3. POST HARVEST TECHNOLOGY MANAGEMENT OF AZADIRACHTA INDICA SEEDS AND TRAINING TO BENEFICIARIES

A revolution in the energy sector is considered possible through Biofuel technology. Biodiesel species plantation is not only important for alternative renewable source of producing transportation fuel but also in maintaining environmental balance and creating rural employment opportunities. The prime aim of the objective is to study and develop the methods for obtaining quality neem seeds through efficient post harvest technology for better yield of oil and biodiesel production and byproduct utilization through scientific methods.

Post harvest handling is the stage of crop production immediately following harvest. It involves stages such as drying, shelling, cleaning, sorting and packing. Post harvest technology involves all treatments or processes that occur from time of harvesting until the foodstuff reaches the final consumer. Proper post harvest processing is critical to maximize yield, longevity, vigor and overall quality of the seed crop. At maturity, seed must be harvested, threshed, cleaned, and fully dried before storage. Each of these steps requires proper timing, skills and in some cases, equipment. Investing in the long growing season of a seed crop, only to lose it with improper harvest and post-harvest handling, is an incredibly frustrating experience. While production of vegetable seeds is similar in many respects to vegetable production, post-harvest practices require knowledge and methods unique to seed production. For organic producers, timing of maturation and harvest can be particularly critical to avoid losses from seed borne diseases or insect pests.

Seed is a biological living entity. Botanically it is a matured fertilized ovule and functionally it is a propagative material consists of dormant plant or a generative part that develop into a new plant. Seed, the basic input of plant regeneration, is used
Molecular studies on Azadirachta indica

both for multiplication and maintenance of generation. Seed, when multiplies through further generation and to maintain its originality, it ought to have some basic characters which are collectively termed as seed quality.

Seed quality is the possession of seed with required genetic and physical purity that is accompanied with physiological soundness and health status. The major seed quality characters are as follows:

3.1 Seed quality characters

3.1.1 Physical Quality

It is the cleanliness of seed from other seeds, debris, inert matter, diseased seed and insect damaged seed. The seed with physical quality should have uniform size, weight and colour, and should be free from stones, debris and dust, leaves, twigs, stems, flowers, fruit walls, other crop seeds and any other inert material. It also should be devoid of shriveled, diseased, mottled, moulded, discolored, damaged and empty seeds. The seed should be easily identifiable as a species of specific category of silvicultural species. Lack of this quality character will indirectly influence the field establishment and planting value of seed. This quality character could be obtained with seed lots by proper cleaning and grading of seed (processing) after collection and before sowing/storage.

3.1.2 Genetic Purity

It is the true to type nature of the seed. i.e., the seedling/plant/tree from the seed should resemble its mother on all aspects. In forestry for maintenance of this purity character, plus tree selection, provenance selection, seed source identification and maintenance of seed source/provenance seed without bulking is important. This quality character is important for achieving the desired goal of raising the plantation either for timber or fruit or tannin or yield of any economic produce.
3.1.3 Physiological Quality

It is the actual expression of seed in further generation/multiplication. Physiological quality characters of seed comprises of seed germination and seed vigour. The liveliness of a seed is known as viability. The extent of liveliness for production of good seedling or the ability of seed for production of seedling with normal root and shoot under favorable condition is known as germinability. Seed vigour is the energy or stamina of the seed in producing elite seedling. It is the sum total of all seed attributes that enables its regeneration under any given conditions. Seed vigour determines the level of performance of seed or seed lot during germination and seedling emergence.

Seeds, which perform well at sowing, are termed as quality seed and based on the degree of performance in production of seedling it is classified as high, medium and low vigour seed. The difference in seed vigour is due to the manifestation of the deteriorative process occurring in the seed before the ultimate loss of ability to germinate. Difference in seed vigour will be expressed as variation in rate of emergence, uniformity of emergence and loss of seed germination. Hence it is understood that all viable seeds need not be germinable but all germinable seed will be viable. Similarly all vigourous seeds will be germinable but all germinable seed need not be vigourous. Physiological quality of seed could be achieved through proper selection of seed (matured seed) used for sowing and by caring for quality characters during extraction, drying and storage. Seed with good vigour is preferable for raising a good plantation as the fruits, the economic outcome are to be realized after several years. Hence selection of seed based on seed vigour is important in silvicultural species for raising future plantation.
3.1.4 Seed Health

Health status of seed is nothing but the absence of insect infestation and fungal infection, in or on the seed. Seed should not be infected with fungi or infested with insect pests, as these will reduce the physiological quality of the seed and also the physical quality of the seed in long-term storage. The health status of seed also includes the deterioration status of seed, which also expressed through low vigour status of seed. The health status of seed influences the seed quality characters directly and warrants their soundness in seed for the production of elite seedlings at nursery/plantation.

