PROVENANCE EVALUATION IN THE SEEDLING CHARACTERS OF NEEM (*AZADIRACHTA INDICA* A. JUSS)

By
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THESIS

Submitted in partial fulfilment of the
requirement for the degree

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DECLARATION

*I hereby declare that this thesis entitled "**Provenance evaluation in the seedling characters of Neem (Azadirachta indica A. Juss.)**" is a bonafide record of research work done by me during the course of research and that this thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title of any other University or Society.*

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We, the undersigned members of the Advisory Committee of Sri. Vinod, S. a candidate for the Degree of Master of Science in Forestry, agree that this thesis entitled "Provenance evaluation in the seedling characters of Neem (Azadirachta indica A. Juss.)" may be submitted by Sri. VINOD, S. in partial fulfilment of the requirement for the Degree.

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Introduction

INTRODUCTION

Neem (*Azadirachta indica* A. Juss) is a fast growing tree of the tropical arid zones of Asia. It belongs to be family Meliaceae and is synonymously called as *Melia azadirachta* L., *Melia indica* (A. Juss) BRANDIS and *Antelaea azadirachta* (L.) ADELB. The scientific name *Azadirachta indica* is in fact derived from the Persian name *Azad - Darakth* or the free tree (Vijayalakshmi *et al.*, 1995).

It is believed that neem originated in Asia and Burma. However, the exact centre of origin is uncertain. The neem tree is widely distributed in most parts of the Indian sub continent in the tropical and subtropical, semi-arid to wet tropical regions. In addition to this it is also widely distributed in other parts of the world viz., Somalia, Nigeria, Mauritiana, Togo, Fiji, Trinidad, West Indies, Puerto Rico, Florida and Southern California.

Neem has got tremendous importance economically and medicinally. The versatility of neem tree is reviewed by Kaul *et al.* (1990). In the field of agriculture neem cakes and neem leaves can be used as a potential fertilizer. It is estimated that 100 kg neem seed cake contains 3.56 kg N, 0.83 kg P and 1.67 kg K. It also contains 0.77 kg Ca and 0.75 kg Mg. Most importantly, neem is a potent insecticide, effective against about 200 insects, including locusts, brown plant hoppers, nematodes, mosquito larvae, colorado beetles and boll weevils. The active ingredients in neem

with medicinal/insecticidal properties are azadirachtin, salanin and malandriol. The tree has been held in high esteem because of its medicinal value in rural areas and has been used in curing many ailments that it has been called "the village pharmacy". The extract from neem was found to cure eczema, ringworm and scabies. Leaf extract has a marked antipyretic effect. Nimbidinic acid, a seed extract is a potent diuretic. Neem seed oil was useful in relieving tissue oedema in congestive heart failure. Extensive work has been done on the antifertility effects of neem oil, sodium nimbidine and neem-leaf extracts. The contraceptive effects of neem oil have been well established. According to Varahamihira's *Brihat Samhita*, neem is one of the trees which should be planted near every one's house (Shiva, 1995). Neem, thus is a species of paramount importance in different fields and can be rightly called as a multipurpose tree species.

Provenance tests are done to screen the naturally available genetic variation within a species and to choose the best available type for reforestation or for future breeding programme. Looking closely at the provenance data will also help to select the best tree or trees of the specific provenance. Additional gain can be achieved by using planting materials from the selected provenances. But in the case of neem no serious attempt have so far been made to exploit the provenance variability in this part of the country.

So a study was conducted with the objective of evaluating the provenance variation for the seed and seedling characters of neem from different regions of peninsular India comprising of Kerala, Tamil Nadu and Karnataka states and thus to identify the most suitable seed source for the eco-climatic condition prevailing in this part of the country.

Review of Literature

REVIEW OF LITERATURE

The term 'provenance' is a synonym for 'origin' or geographic race (Rao, 1992), which usually denotes the original geographic area from where seeds or other propagules have been obtained. The word is being used by tree breeders to mean "ultimate natural origin" (Tewari, 1994). Nevertheless, the term has been variously defined in the literature, Some of the definition of provenance are:

(i) a sub-division of a species consisting of genetically similar individuals, related by common descent and occupying a particular territory to which it has become adapted through natural selection (Wakely, 1959), (ii) the geographical source or place of origin from which a given lot of seeds or plants was collected, the material from such a source or origin , often restricted to imply material from a specified race (Empire Forestry Association, 1953). (iii) the original geographic source of a lot of seed (or pollen) (Wright, 1962). (iv) the geographic area and environment to which the parent trees, etc. are native and within which their genetic constitution has been developed through natural selection, (The Society of American Foresters, 1971) and (v) it is the area on which any stand of tree is growing, the stand may be indigenous or non-indigenous (OECD, 1971).

2.1 Provenance testing and its significance

Success in the establishment and productivity of forest tree plantations is governed largely by the species used and the source of seed within

species (Lacaze, 1978; Kumaravelu *et al.* 1995). No matter how sophisticated the breeding techniques, the largest, cheapest and fastest gains in most forest tree improvement programmes will accure if use of suitable species and seed sources within species is assured (Zobel and Talbert, 1984). Provenance research therefore is having paramount importance.

Provenance test is an experiment in which seeds are collected from a number of widely scattered stands (usually natural) and the seedlings are grown under similar conditions (Wright, 1976). Provenance testing has two main objectives - (i) to select the best performing and adapted seed source/provenance for further use in establishing large scale plantations (Kapur and Dogra, 1987; Tewari, 1994) and (ii) for use as the best adapted and most productive base population for use in selection and further genetic improvement work (Tewari, 1994).

In order to provide a sound choice of species and provenance for planting, extensive systematic exploration and testing are required (Burley, 1980; Palmberg, 1981; Turnbull, 1983). According to Nanson (1972) the first logical step in the breeding programme of any forest tree species is provenance testing. It can be said that the determination of the species or the geographic sources within a species that should be used in a given area is the first step in any tree improvement programme. Forest tree improvement programmes starts with the study of available variations in the entire range of species distribution and delimitation of population capable of providing the best trees. This is done by provenance testing (Suri, 1984). Knowledge

of variability within a species is a pre requisite for developing effective tree - improvement/breeding strategies (Vakshasya, *et al.*, 1992). The significance of genetic variation studies and provenance testing in forest tree improvement is very well realised by different authors like Pryor (1963); Callaham (1964) and Wright (1976).

Provenance test is a good way to start an improvement programme. It is considered to be an essential part of the genetic improvement work. Tree improvement in the sense of species provenance selection and breeding can contribute directly to increased net economic yield through increasing average growth rate (Gupta *et al.*, 1992).

Genetic differences associated with place of origin have often been several times as great as those among individual trees in the same stand. That is why the provenance testing is done. But the thumb rule still existing in forestry is to use local seed - source or provenance until some others prove their superiority than the local source by appropriate testing (Tewari, 1994). The importance of seed source is emphasised by Zobel and Talbert (1984). They are of the opinion that the best information available in the tree improvement field relates to seed sources. The most successful tree improvement programmes are those in which the proper seed sources and provenances are used. The losses from using the wrong source can be great and even disastrous.

Provenance tests in native species are peremptory to screen the naturally available genetic variation for higher productivity (Khosla, 1981; Shivkumar and Banerjee, 1986; Burley, 1987; Balkrishan and Toky, 1995a; Kumar and Toky, 1996) for use in agroforestry (Wood and Burley 1991; Balkrishan and Toky, 1995a) for reforestation (Shivkumar and Banerjee, 1986) and for future breeding strategies (Burley and Wood, 1976; Shivkumar and Banerjee, 1986; Balkrishan and Toky, 1995a; Kumar and Toky, 1996). According to Wright (1976) and Tewari (1994) provenance test is imperative when dealing with an exotic species.

Selection of the best provenance of the best species for a given site or region is necessary for achieving maximum productivity both in plantation forestry and with agroforestry systems (Subramanian *et al.*, 1992). In a provenance trial it is possible to measure many characteristics of nursery traits and subsequently in field - survival, growth pattern, morphology, chemistry and anatomy of trees (Tewari, 1994). Now in the present tree breeding scenario the concept of provenance testing has been well established and demonstrated through nationally or internationally conducted co-ordinated provenance trials.

Substantiating the need for provenance testing Vivekanandan (1975) observed that with large scale planting of various species it is imperative that seeds of genetically improved quality and right provenance be used to raise plantation to produce timber of desired quality in the shortest possible time with minimum input of money and manpower.

2.2 General procedure of Provenance Testing

Zobel and Talbert (1984) has suggested two approaches for doing provenance testing. They are (i) range-wide tests and (ii) limited range tests.

In Range wide test, for a species with a comparatively small range, test trees are selected from 20 to 30 localities, and for species with larger range test trees are selected from 50 to 200 localities.

Limited range tests are often used as follow-ups to range-wide tests to sample intensively the region(s) proved to give the best seed in general.

Different experimental designs are adopted in provenance testing, which include randomised complete block design, incomplete block design, lattice design, fully randomised design, non orthogonal block design, latin squares, family block design and systematic design.

Assessment of provenance trial is a lengthy and expensive process. So only practically important traits with substantial experimental variation are taken for assessment. Assessment in field are usually made on all trees.

Generally in a provenance test no effort is made to maintain separate identity for the offspring of individual trees within a stand. Wells and Switzer (1971) combined the function of provenance and progeny tests into one experiment. Such

combined progeny provenance tests have advantages of providing information about geographic trends and genetic differences among individual trees at the same time. But they are large and expensive. Wright (1978) proposed a simplified design for combined provenance - progeny testing. It is said that with this the combined test can be done as simply as an ordinary provenance.

2.3 Provenance tests on various species

So far provenance trials were undertaken in various tree species in different parts of the world. Species-wise reports of provenance trials are presented below under different heads.

2.3.1 Acacia

2.3.1.1 *Acacia auriculiformis*

A provenance trial was undertaken by Aini *et al.* (1994) using 28 provenances of this species and was assessed for survival and growth at age 12 months in Malaysia. In his study all provenances survived very well, but they differed very significantly in their growth performance. But Awang *et al.* (1994) when using 25 provenances in Thailand, found significant differences among the provenances at each site, as well as between sites and the interaction between provenance and sites. He has analysed the growth and survival for 18 months. Provenance trials were conducted on *A. auriculiformis* in Australia (Huang, 1989), in Jammu-Tawi (Dutt and Jamwal, 1991), in China (Yang and Zeng, 1991), and in Thailand (Luangviriyasaeng *et al.*, 1991).

2.3.1.2 *Acacia nilotica*

Accumulation of free-proline is a general characteristic in this species. A comparative study of different provenances for the free-proline content in leaves of *A. nilotica* was done by Bagchi and Singh (1994). It is concluded that provenance variation in free-proline content exists and it is not related to the geographical distribution.

Twenty one provenances of *A. nilotica* sp. *indica* collected from 11°N to 31°N latitude and 19 m to 650 m altitude in India were grown at Hisar by Balkrishan and Toky (1995a). There were significant variations in stem height, branch, leaf and spine growth, and also found that provenances from North-Western and central India were found to be superior than those from South India.

Balkrishan and Toky (1995b) reported that the carbohydrate, starch, chlorophyll and N, P, K, Ca and Mg contents in leaf varied significantly among 21 provenances of *A. nilotica* spp. *indica*. Nevertheless, the provenance variations were random, and did not show a significant relationship with the latitude of the original source.

Variability study was conducted by Ginwal *et al.* (1995) with seedlings of 32 provenances belonging to 5 sub species from 5 countries viz., India, Sudan, Pakistan, Yamen and Senegal under nursery conditions. The study could bringout the existence of significant genetic variation among seed sources for some growth characters.

Provenance studies are also conducted in *A. nilotica* in Kanpur (Shivkumar and Banerjee, 1986) and in Dehra Dun (Bagchi and Dobriyal, 1991; Bagchi and Dobriyal, 1992; Bagchi, 1994).

2.3.2 Albizia

Protein, oil, carbohydrate, and starch contents of seeds of 12 provenances of *A. lebbeck* collected from sites in North and South India, varied significantly (Kumar and Toky, 1994). Again, in another report of Kumar and Toky (1996) significant differences were found among the 12 provenance selected for the trial. The variations were random and did not show significant relationship with the latitude or longitude of seed source.

2.3.3 Casuarina

Seeds of eight provenances of *Casuarina* were sown in the nursery beds and the height and collar diameter of 25 seedlings of each provenances were recorded after six months. Significant difference among provenances was noticed (Toky and Bisht, 1991).

Mishra and Banerji (1995) gave an account of provenance trial with 19 provenances of *Casuarina* species collected from different countries and found that the overall performance of *C. cunninghamiana* and *C. equisetifolia* collected from Australia, Egypt and Isreal were found to be the best.

2.3.4 Dalbergia

The improvement of shisham (*Dalbergia sissoo)* has remained more or less stationary in India due to non availability of germplasm of divergent forms. A provenance study, of *D. sissoo* have been laidout at Kanpur using six provenances and all belonging to UP region only (Gupta *et al.*, 1992). Data showed marked differences among the provenances. In another study, tree height, crown spread, self pruning ability and age showed variation among provenances (Dhillon *et al.*, 1992).

Provenance trials in Dalbergia were conducted by Neil (1990); White *et al.* (1990); Singh and Danayak (1991); and Dhillon *et al.* (1995) also.

2.3.5 Eucalyptus

2.3.5.1 *Eucalyptus camaldulensis*

Growth and survival of 16 provenances were studied by Jha and Chhimwal (1993) and they showed significant differences. In another study by Chandra, *et al.* (1994), diameter and height growth exhibited considerable variation between provenances. The variation was generally associated with the site and climatic conditions.

Several other workers (Kapur and Dogra, 1987b; Abou-Gazia and El-Baha, 1989; Chaturvedi, *et al.*, 1990; Banerjee and Singh, 1991 have also reported provenance trial of *E. camaldulenis* in different climatic conditions.

2.3.5.2 *Eucalyptus tereticornis*

No significant difference was observed between provenances in a study undertaken in Punjab by Kapur and Dogra (1987). Similar results were also reported by Chaturvedi *et al.* (1985) and Banerjee and Singh (1991).

However, studies conducted by Kumaravelu, *et al.* (1995) in Tamil Nadu revealed significant differences among provenances in respect of the parameters evaluated. But at the same time, another study conducted in Tamil Nadu showed no statistical difference between provenances (Sundararaju *et al.*, 1995). They have used 13 provenances of *E. tereticornis* from Australia, Dehra Dun and local provenances.

Several other workers (Basu, *et al.*, 1989; Banerjee *et al.*, 1990; Otegbey, 1990b) have also reported provenance trials of *E. tereticornis* in different climatic regions.

2.3.5.3 Other species of Eucalyptus

Prominent variations were observed in a trial involving five species of *Eucalyptus* (Aradhya and Phillips, 1993). Reports are also there with *E. brassiana* (Jha, 1991 and Manathuragimath, *et al.*, 1991a); *E. grandis* (Subramanian *et al.*, 1992); *E. hybrid* (Kapur and Dagra, 1987b) and *E. microtheca* (Subramanian *et al.*, 1991).

2.3.6 Pines

Introduction trials on a systematic and regular basis were attempted in different parts of the country beginning from 1958 onwards, to identify the most promising species and their provenances for large scale plantation activity.

2.3.6.1 *Pinus caribaea*

Performance of this species was evaluated in relation to soil properties of each zone by Bari and Prasad (1989). In another species cum provenance study height and diameter were analysed by Dwivedi and Thapar (1990). Among species, *P. caribaea* was found to be the best performer.

Dutt and Jamwal (1995) gave an account of growth and performance of four provenances of this species in Jammu-Tawi. Provenance x Site interaction was estimated for tree height and girth at breast height at three Nigerian Savanna sites and there was no significant Provenance x Site interaction in any of the two growth characteristics examined (Otegbeye, 1995).

Results on seed germination, height growth, diameter growth and survival percentage were evaluated from a provenance trial at Orissa by Swain and Patnaik (1996). It was established that there is a wide variation in germination percentage among the provenances but no corresponding effect on survival percentage.

2.3.6.2 *Pinus oocarpa*

Performances of the different provenances have been reported by Vivekanandan (1977), Greaves (1980), Bari and Prasad (1989) and others.

A study conducted by Otegbeye (1990a) showed no significant difference among provenances. Similar results were also obtained by Zashimuddin, *et al.* (1991) in a trial conducted at Bangladesh. It was concluded that provenance trials with more seed sources may be established at various locations in Bangaldesh to select the better provenances of the species if available.

2.3.6.3 *Pinus patula*

The survival, growth, yield and wood basic density of three provenances of this species revealed no significant difference between provenances (Nasser *et al.*, 1993). But at the same time significant difference were reported by Nshubemuki *et al.* (1996) in a trial conducted at the same place where earlier work was done.

2.3.7 Prosopis

Prosopis cineraria an extremely important agroforestry tree species in the Thar Desert, showed significant variation in seed germination, seedling survival and growth among 31 provenances collected from India (Arya *et al.*, 1995). Variability studies were also undertaken by Bahadur and Hooda (1995) in Hissar and significant difference for all the characters have been reported.

2.3.8 Sesbania

A species-cum-provenance trial was conducted at Karnataka (Nadagoudar and Mutanal, 1994). In respect of germination, survival and height, *Sesbania formosa* was superior to *S. cannabina* among exotics and *S. grandiflora* to *S. rostrata* among indigenous species.

2.4 Neem - General Aspects

Neem is a fast growing (Hoamuangkaew *et al.*, 1990) deep rooted medium sized tree (Gupta, 1993; Ponnammal, 1993), attaining about 20 m height (Shiva, 1995), usually described as evergreen (but deciduous) except for periods of extreme drought (Anon, 1988; Gupta, 1993). It is believed that neem originated in Asia and Burma. However, the exact centre of origin is uncertain. Some believe it to be native to the whole Indian subcontinent whereas others are of the opinion that it belongs to dry forest areas throughout all of Burma, India, Indonesia, Malaysia, Pakistan, Sri Lanka, Thailand and arid regions of India and Africa (Gupta, 1993; Vijayalakshmi *et al.*, 1995).

Govindachari (1992) strongly believes that the neem tree is indigenous to South Asia. It is found in most part of Indian subcontinent in the tropical and subtropical, semi-arid to wet tropical regions. Neem is now widely distributed in many countries of the world by cultivation. Indian settlers have been responsible for its introduction to the African countries, where it is abundant in the tropical belt from Somalia in the East to Nigeria, Mauritiana, Tago, etc. in the west. It was introduced

first to Fiji islands and from there it has spread to other South Pacific islands. From Trinidad, it has spread to other islands in the West Indies and many countries in Central and South America. It is being cultivated in Puerto Rico, Florida and Southern California. Large scale plantations are there in Malaysia and the Philippines as a source of timber and fuel wood. A grove of 50,000 trees has been planted outside Mecca for affording shade to pilgrims (Ahmed *et al.*, 1989). It is also widely cultivated in Haiti (Lewis and Elvin-Lewis, 1983) and in Somalia (Kasmi, 1980).

2.4.1 Environmental needs

Usually it grows at an attitude of 50-1500 m and has a wide climatic adaptability.

2.4.1.1 Rainfall

According to Troup (1986) it is almost successful in a mean average annual rainfall of 450 mm to 1150 mm. But on the other hand it can also tolerate rainfall as low as 130 mm a year (Gupta, 1993). The relative humidity in its natural range of distribution in July varies from 60-90 per cent and in January 40-90 per cent.

2.4.1.2 Temperature

It can withstand high temperature and cannot survive frost. It can stand still at higher temperature up to 40°C and low temperatures near 0°C in some places; however, in its natural range of distribution the minimum varies from 0-15°C.

2.4.1.3 Soils

The tree grows well on moist soils as well as dry, stony, clay and shallow soils, but will not grow on seasonally waterlogged soils or on deep dry sands (Gupta, 1993; Vijayalakshmi, 1995). It grows in almost all kinds of soils but does well on black cotton soil (Anon, 1988). It does not grow well on saline soils but can persist on such soils where a few species do grow (Chaturvedi, 1955). It can adapt to and tolerate a wide range of conditions including shallow, nutrient deficient soils (TDRI, 1989). The optimum pH is 6.2 or above but it can survive pH of 5.0. According to Chaturvedi *et al.* (1985) it can grow fairly well in soils with a pH up to 10.

2.4.2 Neem-polemics on seed

Whether neem is a genuine recalcitrant or short lived orthodox species, however is still nebulous (Willan, 1985). On the basis of low moisture content of seeds (12.5%) from a Haiti plantation it has been argued that neem is not a recalcitrant species (Chaney and Knudson, 1988). Again since neem occurs in dry tropical forests while most recalcitrant tropical species are found in moist tropical forests, it was suggested by Willan (1985) that neem may have short lived orthodox seeds. However, recognizing several facts like advocacy by many for sowing the seeds within two or three weeks after collection (Kamweti, 1982), occurrence of high moisture content (37.2%) in fresh seeds and manifestation of chilling damage by the seeds (Maithani, *et al.*, 1989), the species is indicated to be a recalcitrant one. A study conducted by Venkatesh *et al.* (1990) showed the germination of fresh seed was 91 per cent, but this

value decreased to 50 per cent after 15 days storage and 10 per cent after four months storage. However, by collecting the fruits from the tree when they are greenish-yellow, depulping them, and drying the extracted seed under shade for two days (to a moisture content of 5 per cent), up to 80 per cent germination can be obtained upto four months (Nagaveni *et al.*, 1987). By about 6-7 months germination decreases to about 50 per cent and after this it is very low. In contrast, seeds stored in earthen pots buried in moist sand bed manifested little loss in seed moisture content and recorded a germinability of 62 per cent at the end of three months (Ponnuswamy, *et al.*, 1991).

2.4.3 Pests associated with neem

Though there is presence of azadirachtin, a strong antifeedant and growth retardant in leaves and seed kernel, a number of insect pests have been recorded on neem. According to Tewari (1992) the young neem plants are frequented by not less than five dozen insect species from all over the world. However, plantations of neem are by and large insect free due to its insect repellent nature.

Among defoliators *Phinoroptera gracilis, Holochlora albida, Neortharis acuticeps, Cryptocephalus* sp., *Myllocerus dorsatus, M. discolor, Holotrichia serrata, Adoretus bicolor, Laspereysia aurantiana, Boarmia* sp. *Cleora coronaria* and *Odites atompa* are very serious (Raghunath *et al.*, 1982; Onkarappa and Kumar, 1994).

Among sucking pests *Aphis gossypi, Pulvinaria maxima, Aonidiella orientalis, Ceroplastes pseudoceriferus, Cletus* sp., *Ferona* sp., *Oxyrhachis tarandus, Helopeltis antonii* and *Aspidiotus orientalis* are devastating (Onkarappa and Kumar, 1994).

In the category of bark/stem feeders order isoptera is prominent. In this *Odontotermes horni, O. wallonensis* and *O. obesus* are important (Ahmed,1992). Verma (1989) reported the incidence of *O. redemanni* and Delate and Grace (1995) reported *Captotermes formosanus.*

2.4.4 Diseases associated with neem

The pathogens recorded are *Ganoderma lucidum* causing root rot, *Corticium salmonicolor* causing stem and twig blight, *Cercospora subsessilis* causing leaf-spot, *Oidium* sp. causing powdery mildew and *Pseudomonas azadirachtae* causing leaf-spot blight (Gupta, 1993). In addition to this reports are also available that root rot is caused by *Fusarium solani* in the *A. indica* seedlings in Kerala and Dehra Dun, Shukla (1992); Sankaran *et al.*(1986). Furthermore, root rot of neem is a serious disease caused by *Ganoderma applanatum* as recorded for the first time from Meghalaya by Chakraborty and Kongner (1995). Leaf spot is also caused by another pathogen *Pseudocereospora subsessilis* (Castellani and Mohammed, 1984). An account is given of the foliar disease caused by *Rizoetonia solani* by Mehrotra (1990).

2.5 Provenance studies on neem

Realising the importance of the species an International Conference on Neem was held in Bangkok during January, 1993. A panal comprising of CIRAD - Forets (France), DANIDA Forest Seed Centre (DSFC, Denmark), Forestry/ Fuelwood

Research and Development Project (F/FRER, Bangkok) and FAO was formed to aid and co-ordinate the work on genetic improvement of neem. This panel initiated a full scale neem improvement programme, with 20 participating countries, by forming the "International Neem Network".

An ambitious study utilizing 27 provenances from 11 countries of Asia and Africa is being simultaneously conducted in 20 different countries of the world as a part of an International provenance study of neem (Mishra, 1995). This project is being globally co-ordinated by FAO. International Research Institutes like DSFC (Denmark) and CIRAD - Forets (France) are associated with the various aspects of this trial. AFRI, Jodhpur has been selected to be the National Focal Institution, for this long term project. Seeds received from the various provenance have been planted in nursery beds at Jodhpur. The project is proposed to be taken up at five location in India.

Some reports of provenance evaluation conducted in neem are already available. Jacobson (1981) reported that seeds from Indian origin contained only 1-3 per cent azadirachtin content, where as seeds of African origin averaged 5-6 per cent with few lots exceeding 9 per cent. Similar results were also obtained by Ermel, *et al.* (1986). Provenance variation in seed characters is also reported by Veerendra *et al.* (1996). They found seed characters such as length, width and 100 seed weight having considerable genetic variability.

Significant variation for various biometrical traits namely, height, basal diameter, root length, number of branches and leaves, leaf size and different components of dry weight among twenty eight one parent families of neem was reported by Kumaran (1991).

A provenance evaluation comprising of 39 provenances of neem was conducted at Jodhpur. The growth data at the age of one year indicated that the provenances from Indore, Ujjain, Kuthalia (M.P), Ravinagar (Maharashtra) and Rajkot (Gujarat) performed better at this stage (Tewari, 1994).

Ten provenances of neem were collected from Karnataka and Andhra Pradsh. Analysis of three characters viz., seed length, width and seed weight revealed very good amount of genetic variability among provenances (Veerendra, 1995).

Studies on fruiting in neem in the three year old provenance trial raised at AFRI, Jodhpur, was carried out during June, 1995. Fruiting in the third year with variations depending on different provenances was noticed by Gupta *et al.* (1995).

2.6 Isozyme analysis

The multiple forms of enzyme separable by electrophoretic procedures, occurring within the same organism and having similar or identical catalytic activities, are generally termed "isozymes or isoenzymes". The term "isozyme" was first introduced by Markert and Moller (1956). The technique of isozyme electrophoresis

was developed some 30 years ago, and has since been widely used to study genotypic variation in plants, including conifers and broad leaved trees (Forrest, 1994).

The assessment of genetic variability is key to progress in tree improvement (Zobel, 1981). Isozyme patterns of plants, are considered to be good indicators of their genetic blue-print (Bhargava, *et al.*, 1986). The patterns of bands/isozymes are treated as phenotypes and investigated through genetic tests that determine which bands are coded by allelic genes and which are specified by genes at different loci (Parkash, 1993).

Isozyme studies have become a useful tool in several areas of genetics (Thormann and Stephan, 1993). They posses practical importance for studies of population genetics and phylogenetic traits and also a diagnostic tool for differentiating the various races, sub species, etc. (Parkash, 1993). The basis for a correct interpretation of isozyme patterns obtained from electrophoretical studies provide a complete knowledge of the modes of inheritance (Hattemer, 1991).

Enzyme electrophoresis has been used as an aid to conventional provenance studies in many tree species (Falkenhagen, 1985; Yeh, *et al.* 1986; Aradhya and Phillips, 1993). Information in levels of allozyme diversity can be very useful for determining on which species and provenances to begin genetic improvement through breeding programmes (Adams, 1983), According to Agarwal and Kaul (1993) electrophoretic separation of the seed protein have been used in recent years as a

useful tool in plant taxonomy at generic, specific and varietal levels. So isozyme electrophoresis is having paramount importance in tree breeding.

2.7 Isozyme analysis in provenance studies

A prerequisite of any genetic analysis of isoenzymes is the occurrence of variation among the isoenzyme patterns found in the material under study, ie., the banding pattern of one individual must differ from the pattern of another in the appearance of at least one isoenzyme band. In many instances provenances will differ in allelic frequencies, and perhaps in the presence or absence of specific alleles. Several studies of enzyme activity were made prior to 1970 with forest tree material to elucidate geographic patterns of genetic variation.

The relevance of isozyme analysis as a powerful tool for estimating genetic variability is discussed by many scientists like Feret and Bergmann (1976), Feret (1979), Asiedu, *et al.* (1992) and Forrest (1994).

Rasmuson and Rudin (1971) demonstrated the genetic control of esterase isoenzymes in *Pinus sylvestris* and showed isoenzyme frequency differences among provenances. A study of the genetic variation and genetic structure of 13 populations of *P. sylvestris* from Eastern Europe and Turkey was done by Prus-Glowacki and Bernard (1994) and found that there was significant differentiation of the populations. Nevertheless, the genetic similarities of populations were not correlated with the geographical localities of the studied stands.

Similar results were also obtained in *P. sylvestris* from Central and Western Europe. (Prus-Glowacki *et al.*, 1993; Prus-Glowacki and Stephan, 1994).

Megagametophyte tissue of seeds collected from three natural populations of *Abies mariesii*, a sub alpine conifer sporadic in isolated mountain ranges of Japan, were subjected to PAGE. Of the 14 enzyme system analysed, 23 loci were identified and using this genetic variations of these three populations were analysed and found that most genetic variation (97.4%) resided within population (Suyama *et al.*, 1992). They also found that the genetic diversity within and among population was very low. Another study conducted by Schroeder (1989a and 1989b) revealed a significant variation between provenances of *Abies alba* and exhibited a clear west-east cline in allele frequencies.

Genetic variation and geographic diversity of five natural populations of *Cryptomeria japonica* in Western Japan were investigated by Tsumura and Ohba (1992) using nine allozyme loci. Most of the variation was found within populations (98.16%) rather than between populations (1.84%).

Forty trees of *Ginkgo bilola* L. from Chinese populations were sampled by Wu *et al.* (1992), and allele frequency of four isozymes at eight loci and average heterozygosity per locus were calculated. Further, they indicated that there was limited genetic variation and strong genetic identity in the population.

Aradhya and Phillips (1993) in a study in fourteen provenances involving five species of *Eucalyptus* observed a high level of polymorphism and heterozygosity.

Geographic distribution and provenance studies clearly demonstrate the differences in *Cunninghamia lanceolala* (Chinese fir), an evergreen conifer occurring naturally in the sub-tropical region of Central-Southern China where it has been cultivated as a timber species for over 1000 years (Fung-LE, 1994). Field studies were supported by isozyme and karyotype analysis, which showed distinct provenance differences.

Fourteen isozyme systems were analysed in leaf parenchyma of nine native and introduced populations of teak (*Tectona grandis* L.f.). The genetic differentiation among provenances were varied. The cluster analysis showed two main gene pools, the first consisting of the Indian provenances and the second of African, Indonesian and Thai provenances (Kertadikara and Prat, 1995).

Genetic variation within and among 11 natural populations of *Pterocarpus macrocarpus* from different forest habitats in Thailand was examined for 18 loci coding for 11 enzymes by starch gel electrophoresis (Liengsiri, *et al.*, 1995). The species possesses a high level of isozyme variation. Also, there was a high degree of among population differentiation.

