building. Use a fan to speed drying of the fruit.
  - Dry the seed for at least two more days in the sun. Spread it in a monolayer on a surface which will not absorb heat and cause the seed to become too hot (newspaper, light colored cloth or jute are recommended). Again, supplemental use of a fan is recommended.
  - If it is the rainy season and the sky is cloudy and the air humid, the seed may be dried indoors under a fan for several days more days instead of outside in the sun.
  - After the seed has been dried to at least 20% moisture content (wet weight basis) it may be stored for a short period of time or shipped in a non-airtight container.
  - If a seed dryer or forced air oven is available, the seed should be dried to low moisture content (8 - 10%) at a moderate temperature (40 - 50°C) for several days after it has been reduced to below 20% moisture content. Very dry seed (less than 8% moisture content) needs little oxygen for respiration and is best stored in airtight plastic bags to retain its low moisture content.
  - Do not store or ship seed of intermediate moisture content (8 -20%) in sealed plastic bags or other closed containers. Lack of oxygen can kill the seed.
  - Be sure to keep careful notes and document the procedures used so we can learn which procedures work best. Monitor the moisture content of the seed several times using the procedures detailed in the next section.

Moisture Testing

The moisture testing procedures are based on methods described by the International Seed Testing Association (ISTA). If sufficient seed is available, testing should be done on an individual tree basis. If not, it is recommended to take a representative sample of seed bulked from all trees in a provenance.

- Select 5 g of stones (approximately 25 seeds). Use ISTA testing rules, or take a random sample of bulked seed. A stratified random sampling technique should be used if seed from each provenance is collected and kept on an individual tree basis.
- Remove the endocarps (shells).
- Cut the kernels into 4-6 parts and quickly place them in a previously weighed porcelain container. Immediately put the lid on, and weigh the container + seed kernels to the nearest 1/100th gram. Subtract the weight of the container from the container + seed. The result is the fresh weight (original weight) of the seed.
- Place the container in an oven and dry at 103°C for 17 hours, ± 1 hour. Remove from the oven and immediately place the container plus lid in a desiccator to cool. After they have completely cooled, weigh again. This is the dry weight of the seed.
- Calculate the original moisture content of the seed (fresh-weight basis) as follows:

$$(FW - DW)/FW \times 100$$

(The difference of fresh weight minus dry weight divided by fresh weight, and all multiplied by 100)

Germination Testing

- Test the germination of each seed lot before sending it to other network collaborators. As with moisture testing, germination tests can be conducted on an individual tree basis if sufficient seed exists. Otherwise, test a random or stratified random sample.
- Select 100 stones and remove the endocarps.
- Sow the kernels in sterilized sand (preferred) or another medium. Sand can be sterilized by placing in an oven for four hours at 130° C.
- Conduct the test under daytime temperatures of 26-30°C and night temperature of 16-20°C to prevent fungal growth, if this is possible. Ambient nursery conditions are acceptable if the above controlled conditions cannot be achieved.

- Count the number of seedlings which emerge during the next 12 days. This number is the germination percentage. If less than 80%, increase the quantity of seed you collect and send to network cooperators according to the following formula:

$$Q_0/P = Qn$$

Q_0 = Original quantity of seed

P = Germination percentage

Q_n = Revised quantity of seed

Seed Lot Documentation

Each individual seed lot must be documented to provide information about genetic variation in the lot and the way fruits have been handled. Attachment 4 is an example of the kind of information required.

Packing and Shipping

Preparing to Ship the Seed

Local regulations pertaining to export of seed from your country should be investigated before the seed is collected. There are usually few regulations restricting the export of small quantities of seed for research purposes. However, if local laws governing export do exist, these should be studied well in advance so that provision can be made to abide by the regulations.

After receiving a request for seed, the following documents and information should be obtained:

- Phytosanitary certificate from the exporting country
- Export permit (if required)
- Details of where the shipment should be sent
- Preferred method of shipment

It is important to find out if there are any special documents or information required by the recipient in addition to the above.

Packing and Shipping

Depending upon the recipient's requirements, seed from individual trees may be kept separate or bulked.

Well dried stones (less than 6% moisture) should be packed in sealed plastic bags (3-4 mil) and kept in ambient conditions or in a cold store (if the bags are moisture proof). For short term transport, ambient temperature is sufficient, and is preferable to avoid too much variation in conditions. For longer term storage, air tight cold storage is preferable.

Air-dried stones of provenances with unknown characteristics must be shipped as soon as possible after drying. These seeds should be kept in cotton bags, and a mild fungicide powder may be applied to minimize risk of fermentation. Pack seeds in a stiff cardboard box with holes for adequate ventilation during shipping. Neem stones have a high rate of respiration and require oxygen.

Shipping by courier is preferable and most companies will accept seed. This must be checked in advance.

Notifying the recipient

- Recipients should be sent a telegram, telex, fax, or telephoned with full details regarding dispatch of the seed so that they can clear the shipment as quickly as possible.
- The following information should be supplied: courier service or airline which received the parcel in the country of origin, flight numbers, dates and routes of flights, date of expected arrival at the destination, airway bill number and airport of arrival.

Requesting and Receiving a Seed

In addition to sending seed, you will also be receiving a seed shipment. This section describes the procedures to follow in receiving seed and planting it in the nursery.

Preparing to Receive the Seed

- It is important to request seed shipments as early as possible so that the cooperator who will ship it can plan how much to collect and make the necessary arrangements.
- Before requesting seed, make sure that you have nursery facilities and budget to plant the seed upon its arrival. Once the seed arrives it must be planted immediately; each day of delay can reduce germination percentage.
- Be sure to communicate complete shipping instructions to the sender and advise him or her of any local regulations pertaining to import of seed into your country

Planting Seed in the Nursery

- Germination rate of *Azadirachta indica* can vary from 85% (fresh seed) to 0% (stored seed). To ensure higher viability, the seed should be sown immediately after it is received.
- Remove the endocarp before sowing. Studies have shown that this significantly improves seed germination rates.
- Use 10 x 20 cm nursery bags if seedlings will be kept in the nursery for up to 6 months, or 13 x 25 cm for longer periods. This will depend on when you expect to plant in the field.

- The planting medium (potting mix) should follow local practices but we recommend low fertilization, sterilization, and only maintenance irrigation to avoid too rapid growth.

Care of seedling

Shade

Seedlings should receive about 50% shade. For the last 2-3 weeks prior to planting, seedlings should be exposed to full sunlight.