3.2 Seed Standards

Seed is the consumer material for the farmers, the end users who insist on their quality before the purchase of the same. In agricultural and horticultural crops, the quality of seeds that are delivered to farmers are to possess the above said seed quality characters with minimum/maximum limit in line with their importance and involvement in productivity. This upper and lower limit of seed quality characters that the seed ought to possess is termed as seed standards. The seeds that are produced under the supervision, as per the norms of Indian seed act (1966) are distributed in the market as certified class of seed. Even the seed that is not certified at field level are to be sold only as labeled seed, which also should have the minimum level of seed quality characters. In agricultural crops researches have conducted studies on comparing the performance of farmers saved seed and certified seed without specific standards for seed quality characters and proved that the seed produced by farmers and that are distributed with out any norms on seed quality, perform poorer with more of off types.
3.2.1 Basis for Seed Standards/the Seed Quality Control System in India

Seed Act (1966) of India for distribution of quality seed to farmers, envisaged its jurisdiction through various interlinked components viz., variety release and notification, seed production, seed certification, seed testing and seed quality control system or seed law enforcement. The procedures for implementing this quality control system are described under Seed Rules (1969) (Fig. 8).

In brief, Seed Act (1966) expresses that once the variety is released and notified it comes under the purview of certification, where the seeds are supervised at their production point for adoption of advanced production technique and for the assurance of genetic purity which could be maintained only at field level by proper and honest supervision, as genetic purity is highly controlled by the source seed used for multiplication and to some extent by the environment. The produced seed are again evaluated for its genetic purity through grow out test based on standards that are fixed by Indian minimum seed certification standard as below for the different classes (generation system/stages of multiplication) of seed.

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**Fig 8: Seed Quality Control System in India**
The seed thus produced are also evaluated for its physiological, physical quality characters and their health status at authenticated and accredited seed testing laboratories. In addition, they also checked for their moisture content depending upon the storage containers as moisture content influences both the physical, physiological quality characters and health status of seed. All these quality evaluations are compared with specific seed certification standards described for each and every crop under the Indian Minimum Seed Certification Standard (Tunwar and Singh, 1988). It emphasizes the fulfillment of two types of standards as below to name the seed as certified seed or quality seed.

3.2.1.1 Field Standard

These standards are to be checked or controlled at field level during the production and maintenance of genetic purity. In verification, field standards are looked for Isolation distance.

- Off type percentage
- Designated diseases
- No. of inspections to check the above characters

2.2.1.2 Seed Standard

After harvest, seeds are handled for post harvest seed management techniques viz., grading, seed treatment and before storage the seeds are checked for the following characters at authenticated laboratory.

- Seed germination (with maximum requirement)
- Seed purity (with minimum requirement)
- Other crop seed (with minimum requirement)
- Inert matter (with minimum requirement)
- Seed moisture content (with minimum requirement)
Designated diseases and noxious weeds

GOI (Tunwar and Singh, 1988) has proposed standards for more than 133 crops including vegetatively propagated crops as Seed Act defines seed as below.

Seed means only of the following classes of seed used for sowing or planting.

- Seeds of food crops including edible oil seeds and seeds of fruits and vegetables.
- Cotton seed
- Seeds of cattle fodder
- Jute seed
- Includes seedling and tubers rhizomes, bulbs, roots, cuttings, all types of grafts and other vegetatively propagated material of food crop or cattle fodder.

3.2.2 Applicability of Seed Quality Control System in Forestry

The requirements of seed standards for quality seed production has also been classified as general and specific standards, where general standards like class of seed, applicability of quality requirement are common to all crops and the specific standards like isolation (field standard), germination (seed standard) etc., are specific to crops irrespective of variety. In addition as per seed Act (1966), the varieties are notified for certification but, while for checking the quality characters, the standards are provided only for the variety but also for the kind of crop. So indirectly, all released varieties with crops are comes under the purview of Seed Act.