Materials and Methods

MATERIALS AND METHODS

3.1 Location

The study was conducted at the College of Forestry, Kerala Agricultural University, Vellanikkara, Thrissur district, Kerala (13° 31′ N latitude and 76° 13′ E longitude and at an elevation of 40.29 m above mean sea level), during the period from 1995 to 1996.

The area experiences warm and humid climate with distinct summer and rainy seasons. The climate data for the experimental period are given in Table 1 and Fig. 1.

The soil of the experimental site is oxisols with an average soil pH of 5.8. The soil and sub-soil are porous and extremely well drained.

3.2 Experimental materials

The experimental materials consisted of seeds collected from eight provenances of *Azadirachta indica* A. Juss. from three states viz., Kerala, Karnataka and Tamil Nadu. The two provenances from Kerala are Trichur (local provenance) and Palghat. Two provenances included from Karnataka are Molakalmur and Ghatti Subramanya. A total of four provenances were tested from Tamil Nadu and they are Coimbatore, Nagarcoil, Dindugul and Srivelliputhur. Details of the provenance are given in Table 2 and Fig. 2.

Table 1 Weather parameters for the experimental period

Month	Rainfall (mm)	Temperature (°C)		Relative humidity (%)
		Maximum	Minimum	
1995				
Sep	282.5	30.1	23.5	82
Oct	110.4	33.2	23.2	78
Nov	88.4	31.3	22.5	80
Dec	0.0	32.5	21.3	57
1996				
Jan	0.0	33.1	22.4	53
Feb	0.0	34.7	23.4	53
Mar	0.0	36.4	24.3	60
Apr	152.0	34.6	25.0	73
May	95.4	32.0	25.2	77
Jun	400.3	30.5	23.8	85
Jul	588.7	28.8	23.1	90
Aug	310.0	29.1	23.6	87

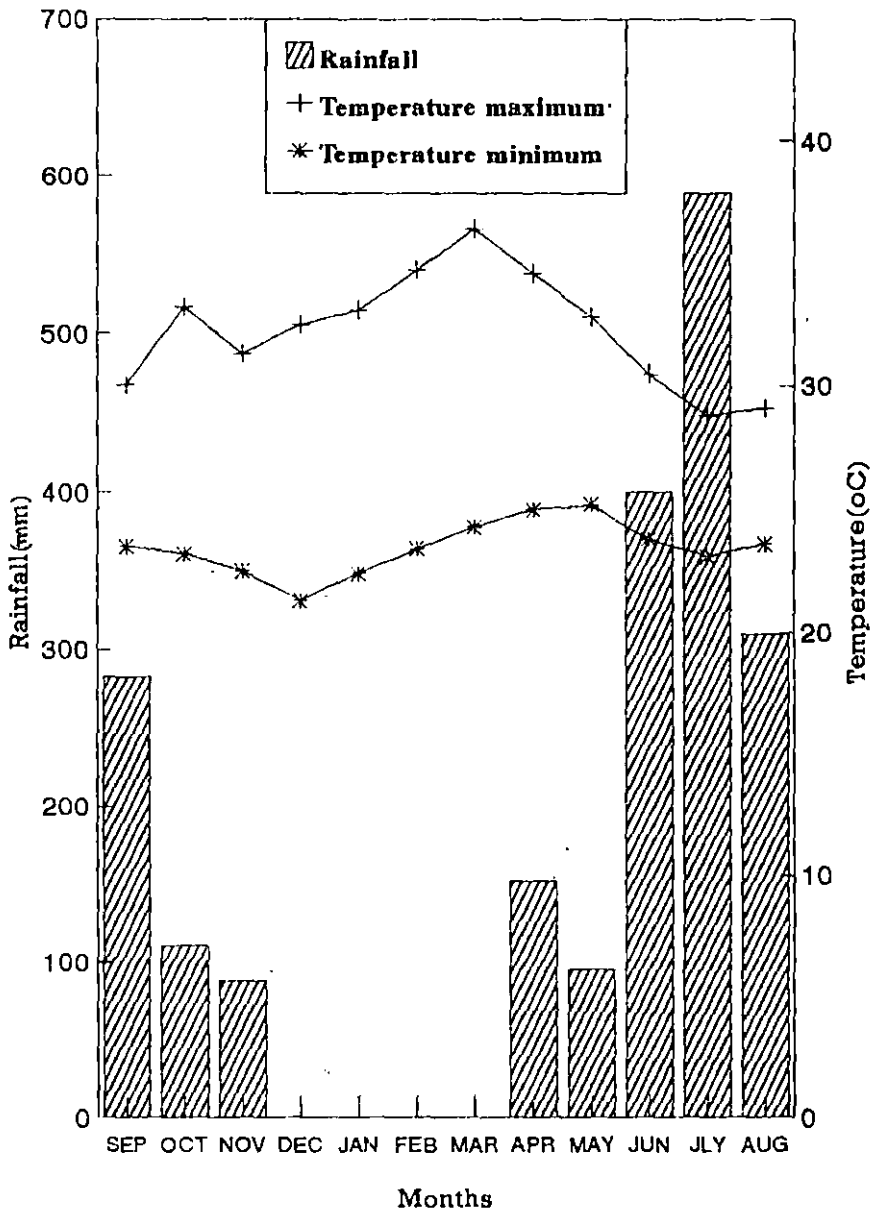


Fig.1 Weather parameters at the experimental location during 1995-96

Table 2 Details of latitude, longitude, rainfall and soil types of different provenances of neem

Sl. No.	Provenances	Latitude*	Longitude*	Rainfall** (mm)	Soil**
1	Dindigul	10°21′N	77°57′E	870	Red sandy
2	Nagercoil	8°10′N	77°26′E	1046	Red sandy
3	Coimbatore	11°17′N	77°7′E	1000	Gravally sandy loam
4	Trichur	10°31′N	76°13′E	2115	Alluvial
5	Srivelliputhur	9°31′N	77°38′E	1000	Red sandy
6	Palakkadu	10°47′N	76°39′E	2058	Red loamy
7	Ghatti Subramanya	12°58′N	77°36′E	800	Red loamy
8	Molakalmur	14°14′N	76°24′E	600	Mixed red and black

Source: * Britannica Atlas, Encyclopaedia Britannica, Inc. Chicago, 1988
** National Atlas of India, Department of Science and Technology, Government of India, Calcutta, 1980

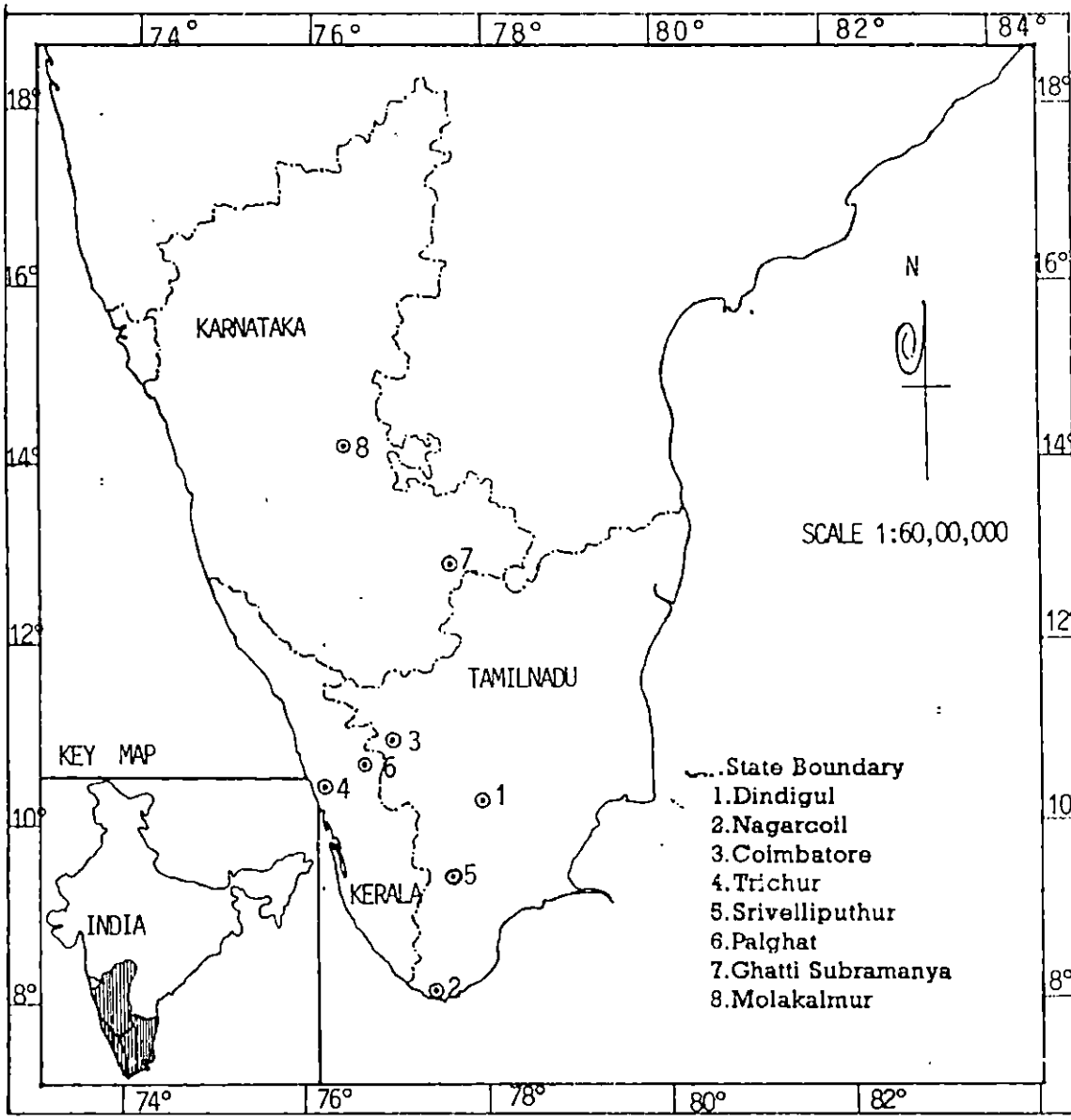


Fig.2. Map showing the location of different provenances of neem

3.3 Nursery

Inorder to obtain good and uniform germination, seeds were subjected to soaking for 24 hrs in water. Seeds floated on upper surface of water were not used for sowing.

Polybags measuring 7" x 9" (250 gauge) were filled with potting mixture containing soil, sand and farm yard manure in the ratio of 1:1:1. The polybags were watered before sowing. The seeds were dibbled at the rate of five seed per polybags. A total of 150 polybags were filled for each provenances. After 150 days of sowing the seedlings were transferred to bigger sized polythene bags of 9" x 18" (250 gauge) at the rate of one seedling per bag. The experiment was laid out in a completely randomized design.

All the seedlings were watered daily. No fertilizer application was done. Management practices were uniformly carried out.

3.4 Observation on seed parameters

Before sowing, the seed parameters viz., seed length, seed diameter, thickness of pericarp and 100 seed weight were measured.

3.4.1 Seed length

The length of 25 seeds each belonging to different provenances were measured individually using a high precision vernier callipers and expressed in millimeters.

3.4.2 Seed breadth

The width of 25 seeds each of different provenances were measured at the centre using a vernier calliper and expressed in millimeters.

3.4.3 Seed length breadth ratio

Length breadth ratio was calculated by dividing the seed length by seed breadth of each seed.

3.4.4 Thickness of pericarp

Pericarp thickness is also measured for 25 seeds using a vernier callipers and expressed in mm.

3.4.5 Seed weight

For each provenance, weight of five replicates of 100 seeds each were taken and repeated 5 times and the mean weight was recorded.

3.4.6 Germination percentage in nursery

Seeds were sown in polythene bags as described in 3.3. The number of seedlings germinated at 30 Days after sowing was recorded for each polythene bags comprising of different provenances. The germination percentage was calculated by considering the number of seeds actually germinated and the total number of seeds kept for germination.

3.5 Observations on seedling parameters

Observations on the biometrical traits namely, shoot length, root length, number of leaves, collar girth and dry weights of leaf, root and stem were made individually in a regular 30 day interval for the experimental period of one year. The observations were taken as described below.

3.5.1 Shoot length

Twenty five seedlings from each provenances were randomly selected and labelled with tin foil. Shoot length was measured at 30 days interval.

The height of the stem from the collar region to the growing tip was measured using a meter scale and recorded in centimetres.

3.5.2 Collar girth

Girth at collar region was measured using vernier callipers and expressed in millimeters. Girth were measured in the same plants in which shoot length were recorded.

3.5.3 Leaf number

Total number of leaves were counted in the seedlings. For this also the same plants mentioned above were used.

3.5.4 Root length

Root length from the collar region to the tip of the longest root was measured using a meter scale and recorded in centimetre.

3.5.5 Dry weight estimations

After biometric observations, stem, leaves and roots were separated and were oven dried at 60°C to 80°C for 6 hours. Then the dry weight was recorded for leaves, stem and roots separately and mean dry weight was expressed in grams. From these values dry weight of shoot and total dry matter production were estimated.

3.5.6 Leaf area

The leaf area of individual plants were measured with an Area meter (Model LI - 3100, LI - cor, USA) and was expressed in cm^2.

3.6 Biochemical studies

3.6.1 Isozyme analysis

Electrophoresis and isoenzyme variation for peroxidase was carried out using vertical slab polyacrylamide gel electrophoresis.

3.6.1.1 Sample collection

Mature leaf samples were collected from each provenances, placed in plastic bags and immediately stowed on ice in an insulated container. The leaves were washed to remove dust, dirt and other extraneous materials. They were then rinsed with distilled water. The rinsed material was gently pressed between blotting paper to remove water.

3.6.1.2 Protein extraction

One gram of mature leaf tissue was weighed and ground in a pre-cooled mortar, along with 4 ml of extraction buffer, below 4°C by keeping in an ice bucket. This was then centrifuged at 15000 rpm for 10 minutes at 4°C in a refrigerated centrifuge (Remi). The clear supernatant was used for electrophoresis.

3.6.1.3 Stock solutions

3.6.1.3.1 Monomer solution

(30.8%T, 2.6% (bis)

Acrylamide	-	30.0 g
Bis acrylamide (N, N′ - methylene bis acrylamide)	-	0.8 g
H_2O	-	to 100 ml

3.6.1.3.2 Polymerization catalysts

a. Ammonium per sulphate (Aps : 1.5% w/v)

   Ammonium per sulphate - 0.15g

b. TEMED (N, N, N′, N′ - tetramethylene - diamine). This was stored at 4°C in an amber coloured bottle.

3.6.1.3.3 Resolving gel buffer

(3M Tris - HCl, pH 8.8)

Tris HCl	-	36.6 g
Adjusted to pH 8.8 with NaOH		
H_2O	-	to 100 ml

3.6.1.3.4 Tank buffer

(0.02 M Tris, 0192 M Glycine, pH 8.3)

Tris HCl	-	6 g
Glycine	-	14.4g
H_2O	-	to 1000 ml

The stock buffer was diluted to 1:9 before use.

3.6.1.3.5 Extraction buffer

(0.1M Tris - HCl; pH 7.6)

Tris-HCl	-	1.211g
H_2O	-	to 100ml

3.6.1.3.6 Staining solution

A.	O. dianisidine	-	0.05g
	IN HCl	-	1 ml
	0.05M sodium acetate buffer	-	3 ml pH - 5.4
	H_2O	-	26 ml
B.	H_2O_2	-	0.01%

Solution A and B were freshly prepared just prior to use.

3.6.1.3.7 Destaining solution I

Acetic acid (7% acetic acid)

Acetic acid	-	14 ml
H_2O	-	200 ml

3.6.1.3.8 Destaining solution II

Acetic acid (2 to 2.5%)

3.6.1.4 Electrophoresis

The recipe for gel preparation using non-dissociating discontinuous buffer system is presented in Table 3.

Table 3 Recipe for gel preparation (8.5%)

Stock solution		Resolving Gel (ml)
Monomer solution	:	8.5
Resolving gel buffer	:	3.5
Distilled water	:	16.0
Ammonium per sulphate	:	0.15 g
TEMED	:	0.015

3.6.1.4.1 Preparation of the resolving gel

1. The glass plates of size 17.3 x 19.3 cm were cleaned thoroughly and sandwiched together with 1 mm spacers in between.
2. In a 250 ml conical flask 28 ml of resolving gel solution was mixed according to the recipe in Table 3 leaving APS and TEMED.
3. The TEMED and APS were then added and mixed well

4. This solution was poured slowly in to the sandwiched glass plates to a level about 1 cm from the top.
5. The comb was inserted in to the sandwich carefully avoiding air bubbles below the teeth of the combs.
6. Polymerisation of the gel was achieved within 15 minutes.

3.6.1.4.2 Sample loading and gel running

1. The combs from the gels were removed slowly prior to sample loading
2. The sandwiched plates were rinsed with distilled water after removing the cellophane tape at the bottom.
3. The water was removed from each well using filter paper strips.
4. The sandwiched plates were then set in the electrophoresis unit of 'Biotech'.
5. Both the lower and upper tanks were filled with tank buffer (pH 8.3) slowly avoiding bubbles.
6. The whole unit was then placed in a refrigerator for some time and then 50 µl of the crude protein extract was loaded in to the wells.
7. The power supply was connected such that the cathode was connected to the upper buffer chamber.
8. The power was set at 30-40mA
9. The power supply was turned off when the dye reached the bottom.

3.6.1.4.3 Staining and destaining of the gel

1. The gel was removed from the glass plates carefully and placed into the staining solution A.
2. The gel was gently shaken for 30 minutes

3. Then this was rinsed with distilled water and staining solution B was added.
4. Brown bands of peroxidase started appearing.
5. Once sufficient staining was achieved, the reaction was stopped by adding destaining solution I.
6. After arresting the reaction destaining solution I was poured off and destaining solution II was added and maintained.
7. The gel was then photographed and used for zymogram preparation and analysis.

3.6.1.5 Measure of similarity

The measurement of electrophoretic similarity between provenances was calculated by making pairwise comparison of the provenances using method of Sokel and Sneath (1963) using the formula:

$$SI = \frac{\text{Number of homologous bands}}{\text{Number of homologus bands + Number of non-homologus bands}}$$

3.6.2 Chlorophyll studies

Chlorophyll content of the leaf was estimated following the method of Starner and Hardley (1967). Five leaf samples were collected from each provenance in a regular interval of 30 days and chlorophyll content was estimated. The selected samples were cut in to pieces and mixed well. From this 0.1 g of the sample was weighed into a mortar and ground with a pestle to extract the chlorophyll using 80 per cent acetone. The extract was filtered using Whattman No.1 filter paper and made up to 25 ml in a volumetric flask using 80 per cent acetone. The absorbance were read

at 663 nm and 645 nm wave length in a spectrophotometer. The chlorophyll-A, chlorophyll-B and total chlorophyll of each samples were calculated using the following formulae.

Chlorophyll-A (mg g^{-1} of tissue)

$$= 12.7 \text{ (OD at 663 nm)} - 2.69 \text{ (OD at 645 nm)} \times \frac{V}{1000 \times W}$$

Chlorophyll-B (mg g^{-1} of tissue)

$$= 22.9 \text{ (OD at 645 nm)} - 4.68 \text{ (OD at 663 nm)} \times \frac{V}{1000 \times W}$$

Total chlorophyll (mg g^{-1} of tissue)

$$= 20.2 \text{ (OD at 645 nm)} + 8.02 \text{ (OD at 663 nm)} \times \frac{V}{1000 \times W}$$

Where,

OD = Optical Density

V = Final volume of chlorophyll extract in 80 per cent acetone

W = Fresh weight of tissue extracted in gram

3.7 Anatomical studies

3.7.1 Stomatal frequency

For counting the stomata, fourth leaf from the top was taken from the seedlings. Five seedlings from each provenance was selected for stomatal count. A

thin film of quick-setting substance (quick - fix) was applied on the lower (dorsal) side of the leaf to get a replica of the leaf surface. After setting the gum, it was peeled off and put on a microscopic slide and observed under a projection microscope. Stomata were counted in 10 microscopic fields for each sample. The frequency is computed and expressed as number per square centimeter.

3.8 Statistical analysis

3.8.1 Analysis of variance

The data were subjected to Analysis of Variance for completely Randomised Design (Panse and Sukhatme, 1989). Analysis of various plant characters were done with PC using MSTAT-C package.

3.8.2 Estimation of genetic parameters

The data were statistically analysed and the variance due to genotype and phenotype, genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV) and heritability in broad sense were workedout as suggested by Singh and Chaudhary (1985). Genetic advance and genetic gain (genetic advance as percentage of mean) were determined following the method of Johnson *et al.* (1955b).

3.8.2.1 Genotypic variance ($\sigma^2 g$)

$$\sigma^2 g = \frac{MS_1 - MS_e}{r}$$

where,

MS_t = Mean sum of squares for treatment

MS_e = Mean sum of squares for error

r = Number of replications

3.8.2.2 Phenotypic variance ($\sigma^2 p$)

$$\sigma^2 p = \sigma^2 g + \sigma^2 e$$

where,

$\sigma^2 e$ = Variance due to error (taken as error mean sum of squares)

3.8.2.3 Genotypic coefficient of variation (GCV)

$$GCV = \frac{(\text{Genotypic variance})^{1/2}}{\text{Grand mean}} \times 100$$

3.8.2.4 Phenotypic coefficient of variation (PCV)

$$PCV = \frac{(\text{Phenotypic variance})^{1/2}}{\text{Grand mean}} \times 100$$

3.8.2.5 Heritability in broad sense (H^2)

$$H^2 = \frac{\sigma^2 g}{\sigma^2 p}$$

3.8.2.6 Genetic advance (GA)

$$GA = \frac{\text{Genotypic variance}}{(\text{Phenotypic variance})^{1/2}} \times K$$

where,

K = 2.06, a selection differential at 5 per cent selection intensity

3.8.2.7 Genetic gain (genetic advance as percentage of mean)

$$\text{Genetic gain} = \frac{GA}{\text{Grand mean}} \times 100$$

3.8.3 Genetic Divergence

3.8.3.1 D^2 analysis

Multivariate analysis was employed to examine the pattern of provenance variation considering a group of characters simultaneously. Mahalanobis' "generalised distance function" (D^2 statistics) as outlined by Rao (1952) was used for estimating the genetic divergence among the eight provenances at 360 days after sowing. D^2 expresses the degree of relationship between two populations, considering simultaneously the group of characters chosen. The formula for two traits is as follows.

$$D^2 = \frac{(\bar{X}_{11} - \bar{X}_{12})^2}{S_1^2} + \frac{(\bar{X}_{11} - \bar{X}_{12})(\bar{X}_{21} - \bar{X}_{22})}{S_{12}} + \frac{(\bar{X}_{21} - \bar{X}_{22})^2}{S_2^2}$$

Where,

X_1 and X_2 = Two characters

$\bar{X}_{11}$ and $\bar{X}_{12}$ = Means of trait 1 for the first and second population respectively

$\bar{X}_{21}$ and $\bar{X}_{22}$ = Means of trait 2 for the same two populations

S_1^2 and S_2^2 = Pooled estimates of the variances of traits 1 and 2

S_{12} = Covariance of traits 1 and 2

For more than two characters the formula is expressed as follows:

$$D^2 = \sum_i \sum_j S_{ij}^{-1} d_i d_j$$

Where,

d_i = the mean population difference for the character i

d_j = the mean population difference for the j^{th} variable

S_{ij}^{-1} = the element in the inverse of the covariance matrix corresponding to the j^{th} and i^{th} variable

D^2 values were computed for 11 characters such as plant height, collar girth, number of leaves, leaf area, total dry weight, root length, root shoot ratio, chlorophyll-A content, chlorophyll-B content; Total chlorophyll-content, and stomatal frequency.

There were eight provenances in this study, so a total of $\frac{8 \times 7}{2} = 28$ values of D^2 were computed.

3.8.3.2 Determination of clusters

Provenances were appropriately grouped into different clusters on the basis of the square root of the average D^2 values which gave the genetic distance 'D' between the provenances. For determining clusters, Tocher's method as suggested by Rao (1952) was followed.

3.8.3.3 Intra and inter cluster distances

After establishing the clusters, the intra-cluster distances were worked out by rating the average of the component provenances in that cluster. The average inter - cluster distance was arrived by taking into consideration all the component D^2 values possible among the members of two clusters considered. The square root of D^2 values give the genetic distance `D' between the provenances.

3.9 Incidence of Pests and Diseases

All the seedlings of neem from different provenances raised in the polythene bags were observed through out the investigation period from 30 to 360 Days after sowing in order to findout the pests and diseases associated with it.

Results

RESULTS

Evaluation of eight provenances of neem collected from three states of the peninsular India, viz., Kerala, Tamil Nadu and Karnataka was carried out for their seed and seedling characters for a period of one year. The results obtained from the study are presented hereunder.

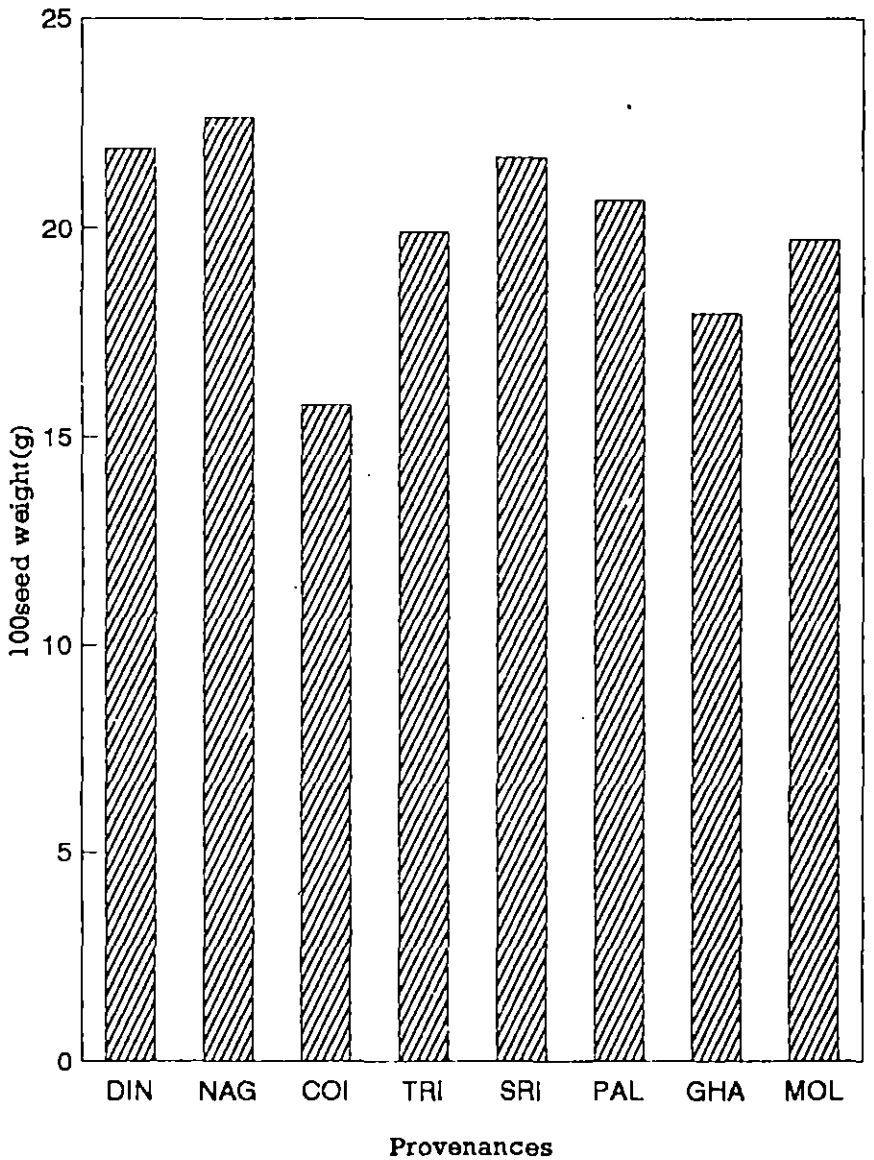
4.1 Seed parameters

4.1.1 Hundred seed weight

Seed weight of the different provenances of neem showed statistically significant variation (Table 4). Four provenances viz., Dindugul, Nagarcoil, Srivelliputhur and Palakkadu recorded higher values over the general mean. The maximum value for 100 seed weight was recorded by Nagarcoil provenance (22.61 g) and the minimum value by Coimbatore provenance (15.79 g). Trichur and Molakalmur provenance recorded 19.91 and 19.71 g, respectively.

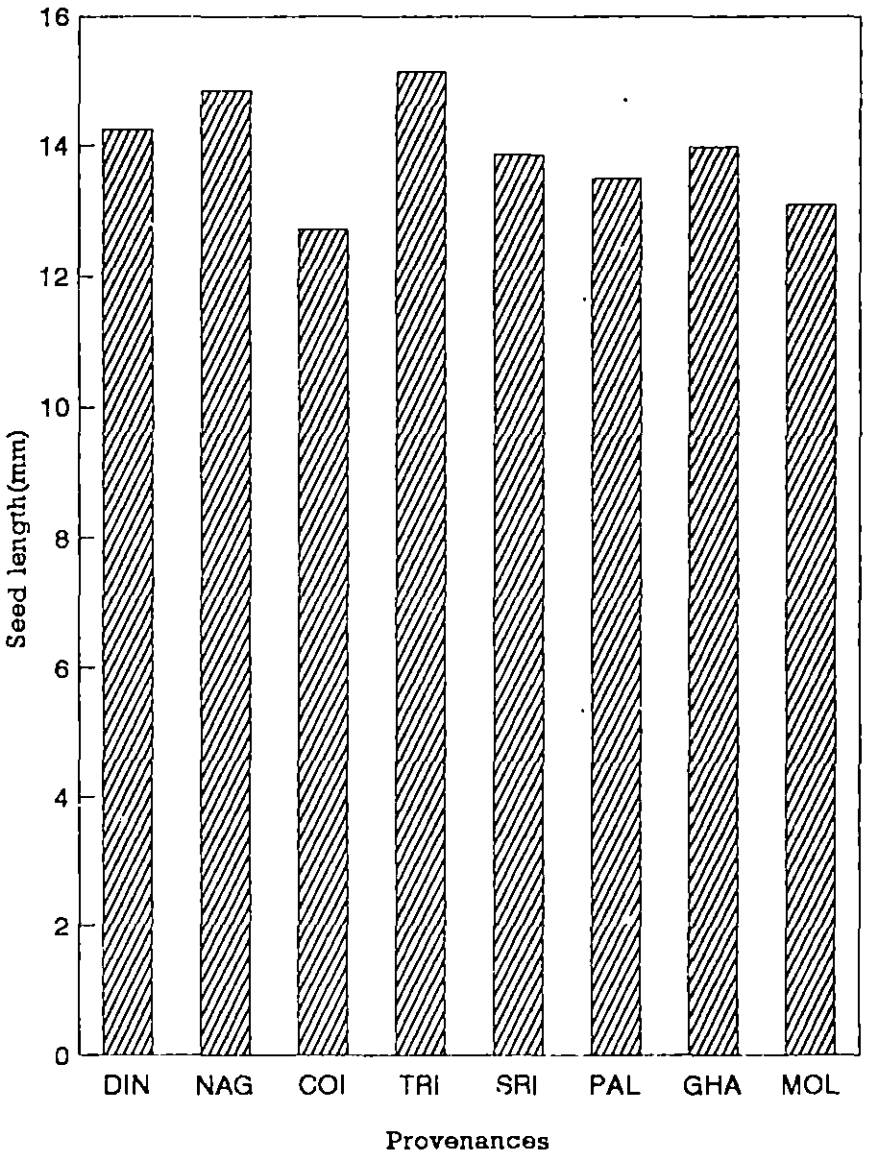
4.1.2 Seed length

Four provenances viz., Dindugul, Nagarcoil, Trichur and Ghatti Subramanya recorded increased seed length over general mean. The seed length was maximum for Trichur (15.13 mm) which was significantly superior to all other provenances tested (Table 4). The lowest seed length was recorded by Coimbatore (12.72 mm). Almost comparable seed lengths were shown by Srivelliputhur and Ghatti Subramanya having their values 13.87 mm and 13.98 mm, respectively.