Watering

Water well in the morning if the upper part of seedling container is dry. Always water seedlings with fine spray, particularly when seedlings are small. Excessive watering should be avoided to prevent excessive growth and pot binding. As a means of "hardening off," watering should be reduced a week or so before the plants are transported to site.

Weeding

Keep seedlings free from weed competition at all stages of development. Manual weeding is recommended.

Grading of beds

To prevent larger seedlings from shading smaller ones, the beds should be graded by placing the larger seedlings on the northern side of the bed and the smaller seedlings at the southern side. Grading may need to be repeated depending on the length of time the seedlings are kept in the nursery.

Root pruning

It is necessary to prune the roots regularly. Do this by cutting roots that emerge from drainage holes. Roots should be pruned at the time the seedlings are graded, and thereafter as necessary.

Pest and fungus control

Control damping off and root rot with fungicides if necessary. Either Blitox or Dithane M-45 can serve this purpose. Similarly, spray pesticides if needed, but exercise great care. Insecticides containing methyl parathion, such as Metacid and Paramar, are usually effective against defoliators.

Field Trial Establishment

Objectives of the Trial

The objectives are to evaluate performance of provenances collected over a number of sites and determine whether there are any provenance x site interactions.

Experimental design

The experimental design is a randomized complete block with 4 replications. Twenty-five provenances (seed lots) will be tested (see Attachment 5 for sites).

Trial Layout

Site selection

The trial site should be representative of the area where the species is likely to be planted. It should be planted under controlled conditions on experimental stations. It is important that there is a weather station at the site or nearby so that meteorological data can be collected.

Site preparation

Clear the site of all vegetation. It should then be tilled adequately. Whenever possible the site should be disc plowed twice in two directions perpendicular to each other.

Block shape and positioning

The block (replication) layout depends on soil variability and practical field considerations. Choose a block shape to minimize variability within the block. All obvious site variation should be carefully evaluated when laying out the trial. If the site is uniform, square blocks are recommended. Where a dominant soil gradient in a particular direction is known (e.g., sloping land), a more rectangular block layout positioned across the slope is a better choice to minimize variability. Blocks do not have to be contiguous or always of regular shape.

Provenances are randomly assigned in complete blocks of 25 plots each. A sample block layout is shown in Figure 1.

23	1	16	8	3
15	18	9	20	25
22	6	24	13	10
5	14	11	7	2
19	4	17	21	12

Figure 1. Example of block layout. Note that each block contains 25 plots. The provenances are randomly assigned to each plot.

Tree spacing is 4 x 4 m. Each plot is uniquely numbered serially and consists of 5 rows spaced 4 m apart. Figure 2 shows a standard plot layout. Each row contains 5 trees, also 4 m apart; 5 rows x 5 trees = 25 trees/plot x 100 plots = 2,500 trees/site. For each provenance, the total number of trees required for planting is 25 x 4 = 100. An extra 50% should be reserved for replacement of dead seedlings.

Plot size is 16 x 16 m = 256 m^2 per plot x 100 plots = 25,600 m^2 (2.56 ha) per site.

In Figure 2, trees are numbered beginning at the near left corner and proceeding right, from 1 to 5. The outer rows of each plot are buffer trees, the interior 9 trees (interior 3 x 3 rows) are samples measured every 6 months after planting.

1	2	3	4	5
6	7	8	9	10
11	12	13	14	15
16	17	18	19	20
21	22	23	24	25

Figure 2. Tree layout within a plot. The 9 trees within the box are for measurement, those outside are buffer rows.

Marking out

Three different sizes of marking stakes will be used:

- Large posts, made of concrete, wood, or PVC pipes to mark block corners.
- Large pegs, small posts, or small PVC plastic pipes (but easily visible) to mark plot corners.
- Small pegs to mark each planting position.

Mapping trial layout

Prepare a map showing trial layout for each trial site. The map should include the following details:

- Name of the trial
- Location of the trial site
- Date of planting
- Trial design
- Illustration of field layout

Planting

Selecting seedlings

From each provenance in the nursery, select 256 healthy seedlings. They should be "hardened off" 1-2 weeks before they are transported to the site. This can be done by gradually reducing the watering and removing shade.

Transport of seedlings

The seedlings should be well watered before transportation. They should be packed in wooden or plastic boxes to avoid damage during transportation. Ensure that the seedlings are not packed loosely. Avoid wind damage during transport by erecting appropriate screens on the truck.

After arriving at the planting site, place the seedlings in a protected, shaded area until planting. Water them thoroughly daily.

Planting seedlings

Planting holes should be 25 x 25 x 25 cm. In addition, cultivate topsoil within 15 cm of the hole. Place locally available compost in the bottom of the hole and cover with soil. Also application of a pesticide is recommended in termite sensitive areas at planting time.

Planting should be done in the morning and/or evening, **not** in the heat of the day. Slit the potting bag with a sharp knife or razor blade and carefully remove the plastic bag without breaking the soil or damaging the roots. Set the seedling in the hole with its root collar level with ground surface. Fill in the soil around the roots of the seedling and firm the soil well down around the seedling to avoid large air pockets in the soil. Do not stamp foot and do not use heel. Mulch with dry grass. Seedlings should be watered with 2 liters. Replace dead seedlings with new ones within 1-2 months after planting.

Post-Planting Maintenance

Weeding

The seedlings should be kept free from weed competition during the initial stages of development, until weeds are outgrown. Manual weeding is recommended. Frequency and method of weed control will depend upon particular site conditions. Intercropping should be avoided to standardize treatments.

Every 2-3 months during the first year, pull all weeds within 50 cm of each seedling, and cut all those in the remainder of the experimental area to prevent competition. For succeeding years, weed as necessary.

Fire protection

Conduct regular weeding and removal to ensure that fuel does not accumulate within the experimental area, especially during the dry season. Prepare a firebreak, 8 - 12 m wide, around the experimental area before the beginning of the dry season. Cut and remove vegetation or plow within the firebreak regularly during the dry season.

Fencing

The experimental area should be fenced to prevent damage, particularly damage caused by animals. To help prevent human damage to trees, communicate to all neighbors the study objectives and long-term importance of the results to the local population. This will help gain support from the local community.

Pest and disease control

Monitor pests and diseases, especially ants, crickets, or other insects. Appropriate control measures should be taken as necessary up to age 1 year. After that, taking effective measures may be difficult and costly.

Data Collection and Exchange

There are two kinds of data that all cooperators must provide: site description information and data which is measured periodically in the field.