Seed Act also deals with seed law enforcement system which insists on verification of quality of seed sold in market (that are billed) that are expressed on the seed container as mark or label or tag through special officers called seed inspectors appointed for the purpose of verification of quality of seed sold in market for any kind of seed. Hitherto specific seed standards are available (implementation of seed law
enforcement) for crops irrespective of variety to which the seed in marketing chain are to be fulfilled.

So all seed sold in the market, irrespective of kind or varieties are indirectly comes under the purview of seed Act and its terms and conditions, necessitating the need for quality testing in each and every crop based on specific standards that are developed by Government of India based on the researchers conducted by researchers of public sector at various places with many a variety and kind in which forestry seeds also not an exemption as the seeds are used as base material for multiplication.

3.3 Seed Collection and Testing

Seed collection is the primary and most important step of initiation of any plantation research programme. Seed is a vital input and quality seeds alone may be instrumental in increasing output up to 20%. As seed quality deteriorates faster, quality control, though costly, is most crucial both in multiplication and distribution of seeds. The effective planning and implementation of research programme depends on the availability of all types of sufficient seeds with right physiological and genetic characteristics. In the first place, the seed must be collected from a genetically proven superior source. Secondly, there must be continuous checking by testing the physical and physiological status of the seed. Finally it is important that seed is stored until required without losing its germinative capacity and quality.

The behaviour of a seed depends on many parameters like species, year of collection, time and the method of collection, transportation, method of cleaning and depulping, storage length and condition, handling of seed during storage and harvesting. Seed testing procedures have, therefore, been developed for maintaining quality control and to provide the prospective seed user the information that has a
bearing on the planting value of the seed. Seed testing and characterization procedures have been standardized.

3.4 Seed Quality Evaluation Technique

Once the minimum requirements are fixed there is a need for standardization of seed quality evaluation technique, as reproducibility is needed on evaluation of seed quality at any point. Knowing the importance of seed quality evaluations, the International Seed Testing Association (ISTA, 1999) has formulated procedures for seed quality evaluations as reproducible seed quality characters are warranted on international movement of seed irrespective of testing laboratories. In ISTA more than 150 countries including India have enrolled as members and are evaluating the seed quality characters of all kinds (agricultural, horticultural shrub, trees, flowers, fruits, medicinal plants) of seeds in movement except individual transaction between the farmers. As per the terms of ISTA, once in 10 years it renews the procedure and methodologies of seed quality evaluations as per the need and lacuna identified on usage. It also has provisions for inclusion of procedures for new species and modification of evaluation techniques already available.

3.5 Methods of Seed Quality Evaluation

ISTA has defined various methods and procedures for analyzing the seed quality evaluations. In seed quality evaluation the basic unit of operation is the seed lot where the size of lot is fixed based on the size of wheat and maize. If the size of seed is more than maize then the size of the lot is 40,000 Kg, but if it is up to the size of wheat then the maximum size of seed lot is 20,000 Kg. From each lot adopting sampling intensity, primary samples are taken either using hand or using triers. From the primary sample composites samples are obtained and from this using dividers submitted sample is obtained as per the recommendations of ISTA and Indian seed
testing procedures. The submitted sample is dispatched to the seed-testing laboratory and again by proper mixing and dividing working sample is obtained. The size of the working sample varies with species and on this sample only all the seed quality evaluations are carried out.

3.5.1 Purity Analysis

This is used or evaluating the physical purity of the sample. The working sample is separated into 3 different fractions viz., pure seed, other crop seed including weed seed and inert matter using purity work board. Based on weight, the seed analyst will report the results of physical purity as pure seed percentage. Samples, which have lesser percentage than the recommended standard, are not accepted as seed. But could be improved further by reprocessing and the quality can be improved on further resubmission of sample for analysis.

3.5.2 Germination Test

Germination test is used for the analysis of physiological purity the seed. Germination is expressed in percentage to the total number of seeds placed for germination, where ISTA specifies that 400 pure seeds are to be evaluated for seed germination. In this test, seeds are germinated in sand or paper media adopting different methodologies (top of the paper, roll towel, inclined plate) and are germinated in a germinator maintained at 250°C and 90-95% RH. On the completion of the germination period, the period specified for evaluation by ISTA for each of the crop, the germination test is terminated adopting the seedling evaluation procedure of ISTA, as normal seedling, abnormal seedling, dead seeds, fresh ungerminated seed and hard seed. Based on normal seedling, the germination is reported in percentage and is compared with required standard germination for seed to categorize as standard or substandard sample/lot.
3.5.3 Moisture Estimation