DIN-Dindigul NAG-Nagarcoil COI-Coimbatore TRI-Trichur
SRI-Srivelliputhur PAL-Palghat GHA-Ghatti Subramanya MOL-Molakalmur

Fig.3 Variation in 100seed weight of different provenances of neem



DIN-Dindigul NAG-Nagarcoil COI-Coimbatore TRI-Trichur
SRI-Srivelliputhur PAL-Palghat GHA-Ghatti Subramanya MOL-Molakalmur

Fig.4 Variation in seed length of different provenances of neem

4.1.3 Seed breadth

This character did not show significant variation between the eight provenances. The maximum value was recorded by Nagarcoil with its value 6.97 mm (Table 4). Four provenances viz., Dindigul, Nagarcoil, Trichur and Srivelliputhur recorded higher values over general mean.

4.1.4 Seed length breadth ratio

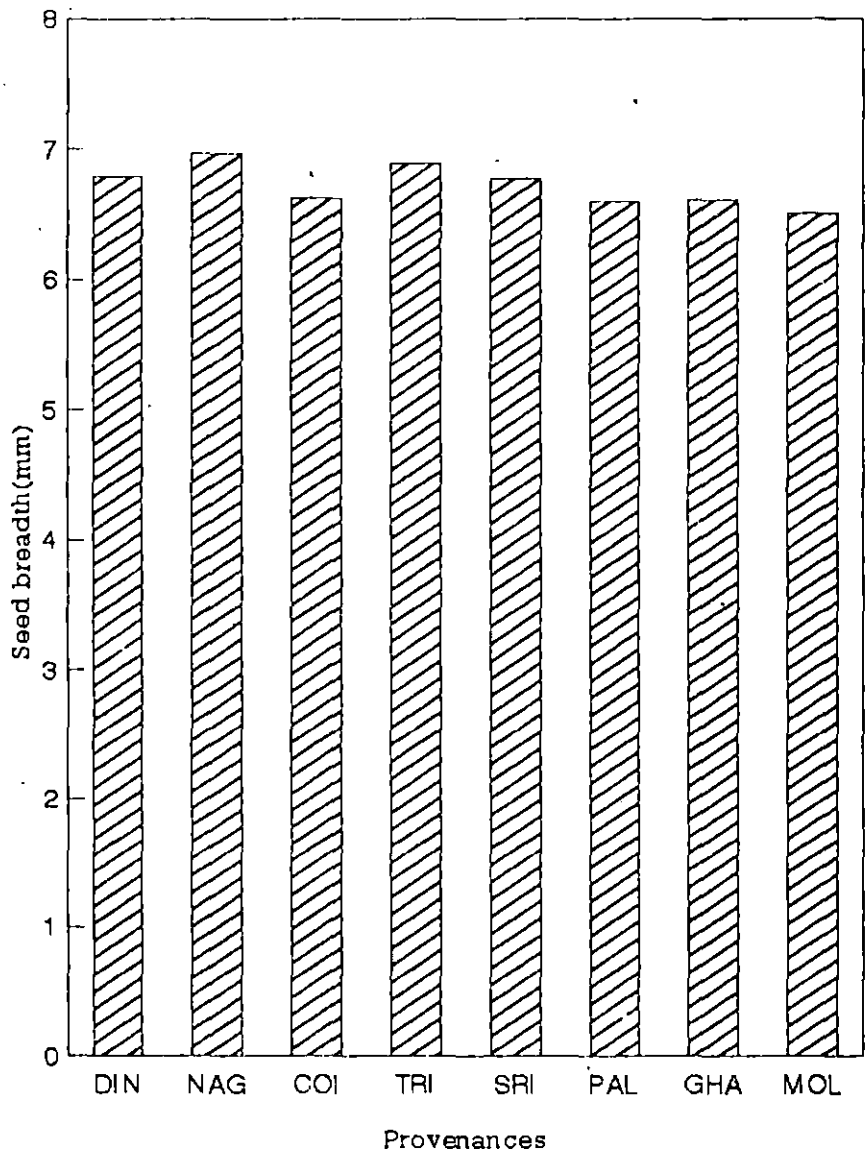
Seed length breadth ratio was maximum for Trichur (2.20) which was statistically superior to all other provenances (Table 4). The minimum value was recorded by Coimbatore (1.92). Four provenances viz., Dindigul, Nagarcoil, Trichur and Ghatti Subramanya recorded higher values of seed length breadth ratio over general mean.

4.1.5 Thickness of pericarp

Thickness of pericarp was maximum for Srivelliputhur (0.43 mm) which was significantly superior to all other provenances tested (Table 4). The minimum value was showed by Molakalmur (0.33 mm). Five provenances viz., Dindigul, Nagarcoil, Coimbatore, Srivelliputhur and Ghatti Subramanya recorded higher values over general mean.

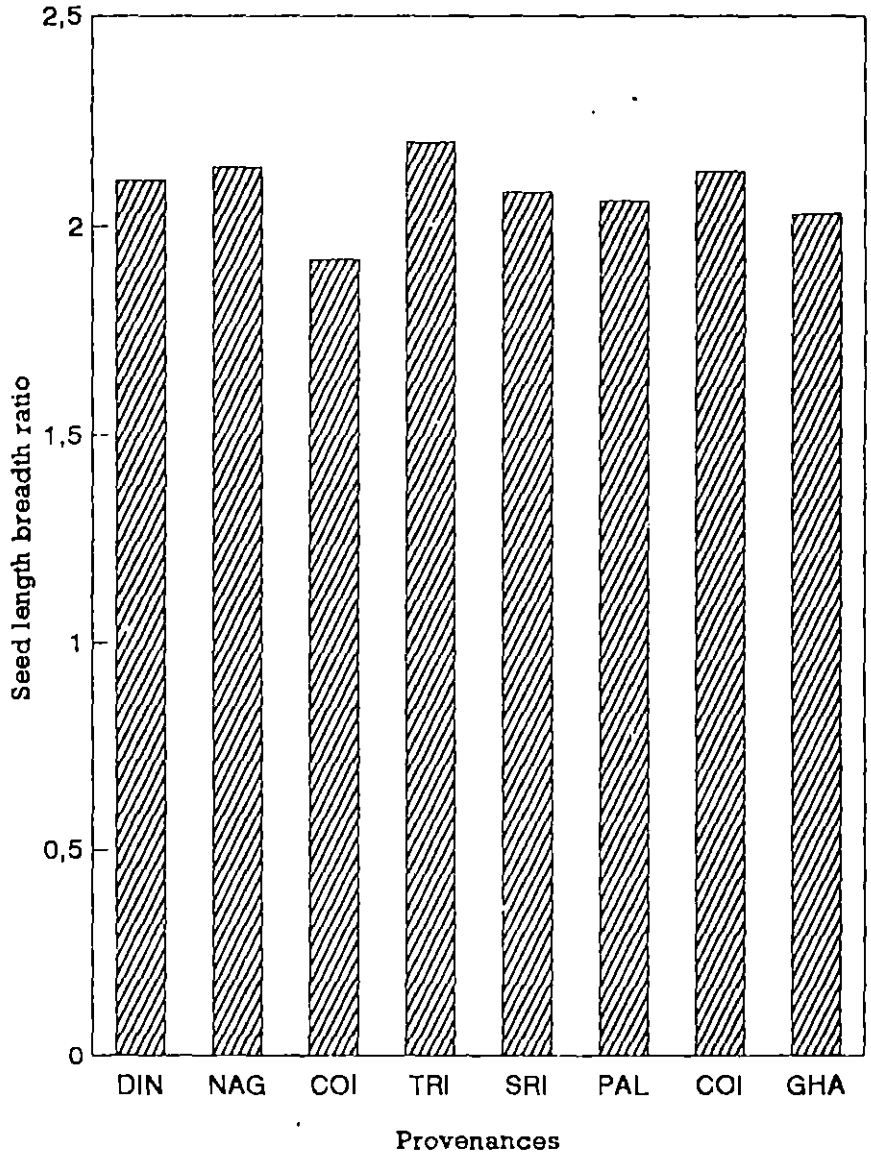
4.1.6 Germination percentage

Table 4 shows that the germination percentage was maximum for Nagarcoil provenance (87.33 per cent) which was statistically superior to all the other



DIN-Dindigul NAG-Nagarcoil COI-Coimbatore TRI-Trichur
SRI-Srivelliputhur PAL-Palghat GHA-Ghatti Subramanya MOL-Molakalmur

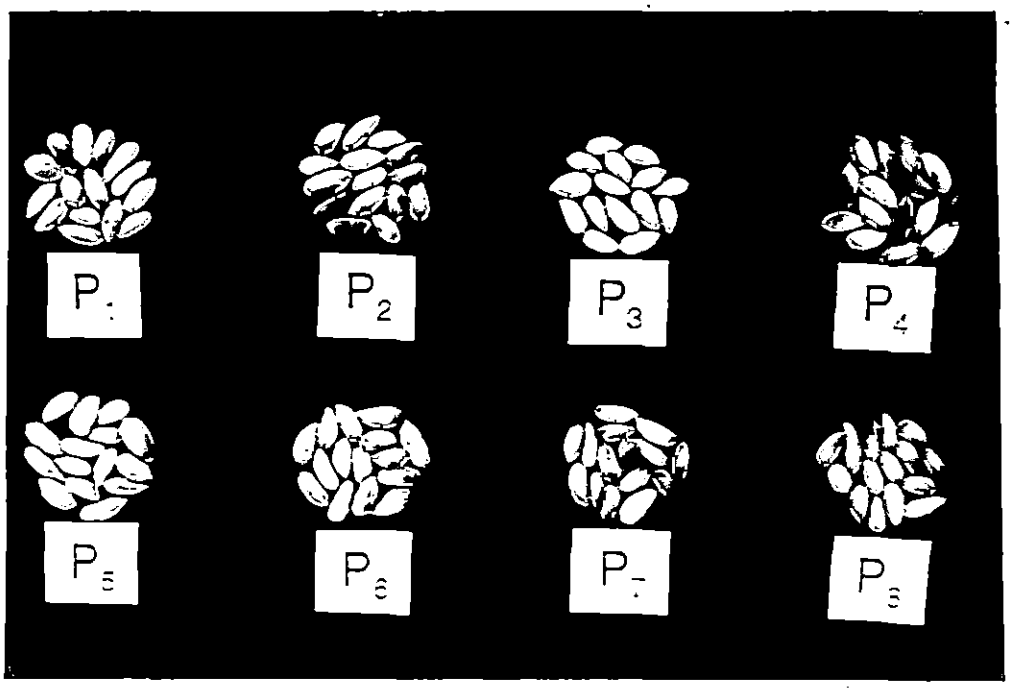
Fig.5 Variation in seed breadth of different provenances of neem



DIN-Dindigul NAG-Nagarcoil COI-Coimbatore TRI-Trichur SRI-Srivelliputhur PAL-Palghat GHA-Ghatti Subramanya MOL-Molakalmur

Fig.6 Variation in seed length breadth ratio of different provenances of neem

Plate 1 Variation in seed size of different provenances of neem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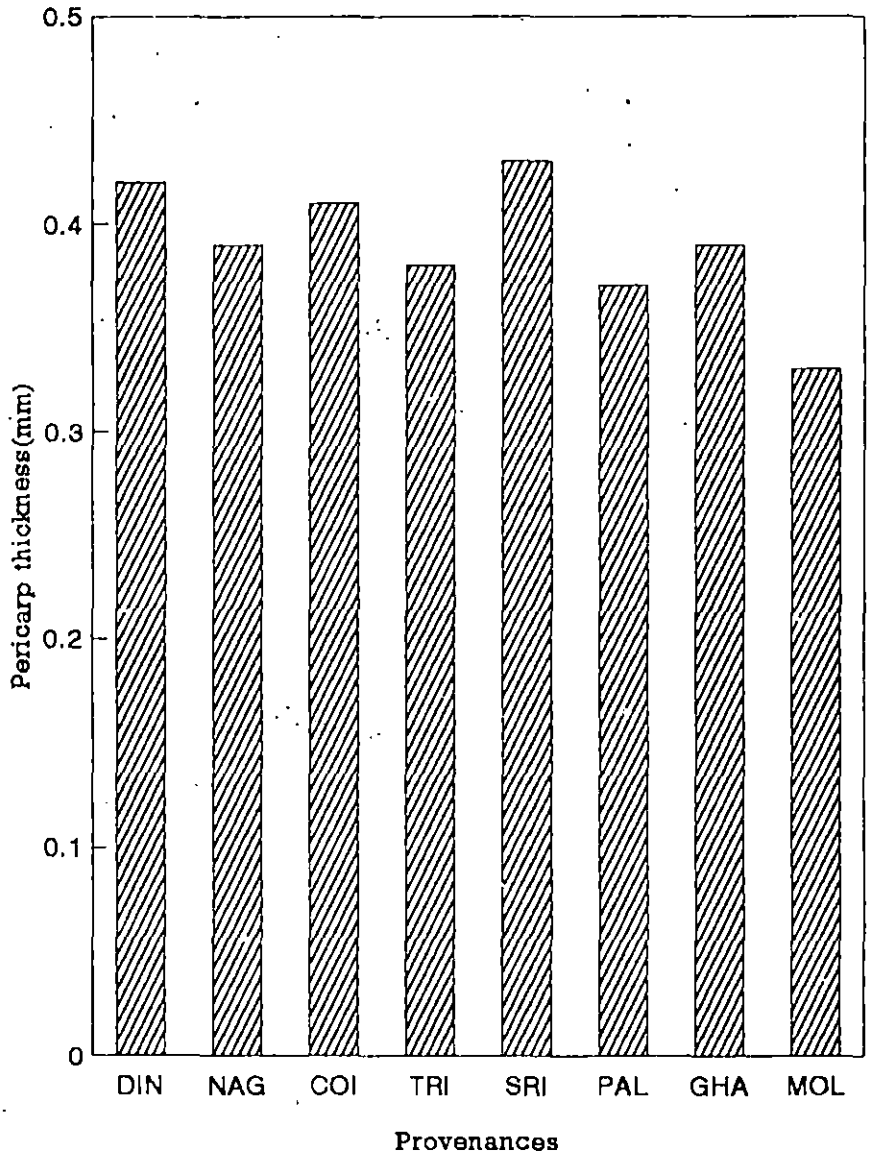
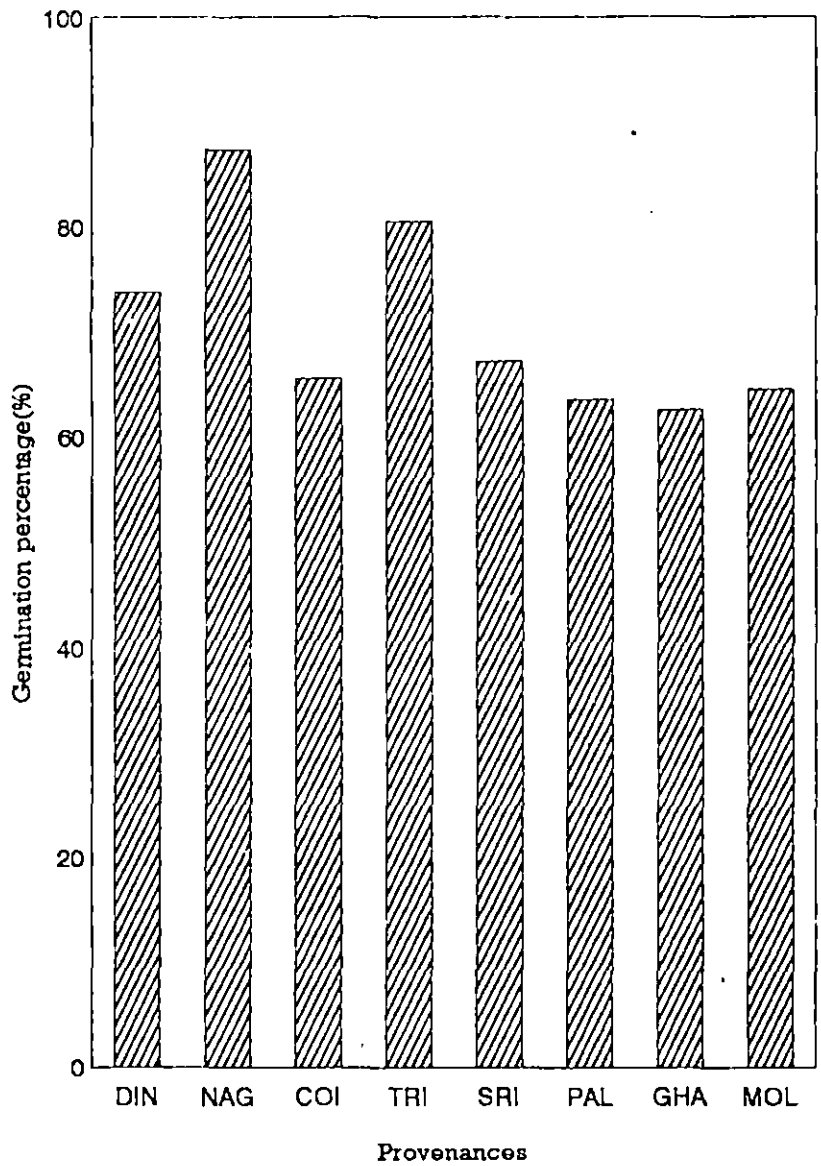


DIN-Dindigul NAG-Nagarcoil COI-Coimbatore TRI-Trichur SRI-Srivelliputhur PAL-Palghat GHA-Ghatti Subramanya MOL-Molakalmur

Fig.7 Variation in pericarp thickness of different provenances of neem



DIN-Dindigul NAG-Nagarcoil COI-Coimbatore TRI-Trichur SRI-Srivelliputhur PAL-Palghat GHA-Ghatti Subramanya MOL-Molakalmur

Fig.8 Variation in germination percentage of different provenances of neem

Table 4 Data on seed characters of different provenances of neem

Provenances	100 seed weight (g)	Seed length (mm)	Seed breadth (mm)	Length breadth ratio	Thickness of pericarp (mm)	Germination percentage
Dindigul	21.85	14.25	6.79	2.11	0.42	73.73
Nagercoil	22.61	14.84	6.97	2.14	0.39	87.33
Coimbatore	15.79	12.72	6.63	1.92	0.41	65.60
Trichur	19.91	15.13	6.89	2.20	0.38	80.40
Srivelliputhur	21.66	13.87	6.78	2.08	0.43	67.07
Palakkadu	20.66	13.50	6.60	2.06	0.37	63.47
Ghatti Subramanya	17.94	13.98	6.61	2.13	0.39	62.53
Molakalmur	19.71	13.11	6.51	2.03	0.33	64.40
Mean	20.019	13.92	6.72	2.09	0 39	70.57
CD (0.05)	0.559	0.787	NS	0.136	0.035	4.86
SEm (±)	0.34	0.11	0.05	0.02	0.00	0.98

provenances. The minimum value for Germination percentage was recorded by Ghatti Subramanya provenance (62.53 per cent). The local provenance (Trichur) was the second best performer. Three provenances viz., Dindigul, Nagarcoil and Trichur recorded higher values over general mean.

4.2 Seedling characters

4.2.1 Biometric Observations

The results of the biometric observations like height, collar girth, leaf area, leaf number and root length of neem seedlings of different provenances at different intervals of time are presented in Table 5 to 9.

4.2.1.1 Height

A highly significant variation was observed in all the growth phases (30 , 60 , 90 , 120 , 150 , 180 , 210 , 240 , 270 , 300 , 330 and 360 Days after sowing) between the provenances in respect of seedling height (Table 5). At 30 days after sowing the maximum height was observed for Nagarcoil (11.4 cm) and the minimum for Molakalmur (6.9 cm). The maximum height growth was later replaced by Coimbatore provenance from 60 days to 360 days after sowing. For the entire growth period of 360 days the minimum growth rate was observed in Molakalmur provenance.

The second maximum height growth was observed for the local provenance from 60 days to 360 days after sowing.

Table 5 Data showing variation in seedling height (cm) of different provenances of neem

Provenances	30 DAS	60 DAS	90 DAS	120 DAS	150 DAS	180 DAS	210 DAS	240 DAS	270 DAS	300 DAS	330 DAS	360 DAS
Dindigal	8.1	16.3	24.3	32.3	40.0	47.8	56.4	64.2	70.6	79.6	88.6	96.8
Nagercoil	11.4	18.4	26.7	36.5	45.7	54.3	63.9	72.2	80.8	89.6	101.3	108.2
Coimbatore	10.3	20.4	30.9	40.7	50.9	60.9	71.6	79.8	90.9	99.3	113.3	119.7
Trichur	9.6	19.1	28.4	38.2	47.6	56.7	66.5	75.6	84.2	93.6	105.4	113.7
Srivelliputhur	8.8	17.6	25.9	34.9	43.6	52.7	60.6	69.4	77.6	85.9	95.7	104.6
Palakkadu	7.9	15.9	23.3	31.5	39.6	47.3	54.9	62.8	69.8	77.1	95.2	94.0
Ghatti Subramanya	8.4	16.7	24.6	32.9	41.6	49.7	57.6	66.2	73.6	82.5	91.8	97.4
Molakalmur	6.9	13.7	20.1	27.4	34.1	41.3	47.4	54.4	60.5	67.9	75.8	81.4
Mean	8.9	17.2	25.5	34.3	43.0	51.4	59.9	68.1	76.1	84.5	95.9	102.0
CD (0.05)	2.365	2.505	3.674	4.867	6.245	7.441	8.732	9.701	9.741	12.04	16.05	13.71
SEm (±)	0.31	0.34	0.51	0.67	0.85	1.01	1.20	1.32	1.50	1.64	2.13	1.89

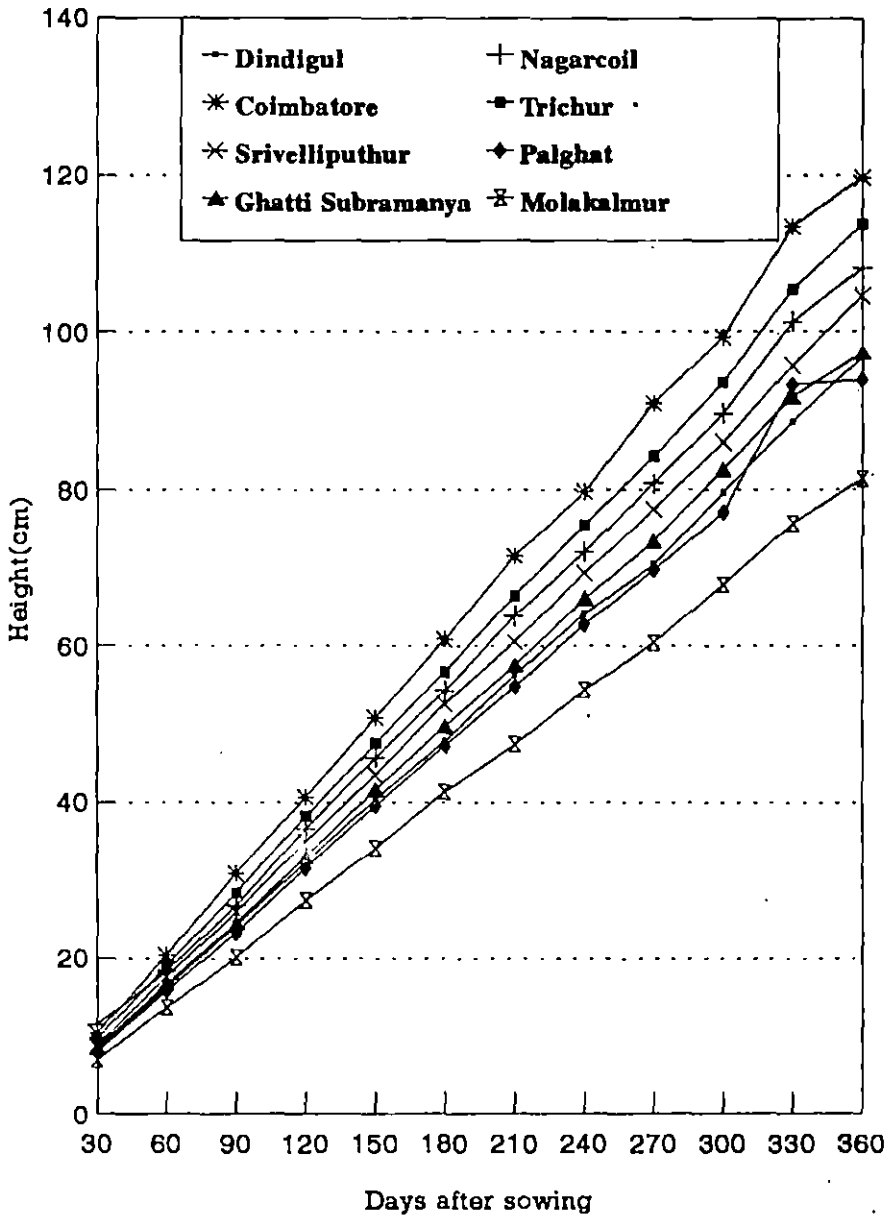
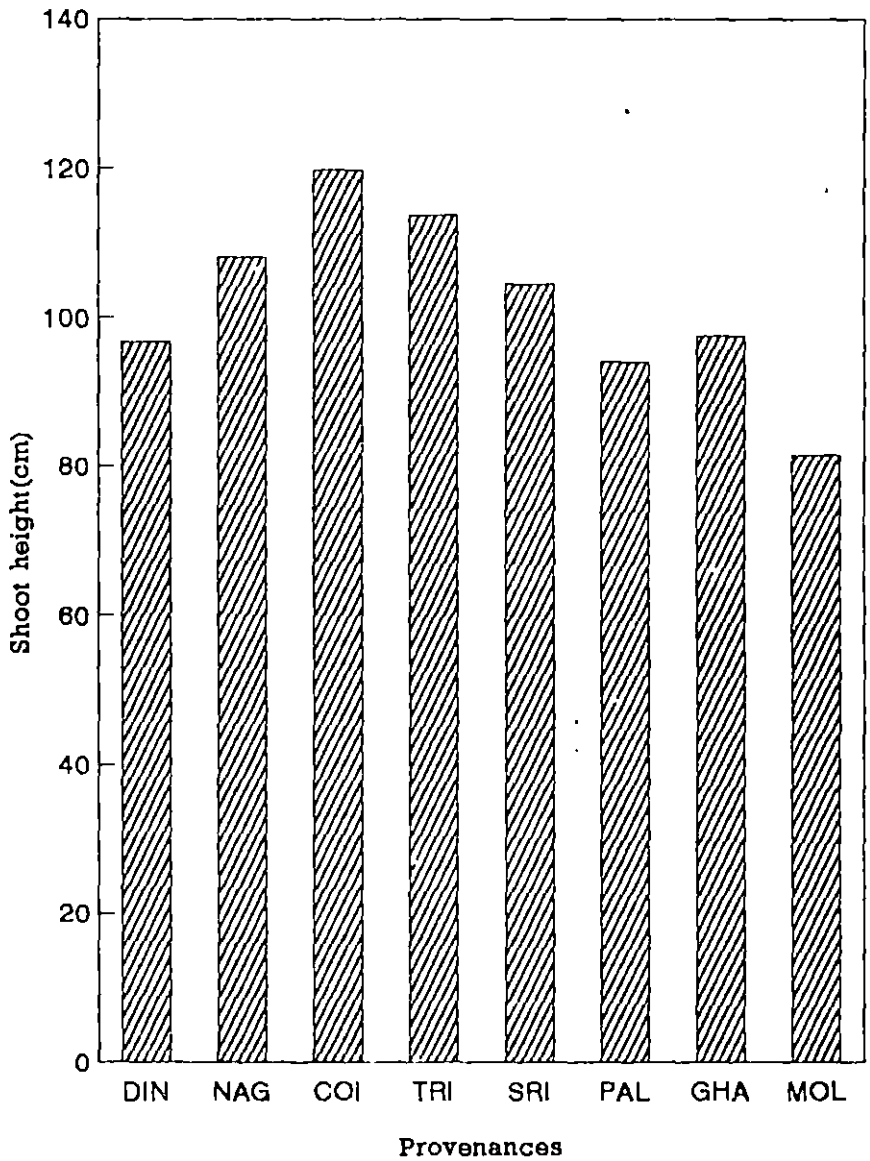


Fig.9 Increase in height of different provenances of neem at different growth phases



DIN-Dindigul NAG-Nagarcoil COI-Coimbatore TRI-Trichur
SRI-Srivelliputhur PAL-Palghat GHA-Ghatti Subramanya MOL-Molakalmur

Fig.10 Variation in shoot height of different provenances of neem at 360DAS

At 30 days after sowing four provenances viz., Nagarcoil, Coimbatore, Trichur, and Srivelliputhur were characterised by increased height growth over the general mean. At 360 days also these provenances were exhibiting the same trend. At 360 DAS the maximum height was recorded for Nagarcoil (108.22 cm) and minimum height was recorded in Molakalmur (81.44 cm). It was seen that the Dindigul provenance is almost on par with Srivelliputhur and Ghatti Subramanya throughout the growth stages.

4.2.1.2 Collar Girth

Data on collar girth recorded at different growth phases are presented in Table 6. Statistically significant difference were observed between provenances throughout the observation periods.

At 30 DAS the maximum girth was recorded for Coimbatore (4.18 mm) and the minimum by Molakalmur (2.72 mm). However, at 360 DAS the maximum girth was recorded for Nagarcoil provenance (39.84 mm) while the minimum was still for Molakalmur (29.93 mm).

From Table 6 it is seen that up to 90 DAS the maximum values were shared by different provenances viz., Coimbatore at 30 DAS, Nagarcoil at 60 DAS and Srivelliputhur at 90 DAS. From 120 DAS to 360 DAS the best performer in maximum girth increment was found to be the Nagarcoil.