Site Description

The site description data is collected at the beginning of the trial and should include the following:

General Information

- Name and address of principal investigator and institution
- Site name, state, country
- Latitude, longitude, and elevation of experiment site

Site Parameters

- Previous vegetation (including existing land use) and method of clearing
- General relief: (flat, rolling, hilly, or mountainous)
- Site relief: (flat, concave, convex, or ridge)
- Position: (upper slope, mid-slope, lower slope, terrace, or valley)
- Slope percentage
- Aspect (N, S, E, W)
- Method of soil cultivation
- Other major site problems (pests, diseases, etc.)

Economics data

- Farmgate prices for fodder, fuelwood, small size timber and tannin.

Climatic data.

This should be obtained from the nearest weather station.

- Name of weather station and straight-line distance from site
- Latitude, longitude, and elevation of weather station
- Mean monthly precipitation, maximum and minimum temperatures and relative humidity for the past 10 years.

Soil data

- Taxonomic classification of soil
- Texture (topsoil and subsoil)
- pH
- Electrical conductivity
- Chemical analysis (soil fertility)
- Drainage, depth to impermeable layer or water table

Field Data Measurement

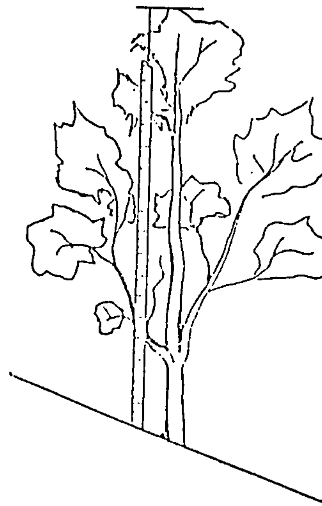
The information described in this section must be recorded periodically during the experiment. Note the frequency of each record where it is mentioned.

Survival

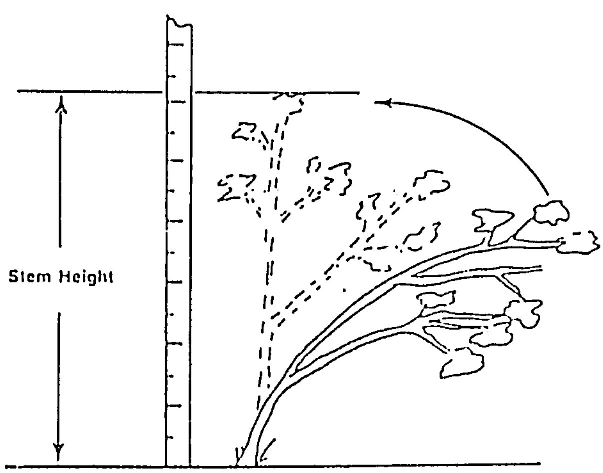
- Count all the trees in each plot and record them. Replace only during the first two months.

Height

- Measure the total tree height (Figure 3a, 3b). If there are multiple stems, measure the tree from ground level to the top of its highest apical bud. If the site slopes significantly, measure the principal stem from the uphill side of the tree. If the stems are bent over, straighten them if possible, so that the actual length of the stem is measured.
- Use a height stick or some type of marked, rigid pole to take this measurement.
- Measure trees numbered 7, 8, 9, 12, 13, 14, 17, 18 and 20 (Figure 2) at the age of 6, 12, 18, 24 months and every 12 months after that. If one of those trees dies or is significantly damaged during the first 24 months, substitute the measurement of an adjacent tree.



(a) Height measurement on a sloping site.



(b) Height measurement of bent or leaning trees.

Figure 3. Measurement of Tree Height

Basal diameter

Basal diameter is defined here as the diameter at 10 cm above ground level (Figure 4). Take measurement on the principal stem (and other stems originating below 10 cm). Mark with paint the point of measurement to ensure that the same point is measured subsequently. The measurement should be made with a metric diameter tape or vernier caliper. For multiple stemmed trees, the combined diameter at a given height is calculated using the following formula:

$$da=\sqrt{d_1^2+d_2^2+....d_n^2}$$

where da = combined diameter and d_1, d_2......d_n are the diameters of the stems.

A branching bole is considered a stem if its diameter is equal to or greater than 50% of the diameter of the principal bole at the same height.

Measurement should be made on the same sample trees used for height measurements at the ages of 6, 12, 18, 24 months and every twelve months after that.

Diameter breast height (dbh)

Take dbh measurement at 1.3 meters above ground level (Figure 4). For forking trees or trees with multiple leaders, take the dbh measurement on all stems if forking or multiple leaders originate less than 50 cm above the ground. Mark with paint all the points of measurement. If there is an abnormality in the tree at 1.3 meters, measure the tree diameter at the point nearest this height which is representative of the stem's diameter.

Take measurement on the same sample trees used for height measurements at the ages of 6, 12, 18, 24, months and every twelve months after that.

Tree form

Take this measurement once on the same 19 sample trees used for height measurements at 24 months. Score the trees into 3 categories (Figure 5).

Class 1: Tree with one main leading stem up to the tip. Branches are small, having basal diameter less than 50% of the diameter of the principal bole at the same height.

Class 2: Tree with more than one leading stem originating at a height above 50 cm above the ground. The branching bole is considered a stem if its basal diameter is equal to or greater than 50% of the diameter of the principal bole at the same height.

Class 3: Tree with more than one leading stem originating below a height of 50 cm above the ground. The definition given above in Class 2 for branching boles considered as stems also applies here.

a. For Class 1 trees, rate them as (1) straight, (2) fair, or (3) crooked.

b. For Class 3 trees, record the number of stems.

c. For trees in Classes 2 and 3, measure the length of the principal bole (i.e., the distance from the ground to the point where multiple stems originate).

Log of experiment operation and monitoring

Keep records of the dates and management operations done. Operations include anything carried out on the trials that may influence growth of the trees (for example, the date of weeding, the type of weeding, or drainage construction).

The trials should also be regularly monitored for other incidences such as wind damage, pest infestation, flooding or diseases.

Data Exchange

One of the main features of these network trials is data exchange for multi site analysis. To facilitate this, the data should be recorded and stored in a standard way. For this purpose, the Experiment Database of the MPTSys computer program will be used by all cooperators. The program will be distributed free of cost to every cooperator by the F/FRED Project.