Moisture content of seed is an important factor that decides the quality of seed both in orthodox and recalcitrant seed. This also helps in maintenance of seed quality in higher order. As per ISTA up to 17% the seeds are shade dried and then are kept at oven adopting low temperature constant method ($103^0\text{C} \pm 1^0\text{C}$ for 17 h) or high temperature constant method ($130^0\text{C} \pm 1^0\text{C}$ for 1-4 h). The moisture content required for storage is specified for each crop above, which hastens the deterioration of seed in storage, which is needed for the maintenance of seed quality up to the validity period.

3.5.4 Seed Weight Determination

It is the determination standard 100 seed weight of the seed lot, which is based on the counting of eight replicates of 100 seeds from the pure seed fraction of working sample. Knowledge on this determination favors to judge the homogenous status of seed lot for seed quality characteristics.

3.5.5 Quick Viability Test

It is also known as topography test or tetrazolium test, were seeds are preconditioned in water and are prepared (longitudinal cut and removal of seed coat) for exposure of embryo to 2,3,5 tetrazolium chloride, which on soaking will stain the viable portion of seed into pink. The nonviable portions will be colorless. This methodology would be highly useful in knowing the physiological status of seed in case of possession of seed dormancy.

3.5.6 Seed Health Test

This test helps in understanding the health status of seed. Seeds are infected by storage and field fungi, and also by primary and secondary insects. Indian seed certification standards expresses that the insect activity in seed should not exceed 1% on any account, otherwise the seed would be discarded. Identification of pathogen
infection is done using blotter technique, were the seeds are placed in blotter paper and are incubated at NUV light for the growth of fungi. Either field or storage fungi, standards for acceptance of seed for sowing purpose is warranted under seed quality evaluations. In agricultural crops in most cases it ranges from 0.1 to 0.5%.

3.5.7 Genetic Purity

In agricultural crops the genetic purity is evaluated through grow out test while ISTA also recommended adoption of electrophoresis (SDS page) technique based on banding pattern of protein or enzyme for identification of purity of gene in the evaluated species, which could be widely adoptable for perennial tree species.

3.5.8 Vigour test

Vigour is the stamina of the seed and various techniques are adopted to evaluate this qualitative factor. Some of the promising tests are brick gravel test, electrical conductivity test, GADA test, dehydrogenase enzyme activity, amino acid, free sugars and free fatty acid content. But even in agricultural crops none of the test is a regular standard test in seed testing. Hence the vigour test along with germination test will express the quality of seed in terms of physiological soundness of the seed.

3.5.9 Other tests

In addition to this for evaluating the quality of seed, other tests such as H₂O₂ test, exercised embryo test, X-Ray test are also recommended by ISTA for the clear understanding of seed quality evaluation.

3.6 Post harvest management of seeds

Postharvest management is a set of post-production practices that includes: cleaning, washing, selection, grading, disinfection, drying, packing and storage. These eliminate undesirable elements and improve product appearance, as well as ensuring that the product complies with established quality standards for fresh and processed
products. Postharvest practices include the management and control of variables such as temperature and relative humidity, the selection and use of packaging and the application of such supplementary treatments as fungicides (FAO, 2009).

A seed has been defined as a 'mature ovule' or a reproductive unit formed from fertilized ovule, consisting of an embryo, reserve food, and a protective cover. The most essential factor for the success of plantation is the ready availability of quality seeds. The quality of seed is totally responsible for the future return/performance of each and every seedling.

3.6.1 Characteristics of poor quality seeds

- low germination percentage
- poor emergence
- poor survival
- poor adaptability to site
- susceptible to disease and pests
- poor growth
- low productivity

3.6.2 Characteristics of good quality seeds

- must be well ripened, healthy and true to type,
- must be pure and free from inert materials and weed seeds,
- must be viable and have good germination capacity,
- must be uniform in its texture, structure and look, and
- must not be damaged, broken and affected by pests and diseases.
3.6.3 Strategies for seed collection, cleaning and upgrading

It requires good planning in advance regarding deployment of trained staff, arrangement of transportation facilities, seed collection equipments, measures to ensure the safety of workers, packing and labeling material, and maintenance of the records, etc. The other important points are:

- information about the location, time of flowering and fruiting,
- information about the periodicity of seed crop,
- prefer seeds of well-adapted local source to the un-adapted sources of different places,
- avoid stands of poorly formed, excessively flimsy, off-color, abnormal or diseased trees,
- change in latitude, humidity, temperature and attack of pests greatly affects the seed quality, yield and periodicity,
- fruit ripening gets delayed due to rains and advanced due to high temperature and drought.