Table 6 Data showing variation in collar girth (mm) of seedlings of different provenances of neem

Provenances	30 DAS	60 DAS	90 DAS	120 DAS	150 DAS	180 DAS	210 DAS	240 DAS	270 DAS	300 DAS	330 DAS	360 DAS
Dindigal	3.12	5.91	5.69	11.65	14.68	17.61	20.67	23.07	23.97	29.17	32.57	34.58
Nagercoil	3.45	6.81	9.81	13.47	16.86	20.08	23.41	26.27	29.62	33.85	37.46	39.84
Coimbatore	4.18	6.58	9.66	13.18	16.40	19.23	22.82	25.41	28.82	32.70	36.18	38.30
Trichur	3.17	6.31	9.21	12.31	15.08	18.64	21.73	25.38	27.27	31.45	34.62	37.25
Srivelliputhur	3.35	6.70	9.92	13.25	16.59	19.86	22.87	25.95	59.55	33.21	36.88	39.39
Palakkadu	3.30	6.64	9.68	13.18	16.42	19.70	22.80	25.40	28.82	32.68	36.51	38.87
Ghatti Subramanya	3.24	6.34	9.36	12.28	15.65	18.91	21.76	24.25	27.71	31.49	34.73	37.17
Molakalmur	2.72	5.08	7.52	10.15	12.62	15.14	17.45	19.45	22.51	25.20	27.98	29.93
Mean	3.32	6.30	9.23	12.43	15.54	18.65	21.69	24.27	31.04	31.22	34.62	36.92
CD (0.05)	0.80	0.78	1.66	1.54	1.95	2.24	2.64	2.93	16.83	3.80	4.19	4.35
SEm (±)	0.10	0.10	0.15	0.21	0.26	0.30	0.35	0.39	2.24	0.51	0.56	0.58

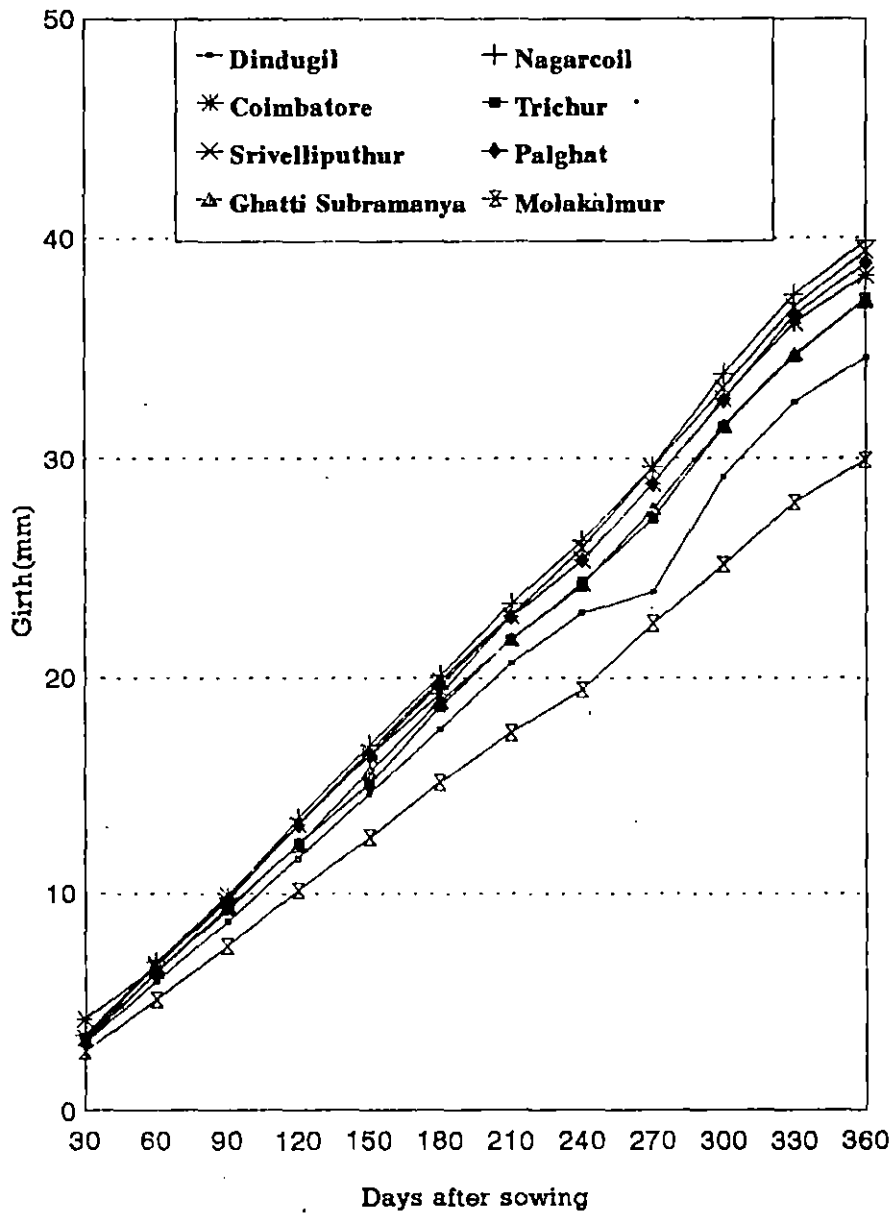
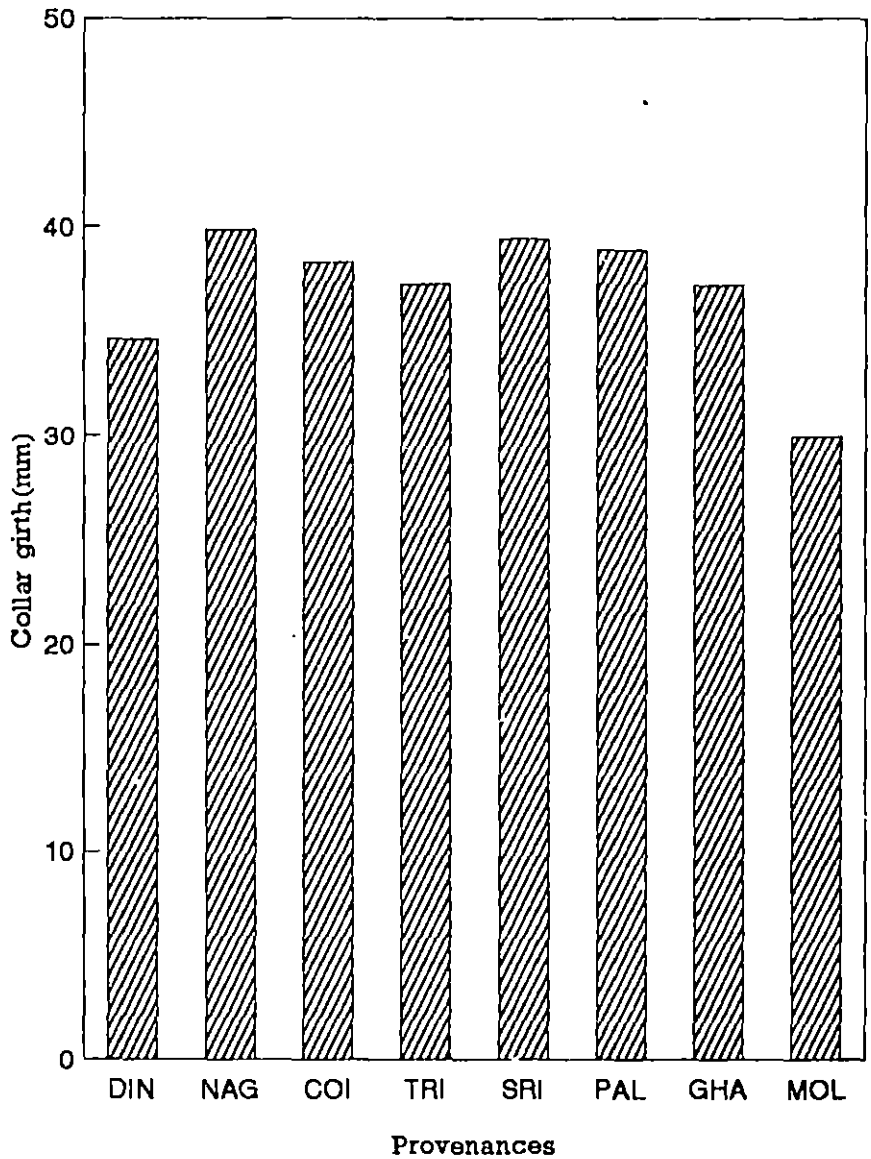


Fig.11 Increase in collar girth of different provenances of neem at different growth phases



DIN-Dindigul NAG-Nagarcoil COI-Coimbatore TRI-Trichur
SRI-Srivelliputhur PAL-Palghat GHA-Ghatti Subramanya MOL-Molakalmur

Fig.12 Variation in collar girth of different provenances of neem at 360DAS

At 30 DAS three provenances viz., Nagarcoil, Coimbatore and Srivelliputhur recorded maximum values over the general mean. But at 360 DAS all provenances except Dindugal and Molakalmur recorded higher values over the general mean.

4.2.1.3 Leaf number

For the entire experimental period Leaf number registered significant variation among provenances (Table 7). Coimbatore registered a high significant value throughout the successive experimental period. The second maximum leaf number was observed for Trichur (local provenance) at 30, 60, 90, 120, 210, 270, 330 and 360 Days after sowing . The second maximum leaf number was also observed for Srivelliputhur at 150, 180, 240 and 300 DAS.

The minimum value for leaf number was observed for Dindugul for the entire successive growth stages up to 330 Days after sowing. And after that the minimum value went to Molakalmur.

At 30 DAS four provenances viz., Nagarcoil, Coimbatore, Trichur and Srivelliputhur recorded higher values over the general mean, to the tune of 7.0, 9.0, 7.1 and 7.0 respectively. At 360 DAS three provenances viz., Nagarcoil, Coimbatore and Trichur recorded higher values over the general mean to the tune of 83.2, 107.0 and 85.0, respectively.

Table 7 Data showing variation in leaf number of seedlings of different provenances of neem

Provenances	30 DAS	60 DAS	90 DAS	120 DAS	150 DAS	180 DAS	210 DAS	240 DAS	270 DAS	300 DAS	330 DAS	360 DAS
Dindigal	5.8	11.6	16.8	23.2	29.1	34.9	40.0	45.3	51.2	55.9	63.9	77.7
Nagercoil	7.0	13.9	20.2	27.6	35.0	41.4	49.2	54.7	61.8	66.6	76.4	83.2
Coimbatore	9.0	17.9	26.8	35.8	44.5	52.7	61.6	70.0	79.5	86.5	98.4	107.0
Trichur	7.1	14.2	20.8	28.8	35.6	41.6	49.3	55.3	62.6	68.7	77.6	85.0
Srivelliputhur	7.0	14.1	20.8	28.1	35.8	41.8	49.0	56.4	62.0	69.0	77.6	82.6
Palakkadu	6.9	13.8	20.2	27.7	34.3	41.5	47.3	54.5	60.7	67.0	75.8	82.3
Ghatti Subramanya	6.2	12.8	18.1	24.6	31.3	36.8	43.0	48.5	54.1	58.9	67.9	74.4
Molakalmur	6.4	12.9	19.0	25.6	32.4	37.9	45.1	50.9	56.8	63.4	70.8	70.4
Mean	6.9	13.9	20.3	27.7	34.8	41.1	48.1	54.5	61.1	67.0	76.1	82.8
CD (0.05)	1.232	2.494	3.479	4.865	5.918	7.441	8.623	9.681	9.741	11.84	13.87	14.69
SEm (±)	0.17	0.33	0.48	0.66	0.80	0.99	1.15	1.30	1.47	1.59	1.86	1.97

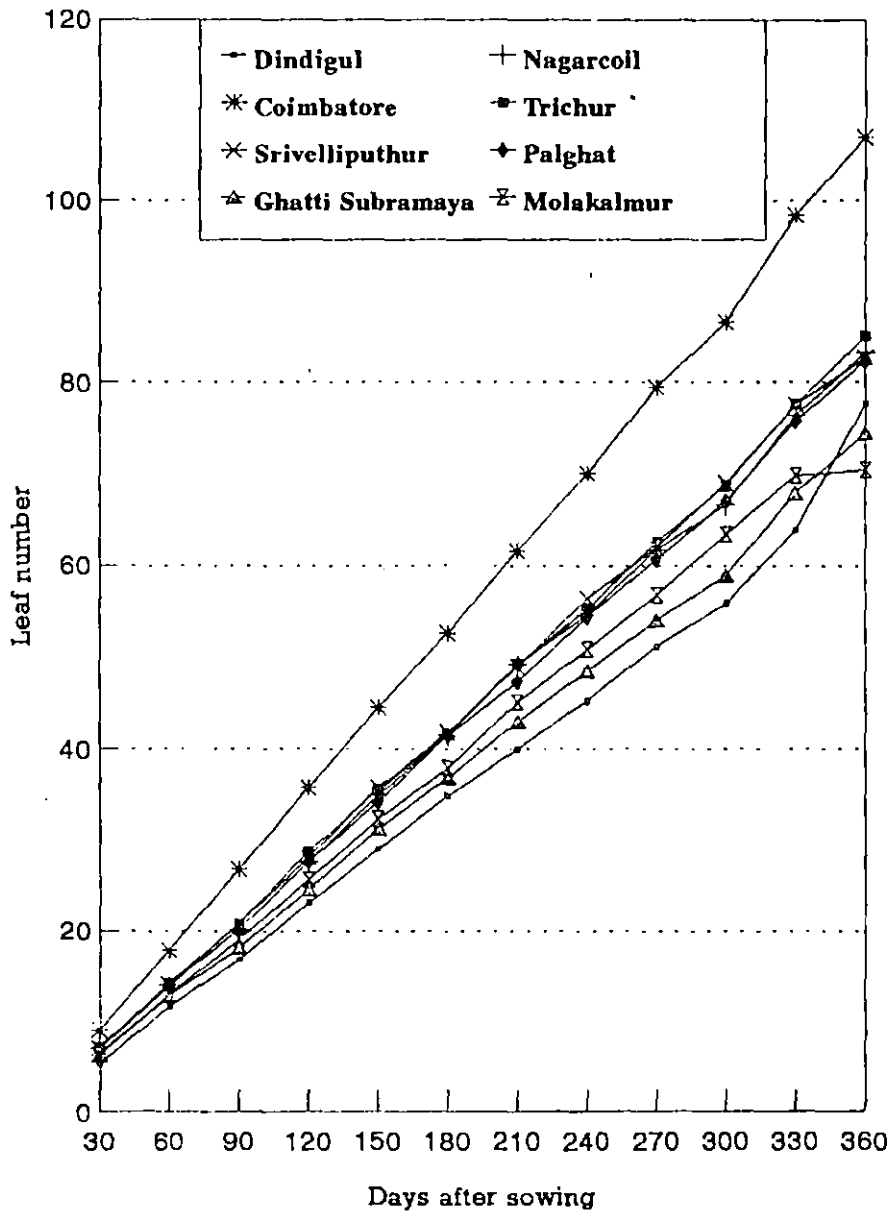
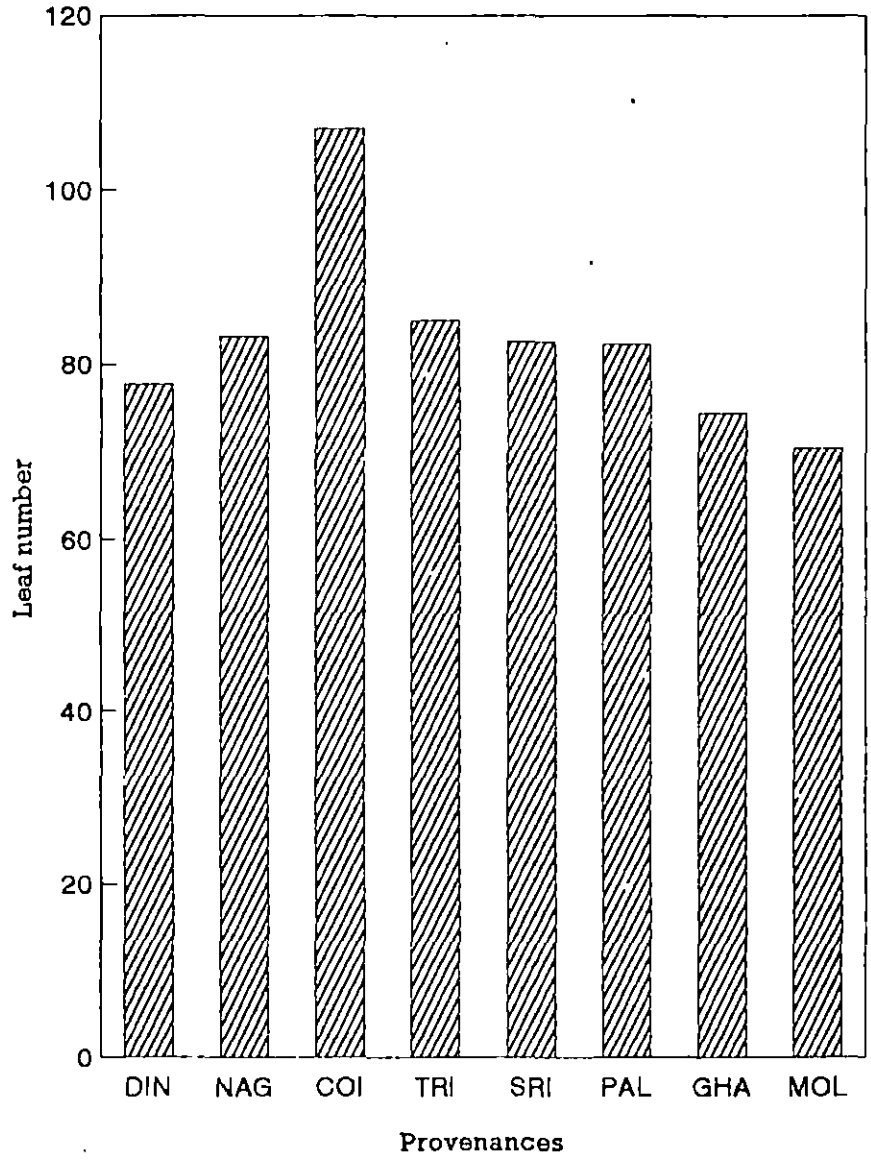


Fig.13 Increase in leaf number of different provenances of neem at different growth phases



DIN-Dindigul NAG-Nagarcoil COI-Coimbatore TRI-Trichur
SRI-Srivelliputhur PAL-Palghat GHA-Ghatti Subramanya MOL-Molakalmur

Fig.14 Variation in leaf number of different provenances of neem at 360DAS

At 30 DAS leaf number was maximum for Coimbatore (9.0) and minimum for Dindugul (5.8). At 360 DAS also Coimbatore had the maximum leaf number (107.0), but the minimum was for Molakalmur (70.4).

Almost similar trend in the increase in leaf number was observed in three provenances viz., Nagarcoil, Trichur and Srivelliputhur for the entire growth period.

4.2.1.4 Leaf area

Leaf area showed a significant variation only for the periods 60, 90 and 120 Days after sowing (Table 8). Nagarcoil, Ghatti Subramanya and Trichur provenances were significantly superior to others in the growth phases 60, 90 and 120 DAS, respectively. At the same time, Molakalmur recorded minimum value at 60 DAS and Dindugul recorded the minimum value for leaf area at 90 and 120 DAS. In all the other growth phases, the provenances were at par with reference to this character.

At 30 DAS the maximum value for leaf area was recorded by Trichur (43.40 cm^2) and the minimum value by Molakalmur (17.80 cm^2). But at 360 days after sowing the maximum value was recorded for Coimbatore (1986.57 cm^2) and the minimum value for Srivelliputhur (1210.11 cm^2).

At 30 DAS four provenances viz., Nagarcoil, Coimbatore, Trichur and Palakkadu recorded maximum values over the general mean. But at 360 DAS Dindugul, Coimbatore, Trichur and Ghatti Subramanya recorded maximum values over the general mean.

Table 8 Data showing variation in leaf area (cm^2) of seedlings of different provenances of neem

Provenances	30 DAS	60 DAS	90 DAS	120 DAS	150 DAS	180 DAS	210 DAS	240 DAS	270 DAS	300 DAS	330 DAS	360 DAS
Dindigal	20.63	140.20	130.60	131.80	785.08	914.45	1024.17	1252.37	1361.84	1550.17	1640.60	1682.25
Nagercoil	34.20	237.00	337.40	369.40	456.38	588.85	728.58	841.96	909.65	1030.47	1111.78	1253.49
Coimbatore	35.80	66.00	183.20	227.60	734.35	885.65	1082.51	983.02	1245.75	1283.95	1740.77	1986.57
Trichur	43.40	81.20	332.95	452.40	587.55	578.16	785.29	1045.35	1059.76	1192.49	1293.01	1922.58
Srivelli-puthur	28.20	93.40	237.20	294.20	523.56	626.24	735.46	752.31	946.02	1085.09	1151.87	1210.11
Palakkadu	34.20	93.00	297.40	312.60	589.05	706.67	823.92	98.82	1237.49	1365.43	1301.61	1452.39
Ghatti Subramanya	20.20	79.00	401.79	401.78	646.15	764.40	957.28	149.66	1079.97	1230.78	1473.04	1586.39
Molakalmur	17.80	61.80	319.97	430.71	526.89	600.67	760.52	847.85	761.12	1074.61	1245.85	1319.65
Mean	29.3	106.5	280.1	327.6	606.1	708.1	862.2	969.3	1100.2	1226.6	1369.8	1552.7
CD (0.05)	NS	80.83	102.6	138.9	NS	NS	NS	NS	NS	NS	NS	NS
SEm (±)	3.14	12.50	17.63	22.47	38.04	39.50	49.79	57.97	60.02	58.94	97.68	99.2

4.2.1.5 Root length

Significant variation in root length was found during the periods from 60 to 180 Days after sowing and also from 270 to 330 Days after sowing (Table 9). At 30, 210, 240 and 360 DAS no significant difference was found between provenances.

During 60 to 120 DAS the maximum value was recorded by Nagarcoil and later replaced by Dindugul up to 180 DAS. At 270 DAS the maximum root length was recorded by Ghatti Subramanya and from 300 to 330 Days after sowing Srivelliputhur provenance was significantly superior to the rest.

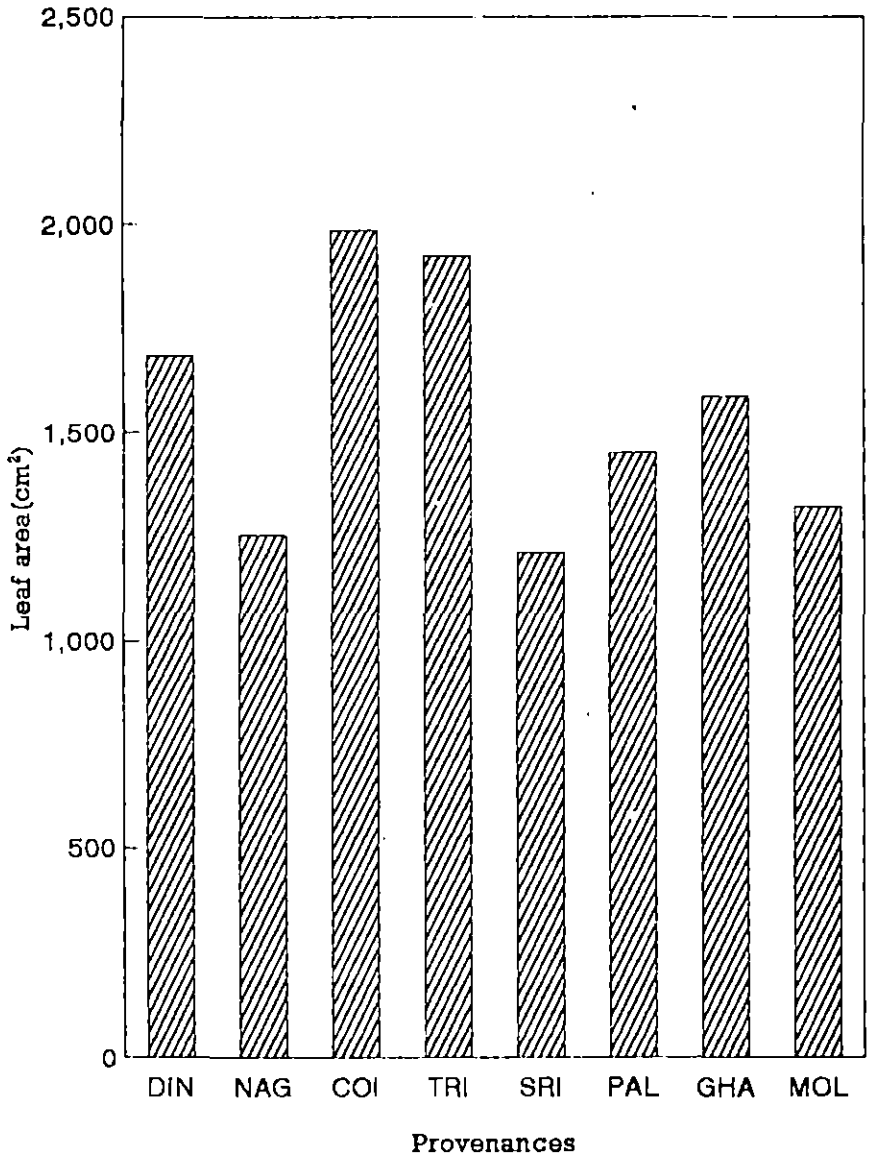
Root length was minimum for the Trichur provenance almost during the entire growth phase. At 360 DAS even though there was no significant difference between provenances, Srivelliputhur (91.30 cm) was found to have the maximum root length compared to others. The minimum value at 360 DAS was recorded by Trichur.

4.2.2 Biomass observations

Results obtained on the biomass characters of neem seedlings of the different provenances are presented in Table 10 to 15.

4.2.2.1 Stem dry weight

Seedlings of different provenances showed a high significant variation in respect of stem dry weight only during the periods of 60, 90, 120, 180, 240, 270, 300 Days after sowing. But no significant difference among provenances was noticed for the periods of 30, 150, 210, 330 and 360 Days after sowing (Table 10).

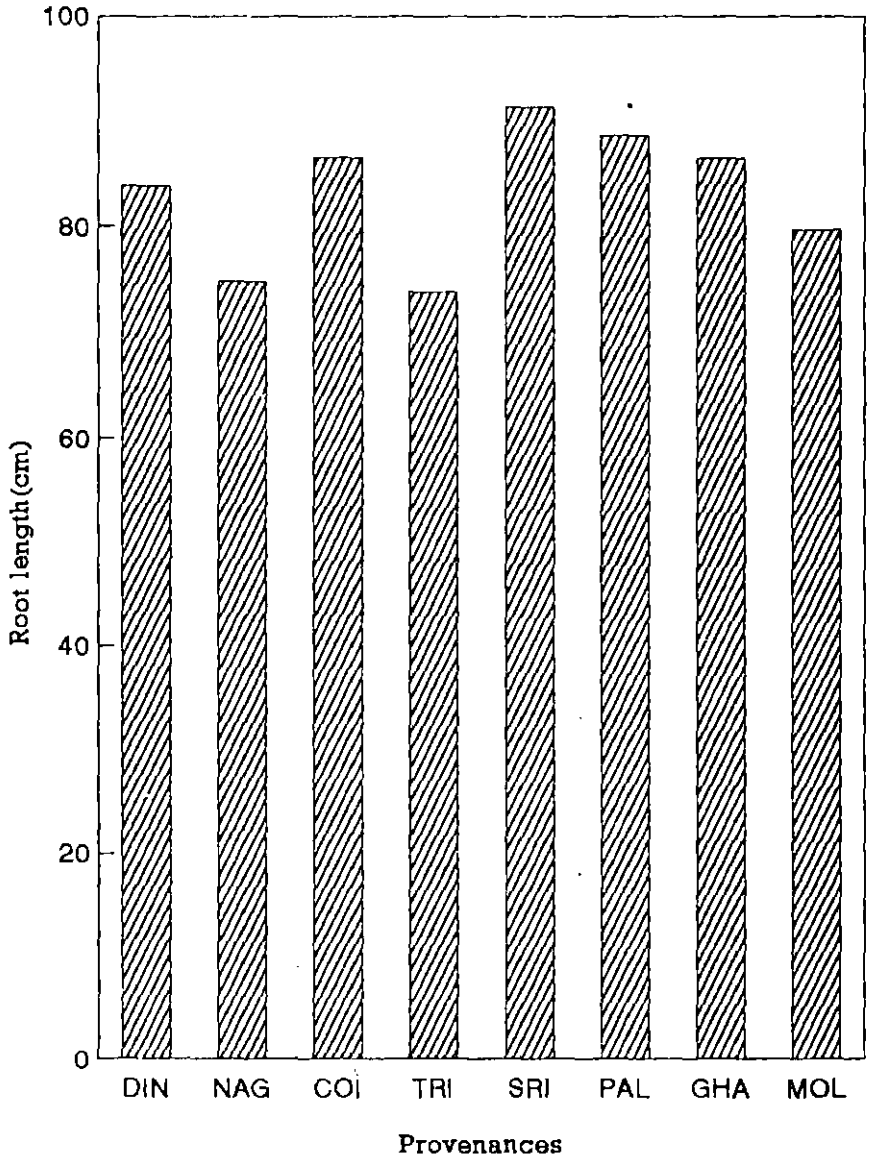


DIN-Dindigul NAG-Nagarcoil COI-Coimbatore TRI-Trichur
SRI-Srivelliputhur PAL-Palghat GHA-Ghatti Subramanya MOL-Molakalmur

Fig.15 Variation in leaf area of different provenances of neem at 360DAS

Table 9 Data showing variation in root length (cm) of seedlings of different provenances of neem

Provenances	30 DAS	60 DAS	90 DAS	120 DAS	150 DAS	180 DAS	210 DAS	240 DAS	270 DAS	300 DAS	330 DAS	360 DAS
Dindigal	18.92	42.40	38.24	37.42	42.98	49.56	46.64	52.02	52.14	58.62	78.18	83.90
Nagercoil	21.40	43.94	43.80	45.30	40.22	47.30	51.10	56.24	58.66	64.78	65.16	74.82
Coimbatore	20.98	23.00	27.50	30.38	32.40	39.92	45.80	52.32	58.92	65.06	72.60	86.50
Trichur	21.92	24.68	13.02	24.50	31.90	38.44	43.82	49.42	54.92	60.54	60.72	73.72
Srivelliputhur	20.36	36.94	22.44	38.74	37.06	44.40	49.96	55.80	61.72	71.46	81.64	91.30
Palakkadu	19.70	27.36	37.62	38.60	35.24	42.28	49.14	55.84	62.86	69.88	77.52	88.62
Ghatti Subramanya	15.06	26.00	21.22	28.48	35.44	42.66	49.38	56.16	65.12	65.58	79.48	86.48
Molakalmur	15.42	23.04	19.54	26.10	32.76	39.36	45.66	52.22	58.52	63.26	71.42	79.60
Mean	19.22	30.9	28.5	33.70	36.00	43.0	47.7	53.8	59.1	64.9	73.3	83.1
CD (0.05)	NS	8.00	5.13	6.86	5.83	6.96	NS	NS	7.75	7.59	11.09	NS
SEm (±)	1.00	1.59	1.60	1.34	0.97	0.97	0.81	0.87	1.07	1.06	1.65	1.97



DIN-Dindigul NAG-Nagarcoil COI-Coimbatore TRI-Trichur
SRI-Srivelliputhur PAL-Palghat GHA-Ghatti Subramanya MOL-Molakalmur