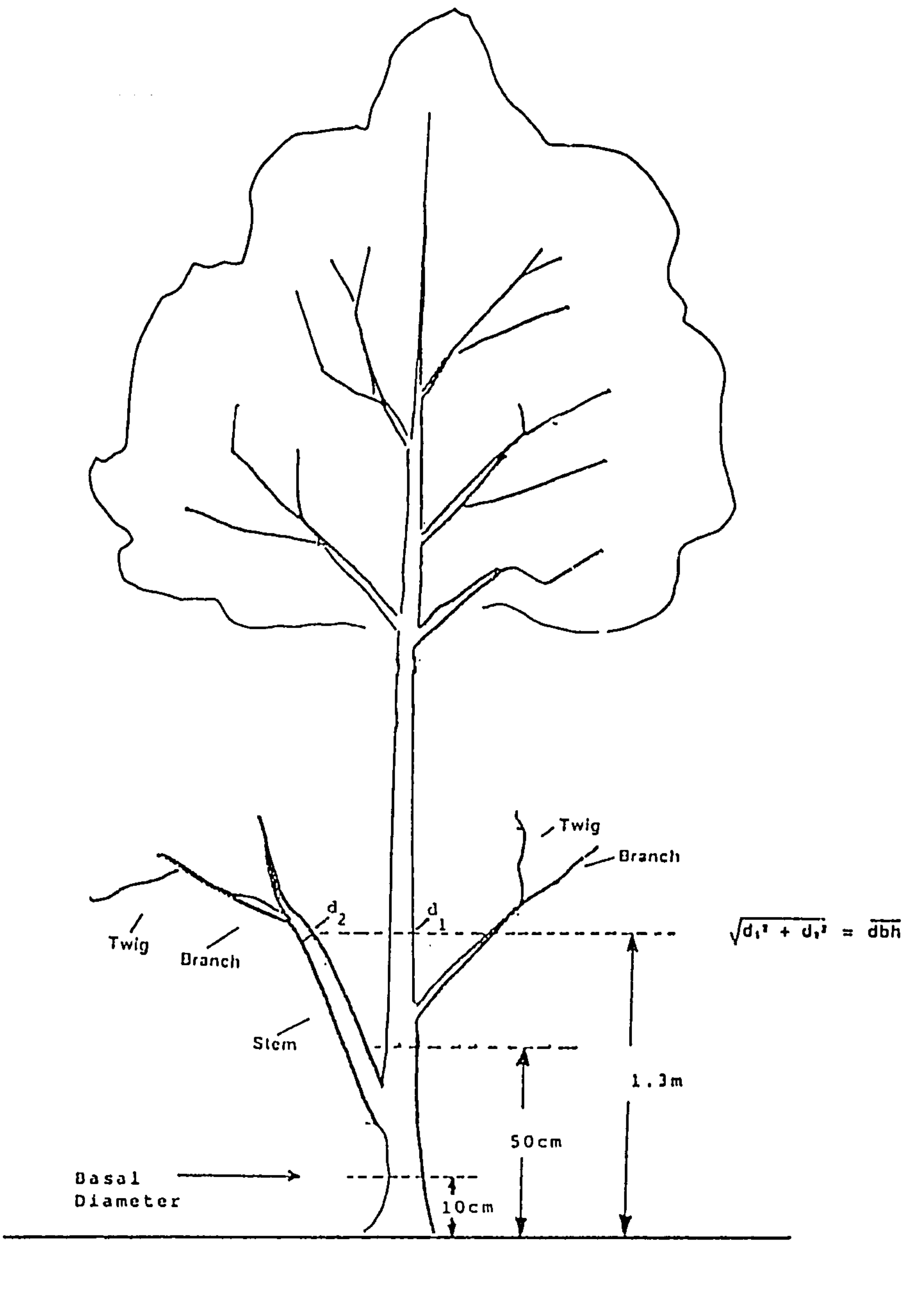


Figure 4. Diameter measurement guidelines

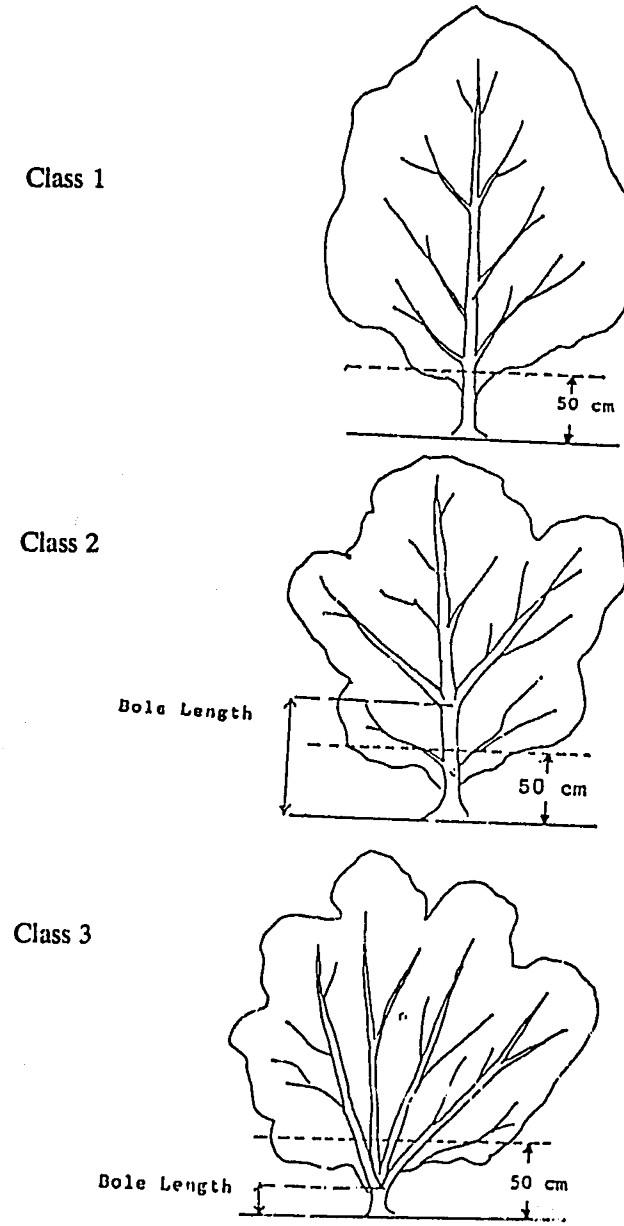


Figure 5. Tree form classes

Summary Checklist of Operations

Before neem flowering

- Communicate with the F/FRED Network Secretariat or FAO Forest Resources Development Branch, Rome if you would like to participate in seed exchange. Remember that a request for seed implies a willingness and commitment to export seed in exchange.
- Study regulations regarding import and export of seed to and from your country.
- Find budget to plant and maintain the neem nursery and field trial
- Identify different neem provenances for collection on the basis of climate, latitude, elevation, etc. Describe the provenance location and characteristics.
- Identify at least 25 neem trees in each provenance, mark them permanently, and identify their location.
- Begin to monitor the phenology of the neem trees and consider participating in the range-wide phenology study.