In most of the tree species seed matures in a phased manner within a few weeks. At first, few seeds ripen and the number gradually increases till it reaches a peak (synchronized maturity) and then there is a gradual decrease. The mature seeds collected during the peak phase give more uniform germination and have greater longevity in storage than immature seeds.
Fig 9: Tree Pruners for Seed Collection

Seed from the standing trees can be collected by following methods:

- by using light weight poles for striking and shaking of branches,
- by using poles with knife and sickle for cutting the small branches,
- by climbing on the tree with the help of tree bicycle, rope-ladder, one-legged ladder, tree pruner, etc. and
- by using nets and other local materials.
3.6.4 Seed Cleaning

It can be done by the following methods: screen cleaning by using sieves of different pore sizes,

- air separation/winnowing or by aspirators,
- de-winging reduces storage volume, make upgrading possible, sowing easier and removes pathogen,
- empty seeds can be removed by liquid floatation, and
- seed drier, seed grader, seed separator, seed blower, seed scarifier, sieves, etc. are some of the useful equipments of seed processing.

3.6.5 Seed Upgrading

It reduces the chances of disease, quantity of the seed to be procured as well as its costs. It is done as follows:

- remove weak and damaged seeds,
- remove empty, immature and discolored seeds.

3.7 Post Harvest Care

The time between collection and extraction of seed is very important to maintain high germination and vigor. Some of the important points to be remembered during seed collection and storage are as follows:

- the freshly collected seed should not be exposed to sun, since heat may kill the seeds,
- the safest drying method is to spread a thin layer of fruits in well ventilated rooms and stirring at regular intervals,
- Seeds should not be left in wet areas otherwise it will rot and die,
- The soft and fleshy seeds such as neem, should not be kept in heap or large sacks/bags immediately after harvest. They can be kept in small-untied
perforated sacks or open basket after cleaning of pulp and drying of seeds. The large and closed sack generates much heat as well as thermophilic fungi that can kill the seeds.

- Seeds should be completely dried and labeled before putting them for storage under species specific conditions

3.7.1 Seed Storage

Seed storage is the preservation of viable seed until their sowing/requirement. It is essential to offset the uncertainty of seed production/availability during bad seed years. It delays deterioration, maintains viability and protects seed from rodent and insect damage. The longevity of seeds is a species specific characteristic. The seed of most of the species can be stored at low temperature and low moisture content in sealed containers. It is important to dry the seed uniformly to prevent fluctuation in moisture content during storage. The moisture content of most of the seeds for storage ranges between 10 to 12%. The respiration continues at low temperature, which is necessary to keep the embryo alive. Polythene bags make good containers because they are impermeable to water but less so to oxygen and carbon dioxide.

3.8 Post harvest management of Neem seeds

The Neem tree (*Azadirachta indica*) has been known as the wonder tree for centuries in the Indian subcontinent. Neem is a medium sized to large tree characterized by its short straight trunk, furrowed dark brown to grey bark and dense rounded crowns of pinnate leaves. Native to India, Neem is widely planted and naturalized in semiarid areas throughout Asia and Africa. Neem is an evergreen of the tropics and sub-tropics.

It belongs to the family Meliaceae and is a cousin of the Chinaberry. With an extensive and deep root system, the hardy Neem can grow luxuriantly even in
marginal and leached soils, and thrives up to an elevation of 1500m. The Neem flowers profusely between February and May. The honey-scented white flowers, found in clusters, are a good source of nectar for bees. Neem fruits are green drupes which turn golden yellow on ripening in the months of June, July and August, in India.

The kernels have about 45% oil. The termite resistant Neem timber is used as a building material and in making furniture and farm implements. The bark yields tannin and gum. The amber hued gum is used as a dye in textiles and in traditional medicines.