Fig.16 Variation in root length of different provenances of neem at 360DAS

Table 10 Data showing variation in stem dryweight(g) of seedlings of different provenances of neem

Provenances	30 DAS	60 DAS	90 DAS	120 DAS	150 DAS	180 DAS	210 DAS	240 DAS	270 DAS	300 DAS	330 DAS	360 DAS
Dindigal	0.07	0.35	0.65	1.08	10.08	18.14	20.59	22.84	25.50	28.15	31.41	33.88
Nagercoil	0.13	0.66	1.47	1.89	8.20	10.92	13.25	16.39	9.24	20.11	23.32	24.84
Coimbatore	0.10	0.17	1.21	2.63	11.88	16.01	17.75	20.29	22.83	25.37	32.29	35.23
Trichur	0.07	0.51	1.79	5.11	10.33	14.56	16.39	16.58	17.45	19.07	28.89	31.49
Srivelliputhur	0.06	0.18	0.33	1.75	8.34	11.29	13.65	17.21	21.52	22.13	22.18	22.36
Palakkadu	0.07	0.43	1.25	1.75	7.09	11.62	13.99	18.53	16.41	15.93	19.67	22.86
Ghatti Subramanya	0.06	0.18	3.39	5.31	7.75	10.20	10.49	13.97	15.88	17.64	18.37	19.23
Molakalmur	0.03	0.19	2.38	5.07	5.73	8.11	9.37	10.59	12.41	13.13	15.60	15.68
Mean	0.07	0.34	1.56	3.07	8.70	12.61	14.44	17.05	17.66	20.19	23.60	25.57
CD (0.05)	NS	0.23	0.69	1.54	NS	6.24	NS	6.89	6.77	7.82	NS	NS
SEm (±)	0.01	0.04	0.17	0.32	0.54	0.85	0.98	0.95	1.11	1.14	2.25	2.12

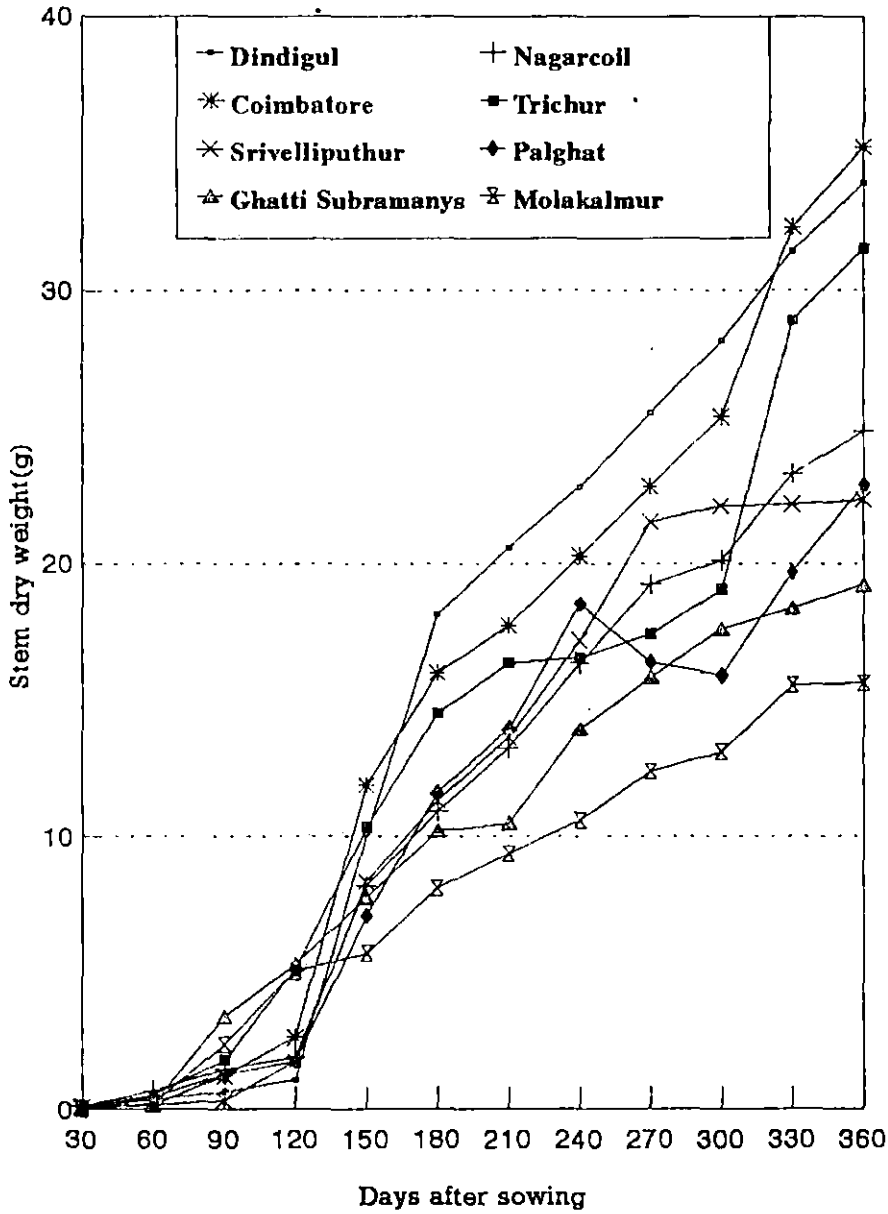
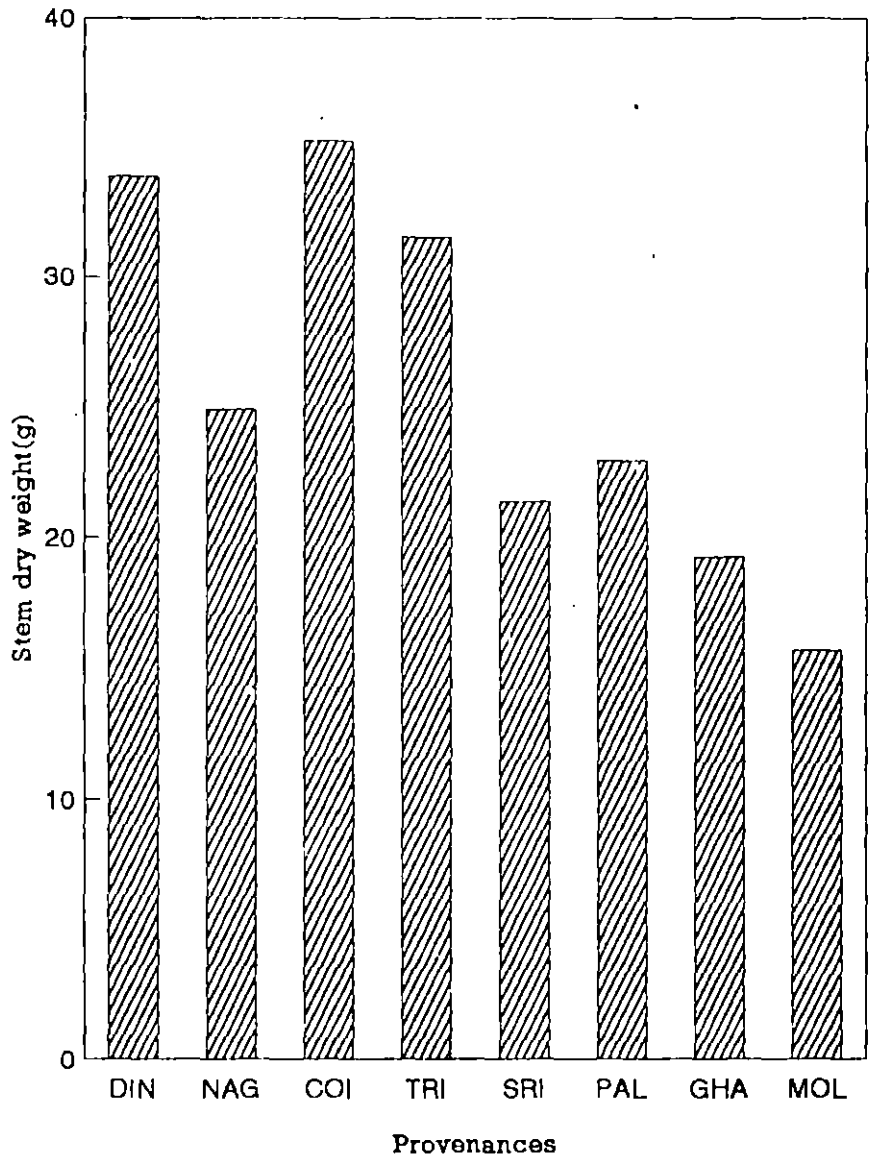


Fig.17 Increase in stem dry weight of different provenances of neem at different growth phases



DIN-Dindigul NAG-Nagarcoil COI-Coimbatore TRI-Trichur
SRI-Srivelliputhur PAL-Palghat GHA-Ghatti Subramanya MOL-Molakalmur

Fig.18 Variation in stem dry weight of different provenances of neem at 360DAS

In the earlier growth stages of 30 and 60 DAS maximum value of dry weight was shown by Nagarcoil and later by Ghatti Subramanya for 90 and 120 DAS. Then Coimbatore showed a maximum value at 150 DAS though statistically at par with the rest of the provenances. From 180 DAS and also from 240 to 300 DAS Dindugul showed the significant stem dry matter accumulation. At the end of the growth phase Coimbatore again showed the maximum value but had no statistical significance.

At 30 DAS two provenances viz., Nagarcoil and Coimbatore recorded higher values over the general mean. But at 360 DAS the situation was that three provenances viz., Dindugul, Coimbatore and Trichur recorded the maximum values over the general mean.

4.2.2.2 Leaf dry weight

Leaf dry weight did not show any significant difference among provenances after 150 DAS to the end of the growing period of 360 DAS. Significant variation among the provenances were found only for the periods of 30, 60, 90 and 120 Days after sowing (Table 11).

The maximum significant value for leaf dry weight was shown by Ghatti Subramanya (4.68 g) and minimum value recorded by Dindugul (1.35 g) at 120 DAS.

Even though there was no statistical difference existing at 360 DAS, the maximum value was recorded by Coimbatore (20.18 g) and minimum value by Srivelliputhur (12.37 g).

Table 11 Data showing variation in leaf dryweight (g) of seedlings of different provenances of neem

Provenances	30 DAS	60 DAS	90 DAS	120 DAS	150 DAS	180 DAS	210 DAS	240 DAS	270 DAS	300 DAS	330 DAS	360 DAS
Dindigal	0.06	0.58	1.48	1.35	7.97	7.97	10.40	12.72	13.83	15.75	16.67	17.88
Nagercoil	0.38	1.26	1.57	1.91	4.64	5.98	7.38	8.55	9.24	10.47	11.29	12.73
Coimbatore	0.14	0.20	0.63	1.43	7.46	8.99	11.00	11.00	12.72	13.04	17.68	20.18
Trichur	0.28	1.36	3.38	4.59	5.97	5.87	7.98	10.62	10.77	12.11	13.13	19.53
Srivelliputhur	0.06	0.35	1.42	1.83	5.32	6.36	7.47	7.64	9.61	14.65	11.70	12.37
Palakkadu	0.07	1.02	1.46	1.89	5.99	7.18	8.37	9.95	10.36	11.95	13.16	14.75
Ghatti Subramanya	0.23	0.12	3.68	4.68	6.46	7.77	9.72	10.66	10.97	12.50	14.96	16.12
Molakalmur	0.06	0.13	3.25	3.78	5.35	6.10	7.26	8.61	9.76	8.98	12.66	13.41
Mean	0.16	0.63	2.11	2.68	6.15	7.03	8.70	9.97	10.91	12.43	13.91	15.87
CD (0.05)	0.195	0.549	1.03	1.42	NS	NS	NS	NS	NS	NS	NS	NS
SEm (±)	0.03	0.10	0.21	0.27	0.39	0.38	0.51	0.57	0.60	0.79	0.99	1.01

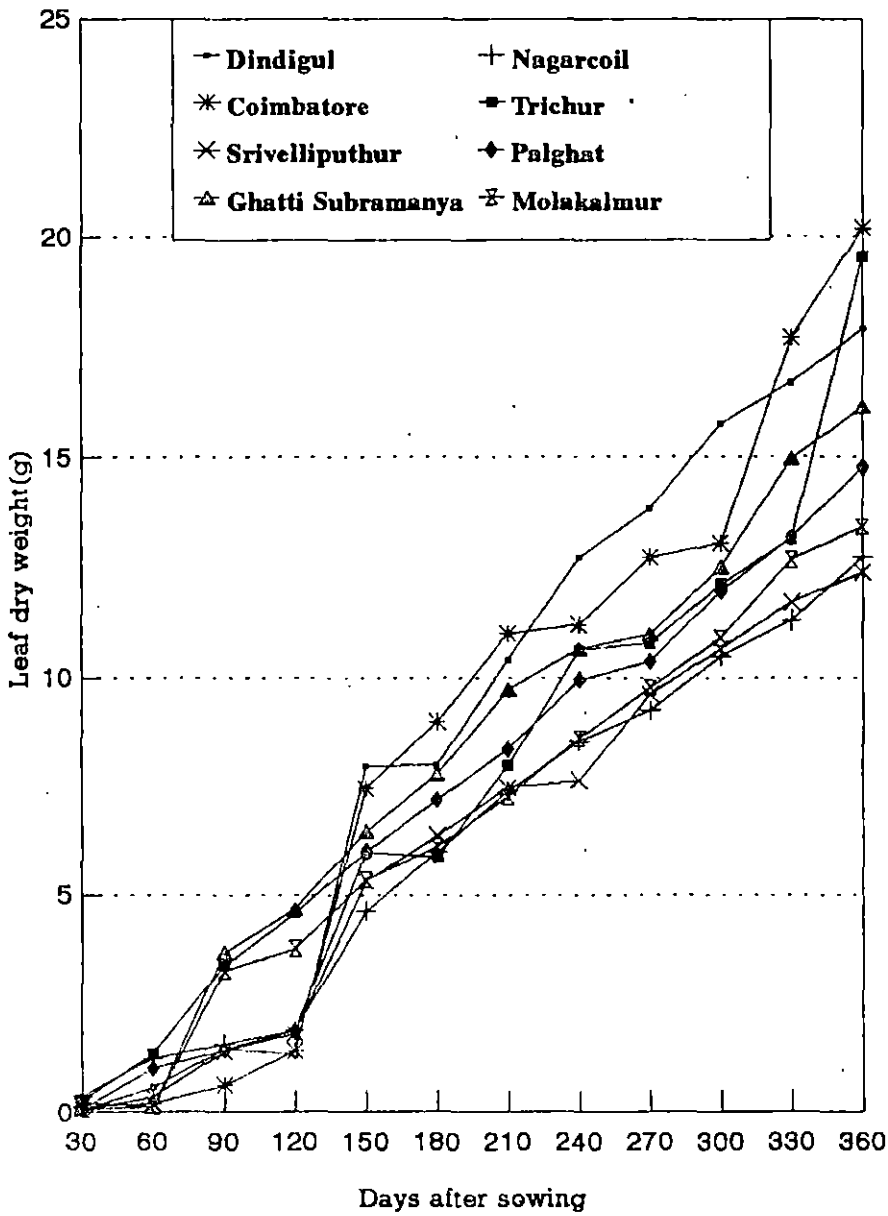
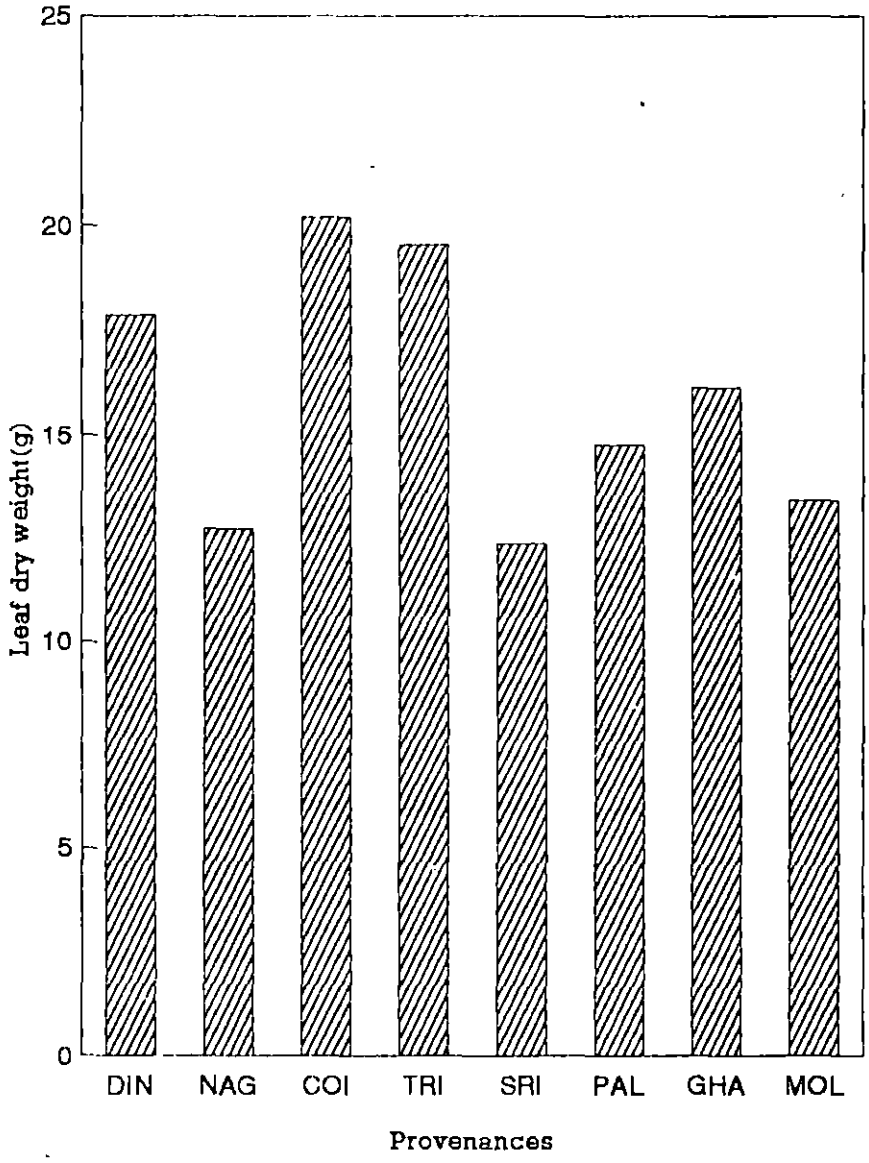


Fig.19 Increase in leaf dry weight of different provenances of neem at different growth phases



DIN-Dindigul NAG-Nagarcoil COI-Coimbatore TRI-Trichur
SRI-Srivelliputhur PAL-Palghat GHA-Ghatti Subramanya MOL-Molakalmur

Fig.20 Variatin in leaf dry weight of different provenances of neem at 360DAS

At 30 DAS three provenances viz., Nagarcoil, Trichur and Gatti Subramanya recorded higher values over general mean and at 360 DAS four provenances viz., Dindugul, Coimbatore, Trichur, and Ghatti Subramanya recorded higher values over the general mean.

At 30 DAS the best performer was Nagarcoil and immediately replaced by Trichur at 60 DAS and again by Ghatti Subramanya at 90 and 120 DAS.

4.2.2.3 Root dry weight

Significant variation in root dry weight was recorded for the entire growth phases except for 150 and 360 DAS as shown in Table 12.

At 30 and 60 DAS maximum root dry weight was observed for Srivelliputhur and replaced by Ghatti Subramanya at 90 and 120 DAS. After that from 150 to 360 DAS maximum values were recorded by Palakkadu.

At 30 DAS the maximum significant value was recorded by Srivelliputhur (0.28 g) and minimum values were recorded by Trichur (0.07 g) and Ghatti Subramanya (0.07 g). At 330 DAS the maximum significant value was recorded by Palakkadu (36.94 g) and the minimum value was recorded by Molakalmur (16.75 g).

At 30 DAS three provenances viz., Nagarcoil, Srivelliputhur and Palakkadu recorded higher values over he general mean. At 360 DAS four provenances viz., Dindugul, Trichur, Srivelliputhur and Palakkadu recorded higher values over general mean.

Table 12 Data showing variation in root dryweight (g) of seedlings of different provenances of neem

Provenances	30 DAS	60 DAS	90 DAS	120 DAS	150 DAS	180 DAS	210 DAS	240 DAS	270 DAS	300 DAS	330 DAS	360 DAS
Dindigal	0.05	0.59	1.11	1.38	10.29	16.42	19.12	22.84	26.35	29.12	29.79	32.12
Nagercoil	0.11	1.35	1.90	2.15	8.26	11.49	13.62	15.61	12.07	20.92	20.84	24.74
Coimbatore	0.09	0.24	1.19	2.64	10.14	14.22	17.07	19.49	21.39	25.09	23.82	29.65
Trichur	0.07	0.72	2.60	4.87	10.54	13.82	16.42	18.67	20.57	22.16	24.93	33.34
Srivelliputhur	0.28	2.54	0.45	2.54	11.39	17.51	19.78	22.65	25.49	28.23	31.16	32.63
Palakkadu	0.12	0.49	1.77	2.32	11.77	18.94	22.46	25.59	28.84	32.16	36.94	39.48
Ghatti Subramanya	0.07	0.23	6.66	8.65	11.56	14.11	16.06	18.83	70.20	21.12	28.07	30.02
Molakalmur	0.08	0.23	3.57	5.89	2.37	8.74	10.18	11.79	13.79	15.31	16.75	18.86
Mean	0.11	0.80	2.41	3.81	10.17	14.41	16.84	19.43	21.84	24.26	26.54	30.10
CD (0.05)	0.07	0.41	1.49	1.67	NS	5.26	5.53	6.10	6.76	6.85	10.56	NS
SEm (±)	0.01	0.13	0.34	0.41	0.43	0.76	0.86	0.94	1.05	1.12	1.51	1.71

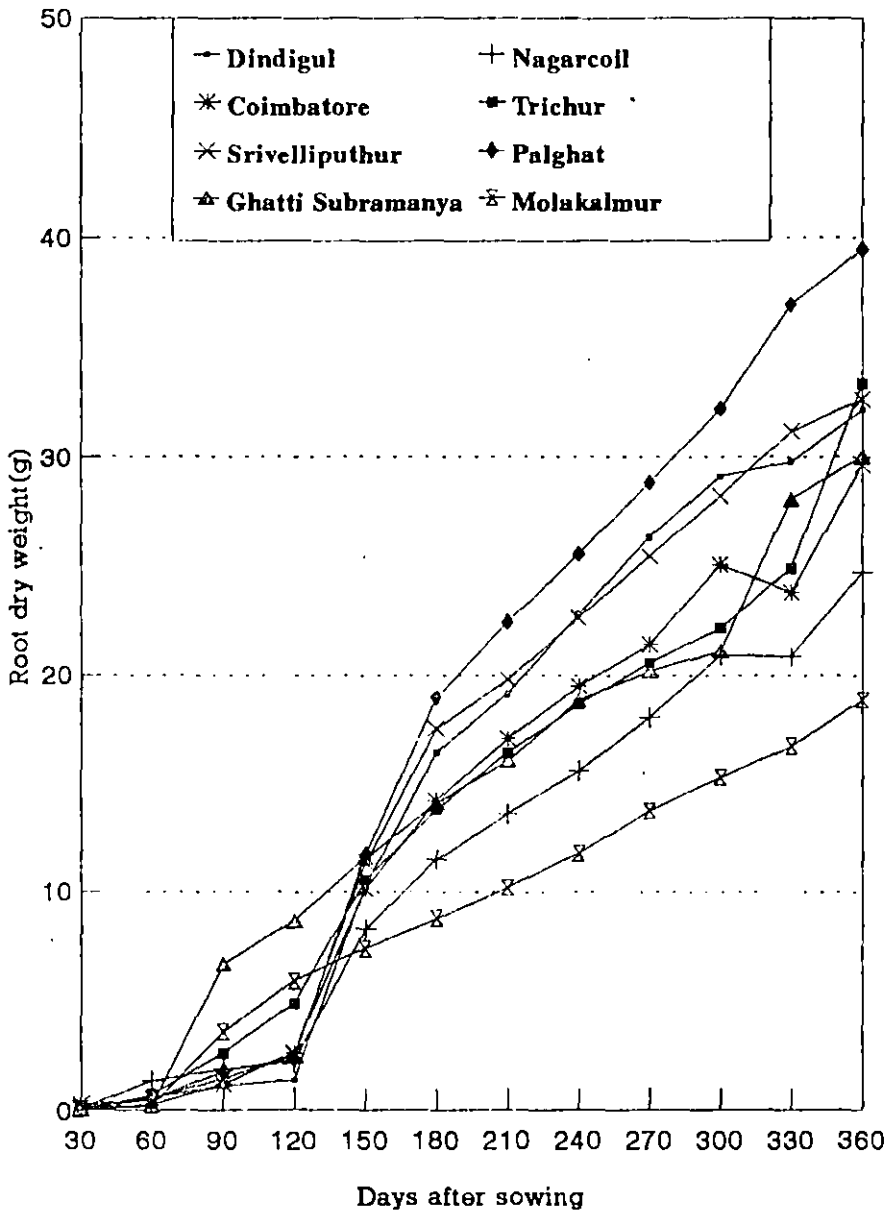
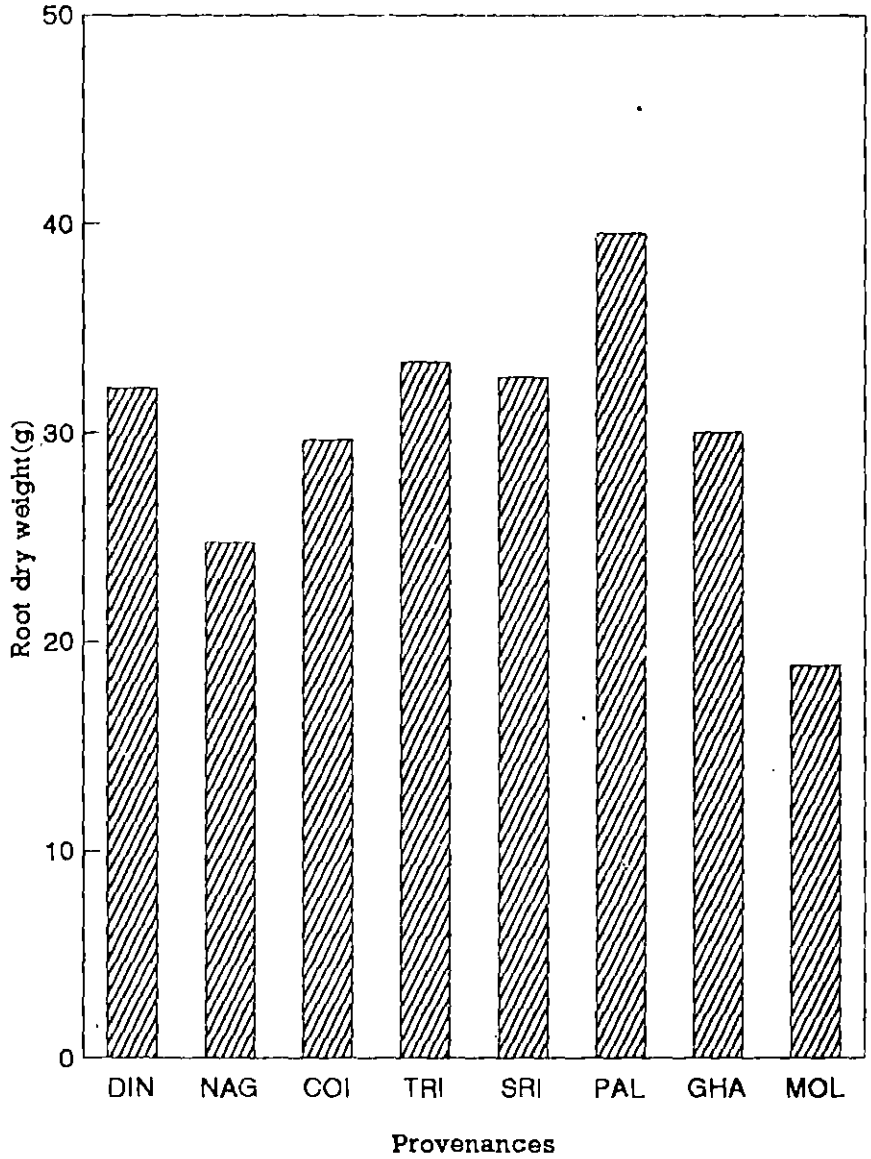


Fig.21 Increase in root dry weight of different provenances of neem at different growth phases



DIN-Dindigul NAG-Nagarcoil COI-Coimbatore TRI-Trichur SRI-Srivelliputhur PAL-Palghat GHA-Ghatti Subramanya MOL-Molakalmur

Fig.22 Variation in root dry weight of different provenances of neem at 360DAS

Local provenance showed better performance in root dry weight and was almost at par with the Ghatti Subramanya from 150 DAS onwards.

4.2.2.4 Shoot dry weight

Significant difference in shoot weight was exhibited by the provenances especially during the earlier growth phase of 30 to 120 Days after sowing. Significant variation was also recorded at 270 and 300 Days after sowing (Table 13).

The data showed that the maximum value at 30 DAS for shoot weight was recorded by Nagarcoil (0.51 g) and the minimum value by Molakalmur (0.09 g). But at the end of the growth phase ie., 360 DAS the maximum value was recorded by Coimbatore (55.4 g) and the minimum value again by Molakalmur (29.09 g).

At 30 DAS four provenances, viz., Nagarcoil, Trichur, Coimbatore and Ghatti Subramanya showed maximum values over the general mean.

In contrary to this only three provenances viz., Dindugul, Coimbatore and Trichur recorded higher values over the general mean.

At 30 and 60 DAS higher values for the shoot weight was recorded by Nagarcoil. Ghatti Subramanya was the best performer at 90 and 120 DAS and later replaced by Dindugul from 150 to 300 DAS. At the end of the growth phase ie. 330 to 360 DAS maximum value was recorded by Coimbatore.