At neem harvest

- Harvest only fully mature green fruit or green-yellow fruit. Yellow fruit still on the tree is acceptable but not preferred. Fallen fruit should **not** be harvested.
- Depulp the fruit as soon as possible. Each day of delay can reduce long-term seed viability.
- Dry the seed in the shade for two days, then in the sun for at least two more. Use a fan to speed drying. Check the moisture content of the seed. If it is 20% or above, continue drying the seed. If a seed dryer is available, seed should be dried to 5 - 8% moisture content.
- Medium moisture seed (8 - 20% moisture content) should be stored and shipped in cloth, fiber, paper or cardboard containers to allow seed respiration. Low moisture seed (less than 8% moisture) should be stored in airtight plastic bags.
- Obtain phytosanitary certificates and ship the seed as soon as it is dried.

Trial planting and maintenance

- Prepare the nursery well ahead of time.
- Plant seed lots in the nursery as soon as you receive them
- Transplant seedlings in the field at the optimum time. The provenances do not all need to be planted in the field together if they were received at significantly different times (e.g. over the course of an entire year).
- Record weather data and summarize on a monthly basis.
- Make periodic field measurements as detailed in the guidelines

Concluding Comments.

Members of the working group who prepared the first draft of these guidelines made the following recommendations:

- Cooperators should meet next year to finalize experimental design.
- Site visits will be useful for final selection/layout of sites and monitoring progress.
- Variation in experimental design should not be allowed for network purposes. However, additional experiments for national purposes may be conducted.
- Trials should continue a minimum of 5 years. After that they can be used for national purposes.
- Adequate seed must be collected to compensate for the possibility of low seed viability, even if it means collecting from many trees and bulking the seed

Attachment 1

Neem Provenances

Country	Ecoclimatic Zones	Number of Provenances Identified	Number of Provenances for Inter.Trials	Core Groups
Pakistan	3		5	1
India	8	60	8	3
Sri Lanka	2		3	
Nepal	1		1	
Bangladesh	1		1	1
Myanmar	4		4	2
Thailand	4		12	2
Laos	2		2	
Cambodia	2		2	1
Vietnam	2		2	
Indonesia	1		1	
Malaysia	1		1	
Africa (including Senegal, Burkina, Nigeria, Sudan, Tanzania, Mali, Niger, and Chad)			8	
Total			**50**	**10**

Attachment 2

Provenance Description

Provenance Name: Provenance Code:

Species (botanical): *Azadirachta indica var. siamensis Val.*
Azadirachta indica A. Juss.

Species (vernacular): *Neem, Nim, Nimba, Limbo, Margosa, Mai Sadao, Mai Saliam,*

Local Uses:

1. Original Provenance

   1.1 Location

   Ref.:Maps for 1) country location, 2) for reaching area, and 3) details of area 1:10,000 - 1:50,000 to be attached.

   Latitude:................N/S Longitude:.........E/W..................Altitude................

   Country:................Province:.....................State:...............

   Administrative unit:......................

   Detailed description fro reaching of location:

   The total area is estimated at.............hectares.

   1.2 Origin

   1.3 Site description: Topographical aspect, soil.

   The rainfall is...........mm per year, with a pronounced dry season during the months of................

   A detailed description of the climatic conditions is attached as Annex B. The nearest weather station is at...............................

   1.4 Stand description

   1.5 Boundaries, demarcation, isolation

2. Extension of Provenance

3. Seed Collection

   3.1 Flowering period-Seed ripening period
   3.2 Best seed collection period
   3.3 Seed production
   3.4 Accessibility
   3.5 Labor availability
   3.6 Contacts for access to area

4. Conservation Needs

   Description on...................................by...

Attachment 3

Tag Description

One tag should be placed inside the sack and one tied outside. It should be made of tear resistant cardboard. A sample is shown below:

Provenance Name: ______________________
Provenance Code: ______________________
Collector's Lot Number: ______________________
Tree Number: ______________________
Grams of Fruits: ______________________
Number of Fruits: ______________________
Collection Date(s): ______________________
Collector's Name: ______________________

Attachment 4

Neem Fruit Collection Data Form

Provenance Name:

Seed Lot No.for Bulk:

Collectors Name:

Provenance Code:

Dates of Collection:

Collector Number	Tree No. In Provenance	Height	Girth Diameter	Quantity Collected (estimated)	Observation
Total					

Area Representation: Are trees spread well over the whole area?
Average spacing of trees collected from:

Processing: Dates for depulping:
Dates for drying:
Date received in seed center:

Attachment 5

Network Cooperators

Region	Countries	Site	Rainfall (mm)	Institute	Responsible Researcher
Asia and Pacific	Bangladesh	Rajshahi	1200	BARI, Joydebpur	Ahmed
	India	Pune Jodhpur Jabalpur Banglore Coimbatore	400-500 300-400 1000-1200 1000-1100 800-1000	BAIF ICFRE/AFRI ICFRE/TFRI ICFRE/IWS ICFRE/FGTB /TNAU	Hegde Dwivedi Gupta Srinivasan Subrahmaniam /Surendran
	Indonesia	East Java	400-1000	FRDC, Bogor	Syamsuwida
	Malaysia	Trengganu	1800	FRIM	Zakaria
	Myanmar	Thazi	1100	FRI, Yezin	Ko Ko Gyi
	Pakistan	PFI, Peshawar	350	PFI	Khan
	Nepal	Hetauda	1800-2000	IOF/TU	Karki
	Sri Lanka	Maha Illuppallama Kundasala	600-1000 1000-1200	FOA MPTS Group	Gunasena Gunasena
	Thailand	Lad Krating Kanchanaburi Kampaengpet	1200 1000 800	KUFF/TPC/ NRCT RFD RFD	Bhumibhamon Boontawee/ Kanchana Boontawee/ Wasuwanich
Africa	Burkina Faso	Kaya Ouagadougou	500 800	IRBET IRBET/CNSF	Diallo Diallo/ Ouedraogo
	Chad	N'Dyanea	600	DFPE/MTE	Tal Moulnang

Region	Countries	Site	Rainfall (mm)	Institute	Responsible Researcher
	Mali	Sotuba	900	IER	Sidibe
	Niger	Niamey	600	DE/INRAN	Habi/Souley
	Nigeria	Iseyin Kaduna	800 500	FRIN	Olapade
	Senegal	Bambey Nioro	400 800	ISRA ISRA	Gaye Gaye
	Sudan	1 site		FRC NTSP	M. Hassan A. Elsheik
	Tanzania	1 site		NTSC/ TAFORI	A. Kalaghe
Latin America	Nicaragua	1 site		TSP	Project Manager

Attachment 6

Sample Trial Site Description Sheet

Part I - General Information

Trial Site Name: Lad Krating Plantation Site ID: LKT-01

Elevation: 150 m

Aspect: 20 degrees N

Latitude: 13 degrees 30 minutes N

Longitude: 101 degrees 32 minutes E

Trial Site Topography:

Slope Gradient: 3%

Slope Position: Middle

General Topography:

Slope Gradient: 3%

Length of Slope: 100 m to 500 m

Existing Vegetation:

Plantation of *Melia azedarach* was burned previously and poor growing stock remains.