**Local Names of Neem in India & around the world**

<table>
<thead>
<tr>
<th>Language</th>
<th>Local Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hindi</td>
<td>Neem</td>
</tr>
<tr>
<td>Bengali</td>
<td>Nim, Nimgachh</td>
</tr>
<tr>
<td>Konkani</td>
<td>Beva-rooku</td>
</tr>
<tr>
<td>Marathi</td>
<td>Kadunimb</td>
</tr>
<tr>
<td>Gujarati</td>
<td>Leemdo</td>
</tr>
<tr>
<td>Tamil</td>
<td>Vembu, Vempu</td>
</tr>
<tr>
<td>Punjabi</td>
<td>Nimb</td>
</tr>
<tr>
<td>Malayalam</td>
<td>Veppu, Aryaveppu, Aruveppu, Kaippan, Veppu, Vepa</td>
</tr>
<tr>
<td>Simhalee</td>
<td>Nimu</td>
</tr>
<tr>
<td>Oriya</td>
<td>Nimo</td>
</tr>
<tr>
<td>Telegu</td>
<td>Vepa</td>
</tr>
<tr>
<td>Kannada</td>
<td>Bevinmara, Kahibevu</td>
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</tbody>
</table>

Neem, starts bearing fruits after 3-5 years and comes to full bearing at the age of 10-12 years. Fruit yield is 10-25 kg per tree per year in the initial years. A mature tree produces 35-50 kg fruit/year. Oil yield varies from 40-43% of seed on dry weight
basis. However we presume a conservative yield of 5, 7, 10, 15, 20 Kg/tree respectively from 5th year onwards. Yield generally stabilizes from 9th year.

![Fruits, Seeds (with endocarp), Seeds (without endocarp)]

**3.8.1 Harvesting and processing**

In many areas seeds are easy to collect from the ground because birds or fruit bats eat the juicy as well as sweet fruits and spit out the kernels. Where this does not occur, the harvested ripe fruits need to be depulped. If water is available, the risk of infection by fungi can be reduced by washing the grains after collecting them. For further processing, use oil or water extracts. For storage, the kernels should be well dried by spreading them on hand ground in the shade. To avoid moulding, kernels should always be stored in a well-aerated container such as a jute sack. Never store them in a plastic bag. Moulding can be due to fungi which produce aflatoxin, a substance which is highly toxic to human beings even in low concentrations. To prepare seeds for planting, dry them carefully, if possible, in the shade because temperatures above 45°C will reduce germination. Storage for more than one month will also decrease the rate of germination. For immediate sowing, kernels need not to be dried.
3.8.2 Seed collection and storage

Only fruits at the yellow green color stage are pricked from the branches by hand or by using ladder. After collection, the fruits are depulped immediately. Soaking in cold water for a few hours helps in removing pulp. Fruits are then rubbed over a coffee weir and floated in water to separate seed from pulp. Storing neem seed for 5 months at 40% natural moisture content at 16\(^\circ\)C is possible. For short storage, the seeds are closed in polythene bags and exposed to air once in a week to keep them viable. Long term storage of neem seeds for more than 10 years is done at 4% moisture content and -20\(^{\circ}\)C temperature. For this purpose, seeds are dried very quickly i.e. within a few hours after depulping in a mono layer at temperature more than 20\(^{\circ}\)C to prevent chilling damage under a fan. Shade drying and storage of seed in cloth bags at a temperature upto 4\(^{\circ}\)C is also done to improve seed viability. Storage of seed in earthen pot containing wet sand (30% moisture) helps to retain viability upto 60% at the end of 3 months. On an average 5000 seeds weigh one kilogram.

3.8.3 Post harvest pre-treatment and processing

It has been found that between 2-5 months after harvesting, the oil content of neem seeds rises to a maximum. Its harvest also coincides with the monsoon in India, making drying difficult. After collection, the fruits are depulped, usually manually, although small mechanical depulpers are available. In some areas, the fruits are buried for a few days to facilitate pulping.

The pulped seeds are then either sundries or piled in heaps and turned periodically. Small artificial tray driers are also used. The dry seeds are de-corticated, usually manually. Hand and power decorticators have also been developed by the Khadi and Village Industry Commission of India which is a machine that processes dry fruit through to kernels.
In the present study, the attempt is made to obtain quality neem seeds for further processing like oil extraction, biodiesel production and characterization of seeds at molecular level. During the attempt, the beneficiaries were trained on post harvest management of neem seeds for quality seed isolation starting from the seed collection from tree to seed transport and storage.

3.9 Post harvest technology developed/transfered

- Technologies for seed quality testing, Biodiesel quality testing and biodiesel production were standardized.