Table 13 Data showing variation in shoot weight (g) of seedlings of different provenances of neem

Provenances	30 DAS	60 DAS	90 DAS	120 DAS	150 DAS	180 DAS	210 DAS	240 DAS	270 DAS	300 DAS	330 DAS	360 DAS
Dindigal	0.13	0.94	2.13	2.43	18.06	26.11	30.10	35.57	39.33	43.90	48.07	51.76
Nagercoil	0.51	1.92	3.05	3.79	12.84	16.90	20.63	24.94	18.48	30.58	34.61	37.57
Coimbatore	0.24	0.37	1.84	4.06	19.34	25.01	28.76	31.30	35.55	38.42	49.98	55.40
Trichur	0.35	1.88	5.18	9.71	16.30	20.44	24.37	27.20	28.21	31.18	42.03	51.02
Srivelliputhur	0.12	0.53	1.75	3.58	13.66	17.65	21.12	24.85	31.13	36.78	30.94	33.70
Palakkadu	0.14	1.45	2.71	3.64	13.08	18.80	22.36	28.49	26.77	27.88	32.83	37.61
Ghatti Subramanya	0.29	0.30	7.07	9.99	14.21	17.98	20.21	24.53	26.85	30.15	33.33	35.34
Molakalmur	0.09	0.32	5.63	8.85	11.09	14.21	16.63	19.20	22.17	22.11	28.26	29.09
Mean	0.23	0.96	3.67	5.76	14.82	19.64	23.13	27.02	28.56	32.62	37.51	41.44
CD (0.05)	0.22	0.75	1.22	2.40	NS	NS	NS	NS	9.67	11.81	NS	NS
SEm (±)	0.03	0.13	0.33	0.54	0.76	1.08	1.34	1.37	1.48	1.64	2.83	2.95

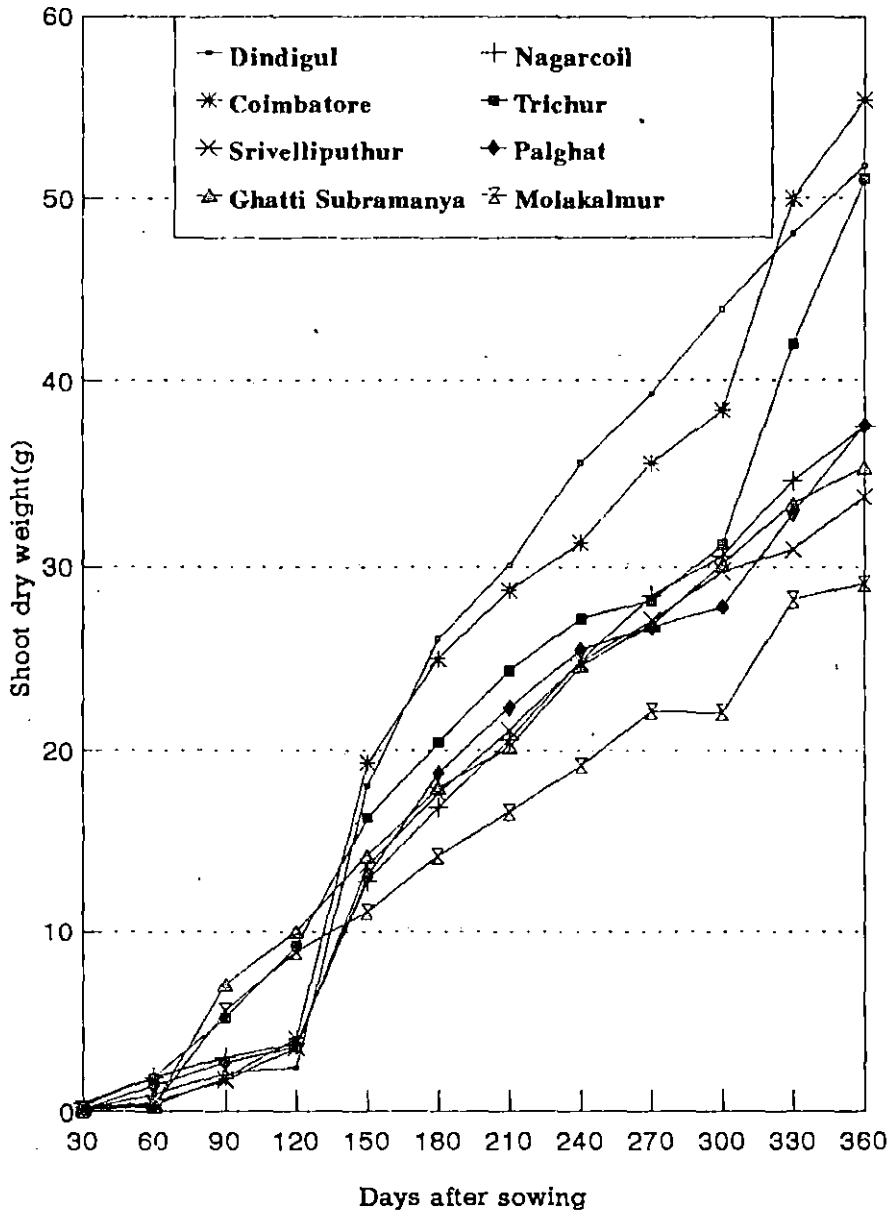
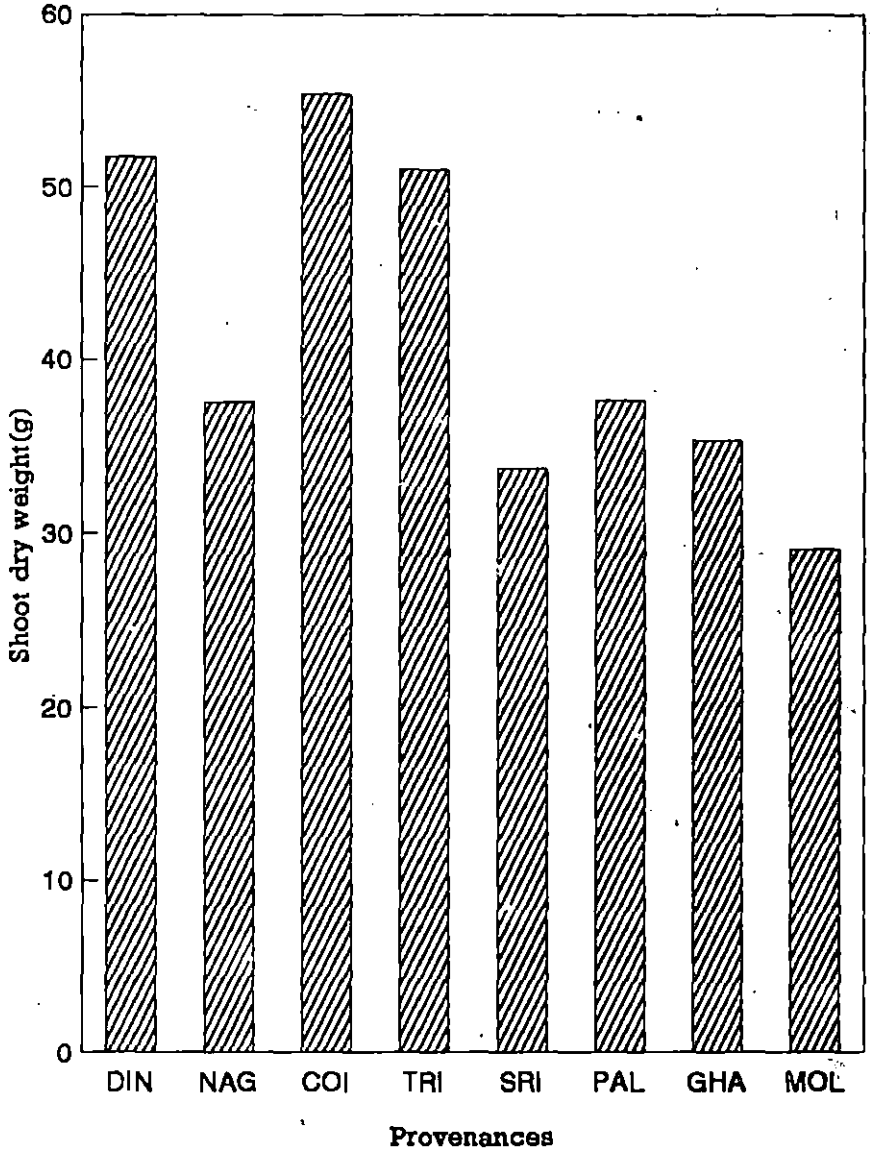


Fig.23 Increase in shoot dry weight of different provenances of neem at different growth phases



DIN-Dindigul NAG-Nagarcoil COI-Coimbatore TRI-Trichur SRI-Srivelliputhur PAL-Palghat GHA-Ghatti Subramanya MOL-Molakalmur

Fig.24 Variation in shoot dry weight of different provenances of neem at 360DAS

4.2.2.5 Total dry weight

Statistically significant variation in terms of total dry weight was recorded between provenances except for the growth phases of 150, 180, 210, 330 and 360 Days after sowing (Table 14).

Maximum significant values for total dry weight was recorded by Nagarcoil at 30 and 60 DAS. From 90 to 120 DAS this was replaced by Ghatti Subamanya which was significantly superior to the rest. The values for Coimbatore provenance was maximum at 150 DAS and 360 DAS though not found significant. From 240 DAS to 300 DAS Dindugul was having the maximum values and were statistically superior.

At 30 DAS four provenances viz., Nagarcoil, Trichur, Srivelliputhur and Ghatti Subramanya recorded higher values over the general mean. And at 360 DAS other four provenances viz., Dindugul, Coimbatore, Trichur and Palakkadu recorded higher values over the general mean.

Three hundred days after sowing was the maximum period after which significant difference was found. During this period four provenances viz., Dindugul, Coimbatore, Srivelliputhur and Palakkadu recorded maximum values over the general mean.

4.2.2.6 Root : Shoot ratio

As far as root shoot ratio is concerned, provenances showed significant differences for all the periods except 90 and 120 DAS (Table 15).

Table 14 Data showing variation in total dryweight(g) of seedlings of different provenances of neem

Provenances	30 DAS	60 DAS	90 DAS	120 DAS	150 DAS	180 DAS	210 DAS	240 DAS	270 DAS	300 DAS	330 DAS	360 DAS
Dindigal	0.18	1.52	3.24	3.81	28.34	42.53	50.11	58.40	65.69	73.02	77.87	83.88
Nagercoil	0.62	3.27	4.95	5.94	21.10	28.40	34.25	40.55	36.55	51.49	55.45	62.31
Coimbatore	0.33	0.61	3.03	6.69	29.47	39.23	45.83	50.79	56.95	63.51	73.79	85.06
Trichur	0.42	2.59	7.78	14.57	26.84	34.26	40.79	45.87	48.78	53.34	66.97	84.36
Srivelliputhur	0.39	3.07	2.20	6.11	25.06	35.17	40.90	47.49	56.62	65.01	62.11	66.33
Palakkadu	0.26	1.95	4.48	5.96	24.85	37.73	44.81	54.08	55.61	60.03	69.77	77.09
Ghatti Subramanya	0.35	0.53	13.73	18.64	25.77	32.04	36.27	43.46	47.05	51.27	61.39	65.36
Molakalmur	0.18	0.54	9.20	14.75	18.46	22.94	26.81	30.99	35.95	37.41	45.00	47.94
Mean	0.34	1.76	6.08	9.56	24.99	34.04	39.97	46.45	50.40	56.89	64.04	71.54
CD (0.05)	0.25	1.08	1.98	3.57	NS	NS	NS	15.32	15.01	17.38	NS	NS
SEm (±)	0.04	0.21	0.63	0.92	1.06	1.65	1.98	2.13	2.28	2.53	3.79	4.24

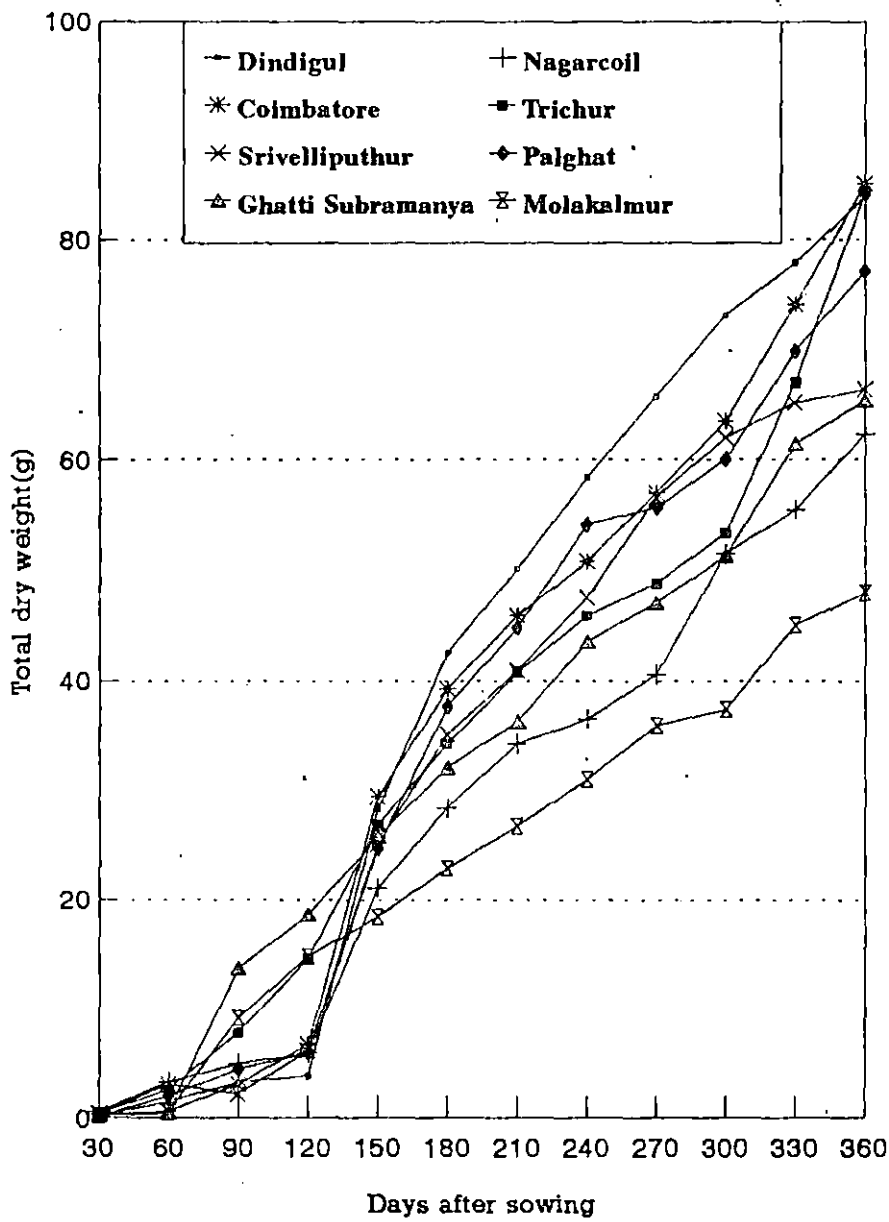
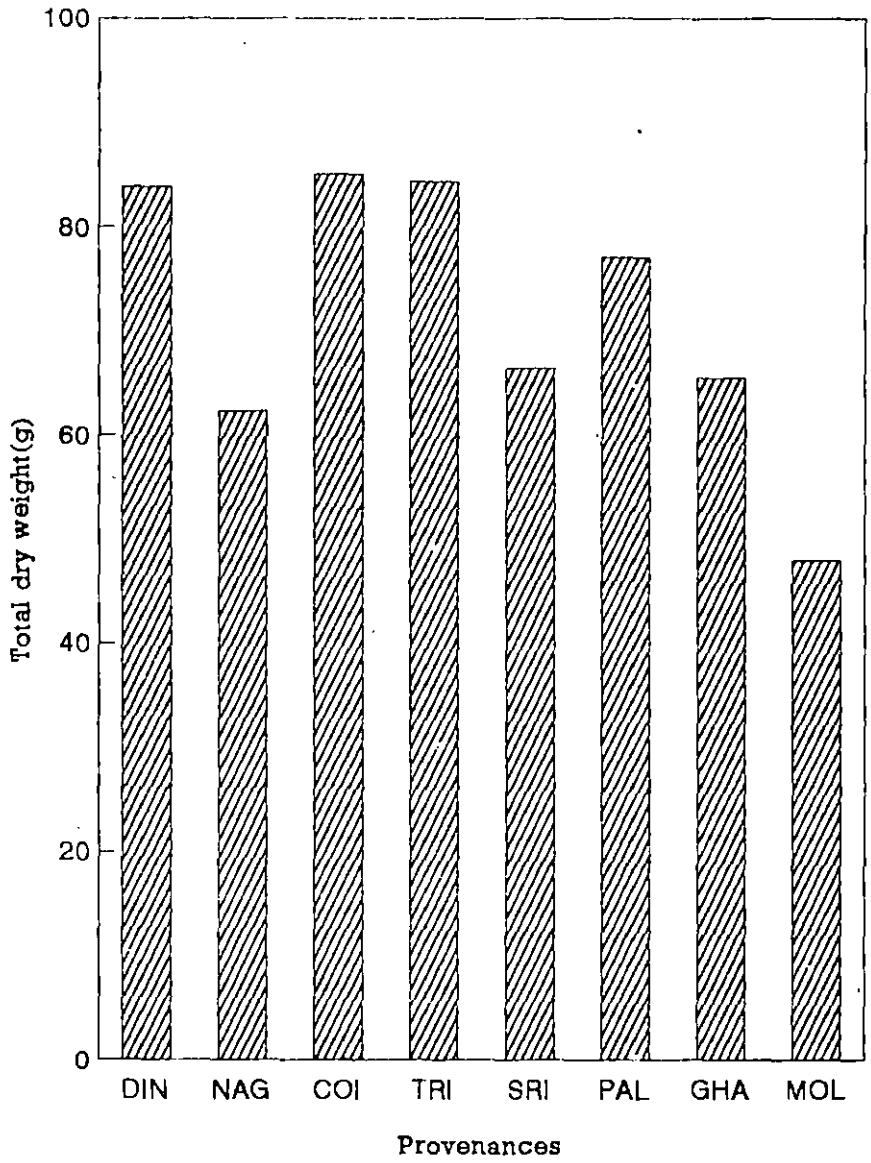


Fig.25 Increase in total dry weight of different provenances of neem at different growth phases

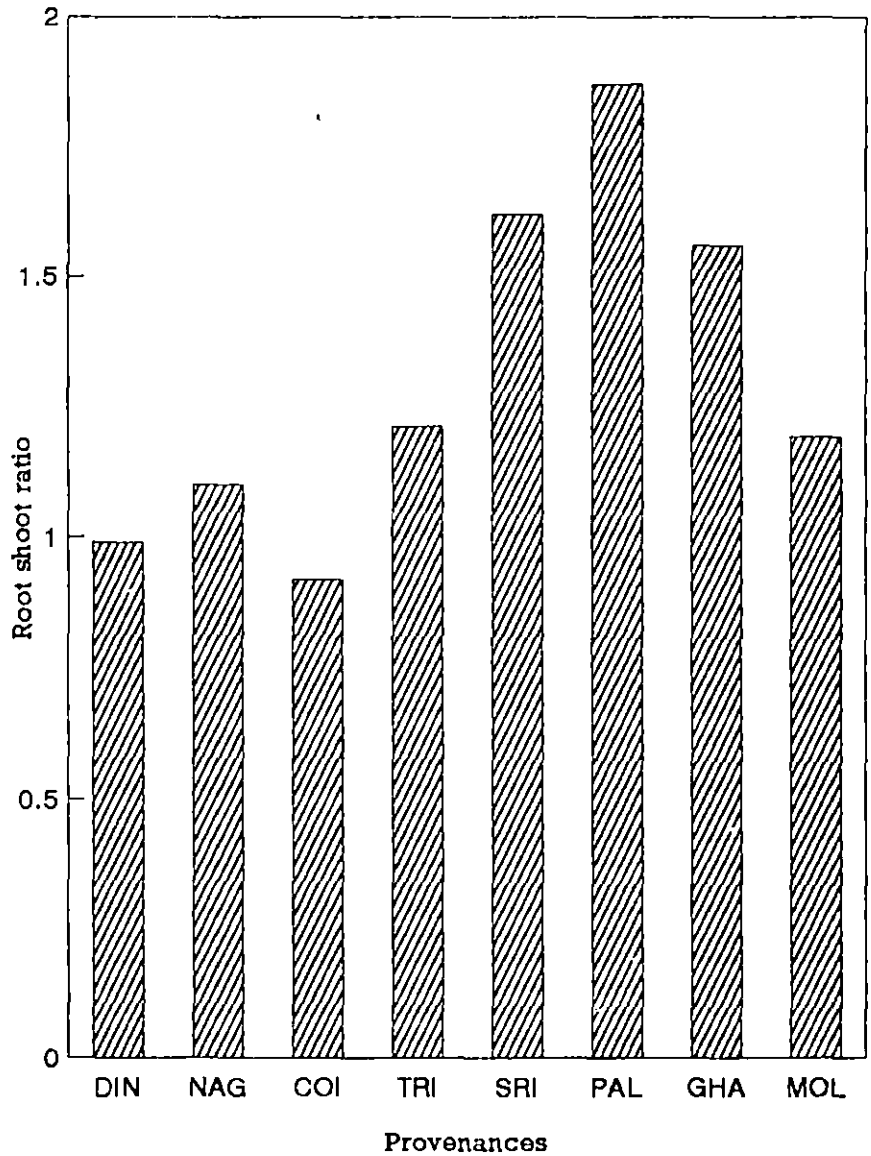


DIN-Dindigul NAG-Nagarcoil COI-Coimbatore TRI-Trichur SRI-Srivelliputhur PAL-Palghat GHA-Ghatti Subramanya MOL-Molakalmur

Fig.26 Variation in total dry weight of different provenances of neem at 360DAS

Table 15 Data showing variation in root-shoot ratio of seedlings of different provenances of neem

Provenances	30 DAS	60 DAS	90 DAS	120 DAS	150 DAS	180 DAS	210 DAS	240 DAS	270 DAS	300 DAS	330 DAS	360 DAS
Dindigal	0.77	1.75	3.49	1.32	1.05	0.90	0.94	1.02	1.07	1.08	1.01	0.99
Nagercoil	1.00	2.13	1.30	1.14	1.08	1.17	1.15	1.01	2.04	1.07	1.11	1.10
Coimbatore	0.80	1.39	1.03	1.06	0.92	0.96	1.03	1.00	0.98	1.03	0.85	0.92
Trichur	0.94	1.45	1.51	1.05	1.09	1.04	1.13	1.16	1.21	1.20	1.25	1.21
Srivelliputhur	4.87	18.26	1.44	1.53	1.41	1.60	1.50	1.36	1.19	1.28	1.71	1.62
Palakkadu	1.63	1.16	1.43	1.36	1.68	1.71	1.67	1.42	1.79	2.12	1.91	1.87
Ghatti Subramanya	1.13	1.24	2.09	1.65	1.52	1.38	1.54	1.34	1.27	1.19	1.53	1.56
Molakalmur	4.46	1.25	1.53	1.19	1.28	1.09	1.10	1.12	1.13	1.17	1.10	1.19
Mean	1.95	3.58	1.73	1.29	1.25	1.23	1.26	1.18	1.34	1.27	1.31	1.31
CD (0.05)	1.47	4.59	NS	NS	0.35	0.38	0.41	0.28	0.32	0.31	0.48	0.48
SEm (±)	0.30	1.03	0.26	0.06	0.06	0.36	0.06	0.04	0.07	0.06	0.08	0.07



DIN-Dindigul NAG-Nagarcoil COI-Colmbatore TRI-Trichur
SRI-Srivelliputhur PAL-Palghat GHA-Ghatti Subramanya MOL-Molakalmur

Fig.27 Variation in root shoot ratio of different provenances of neem at 360DAS

At 30 DAS root shoot ratio was maximum for Srivelliputhur (4.87) and minimum for Coimbatore (0.80). But the situation was entirely different at the end of the growing phase of 360 DAS as the maximum value was recorded by Palakkadu (1.87) and the minimum value was recorded by Coimbatore (0.92).

It is transparently clear from the table that the different provenances recorded maximum values at different phases of growth. At the initial growth phases Srivelliputhur (at 30 and 60 DAS), Dindugul (at 90 DAS), and Ghatti Subramanya (at 120 DAS) showed maximum root shoot ratio. But in the later growth phases Palakkadu was found to be the better performer from 150 DAS until the end of the growth phase of 360 DAS in terms of root shoot ratio.

4.3 Biochemical studies

4.3.1 Isozyme analysis

4.3.1.1 Peroxidase isozyme banding pattern of neem

The eight provenances of neem from Kerala, Tamil Nadu and Karnataka were analysed for isozyme variation of peroxidase. Three isozymes were observed in the eight provenances studied. They were named PRX-1 to PRX-3 (Fig.28 and Plate 2).

PRX-1 and PRX-3 were found common in all the provenances. PRX-2 was present only in three provenances viz., Dindigul, Nagarcoil and Trichur. The provenances were classified into two groups based on the presence or absence of PRX-2 (Table 16).

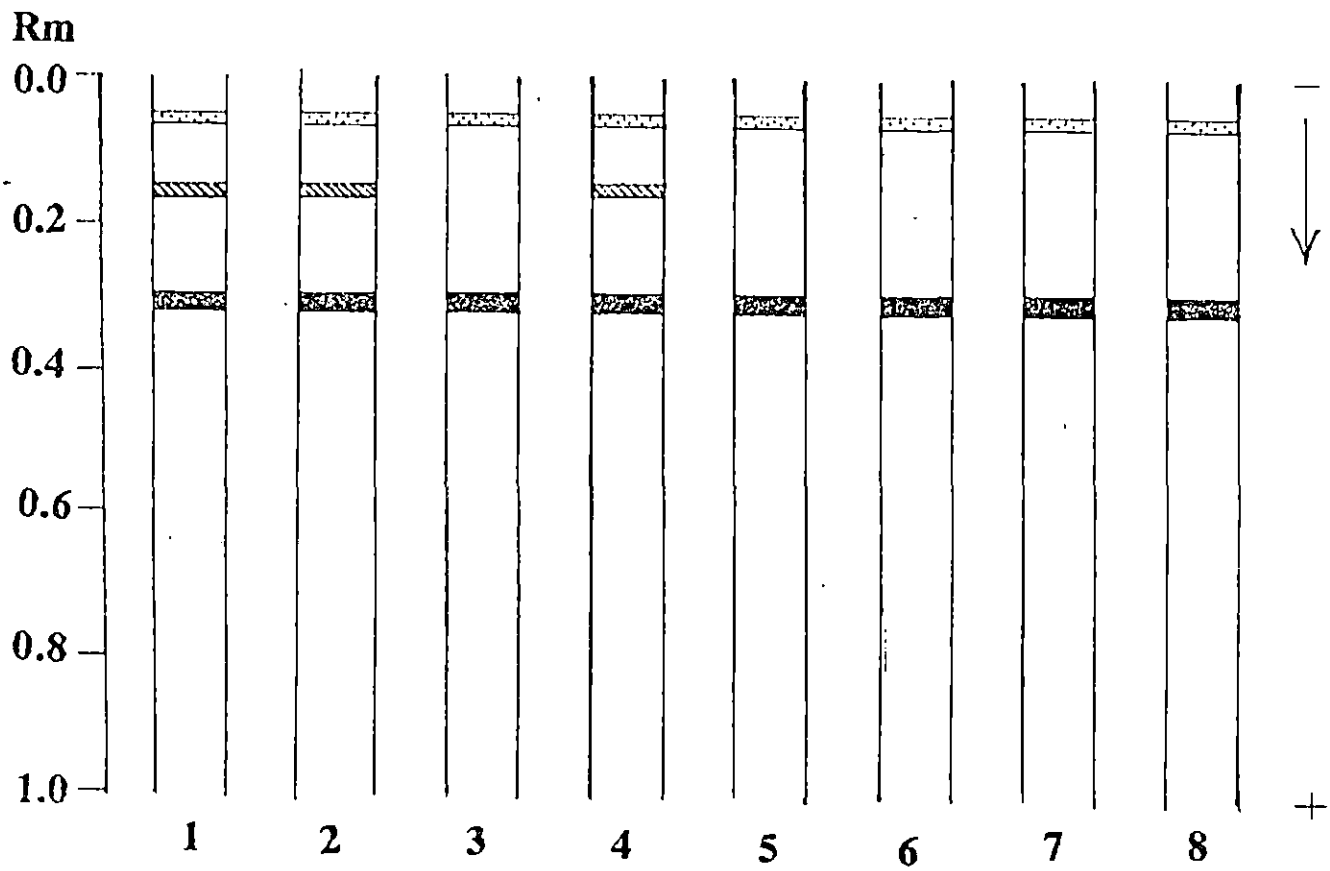


Fig. 28 Peroxidase zymogram of different provenances of neem

Plate 2 Peroxidase banding pattern of different provenances of neem

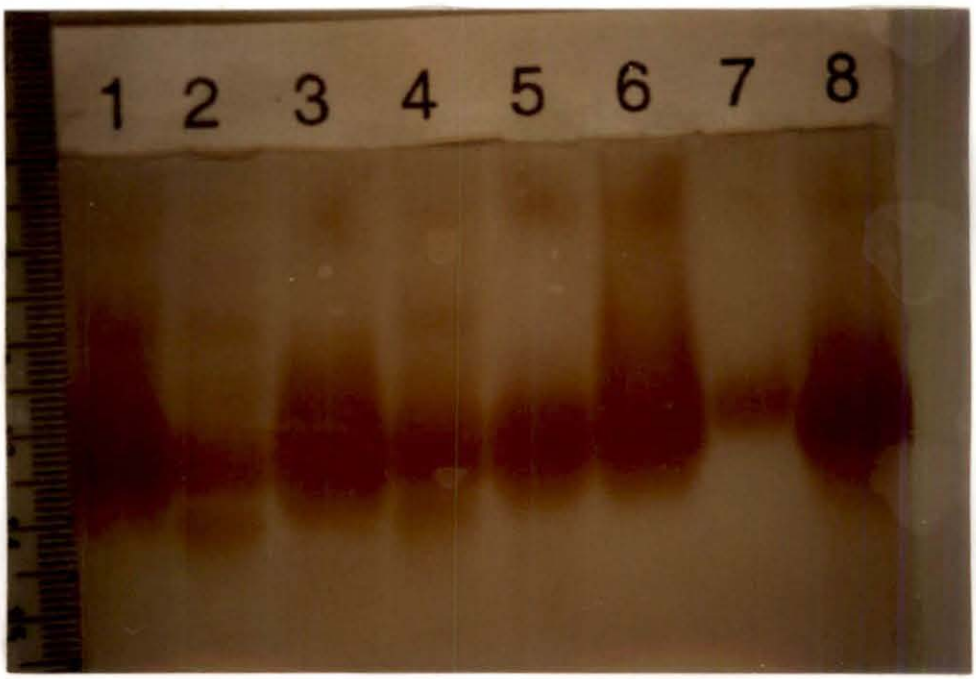

1 2 3 4 5 6 7 8

Table 16 Grouping of neem provenances based on isozyme banding pattern of peroxidase

Group I	Group II
Provenances lacking PRX-2	Provenances with PRX-2
Coimbatore	Dindigul
Srivelliputhur	Nagarcoil
Palghat	Trichur
Ghatti Subramanya	
Molakalmur	

4.3.1.2 Similarity among provenances of neem

The similarity indices among the different provenances for peroxidase isozyme banding pattern was calculated and presented in Table 17. The range of similarity among the provenances for peroxidase zymogram was 0.67 to 1.0.

4.3.2 Chlorophyll content

4.3.2.1 Chlorophyll-A

Significant difference in chlorophyll-A content was noticed only at 180 DAS (Table 18). At this growth stage the maximum value for chlorophyll-A was recorded by Srivelliputhur (1.17 mg/g) and the minimum by Trichur (0.96 mg/g).

Dindugul recorded the maximum value at 60 DAS. Nagarcoil recorded the maximum values at 270 and 360 DAS. Coimbatore recorded highest at 150 DAS and Trichur at 240 and 330 DAS. Srivelliputhur showed higher values at 90, 180, 300 and 360 DAS. Palakkadu recorded higher values at 120 DAS. Molakalmur recorded higher values at 30 DAS. Mean while Ghatti Subramanya didn't show higher value at any of the observation period.