Method of Clearing:

Cut down big tree with chainsaw, all stumps were removed with bulldozer, land was cleared afterward.

Residue:
Removed

Percentage of surface free of residues after preparation: 40%

Method of Soil Cultivation:

Plowing with tractor was done 3 times prior to the planting, to a depth of 50 cm

Attachment 6 (cont.)

Part II - Climate

Climate Station Name: Sanam Chai Khet   Climate Station ID: 423006

Years of Records: 79

Latitude: 13 degrees 30 minutes N

Longitude: 101 degrees 32 minutes E

Elevation: 150 m

Distance of Experiment Site: 5.0 km

Monthly Climate Data:

Month	Maximum Temp (C)	Minimum Temp (C)	Precipitation (mm)
January	32.1	21.8	143
February	33.1	22.3	188
March	33.3	22.6	229
April	33.4	22.2	214
May	33.5	22.5	215
June	33.1	22.1	127
July	32.5	21.6	138
August	32.9	21.6	88
September	32.7	21.4	150
October	32.5	22.1	198
November	32.4	22.1	277
December	32.2	21.8	174
Mean	32.8	22.0	**Total:** 2141

Supplemental Studies

Section 5

Gaps in the Knowledge: Supplemental Studies Needed To Support International Provenance Trials

M.D. Read

Winrock International - Forestry/Fuelwood Research and Development Project, Bangkok, Thailand

Genetic improvement of neem has been cited by a number of authors and international agencies as an activity which should receive attention (Lauridsen *et al.*, 1991; National Academy of Science, 1992; Tewari, 1992). To date, however, there has been no successful international effort to collect, exchange and evaluate germplasm from various sources. This consultation offers the hope that such an activity may at last come to fruition. There are obstacles, however. This paper will attempt to discuss several of those obstacles in detail.

The FAO recently commissioned four country studies and seed collections of neem in Asia (O. Souvannavong, personal communication). Supplemental studies on key issues can enhance the usefulness of seed collections and improve the efficiency of resources devoted to neem improvement in the future. This paper does not pretend to present an exhaustive discussion of the topics which should be investigated. Instead, it will focus on three research themes which are considered critical to the success of neem provenance collection and exchange. These three themes are seed viability and storage, botany, and provenance delineation.

Seed Viability and Storage

Whether neem seed is orthodox or recalcitrant is moot. The fact is that it has proven to be an extremely difficult seed to store for long periods of time. The conventional wisdom is that neem should be planted soon after seed harvest and that the viability of seed declines rapidly with storage. Most authors report little or no germination 2-6 months after harvest (National Academy of Science, 1992; Tewari, 1992). Obviously, such a situation is not conducive to a systematic evaluation of seed collected across neem's naturalized range. Time of fruiting varies considerably, and so assembling and shipping seed requires additional time. It would be most desirable if seed could be collected one year, dispatched and assembled into provenance trials later that year, then planted the next. However, seed viability would have to be maintained longer than several months for this to be possible.

Ponnuswamy *et al.* (1991) recommend storing seed in earthen pots buried in moist sand. They recorded germination of 62% after 3 months storage (Table 1). This study compared seed stored at high moisture (no appreciable loss of seed moisture content), vs. seed stored at ambient conditions (average temperature of 33.8 ° C). The relative humidity inside pots stored in moist earth was close to 100%, while humidity under ambient conditions was not noted in the paper. It was not dry, however, because at the end of 3 months storage under ambient conditions the seed still had 15.5% moisture. The authors attributed low germination following storage under ambient conditions to rapid dehydration.

Table 1. Effect of wet storage on seed germination in neem.

	Seed moisture content (%)				Seed germination (%)			
Months after storage	0	1	2	3	0	1	2	3
Earthern pot	30.8	30.5	30.4	30.1	90.0	86.0	78.0	62.0
Ambient	30.8	22.6	17.4	15.5	90.0	74.0	26.0	8.0

	SEd	LSD (0.05)	SEd	LSD (0.05)
Method	0.05	0.10	0.52	1.11
Time	0.07	0.15	0.73	1.58
M * T	0.10	0.21	1.04	2.23

(Figures in parentheses are arc-sin transformations)
Source: Adapted from Ponnuswamy *et al.*, 1991.

Several researchers have reported that ingestion of seeds by wild animals increased germination percentage (Surendran, personal communication; Laurie, 1939; Lieberman *et al.*, 1979). The practical significance of this finding is limited, but the results are consistent.

Chaisurisri *et al.* (1986) investigated the effects of three drying treatments, two storage temperatures and two types of storage containers on seed viability. For each drying method, a number of drying times (durations) were studied. Treatments are summarized in Table 2.

Table 2. Drying treatments, storage temperatures, and storage containers investigated in the study on neem seed storage by Chaisurisri *et al.*, 1986.

Drying treatment	
D	Dried 25°C in a dehumidified air-conditioned room for 4 hours, 1, 2 or 11 days.
A	Dried in the laboratory under ambient conditions for 1, 2, 3 or 7 days
S	Dried in sunlight in a glass house for 1, 2, 3 or 7 days.
Storage conditions	
T	Stored under ambient conditions, 15°C or 2°C.
Container	
C	Stored in double sealed polyethylene bags or in cotton sacks

The conclusions drawn from this study were:

- None of the treatments maintained high germination beyond 12 weeks and almost all seed was dead after 24 weeks of storage.
- The most effective drying treatment was sun drying in a glass house. This resulted in a steady reduction in moisture content at a faster rate than the other two drying conditions. Drying for two days is recommended as this resulted in high initial germination, which was maintained in storage for up to 12 weeks with minimal loss in viability.
- Nuts stored in cotton gunny sacks at 15° C maintained better germinative quality than those in airtight containers, in any of the three storage conditions, or either container at 2° C, or ambient storage temperatures.
- Nuts in ventilated storage continued to lose moisture content. Storage for 4-6 weeks subsequent to drying enhanced germination regardless of drying conditions.