- Protocols for healthy and mature seed harvest, processing and storage technologies standardized.

- Information booklets/CD in Kannada is prepared on demonstration on Post harvest technologies of seed harvest, seed processing and conservation.

- Byproduct has been utilized for the biofertilizer and biogas production.

- Selection of the elite clones and propagation technologies were standardized.

- The marketing point is established in Gulbarga university campus, Chincholli and Basavakalyan.
### Table 8: Awareness and training program conducted to beneficiaries on "Post harvest management of neem seeds".

<table>
<thead>
<tr>
<th>Sl.No.</th>
<th>Beneficiaries</th>
<th>No. of Beneficiaries</th>
<th>Technology trained</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Agricultural Womens</td>
<td>50</td>
<td>Seed collection and Seed Processing</td>
</tr>
<tr>
<td>2</td>
<td>Farmers</td>
<td>2500</td>
<td>Seed collection</td>
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<tr>
<td>3</td>
<td>Engineering Students</td>
<td>30</td>
<td>Biodiesel production and quality testing</td>
</tr>
<tr>
<td>4</td>
<td>Entrepreneurs</td>
<td>10</td>
<td>Oil extraction, Biodiesel production and quality testing</td>
</tr>
<tr>
<td>5</td>
<td>Govt. employs</td>
<td>15</td>
<td>Biodiesel quality testing</td>
</tr>
<tr>
<td>6</td>
<td>NGOs</td>
<td>12</td>
<td>Seed processing and Biodiesel production</td>
</tr>
<tr>
<td>7</td>
<td>Forest employs</td>
<td>35</td>
<td>Cultivation and post harvest care</td>
</tr>
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<td>8</td>
<td>MSc Students</td>
<td>100</td>
<td>Oil extraction by soxhlet method and biodiesel production</td>
</tr>
<tr>
<td>9</td>
<td>School Students</td>
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<td>Demonstration on Oil extraction, Biodiesel production and quality testing</td>
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<td>10</td>
<td>Self Help Group</td>
<td>10</td>
<td>Oil extraction</td>
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<td>11</td>
<td>Executive officers</td>
<td>25</td>
<td>Quality testing</td>
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<tr>
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<td>Zilla Panchyat Members</td>
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<td>Quality testing</td>
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<td>13</td>
<td>PDO's</td>
<td>136</td>
<td>Biodiesel production and quality testing</td>
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<td><strong>Total</strong></td>
<td></td>
<td><strong>3034</strong></td>
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</tr>
</tbody>
</table>
Fig 10: Information booklets/CD in Kannada on Post harvest technologies of seed harvest, seed processing and conservation.

Fig 11: Cultivation of Neem plants on wasteland at Biodiesel Technology Park, Gulbarga University, Kalaburagi.
Fig 12: 1- Biodiesel Information and Demonstration Center at Gulbarga University campus; 2- Quality Testing Lab; 3- Transestrification Unit.
Fig 13: Training program conducted to beneficiaries on Post harvest management of seeds; 1 & 2- Awareness on the post harvest management of seed processing; 3- Seed collection from the standing tree; 4- Seed drying in shade; 5- Oil extraction from seed oil and 5- Biodiesel production.
3.10 Methods developed for Obtaining Quality Neem Seed

3.10.1 Collection

1. Naturally Ripened fruits drop on the ground are to be collected within 1-2 days for further processing.
2. Fruits with yellowish color should be harvested.
3. Fruits should be harvested by shaking tree branches as well as by plucking fruits and if possible spread a cloth, tarpaulin under the tree.
4. Fresh fruits should be collected in the morning.
5. Fruit should be transported in baskets and or gunny bags.
6. These fruits should not be mixed up with semi dried fruits collected through sweepings. Moisture in Commercial Neem Fruits to be 6-9% and after drying 5%.

3.10.2 Depulping

1. Collected fruits should be kept immediately in warm water to avoid fungal growth. These fruits may be kept in water from 12-24 hours to further soften the pulp.
2. Such Water soaked fruits should be macerated with the help of gunny bags.
3. Mechanized scrubbers, macerators and washers can also be used for larger quantities.
4. Depulping can also be done manually by rubbing and washing the detached pulp.
5. A simple alternative is to dump the fruits in ash or soil for a couple of days followed by trampling, rubbing and winnowing.
3.10.3 Drying

1. Seed should be spread in thin layers for drying.
2. Jute gunny bags or perforated sheets should be used for drying seed.
3. Small tray driers using hot flue gases from a furnace have also been fabricated.
4. Sun drying in open space or partially covered space is preferable. However, in cloudy, rainy weather use of fans and hot air blowers are suggested.
5. In dried seed moisture level of 9-15% is reasonable (as against 40-50% moisture at the time of collection of fruits).
6. Germination rate of fresh seed is 90% which would drop to 40% in 30 days and less than 5% in 60 days.