4.3.2.2 Chlorophyll-B

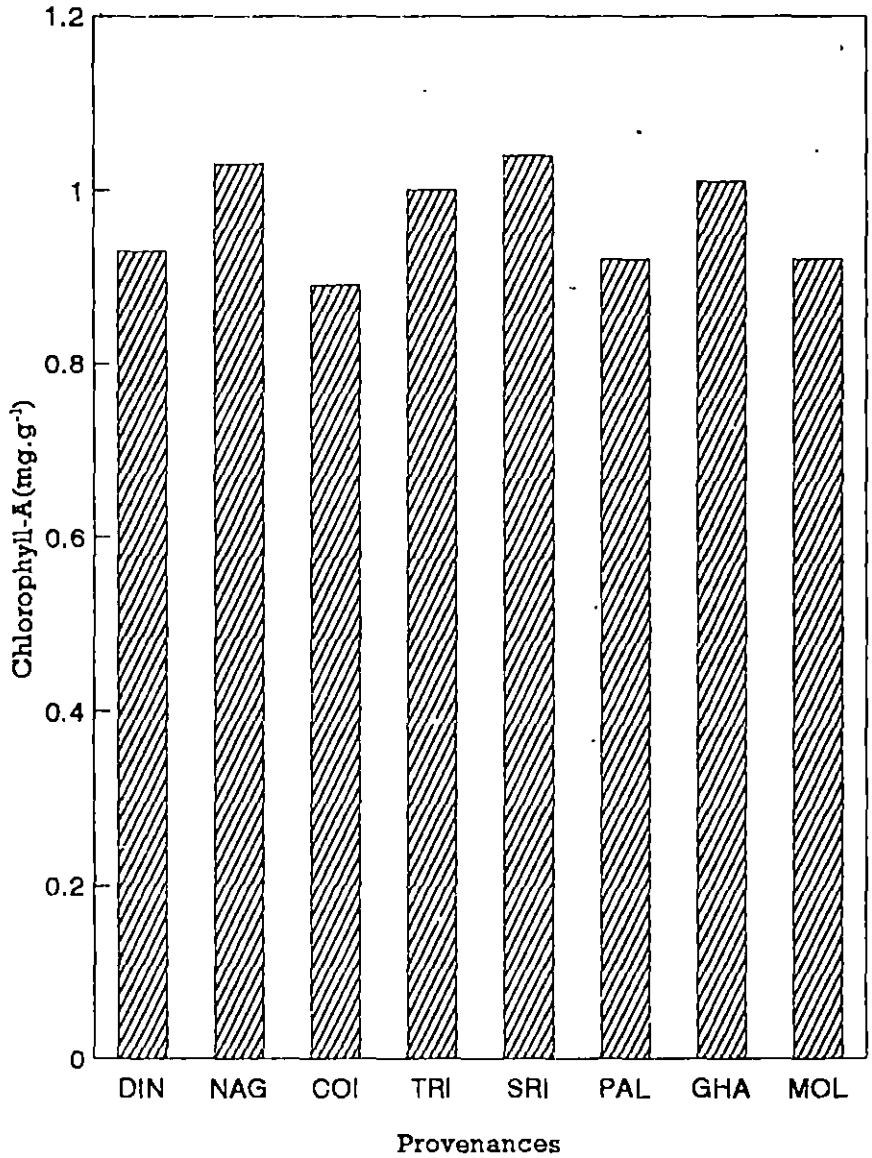
Significant difference for chlorophyll-B content was noticed only at 120 DAS (Table 19). At this period the maximum value was recorded by Trichur (0.91 mg/g) and was significantly superior to other provenances in terms of chlorophyll-B. At the same period the minimum value was recorded by Palakkadu (0.37 mg/g).

Table 17 Similarity indices for peroxidase in different provenances of neem

	1	2	3	4	5	6	7	8
1	1.00							
2	1.00	1.00						
3	0.67	0.67	1.00					
4	1.00	1.00	0.67	1.00				
5	0.67	0.67	1.00	0.67	1.00			
6	0.67	0.67	1.00	0.67	1.00	1.00		
7	0.67	0.67	1.00	0.67	1.00	1.00	1.00	
8	0.67	0.67	1.00	0.67	1.00	1.00	1.00	1.00

Table 18 Data showing variation in chlorophill-A (mg/g) of seedlings of different provenances of neem

Provenances	30 DAS	60 DAS	90 DAS	120 DAS	150 DAS	180 DAS	210 DAS	240 DAS	270 DAS	300 DAS	330 DAS	360 DAS
Dindigal	0.91	1.75	0.95	0.92	0.92	1.14	1.13	1.05	0.93	0.89	1.02	0.93
Nagercoil	0.91	0.66	0.99	0.91	0.98	1.13	1.04	1.07	1.03	0.95	0.98	1.03
Coimbatore	0.89	1.16	0.88	0.92	1.06	1.06	1.04	1.05	0.95	1.00	0.98	0.89
Trichur	0.88	0.58	0.93	0.85	0.91	0.96	1.07	1.09	0.92	0.99	1.05	1.00
Srivelliputhur	0.92	0.64	1.03	0.94	1.02	1.17	1.13	1.08	0.94	1.05	0.95	1.04
Palakkadu	0.94	1.26	0.89	0.98	0.95	1.10	1.21	0.95	0.96	0.95	1.02	1.92
Ghatti Subramanya	0.93	0.48	0.90	0.93	0.94	1.01	0.98	0.90	0.95	0.89	0.97	1.01
Molakalmur	0.95	0.52	2.59	0.96	0.96	1.08	1.07	0.95	1.00	0.96	1.02	0.92
Mean	0.92	0.57	1.34	0.93	0.97	1.08	1.08	1.02	0.96	0.96	1.00	0.97
CD (0.05)	NS	NS	NS	NS	NS	0.13	NS	NS	NS	NS	NS	NS
SEm (±)	0.01	0.03	0.22	0.01	0.02	0.02	0.03	0.03	0.02	0.02	0.02	0.02

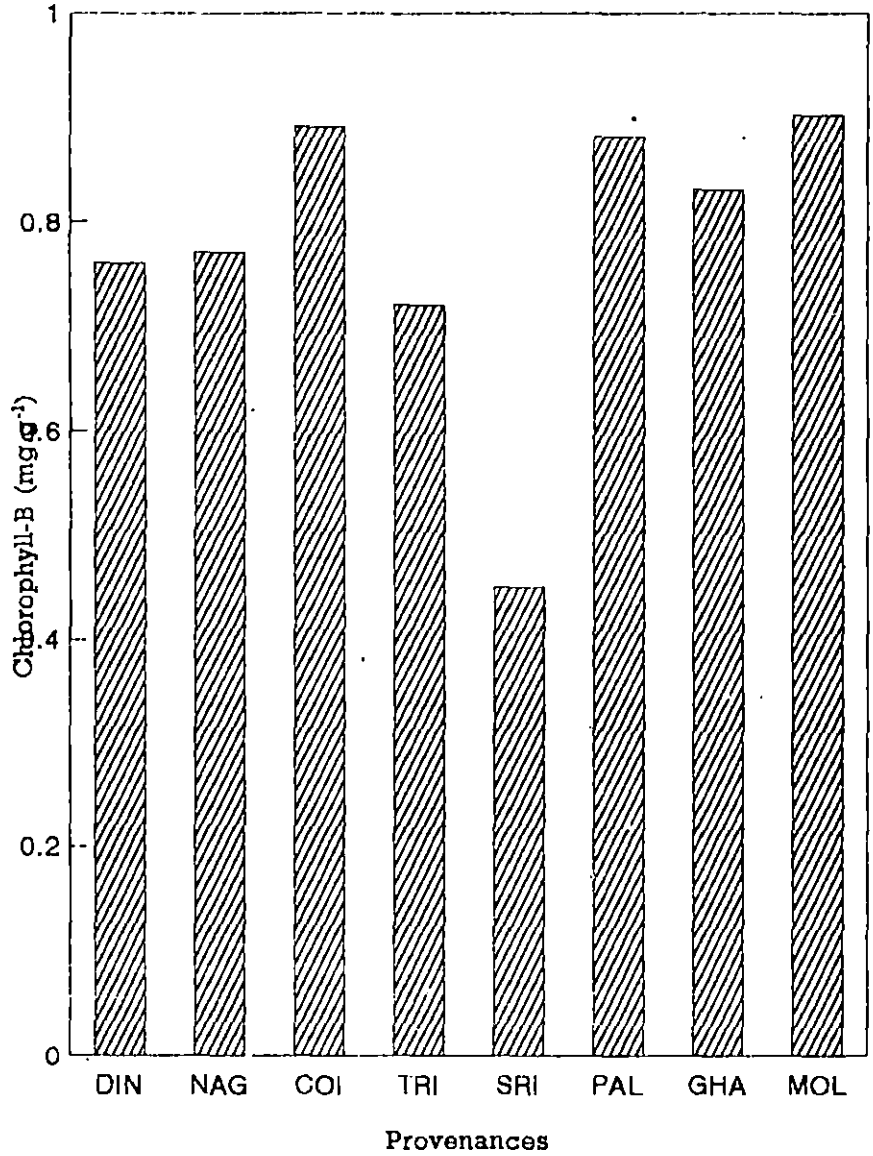


DIN-Dindigul NAG-Nagarcoil COI-Coimbatore TRI-Trichur SRI-Srivelliputhur PAL-Palghat GHA-Ghatti Subramanya MOL-Molakalmur

Fig.29 Variation in chlorophyll-A content of different provenances of neem at 360DAS

Table 19 Data showing variation in chlorophill-B (mg/g) ofseedlings of different provenances of neem

Provenances	30 DAS	60 DAS	90 DAS	120 DAS	150 DAS	180 DAS	210 DAS	240 DAS	270 DAS	300 DAS	330 DAS	360 DAS
Dindigal	0.40	0.71	0.63	0.86	0.65	1.11	0.77	0.76	0.54	0.84	0.68	0.76
Nagercoil	0.63	0.58	0.32	0.43	0.53	0.99	0.77	0.80	0.52	0.88	0.67	0.77
Coimbatore	0.65	0.29	0.51	0.62	0.87	0.97	0.98	0.73	0.58	0.52	0.62	0.89
Trichur	0.55	0.65	0.43	0.91	0.56	1.06	0.93	0.86	0.67	0.70	0.71	0.72
Srivelliputhur	0.63	0.53	0.51	0.87	0.63	0.93	1.00	0.63	0.57	0.72	0.53	0.42
Palakkadu	0.46	0.88	0.53	0.37	0.61	0.95	0.87	0.67	0.62	0.58	0.61	0.88
Ghatti Subramanya	0.57	0.84	0.61	0.65	0.62	1.05	0.98	0.84	0.73	0.68	0.45	0.83
Molakalmur	0.66	0.70	0.20	0.38	0.46	1.06	0.86	0.86	0.73	0.63	0.55	0.90
Mean	0.57	0.65	0.45	0.64	0.62	1.01	0.92	0.77	0.62	0.70	0.60	0.78
CD (0.05)	NS	NS	NS	0.23	NS	NS	NS	NS	NS	NS	NS	NS
SEm (±)	0.03	0.08	0.09	0.05	0.04	0.02	0.03	0.04	0.03	0.04	0.04	0.04



DIN-Dindigul NAG-Nagarcoil COI-Coimbatore TRI-Trichur
SRI-Srivelliputhur PAL-Palghat GHA-Ghatti Subramanya MOL-Molakalmur

Fig.30 Variation in chlorophyll-B content of different provenances of neem at 360DAS

Dindugul recorded maximum values at 90 and 180 DAS. Other provenances which recorded higher values were Nagarcoil at 300 DAS, Coimbatore at 150 DAS, Trichur at 120, 240 and 330 DAS, Srivelliputhur at 210 DAS, Palakkadu at 60 and 90 DAS, Ghatti Subramanya at 270 DAS and Molakalmur at 30, 240, 270 and 360 DAS.

4.3.2.3 Total Chlorophyll

Significant variation in total chlorophyll content was recorded at 60, 120, 150 and 180 DAS (Table 20). At 60 DAS the maximum value was recorded by Dindugul (2.46 mg/g) and the minimum value by Srivelliputhur (1.17 mg/g). From 120 to 180 DAS the maximum values were recorded by Srivelliputhur at 120 DAS, Coimbatore at 150 DAS and Dindugul at 180 DAS. During these periods the minimum values were recorded by Molakalmur at 120 and 150 DAS and by Coimbatore at 180 DAS.

4.4 Anatomical studies

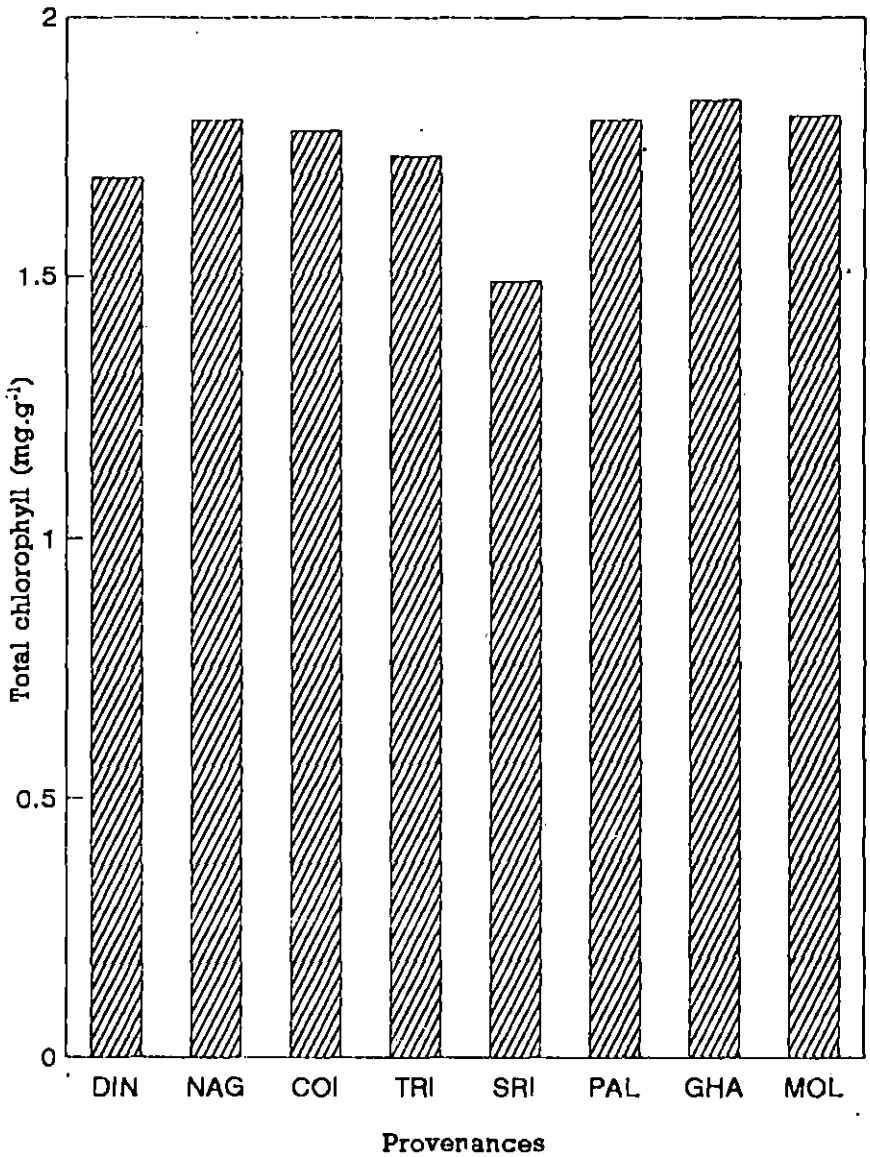
4.4.1 Stomatal frequency

From Table 21 it is obvious that the stomatal frequency was not having any significant difference for most of the experimental period except for 30, 60, 120, 330 and 360 days after sowing.

At 30, 60 and 120 DAS maximum stomatal frequency was recorded by Nagarcoil. At the later stage of the observation significant difference was noticed at 330 and 360 DAS. During these periods the maximum value was shown by Coimbatore and was statistically superior to the other provenances.

Table 20 Data showing variation in total chlorophyll (mg/g) of seedlings of different provenances of neem

Provenances	30 DAS	60 DAS	90 DAS	120 DAS	150 DAS	180 DAS	210 DAS	240 DAS	270 DAS	300 DAS	330 DAS	360 DAS
Dindigal	1.31	2.46	1.57	1.79	1.56	2.26	1.90	1.82	1.48	1.73	1.69	1.69
Nagercoil	1.51	1.24	1.31	1.35	1.50	2.13	2.00	1.86	1.55	1.83	1.65	1.80
Coimbatore	1.54	1.46	1.39	1.54	1.93	2.03	2.01	1.78	1.53	1.52	1.61	1.78
Trichur	1.44	1.24	1.37	1.76	1.47	2.02	2.00	1.95	1.59	1.69	1.76	1.73
Srivelliputhur	1.54	1.17	1.54	1.83	1.65	2.10	2.13	1.71	1.51	1.80	1.47	1.49
Palakkadu	1.39	2.15	1.47	1.36	1.56	2.05	2.08	1.62	1.58	1.49	1.63	1.80
Ghatti Subramanya	1.41	1.32	1.51	1.57	1.56	2.05	1.97	1.74	1.68	1.57	1.46	1.84
Molakalmur	1.61	1.22	2.79	1.34	1.42	2.14	1.92	1.80	1.73	1.59	1.58	1.81
Mean	1.48	1.53	1.59	1.57	1.58	2.10	1.10	1.78	0.58	1.65	1.60	1.74
CD (0.05)	NS	0.83	NS	0.26	0.24	0.13	NS	NS	NS	NS	NS	NS
SEm (±)	0.03	0.12	0.14	0.04	0.04	0.02	0.03	0.04	0.03	0.04	0.04	0.03

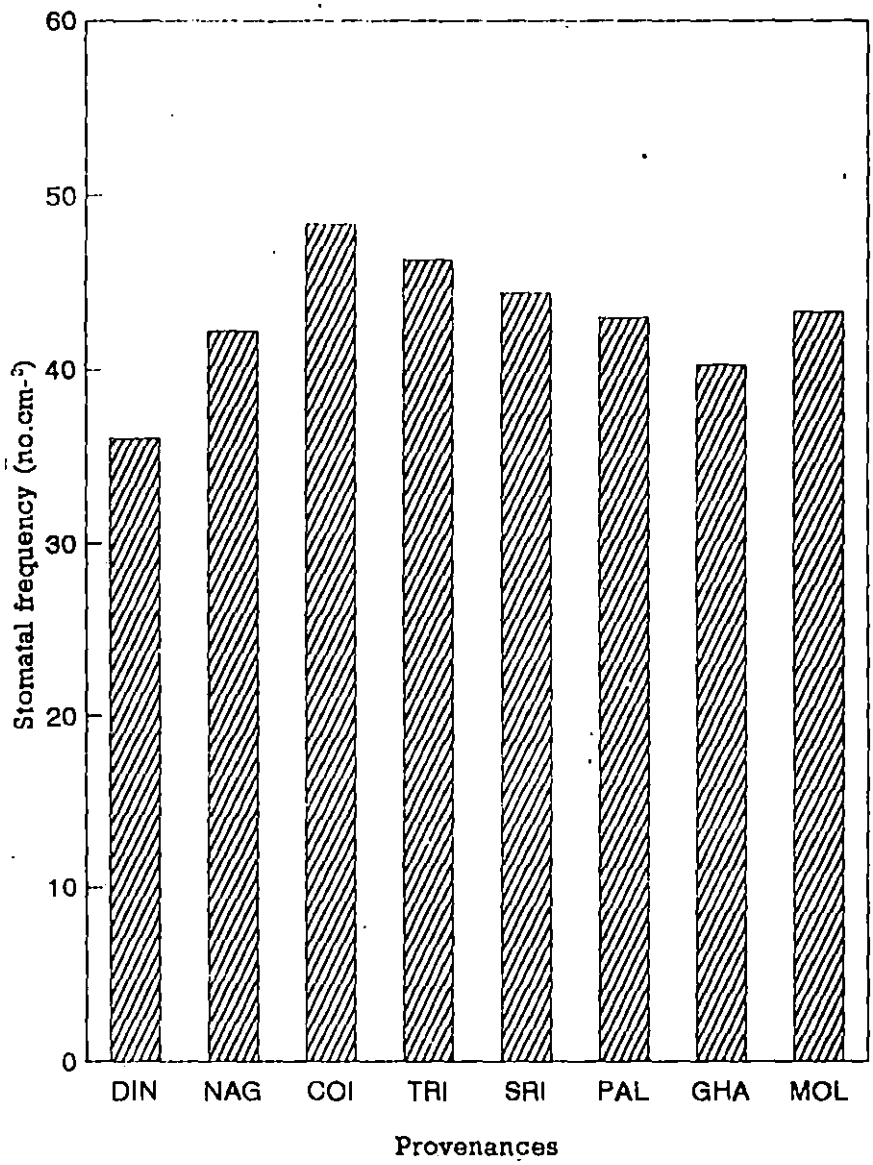


DIN-Dindigul NAG-Nagarcoil COI-Coimbatore TRI-Trichur SRI-Srivelliputhur PAL-Palghat GHA-Ghatti Subramanya MOL-Molakalmur

Fig.31 Variation in total chlorophyll of different provenances of neem at 360DAS

Table 21 Data showing variation in stomatal frequecy (No/cm^2) of seedlings of different provenances of neem

Provenances	30 DAS	60 DAS	90 DAS	120 DAS	150 DAS	180 DAS	210 DAS	240 DAS	270 DAS	300 DAS	330 DAS	360 DAS
Dindigal	26893.1	32922.8	40509.6	41413.7	40962.5	41924.9	36036.8	35187.5	35470.6	35583.8	36178.3	36008.4
Nagercoil	33970.3	35498.9	47501.7	46709.1	46963.9	44557.7	34989.3	35215.8	35527.2	36716.2	40962.4	42208.0
Coimbatore	23184.7	31139.4	36150.0	37735.3	40424.6	40509.5	37820.2	36772.8	37112.5	38301.5	48152.8	48351.0
Trichur	28874.7	29129.5	39094.1	41358.7	42236.3	42208.0	38754.3	38216.5	39094.1	39943.3	46397.7	46284.5
Srivelliputhur	33375.8	33857.0	44189.6	44076.4	44217.9	44302.8	38612.8	38159.9	39886.7	41443.7	43340.4	44387.8
Palakkadu	26496.7	28223.6	37876.8	38584.5	41726.8	39801.8	37508.8	37675.7	37876.8	38952.5	42887.4	42944.0
Ghatti Subramanya	27600.8	27515.9	41641.9	41273.8	41953.2	40877.5	37820.2	38499.6	38188.2	40622.8	39632.0	40198.1
Molakalmur	28789.8	31790.5	37773.5	40368.0	41042.4	42208.0	40849.2	42094.8	42576.0	42689.3	43255.4	43227.1
Mean	28648.3	31259.7	40842.2	41443.7	42441.6	42048.8	37798.9	37728.2	38216.6	39281.7	42600.8	42951.2
CD (0.05)	3652	3211	NS	4298	NS	NS	NS	NS	NS	NS	6988	6752
SEm (±)	671.87	552.69	953.32	643.07	586.02	572.81	618.9	689.53	685.20	703.18	959.65	938.07



DIN-Dindigul NAG-Nagarcoil COI-Coimbatore TRI-Trichur SRI-Srivelliputhur PAL-Palghat GHA-Ghatti Subramanya MOL-Molakalmur

Fig.32 Variation in stomatal frequency of different provenances of neem at 360DAS

Coimbatore has recorded the minimum value at 30 and 120 DAS and at 60 DAS the minimum value was recorded by Ghatti Subramanya. At the later stage Dindugul was the lowest performer at 330 and 360 DAS.

4.5 Genetic parameters

4.5.1 Seed characters

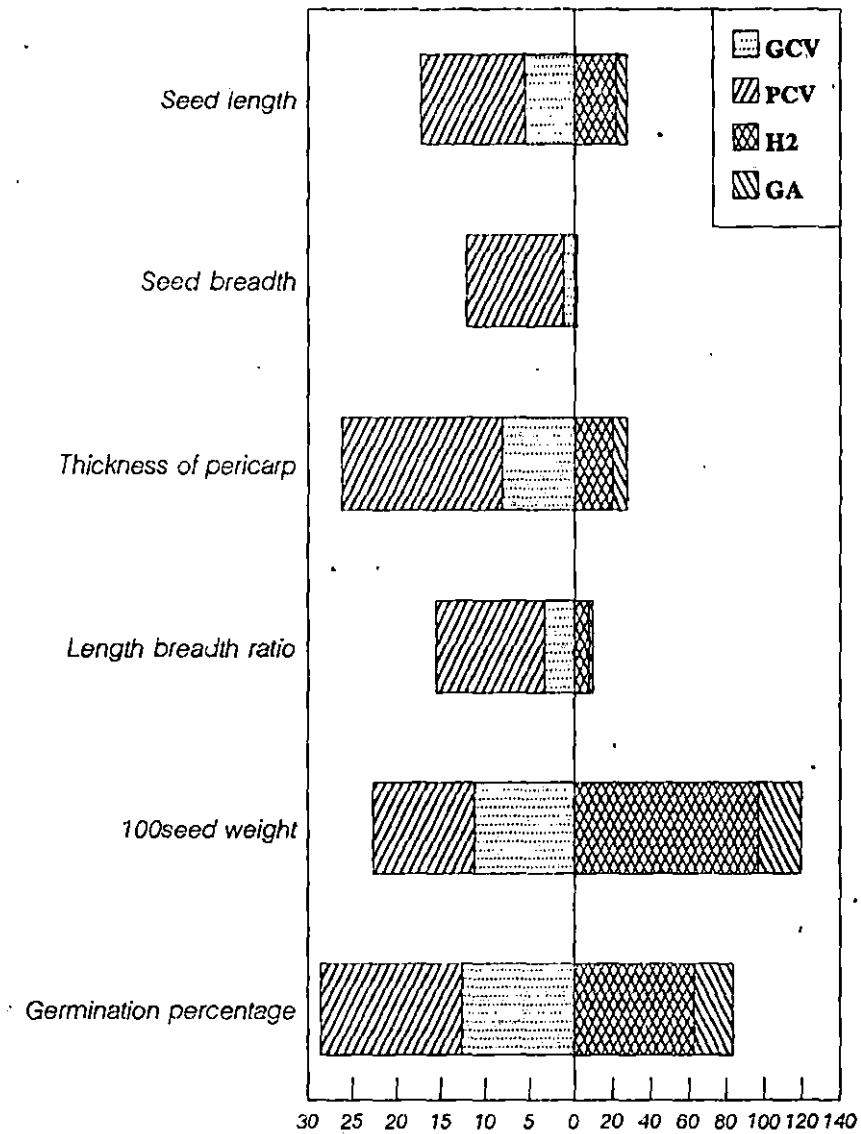
The results of the study on the genotypic and phenotypic variation, heritability in broad sense, genetic advance and genetic gain for the seed characters are furnished in Table 22. It is clear from the table that the genetic variability expressed as genotypic coefficient of variation (GCV) was the highest for the germination percentage (12.63%) followed by 100 seed weight (11.23%) and thickness of pericarp (8.13%). Very low values for GCV was recorded for seed length (5.52%) followed by seed length breadth ratio (3.39%) and seed breadth (1.25%).

Similar to the GCV, the phenotypic coefficient of variation (PCV) was the highest for thickness of pericarp (18.11%) followed by germination percentage (15.95%), seed length breadth ratio (12.19%), seed length (11.75%), 100 seed weight (11.42%) and seed breadth (10.89%). By and large, it was seen that the estimate of genotypic coefficient of variance (GCV) was less in magnitude than the phenotypic coefficient of variance.

The heritability in broad sense was highest for the 100 seed weight (97.0%) followed by germination percentage (63.0%). Moderate values were recorded for seed

Table 22 Genetic parameters for the seed characters of different provenances of neem

Sl. No.	Characters	GCV	PCV	H^2 (%)	Genetic advance	Genetic gain
1	Seed length	5.52	11.75	22.00	0.74	5.33
2	Seed breadth	1.25	10.89	1.30	0.02	0.29
3	Thickness of pericarp	8.13	18.11	20.00	0.03	7.49
4	Seed length breadth ratio	3.39	12.19	7.70	0.04	1.94
5	100 seed weight	11.23	11.42	97.00	5.55	22.73
6	Germination per cent	12.63	15.95	63.00	14.53	20.59



GCV-Gentypic coefficient of variation H^2**-Heritability**
PCV-Phenotypic coefficient of variation **GA-Genitic advance**

Fig.33 Genetic parameters for the seed characters of neem

length (22.0%) and thickness of pericarp (20.0%). The low value for heritability was recorded in case of seed length breadth ratio (7.7%) and seed breadth (1.3%).

The genetic advance in percentage of mean was found to be high for 100 seed weight (22.73%) followed by germination percentage (20.59%). Characters like thickness of pericarp, seed length, seed length breadth ratio and seed breadth recorded low values such as 7.49 per cent, 5.33 per cent, 1.94 per cent and 0.29% respectively.

4.5.2 Seedling characters

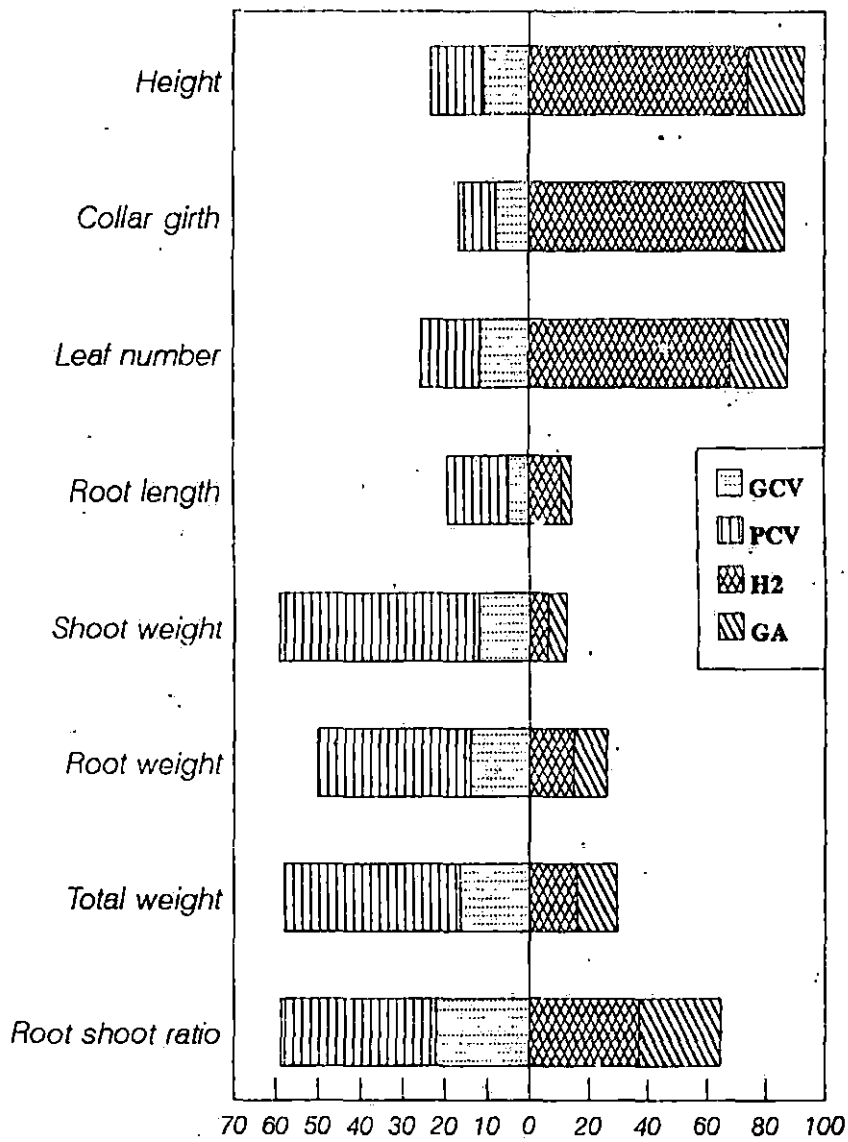
A perusal of Table 23 reveals that the genotypic coefficient of variation (GCV) was always lower in magnitude than the phenotypic coefficient of variation (PCV) in all the eight characters studied in seedlings of neem. The highest values for GCV was recorded by root shoot ratio (22.18%) followed by total weight (16.44%), root weight (13.88%), shoot weight (11.92%), leaf number (11.57%) and height (10.7%). Characters like collar girth (7.75%) and root length (4.75%) recorded low GCV.