Although the study was comprehensive, even the most intense drying treatment (sunlight in the glass house for 7 days) only reduced moisture to 35%. None of the treatments investigated the effect of low seed moisture and cool temperatures on seed viability.

Troup (1981) reported that seed stored better in gunny bags than in sealed tins. Moisture content of the seed is not reported.

Maithani *et al.* (1989) studied storage of neem seed in a 3 x 4 factorial experiment with 3 temperatures (room temperature, 15°C and 5°C), and four containers (sealed polyethylene bag, perforated polyethylene bag, perforated cardboard box, and over silica gel in a desiccator). The following conclusions were drawn:

- Seeds stored in sealed poly bags exhibited rapid loss of viability at all temperatures. High temperatures were the worst; there was no viability after 15 days. Seed retained viability for 2 months when stored at 15°C and 1.5 months when stored at 5°C.
- Perforated poly bags were better than non-perforated ones, and 15°C was better than 5°C. Seed was still viable after 6 months (12% viability compared to an initial viability of 58% at the beginning of the experiment).
- Seed stored in perforated cardboard boxes lost viability after 3.5 months and 6 months when stored at 5° and 15°C, respectively. Room temperature was the best, but was not significantly different from 15°C.
- Seed stored in the desiccator over silica gel was viable for only 3.5 or 4 months at 5°C or 15°C. When stored at room temperature, it lost viability after 1.5 months.

In this study, as in many others, seed deterioriated much faster when stored in an airtight plastic bag than in a ventilated container. The authors attributed this to the fact that seed with relatively high moisture content requires oxygen for respiration. It was noted that seed stored at the coolest temperature lost germination earlier than that at higher temperatures. The authors speculate that this could be due to chilling damage. The initial moisture of the seed in this study was 37%, but no data on final seed moisture contents were reported.

Troup (1981) cites a study conducted at Dehra Dun in 1939 which found that seed stored better in gunny bags than in sealed tins, but it could still not be stored longer than 10 weeks. The moisture content of the seed was not given.

Oo (1987) studied neem in Myanmar and found that the average life of neem seed, provided it is initially sound, thoroughly mature and of high viability, is largely dependent on environment. The principal factors influencing the viability of such seeds in storage were found to be moisture content of the seed and temperature. She found significant differences in the medium term viability of fungus-free seed and randomly selected seed, and recommended discarding seeds with fungal hyphae. Even selected seed lost viability after 50 days, however. Seed was harvested, depulped and dried under a ceiling fan for five days, then spread on a bamboo mat and kept in the laboratory at room temperature. The moisture content of the seed was not reported.

A few studies suggest that neem can be stored for much longer periods of time. Roederer and Bellefontaine (1989) report that seedlots collected from "various sources" were stored for over five years at CTFT and still germinated. In one seedlot from Cameroon, a germination percentage of 42% was recorded for seed which had been stored for 64 months. The seed was stored in a cold room at 5°C and 30% moisture. The moisture content of the kernels ranged from 5-8%, while that of the endocarp was 11%. Drying method was not reported in this study.

Tompsett *et al.* (1991) report that neem seed lots were stored for 10 years and still remained viable. Germination rates of up to 70% were recorded. They achieved this by harvesting yellow-green fruits, drying the stones in the shade for 2-4 days, desiccating the stones to 8% moisture content or lower in a drying room at 15°C and 15% relative humidity or in a fan ventilated oven at 40°C, and storing at -20°C and low relative humidity. Desiccation of stones to 4-5% moisture content without severe damage was possible, indicating the seed batches tested were orthodox and not recalcitrant.

Maithani *et al.* (1989) studied the effect of time of harvest on seed viability and concluded that seed should be harvested 10-12 weeks after flowering, when some of the fruits turn yellow and start falling on the ground. This observation is supported by other researchers (Roederer and Beliefonatine, 1989). Souvannavong (1992) recommends harvesting when fruit is yellow-green and still on the tree.

Chany and Knudson (1988) recommend removal of the endocarp for optimum germination of neem seed. Fagonne (1983) and Thompsett *et al* (1991) also found that this enhances germination. Fagonne recommends soaking the kernels in water overnight before sowing directly in damp soil. If it is too time consuming to remove the endocarp, seed can be soaked in water for 3 days instead.

To summarize the results of seed viability studies, the optimum time for harvest is when the seed is just ripening. Seed should be plucked from the tree at this time and thoroughly cleaned. Most authors report that neem retains its viability only a few months (maximum 6), but there are several intriguing studies which suggest that long term storage is possible. Few researchers have studied the effect of seed moisture on long term viability, and fewer still have tried rapidly drying it to a low moisture content, then storing under cool temperatures. Perhaps this is because the seed is presumed recalcitrant. In any case, more research is needed.

Botany and Taxonomy

Lauridsen (1991) reports there are two varieties of neem in Thailand - *Azadirachta indica,* known locally as Indian neem or quinine neem, and *A. indica* var. *siamensis.* The authors of a recent booklet on neem (National Academy of Sciences, 1992) consider var. *siamensis* to be a separate species but do not say why. Lauridsen *et al.* (1991) report that *indica* neem is found primarily in southern Thailand but that it overlaps with *siamensis* near Prachuab Khiri Khan on the peninsula. The two varieties or species hybridize there and give progeny of intermediate characteristics. He states that, north of that location, no hybridization occurs because of differences in phenology between the two varieties. For this reason, Thai neem will be referred to as a variant only and not a separate species in this paper. However, more work on the botany of these two species or sub-species is needed.

Hegde (personal communication) reports that *A. indica* var. *siamensis* growing at Poona (India) has leaves which are less bitter and darker green color than the local neem. He maintains the *siamensis* grows faster than Indian neem in that area. Lauridsen (1991) notes that the Indian neem is particularly valued for its medicinal uses in Thailand and that the two species of *Azadirachta* can be differentiated based on differences in pollen morphology and isozyme patterns. Oo (1987) observed in

Myanmar that local people differentiate edible (*Sartamar*) and non-edible (*Tamarkha*) neem trees on the basis of bark, slash, leaf, smell and other characteristics. It is probable that the sartamar and tamarkha neem of Myanmar are equivalent to the quinine neem and *siamensis* neem of Thailand, the former being *Azadirachta indica* and the latter *Azadirachta indica* var. *siamensis*.