3.10.4 Storage

1. Cold Storage of seed prolongs its shelf life.
2. Dried Seed should be stored in airy containers, jute bags or perforated bags at room temperature under moisture free conditions: can be stored up to one year but never store in plastic bags. After collection and drying of seed immediate transport arrangements should be made for it’s processing.
4. CHARACTERIZATION OF DIVERSITY FROM AZADIRACHTA INDICA OF HYDERABAD KARNATAKA (HK) AREA

Molecular markers are commonly used by plant biologists to perform a number of tasks, including the genetic fingerprinting of plant varieties, determining similarities among inbred varieties, mapping of plant genomes, and establishing phylogeny among plant species. New techniques for the extraction, purification and amplification of plant DNA are being developed on a regular basis, enabling researchers to decrease preparation time and obtain readily reproducible results. Plants can now be compared at the molecular level in several ways, via examination of restriction fragments, identification of isoenzymes (protein/gel electrophoresis) or products of the polymerase chain reaction (PCR).

A large number of methodologies exist for the assessment of genetic diversity in plant species. The relative genetic diversity among the individuals or populations can be determined using morphological and molecular markers. Phenotypic characters have a limited importance since they are considerably influenced by environmental factors and developmental stages of the plant and also due to the fact that in some species adequate levels of phenotypic polymorphism are not available (Tatineni et al., 1996). On the contrary, molecular markers, based on DNA sequence polymorphism, are independent of environmental conditions and show high levels of polymorphism (Choudhary et al., 2001). Several markers like protein and isozyme electrophoresis have been used in many cultivated crops (Tanksley et al., 1983; Soltis et al., 1990).

The major limitation of these techniques is insufficient polymorphism among closely related cultivars. Because proteins are the products of gene expression, they may vary in different tissues, developmental stages and environments. On the other hand, DNA based markers avoid such difficulties. Of the various kinds of DNA
markers characterized so far, restriction fragment length polymorphisms (RFLPs) were the first to provide the means to directly detect variations present at the DNA level. RFLPs give a much higher degree of polymorphism and stability.

RFLPs have been used to document the genetic diversity in many cultivated plant species (Tanksley et al., 1989; Russell et al., 1997). Although highly specific, performing RFLPs is quite laborious and expensive since it requires large amounts of pure DNA and needs an expertise in handling radioactivity. As an alternative, randomly amplified polymorphic DNAs (RAPDs), a PCR based technique, resolved most of the technical impediments owing to its cost-effective and easy to perform the approach (Welsh and McClelland, 1990; Williams et al., 1990). This efficient technique obviates the need to work with radioisotopes and gives good results even with crude DNA preparations. RAPDs have, therefore, been used in assessing genetic variations in several agricultural crops (Tatineni et al., 1996 and Mackill, 1995). We report here the application and reliability of RAPD markers to investigate the extent and distribution of genetic diversity in *Azadirachta indica* from different eco-geographical regions.

Biochemical markers such as proteins and isozymes have served as an important tool to detect genetic relationships in plants (Mukhlesur et al., 2004). Protein polymorphism serves as genetic markers as they are direct products of active genes and are quite polymorphic and generally heritable (Gepts, 1990). The polymorphism observed in the protein profiles reflects the changes in the active part of the genome. Although protein polymorphism can be analyzed through a variety of techniques, polyacrylamide gel electrophoresis (PAGE) is generally favored technique for rapid analysis (Fergusan and Grabe, 1986; Smith and Smith, 1986; Raymond et al., 1991) due to its validity and simplicity for describing genetic
Molecular studies on *Azadirachta indica*

variations (Ahmed and Slinkard, 1992). This technique has been used effectively to
decipher genetic diversity among/ between genotypes in different plant species (Cook,
1984; Mukherjee and Datta, 2008).

The objective of this study is to evaluate the genetic variations and genetic
relationships among the neem accessions collected from four ecological regions on
the basis of RAPD and protein profiles.