PCV was highest for shoot weight (47.21%) followed by total weight (41.55%) and root weight (36.23%). Collar girth shows lowest value for PCV (8.82%).

Height recorded high heritability (74%) followed by collar girth (73%), leaf number (68%) and root shoot ratio (37%). Heritability was low for the characters such as total weight (16%), root weight (15%), root length (11%) and shoot weight (6%).

Table 23 Genetic parameters for the seedling characters of different provenances of neem

Sl. No.	Characters	GCV	PCV	H^2 (%)	Genetic advance	Genetic gain
1	Height	10.70	12.47	74.00	19.02	18.92
2	Collar girth	7.75	8.829	73.00	4.91	13.34
3	Leaf number	11.57	14.02	68.00	16.39	19.67
4	Root length	4.75	14.48	11.00	2.67	3.21
5	Shoot weight	11.92	47.21	6.00	2.57	6.20
6	Root weight	13.88	36.23	15.00	3.30	10.96
7	Total weight	16.44	41.56	16.00	9.59	13.40
8	Root shoot ratio	22.18	36.55	37.00	0.36	27.60



GCV-Genotypic coefficient of variation H^2-Heritability
PCV-Phenotypic coefficient of variation GA-Genetic advance

Fig.34 Genetic parameters for the seedling characters of neem

The highest genetic advance (% of mean) was observed in root shoot ratio (27.6%) followed by leaf number (19.67%), height (18.92%), total weight (13.4%) and collar girth (13.34%). Very low genetic advance was recorded by root length (3.21%).

4.6 Genetic Divergence

4.6.1 D^2 analysis

Application of Mahalanobis' D^2 analysis and the Tocher's clustering method has resolved the eight provenances in to four clusters (Table 24). Clusters I and IV are having three provenances each and the clusters II and III are having only one provenance each. Three provenances included in the cluster I are Dindigul, Palakkadu and Ghatti Subramanya. Three provenances included in the cluster IV are Nagarcoil, Trichur and Srivelliputhur. Single provenances included in cluster II and III are Coimbatore and Molakalmur, respectively.

4.6.2 Average inter-cluster distances

Table 25 shows the inter-cluster distances based on the average of D^2 and D values. The same data have been diagrammatically furnished in Fig.36. The inter-cluster distance revealed that the maximum divergence occurred between cluster II and III (D^2 = 117.79, D = 10.9) and the minimum genetic distance was noticed between cluster I and IV (D^2 = 19.96, D = 4.5). Genetic distance was almost similar between cluster II and IV (D^2 = 32.25, D = 5.7) and between I and III (D^2 = 33.71, 0 = 5.8). Inter cluster distances between I and II (D^2 = 58.56, D = 7.7) and between III and IV (D^2 = 74.69, D = 8.6) were found to be moderately high.

Table 24 Clustering of eight provenances of neem

Cluster	No. of provenances	Designation and source
I	3	1 Dindigul
		6 Palakkadu
		7 Ghatti Subramanya
II	1	3 Coimbatore
III	1	8 Molakalmur
IV	3	2 Nagercoil
		4 Trichur
		5 Srivelliputhur

Table 25 Average intra and inter cluster D^2 and D values (in parenthesis)

Cluster	I	II	III	IV
I	10.97 (3.3)			
II	58.56 (7.7)	0.00 (0.0)		
III	33.71 (5.8)	117.79 (10.9)	0.00 (0.0)	
IV	19.96 (4.5)	32.25 (5.7)	74.69 (8.6)	12.48 (3.5)

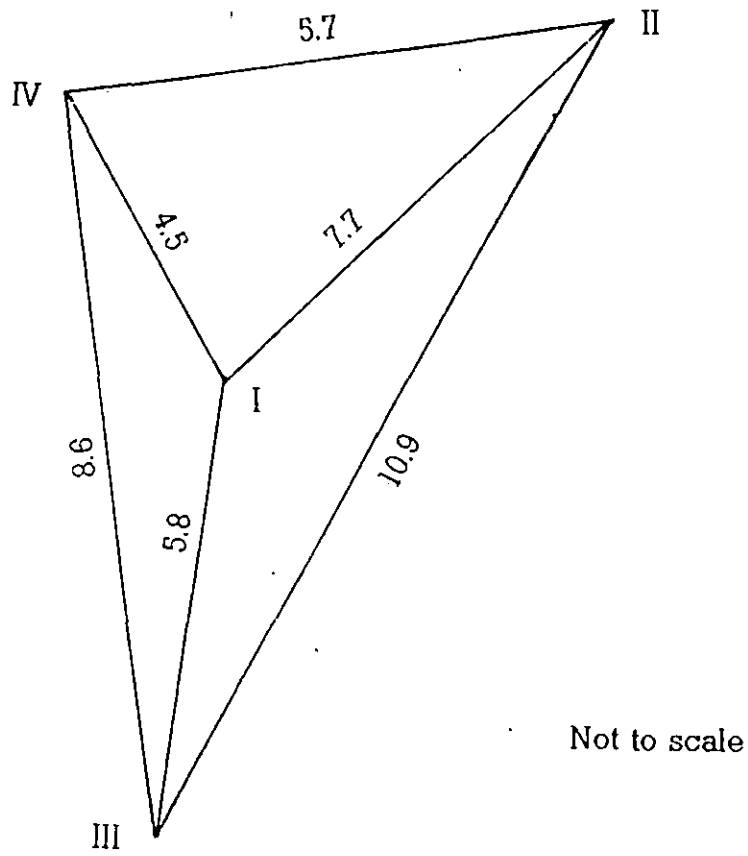


Fig.35 Diagrammatic representation of the approximate degree of similarity among clusters of provenances according to average between-cluster D values

4.6.3 Average intra cluster distances

Average D^2 and D values showing the genetic distance between provenance in furnished in Table 26. From this it is clear that the distance between provenances Coimbatore and Molakalmur (D^2 = 117.78, D = 10.9) is maximum characterised by cluster II and III, while close proximity existed between provenances Palakkadu and Ghatti Subramanya (D^2 = 8.07, D = 2.8). The second maximum intra cluster distance was recorded between provenances Srivelliputhur and Molakalmur (D^2 = 94.39, D = 9.7). The minimum intra cluster distance was shown by cluster I between Palakkadu and Ghatti Subramanya (D^2 = 8.07, D = 2.8).

4.7 Incidence of pests and diseases

Incidence of pest was not observed on neem seedlings of different provenances, in any of the investigation period from 30 to 360 days after sowing. As far as disease incidence is concerned all the provenances were found affected by sooty mold caused by *Capnoidium sp.* only during the rainy seasons.

Table 26 D^2 values and D values (in parenthesis) between provenances of neem

Provenances	8	7	6	5	4	3	2
7	23.53 (4.9)						
6	39.85 (6.3)	8.07 (2.8)					
5	94.39 (9.7)	30.18 (5.5)	24.00 (4.9)				
4	67.87 (8.2)	21.51 (4.6)	26.10 (5.1)	16.06 (4.0)			
3	117.78 (10.9)	68.25 (8.3)	66.50 (8.2)	39.53 (6.3)	23.30 (4.8)		
2	61.81 (7.9)	16.65 (4.1)	14.43 (3.8)	10.67 (3.3)	10.71 (3.3)	33.92 (5.8)	
1	37.73 (6.1)	10.46 (3.2)	14.38 (3.8)	21.30 (4.6)	16.20 (4.0)	40.92 (6.4)	9.30 (3.0)

Discussion

DISCUSSION

Evaluation of neem (*Azadiracta indica* A. Juss.) collected from eight provenances of the peninsular India was carried out at Vellanikkara, Thrissur during 1995-96 to compare variations, if any, in their seed characters and seedling growth parameters including biometrical, anatomical and biochemical characteristics. The important findings obtained in this study are discussed hereunder.

5.1 Seed characters

Significant variations were observed among the eight provenances of neem for their seed characters viz., hundred seed weight, seed length, seed breadth, length-breadth ratio, thickness of pericarp and germination percentage.

The maximum seed length was observed for Trichur provenance and the minimum for Coimbatore provenance. Provenances Nagarcoil, Trichur and Srivelliputhur showed higher values for seed breadth, length-breadth ratio and thickness of pericarp. The results on seed length and breadth are in confirmity with the work done on neem by Veerendra *et al.* (1996) in Bangalore who have recorded genetic variability for these traits between 12 provenances from Karnataka, Tamil Nadu and Andhra Pradesh. Toon *et al.* (1990) in a study revealed that seed size is an indication of the quality of the seeds and genetic potentialities. By and large, in a broader perspective, it is said that the larger sized seeds have greater advantages over the smaller sized seeds with reference to germination and further performance of the

seedlings. The maximum value for hundred seed weight was recorded by Nagarcoil provenance and the minimum value by Coimbatore provenance. The higher seed weight could be attributed to better differential seed filling based on locality or site factors. Variations in seed weight was reported in *Azadirachta indica* by Kumaran (1991), Ponnammal *et al.* (1993) and Veerendra *et al.* (1996). Provenance variation in seed weight are available with other species like *Dalbergia sissoo* (Dhillon *et al.*, 1995), *Bassia latifolia* (Jenner, 1995) and *Prosopis cineraria* (Raj Bahadhur and Hooda, 1995) also.

In this study it was observed that Nagarcoil provenance recorded the highest germination of 87.33 per cent and was immediately followed by Trichur provenance with 80.4 per cent. The provenance Nagarcoil recorded higher values for 100 seed weight and seed breadth which might be attributed to its higher germination percentage. Similar results were reported in teak by Kumar (1979) and Prasad (1996). However, according to Ponnammal *et al.* (1993) the medium sized seeds showed higher percentage of germination than that of large and small seeds in neem. Significant variation in seed germination among provenances was also reported *Pinus brutia* (Isik, 1986) *Acacia mangium* (Salazar, 1989), *Gliricidia sepium* (Ngulube, 1989), *Prosopis cineraria* (Arya *et al.*, 1995), *Dalbergia sissoo* (Dhillon *et al.*, 1995) and *Pinus caribaea* (Swain and Patnaik, 1996). Some authors are of the opinion that provenances with higher germination percentage also have greater potential for height growth (Ngulube, 1989). The low germination may be due to defective seed

collection, such as using unripe seeds. It may also occur when seeds are collected from naturalised stands outside the natural distribution range of the species (Hughes, 1987).

From the above observations, it is seen that the magnitude of provenance variation in seeds is significantly high for most of the characters studied. It is interesting to note that none of the provenances showed highest value for all the characters, suggesting wide genotypic variations among populations in each provenances. However, Nagarcoil recorded highest values for 100 seed weight, seed breadth and germination percentage, Trichur recorded highest values for seed length and seed length-breadth ratio and Srivelliputhur recorded highest value for thickness of pericarp. Considering the fact that seed characteristics especially seed size has a direct bearing on germination and subsequent seedling characteristics it can be presumed that the above two provenances will surpass the rest of the provenances in further evaluation also.

5.2 Biometrical characters

Significant variation in plant height was observed between the eight provenances, at all the growth phases from 30 to 360 days after sowing (DAS). At 30 days after sowing the maximum height was observed for Nagarcoil and the minimum height by Molakalmur. The maximum growth in height was later replaced by Coimbatore provenance from 60 to 360 DAS. Molakaimur provenance remained consistently inferior throughout the entire growth phases. Tree height has been

observed to exhibit pronounced variation in neem (Kumaran, 1991; Tewari, 1994). However, provenance studies in respect of seedling height growth in neem is scant. But, referring back, such variation among provenances have been reported for height in many other tree species viz., *Eucalyptus tereticornis* (Otegbeye, 1990b); *Pinus oocarpa* (Zashimuddin *et al.*, 1991); *Acacia auriculiformis* (Awang, 1994; Nor Aini *et al.*, 1994), *Acacia nilotica* (Balkrishan and Toky, 1995a; Ginwal, *et al.*, 1995) and *Pinus caribaea* (Swain and Patnaik, 1996).

Provenances exhibiting faster height growth are generally regarded as suitable for areas where weed competition is severe. Gupta *et al.* (1991) while discussing juvenile-adult correlation reported that the provenances which showed better performance at the seedling stage, proved to be consistently best performers over a period of seven years. Reports of Burdon and Sweet (1976) and Rai *et al.* (1982) on juvenile adult correlation also show that one which performs well at juvenile stage may perform equally well at adult stage also. In this context, in the present study Coimbatore provenance render more promise.

The results showed marked differences among provenances in their performance in terms of collar girth. At 30 DAS the best performing provenance was Coimbatore. At 60 DAS the best performer was Nagarcoil and Srivelliputhur at 90 DAS. From 120 to 360 DAS the single best performer was again Nagarcoil which was the best performer at 60 DAS. In fact, the worst performing provenance with the slowest recorded girth increment was Molakalmur provenance. Provenance variation

in neem with respect to collar girth has been reported earlier by Kumaran (1991). According to Awang (1994) with time the performance of the provenance may change. Wide variation among provenances and in between the growth stages were also reported in *Tectona grandis* (Kedharnath and Matthewa, 1962), *Pinus sp.* (Namkoong and Conkle, 1976), *Leucaena leucocephala* (Palit, 1980) and *Populus sp.* (Jha *et al.*, 1991).

Amara (1987) reported that provenance which exhibited faster height growth also characterised quicker radial expansion. In contrast, observations in this study show that the Coimbatore provenance, which was best in terms of height growth at 360 DAS was behind three other provenances with reference to radial growth at 360 DAS. This is in agreement with the results obtained in *Gliricidia* by Krishnan (1990). This indicates that height growth the radial growth need not always be directly related.

Root growth varied significantly among provenances and also between stages from 60 to 180 DAS and also from 270 to 330 DAS. The provenance with faster root growth at 30 DAS (Trichur) did not maintain its ranking at 360 DAS. The best performer at that stage was Srivelliputhur provenance. Significant variation in root length was recorded earlier in provenance evaluations of neem (Kumaran, 1991) and *Acacia nilotica* (Ginwal *et al.*, 1995).

Leaf area was statistically nonsignificant at 30 DAS and also from 150 DAS till the end of the growth phase. At 30 DAS the maximum leaf area per plant was

recorded by the Trichur provenance but at the end of the growth phase it was replaced by Coimbatore provenance. At the same time, leaf number showed statistical difference throughout the growth phases. Leaf number per plant was the largest in Coimbatore provenance at 30 DAS, and consistently maintained its superiority throughout the growth phases upto 360 DAS.

Variation in number of leaves was reported in neem (Kumaran, 1991). The performance of the different provenances on leaf number have been reported in *Gliricidia* (Rajaram, 1990), *Bassia* (Jenner, 1995) and *Acacia nilotica* (Balkrishan and Toky, 1995a). Variation in leaf area is reported by Balkrishan and Toky (1995) in *Acacia nilotica*. More number of leaves and leaf area presumably indicate that more biomass is allocated to leaves, in order to increase the photosynthetic efficiency of plants. Results showed that at 360 DAS the number of leaves and leaf area are maximum with the Coimbatore provenance. This can be attributed to the increased height growth of this provenance at 360 DAS. This is in consonance with the results obtained by Jayasankar (1996) in teak.

5.3 Biomass characters

As estimate of the biomass indicates the total biological matter produced by the plant and how much is contributed by the various parts of the plant to the ultimate biological yield. Total biomass production is known to be strongly genetically controlled and can be considered as a parameter for selection of superior geno types.

In the present study, variation between provenances existed for the various biomass characters such as shoot dry weight, root dry weight and total dry weight at different growth stages. At 360 DAS Coimbatore recorded maximum values for shoot dry weight and total dry weight, but root dry weight was maximum for Palakkadu provenance. At this stage, however, none of the biomass characteristics exhibited significant variation among the provenances.

The morphological characters such as seedling height and other crown characters are known to be strongly inherited (Shivkumar and Banerjee, 1986). Therefore, these characters may be considered as the most useful for early selection of superior provenances. However, selection of provenances on the basis of one character alone may some time do not give the desired level of superiority (Ginwal *et al.*, 1995). This indicates that seed source selection should be on the basis of multi-trait consideration. This has been also emphasised by Vakshasya *et al.* (1992). In this perspective Coimbatore provenance with its superior height growth coupled with superior shoot dry weight and total dry weight holds more promise.

The root shoot ratio varied significantly between provenances almost during the entire growth phase except at 90 and 120 DAS. Results indicated that most of the seedlings allocated more root than shoot.

5.4 Biochemical characters

5.4.1 Chlorophyll content

Chlorophyll content in plants decides their photo-synthetic potential. Therefore an estimate of chlorophyll A and B of the different provenances will provide information regarding this varying biochemical parameter which ultimately will be contributing to the total biomass.

Reports on chlorophyll content among provenances of arid and semi-arid tree species in India is very scant. However, provenance variation in terms of chlorophyll content has been reported in *Acacia nilotica* (Balkrishan and Toky, 1995b). The eight provenances of neem evaluated during the present study did not differ significantly with respect to chlorophyll A, B and total chlorophyll. However, at certain growth phases they exhibited significant variation (180 DAS - chlorophyll A; 120 DAS - chlorophyll B). This indicates that the total photosynthates as well as biomass production were more or less equal in all the provenances during most of the growth phases. N and Mg are greatly concerned with chlorophyll biosynthesis, and a deficiency of these elements is responsible for significant decrease in chlorophyll content. Significant variations in chlorophyll content were also observed in six provenances of *Abies alba* in Czechoslovakia (Paule, 1977), and of *Pinus caribaea* in Central America (Gerhold, 1959; Venator *et al.*, 1977).

5.4.2 Isozyme analysis

Isozymes are direct products of single locus and therefore phenotypic variations can be related to genotypic charactors. In this experiment, two bands were found common in all the provenances. Provenances viz., Dindigul, Nagarcoil and Trichur were characterised by three isozyme bands. Enzymes are primary gene products, and variation in their structure should give reliable information about the variability in the genome itself (Forrest, 1994). In the present study, it can be emphasized that three provenances viz., Dindigul, Nagarcoil and Trichur were different in terms of peroxidase isozyme from other provenances studied.

Variation in isozyme banding pattern between provenance is also reported in *Eucalyptus* species in Hawaii (Aradhya and Pillips, 1993) and in *Pinus sylvestris* in Poland (Prus-Glowacki and Bernard, 1994). In some cases locally common rare alleles occur, which can then be used in the identification of seed origin or in the delimitation of regions of provenance (Forrest, 1994). But in this experiment no such rare alleles were found. Nevertheless, inorder to estimate genetic variation pattern at all reliabily, many isozyme loci in the entire germplasm should be surveyed.

5.5 Anatomical characters

5.5.1 Stomatal frequency

The eight provenances of neem differed significantly with reference to the number of stomata per square cm during many of the growth stages. At the earlier stages of growth Nagarcoil provenance recorded increased number of stomata per

square centimetre. But at the later stages of growth, ie, at 330 and 360 DAS the ranking goes to Coimbatore provenance. Stomata are important in tree seedling establishment and growth because most of the water lost by transpiration escapes and most of the carbondioxide used in photosynthesis enters through them. Gopikumar *et al.* (1995) have reported that the number of stomata per unit area of leaf surface varies with species and environmental conditions.

Winnerberger (1958) has obtained some indirect evidence indicating that transpiration may have an influence on the growth of some plants. It can also be inferred that more transpiration means more number of stomata per square centimetre. In this study also the provenance which is having high stomatal frequency at 360 DAS (Coimbatore) also showed better growth attributes supports the findings of Winnerberger (1958). It can be tentatively inferred that provenances with higher stomatal number in the seedling may have better establishment of seedlings and tend to grow more vigorously.

5.6 Genetic characters

5.6.1 Genetic divergence

Mahalanobis' D^2 Statistics was applied to evaluate the genetic divergence among the eight provenances included in this study. This method has been extensively used in annual crops as an efficient tool to find out the genetic diversity (Murthy and Arunachalam, 1966; Subramanian, 1979). But its application in tree crop is of very recent origin (Rajaram, 1990; Paramathma, 1992).

In order to examine the genetic relationship among provenances the cluster technique was used. A total of four clusters were formed. Cluster I and IV consisted of three provenances each from different geographic sources while cluster II and III have only one provenance each. The inclusion of geographically different provenances of neem in the same cluster was attributed to the fact that the factors other than geographic diversity might be responsible for their genetic uniformity (Kumar, 1991). This is in consonance with the studies of Bagchi (1992) and Manojkumar (1994) in *Santalum album* and Singh and Chaudhary (1992) in *Prunus armeniaea.*

The highest intra-cluster distance characterised by the cluster I, reveals that the existing variability was high in this cluster. The highest inter-cluster difference was recorded between clusters II and III followed by III and IV and I and II. The inter-cluster difference was minimum between I and IV. Clusters appearing close together in the figure are relatively similar, while those far apart are dissimilar.

5.6.2 Genetic parameters

The genotypic coefficient of variation was always lower in magnitude than the phenotypic coefficient of variation in all the seed and seedling characters studied. This indicates that environment is greatly influencing the expression of these characters. Similar results were reported by Srivastava *et al.* (1993a,b), Dhillon *et al.* (1995) and Reddy *et al.* (1996). The difference between GCV and PCV for 100 seed

weight, germination percentage (of seeds) height, collar girth and leaf number (of seedlings) were very small indicating less influence of environment on these characters. The difference in variance was to the extent of 50 per cent and above for the seed characters such as seed length, seed breadth, thickness of pericarp and seed length breadth ratio and for seedling characters such as root length, shoot weight, root weight and total weight indicating the greater influence of environment in the expression of these characters. According to Burton (1952) the study of the GCV together with heritability estimates would give the best picture of progress to be expected from selection.

Heritability indicates how much of the phenotypic variability is heritable. According to Srivastava *et al.* (1993b) heritability estimates enable a plant breeder to base his selection on the phenotypic performance for further improvement of specific characters. With respect to seed characters heritability in broad sense was highest for 100 seed weight followed by germination percentage and with seedling characters high heritability was observed for height followed by collar girth and leaf number. The heritability, however, indicates only the effectiveness with which selection of genotype can be based on the phenotypic performance and this fails to show the genetic progress (Johnson *et al.*, 1955). Therefore, high heritability estimates does not mean that the greater genetic gain and this was emphasized by Ramanujam and Tirumalachar (1967). They also regarded that heritability estimates are reliable only when they are accompanied by a high genetic gain. Johnson *et al.* (1995) also believe that high heritability estimates, if considered together are more successful than alone.

In this investigation for seed characters high heritability in conjunction with high genetic gain was observed for 100 seed weight and for seedling characters this kind of situation was observed for height and leaf number. Such a nature was attributed to additive gene action by Panse (1957). Again, for the seed characters low heritability with low genetic gain was observed for seed length, seed breadth, seed length-breadth ratio and thickness of pericarp and for seedling characters the same kind of situation was observed for root length, shoot weight, root weight and total weight. Low heritability coupled with low genetic gain is indicative of non-additive genetic effects as explained by Srivastava (1993a).

5.7 Incidence of pests and diseases

Pest incidence was not observed on neem seedlings of different provenances, in any of the investigation period from 30 to 360 DAS. The possibility is that all the provenances used in the study were resistant to pests. According to Onkarappa and Kumar (1994) there are about 20 ingredients in neem and it is difficult for any insect to develop resistances to all of them. Also the presence of azadirachtin in the leaves acts as a strong antifeedant and growth retardant of insects. Tewari (1992) reported that the young neem plants are frequented by not less than five dozen insect species from all over the world. Contrary to this no insect pest was recorded in the present study.

With reference to the disease incidence on neem all the provenances were found affected by sootymold caused by *Capnodium sp.* This was found only during the rainy seasons. This leads to the conclusion that none of the provenances under this study was resistant to this disease. Any way, no seedling mortality was observed in any of the provenances due to this disease. Reports are not available with sootymold causing disease on neem. But, Butler and Bisby (1960) reported that *Capnodium* causing sootymold on *Bombax ceiba* in India.

Summary and Conclusions

SUMMARY AND CONCLUSIONS

The experiment was carried out at College of Forestry, Vellanikkara to study the performance of eight provenances of neem (*Azadirachta indica* A. Juss) from different agroclimatic regions of peninsular India for a period of one year during 1995-96. The provenances under study were Dindigul, Nagercoil, Coimbatore, Trichur, Srivelliputhur, Palghat, Ghatti Subramanya and Molakalmur. The various characters studied included seed parameters such as seed length, breadth, length breadth ratio, 100 seed weight and pericarp thickness as well as seedling parameters such as biometrical, biochemical, anatomical, genetic and isozyme characters. The pot culture experiment was laidout in a Completely Randomized Design. The salient results are summarised below:

1. Seed weight differed considerably between provenances. The superior provenance in terms of 100 seed weight was Nagarcoil provenance.

2. Individual seed characters like length, breadth, length breadth ratio and thickness of pericarp were found to vary significantly between the provenances. Maximum seed length and seed length breadth ratio were recorded by Trichur provenance. Nagarcoil recorded maximum value for seed breadth and Srivelliputhur recorded the maximum value for thickness of pericarp.

3. Germination percentage in the nursery differed considerably between provenances. Nagarcoil provenance was found to be superior in this respect.

4. The best performer in terms of height growth was Coimbatore; and in terms of collar girth it was Nagarcoil.

5. Coimbatore registered a significantly high performance throughout the successive experimental periods in the case of leaf number. But in case of leaf area the significant superiority was observed only during the earlier growth phases.

6. The maximum root length was recorded by Srivelliputhur at the later stages of growth.

7. Total dry matter accumulation was maximum for Dindigul provenance.

8. Root shoot ratio was maximum for Palakkadu provenance for most of the growth phases.

9. Provenances did not differ significantly with respect to chlorophyll-A, B and total chlorophyll.

10. At the earlier stages the maximum number of stomata per square centimetre was recorded by Nagarcoil and at the later stage by Coimbatore.

11. Hundred seed weight was found to possess high values for heritability and genetic gain. Germination percentage was having high values of GCV sand PCV.

12. Among the seedling character root shoot ratio was having high GCV and PCV followed by shoot weight.

13. High heritability coupled with high genetic gain were in favour of height followed by collar girth.

14. Application of Mahalanobis D^2 statistics and Tocher's method of clustering resolved the eight provenances in to four distinct clusters. The first and fourth cluster comprising of three provenances each and the second and third cluster comprising of only one each.

15. The inter cluster distance was highest between the clusters II and III. The distance between provenances Coimbatore and Molakalmur was the maximum.

16. Isozyme banding pattern of peroxidase in the eight provenances of neem showed two bands PRX-1 and PRX-3 in common. PRX-2 was found only in three provenances viz., Dindigul, Nagarcoil and Trichur. Eight provenances were classified in the two groups based on the presence or absence of PRX-2. Similarity index for peroxidase ranged between 0.67 to 1.0.

17. Genetic distance measured by D^2 method and isozyme analysis did not correlate with each other. This may be due to the reason that the study was confined only to one enzyme.

18. No incidence of pests was noticed during the entire period of investigation. But, as far as incidence of disease is concerned all provenances were found affected by sooty mold caused by *Capnodium sp.* during the rainy seasons. However, this in no way affected the growth of the seedlings.

The results lead to the conclusion that the neem seedlings exhibited significant variation between the provenances in most of the characters studied. Among the eight provenances studied, four provenances viz., Nagarcoil, Trichur, Coimbatore and Dindigul were found to be better in terms of seed and seedling characters studied. With in these above mentioned provenances Coimbatore was found to be the outstanding performer in most of the characters studied.

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PROVENANCE EVALUATION IN THE SEEDLING CHARACTERS OF NEEM (*AZADIRACHTA INDICA* A. JUSS)

By
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ABSTRACT OF A THESIS

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ABSTRACT

A study was conducted at College of Forestry, Vellanikkara, Trichur on provenance evaluation in the seedling characters of neem (*Azadirachta indica* A. Juss) for a period of one year from 1995 to 1996. The experiment was laid out in a completely randomised design. A total of eight provenances were used in this study they are Dindigul, Nagarcoil, Coimbatore, Trichur, Srivelliputhur, Palghat, Ghatti Subramanya and Molakalmur.

In respect of various seed characters studied Nagarcoil was the best performer in characters like 100 seed weight, seed breadth and germination percentage. Trichur was the best performer in seed length and seed length breadth ratio and Srivelliputhur was the best performer in thickness of pericarp.

In terms of various seedling characters studied Coimbatore was the best performer in height, leaf number, leaf area, stem dry weight, leaf dry weight, and shoot dry weight. Palghat provenance was superior with respect to root dry weight, and root shoot ratio; Nagarcoil was the best in terms of collar girth and Dindigul in total dry weight. Nevertheless, Coimbatore provenance was found to be the best performer in most of the seedling character studied.

Isozyme banding pattern for peroxidase showed two bands PRX-1 and PRX-3 in common. Eight provenances were grouped in to two based on the presence or absence of PRX-2.

No significant variation was observed in terms of chlorophyll - A, B and total chlorophyll content between provenances. Number of stomata per square centimetre was maximum in Coimbatore provenance.

For the genetic characters studied 100 seed weight and germination percentage recorded maximum GCV and PCV for seed characters and shoot weight and shoot root ratio for seedling characters. Maximum heritability and genetic gain were recorded in germination percentage and height and collar girth in seedling characters.

Application of Mahalanobis' D^2 statistics and Tocher's method of clustering resolved eight provenances in to four distinct clusters. Cluster I comprising of three provenances (Dindigul, Palakkadu and Ghatti Subramanya). Cluster II comprising of only one cluster (Coimbatore), cluster III comprising of again only one cluster (Molakalmur) and cluster IV comprising of three provenances (Nagarcoil, Trichur and Srivelliputhur).

Incidence of pests was not noticed during the entire period of investigation. All provenances were found affected by sooty mold caused by *Capnodium sp.* during the rainy seasons with out affecting the growth of the seedlings.