Oo investigated taxonomy, wood anatomy and chromosome number in edible and non-edible neem and found them to be identical in these respects. The chromosome number was 14 in both cases and there was no difference in azadirachtin content between the two.

Willan *et al.* (1990) report that *Azadirachta indica* var. *siamensis* has a wide natural distribution in Thailand at altitudes below 200 meters. It extends south on the peninsula to the ninth parallel north. His observations regarding growth are in agreement with those of Hegde cited above; namely that *var. siamensis* is more robust than the *A. indica* found in India.

A. excelsa (A. integrifolia) is a relative of neem which is found in Thailand and other countries of southeast Asia. It is more distinct and less likely to hybridize with *A. indica*. This species contains azadirachtin but in lesser quantities than neem. It also contains other chemical constituents which may prove useful in controlling insect pests (Mungkorndin, 1993).

Provenance Delineation.

The above discussion of *Azadirachta indica* and *A. siamensis* emphasizes the need to define neem provenances on the basis of biophysical and genetic determinants. MacQueen (1992) discusses the differentiation of *Calliandra calothyrsus* provenances on the basis of such criteria and recommends a series of nine steps which may be used as a framework for the pragmatic delineation of provenances in other species. A brief review of these nine steps reveals additional "gaps in the knowledge" which need to be investigated prior to, or in conjunction with, seed collection and exchange.

- Make an accurate map of the distribution of the species. This begins with individual country maps. Regional maps are created from the country maps.
- Draw in any key topographic features which might impede gene flow (for example, mountains).
- Add other geographical features which may impede gene flow. For example, in some cases, political boundaries may restrict collection efforts.
- Map the major soil orders. Soil characteristics may serve to delimit provenances if they exert significant selection pressure.
- Delineate the major vegetation types. The vegetation of an area reflects climate, soil, and land use.
- Map the gradients in annual precipitation.

- Differentiate between areas with similar annual rainfall. but significantly different distribution patterns.
- Identify areas which will be easier to collect seed from and which are most likely to be able to furnish the required quantities of seed.
- Keep the number of provenances to a manageable number. Between 10 and 20 is recommended.

Only after these steps have been followed can a logical decision be taken regarding the number of provenances to collect for an international provenance trial to include the greatest range of neem biotypes.

A study of neem phenology throughout its naturalized range should be undertaken to complement the above steps and ensure that collections are made at the optimum time. This could be accomplished by having strategically located cooperators fill in a standardized data sheet at regular intervals and send the data to a central location for processing. We also need to collect information on where neem occurs naturally and where it has been introduced relatively recently as a roadside tree, in villages, or on farmers' fields.

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Priority Topics for Supplemental Studies on Neem Improvement

Report of Working Group Two

The primary justification for holding the First International Consultation on Neem Improvement was to arrange for seed exchange among participating countries and to plan a set of international provenance trials. It is difficult to establish provenance trials of uniform age because of a number of problems pertaining to seed handling and germination which need further investigation. Research is also needed on population genetics, reproductive physiology, pathology, entomology, and vegetative propagation to complement the provenance trials.

Working Group Two was asked to define research priorities for supplemental studies on neem. The studies were meant to complement the provenance trials by providing additional data to facilitate seed collection and evaluation and to ensure that the widest range of available biotypes was collected. Discussion was restricted to topics in the biological and physical sciences. This was not because socioeconomic studies are not needed. It is, rather, a reflection of the focus on genetic improvement of neem.

The results of discussions are presented as a list of priority research topics followed by a number of complementary studies which could also be addressed. No funding for the research was committed during the meeting.

Priority Studies

Taxonomy and Population Genetics

Taxonomy

- Evaluate genetic diversity through herbarium samples and determine zones of introgression.
- Sample, identify and categorize trees using chemio-taxonomy techniques.
- Look into the possibility of using genetic markers for identification of species.

Population genetics

- Study diversity of the species (*A. indica*).
- Evaluate the relative importance of within and between population variations.
- Assess movement of seed and the genetic origin of man-made populations.
- Examine isoenzymes and chloroplast DNA.

Phenology and Reproductive Biology (2-3 years)

Phenology:

- Evaluate time of flowering and fruiting as well as variation over years with simple environmental parameters.
- Define the stages to be observed and determine periodicity of the observations required.

Reproductive biology

- Study factors affecting seed production, record variation of this production and examine the mechanisms behind organization of genetic diversity of neem;
- Identify subsets of populations through quantification of flowering and fruiting, study of the effect of environmental parameters on production, flower anatomy; pollen morphology, ovule and pollen development and viability.
- Collect seed on a single tree progeny basis in order to estimate out-crossing levels using isoenzyme techniques.
- Carry out controlled crosses — inter-taxa for taxonomic purposes and intra-taxa for better knowledge of the breeding system.

Loss of genetic diversity during seed storage: (24 months)

- Determine how storage conditions affect genetic diversity between and within progenies.
- Evaluate the effect of alternative storage containers, storage temperature, moisture content and relative humidity.
- Investigate seed morphological development.

Vegetative propagation techniques: (24 months)

- Carry out trials to standardize techniques.
- Evaluate the relative effectiveness of stem cutting, grafting, air-layering and tissue culture.
- Confirm general applicability of methods developed in Thailand.

Seed Physiology and Vegetative Propagation

Seed viability: (6-8 months)

- Examine the effect of drying conditions on seed viability.
- Examine the effect of temperature, seed moisture content, relative humidity and speed of drying on seed moisture content and viability.
- Establish standardized procedures for seed storage.

Effect of depulping time and endocarp removal on germination

- Evaluate the effect of post-harvest fruit treatment on seed viability.
- Determine germination rates with and without endocarp and the influence of time of depulping.

Seed pest and diseases: (2-24 m)

- Identify the general types of seed pests and diseases with special attention to seed borne diseases.
- Develop a protocol for seed handling to minimize disease problems (some work is being carried out in India)

Complementary Studies

Socioeconomic Aspects and Extension Work

- Examine alternative uses of neem.
- Evaluate domestic and foreign market potential.
- Establish criteria for selection and improvement.

Physiological Studies in Relation to Stress

- Evaluate production of chemicals under different stress conditions
- Evaluate biomass production under different conditions.

Silviculture and Tree Management

- Evaluate alternative nursery management procedures
- Evaluate alternative plantation management schemes

General Remarks

Institutional arrangements must be made to carry out supplemental studies. Specialized topics may be handled by the ASEAN-Canada Tree Seed Center, CIRAD-Forêt and other international and regional organizations.

Training will be needed in special areas to strengthen capabilities of persons working with neem. Finally, additional funding must be organized to carry out the planned supplemental research.

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