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**DEVELOPMENT OF PROTOCOLS FOR  
PROCESSING AND TESTING OF FOREST SEEDS**

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**March 2009**

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TESTING OF FOREST SEEDS**

*(Final Report of the Project No: KFRI 389/03)*

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## ABSTRACT OF PROJECT PROPOSAL

1. Project No. : KFRI 389/03
2. Title of the project : Development of protocols for processing and testing of forest seeds.
3. Objectives :
  1. To standardise seed processing techniques for important indigenous forest species of Kerala
  2. To assess the storage physiology of forest seeds for which information is not available.
  3. To develop appropriate procedures for seed viability and purity testing for certification.
4. Date of Commencement : April 2003
5. Date of Completion : March 2006 (extended up to June 2006)
6. Funding Agency and Total amount of grant sanctioned : Plan Funds  
Rs. 3 lakhs + 0.4 lakhs
7. Name of the principal investigator : K. C. Chacko

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Dr. M. Sivaram was helpful in statistically analyzing the results of the teak cutting test and correlating it with germination percentage through a prediction equation, and also arriving at sample sizes for purity test for *Neolamarckia cadamba*, *Cassia fistula*, *Oroxylum indicum* and *Albizia lebbek*.

The voluntary technical help rendered by Mr. Viju Varghese, Range Officer and Mr. M. K. Ambujakshan, Forester, Kerala Forest Seed Centre, is acknowledged with thanks. Thanks are also due to Mr. C. B. Santhoshkumar for technical assistance and help rendered in finalizing the report. Smt. E. V. Thanka, Helper, provided help in laboratory works.

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## ABSTRACT

Various aspects of seed handling such as processing, storage physiology, pre-sowing treatments, viability and purity testing were studied for twelve indigenous forest tree species, viz., *Albizia lebbbeck*, *Aegle marmelos*, *Artocarpus hirsutus*, *Cassia fistula*, *Dysoxylum malabaricum*, *Gluta travancorica*, *Gmelina arborea*, *Neolamarckia cadamba*, *Oroxylum indicum*, *Syzygium cumini*, *Tectona grandis* and *Terminalia bellirica* of Kerala State in Southern India.

For extraction of seeds of *C. fistula*, the pods are broken open to release the seeds by hitting along suture line of pods with wooden mallet. For *N. cadamba*, 'froth method' is effective; the fruits are fermented and made into slurry by crushing in water and the seeds floating with the froth, when the diluted slurry is agitated, are skimmed off and dried. For *A. lebbbeck* pods and similar dehiscent fruits and all-weather solar seed drier has been designed for seed extraction. For size-grading of *T. grandis* (with mesocarp) above 9 mm, a seed grader was designed.

Storage physiology studies revealed that *G. arborea* seeds are intermediate and *A. marmelos*, *N. cadamba*, *O. indicum* and *T. bellirica* are orthodox. As regards to shelf life of seeds, *A. hirsutus* and *D. malabaricum* seeds store well only up to two weeks under ambient well-ventilated conditions.

Pre-sowing treatment studies revealed that removal of mesocarp followed by weathering of seeds (wetting with water and drying under sun) for a week improves germination of *T. bellirica*. For *G. travancorica* partial or complete removal of seed coat enhances germination.

Studies on seed viability, cutting test, and germination of *T. grandis* revealed a strong correction ( $G\% = [\exp(b \text{Log } D + c (\text{Log } D)^2) - 2] * V\%$ ); where  $G\%$  is germination percentage,  $V$  is the viability percentage and  $D$  is the days after sowing and  $b$  and  $c$  are constants) between cutting and germination tests. This equation is recommended for

predicting germination from results of cutting test, for seeds humid tropical regions of Kerala. Topographical Tetrazolium staining test can be employed to determine viability of *O. indicum* seeds. However, further studies are required in this regard.

For purity test, sample sizes suggested for different species are: 50 g for *A. lebbbeck*, 100 g for *C. fistula*, 1.5 g for *N. cadamba* and 10 g for *S. cumini*.

## 1. INTRODUCTION

Good seeds produce good planting stock. Quality of seeds can be ensured through seed collection from genetically superior stands/trees and scientific handling practices till sowing. Once the seeds have been collected from superior trees/stands, they need to be handled properly to maximize production of good quality seedlings. Therefore, a thorough knowledge of seed handling, which includes proper seed collection and extraction methods, drying and storage process, appropriate pre-sowing treatments, reliable testing methods, etc. is necessary. Although several methods of seed handling are known, they need standardization for many of our indigenous species. For this, an understanding of the storage physiology, as to whether it is orthodox, intermediate or recalcitrant, is necessary for improving processing and storage technology. Similarly standards for quick viability testing by topographical tetrazolium test or even the simple, yet efficient, cutting test need to be standardized. Thus the project envisages standardization of seed handling procedures for important indigenous forest species for which several details are not available for practice.

The project has the following specific objectives.

- To standardize seed processing techniques for 12 indigenous forest species of Kerala.
- To assess the storage physiology of forest seeds for which information is not available.
- To develop appropriate procedures for seed viability and purity testing.

## 2. MATERIALS AND METHODS

Various aspects studied include processing technology, storage methods, pre-sowing treatments, determination of storage physiology, and standardization of seed viability testing and seed purity testing. Since some of the details are available for some species, studies were limited to aspects for which information was not available. The list of the twelve species and aspects studied are given in Table 1.

Table 1. Species and aspects studied for developing protocols of seed handling

Sl. No.	Species	Aspect studied					
		Processing techniques	Storage methods	Storage physiology	Presowing treatments	Viability testing	Seed purity testing
1.	<i>Albizia lebbek</i>	*	*			*	*
2.	<i>Aegle marmelos</i>	*		*			*
3.	<i>Artocarpus hirsutus</i>	*		*			
4.	<i>Cassia fistula</i>	*				*	*
5.	<i>Dysoxylum malabaricum</i>	*	*	*			
6.	<i>Gluta travancorica</i>		*	*	*		
7.	<i>Gmelina arborea</i>	*				*	
8.	<i>Neolamarckia cadamba</i>	*		*		*	*
9.	<i>Oroxylum indicum</i>	*				*	*
10.	<i>Syzygium cumini</i>	*				*	
11.	<i>Tectona grandis</i>	*				*	
12.	<i>Terminalia bellirica</i>	*			*	*	

Various techniques and technologies specifically applicable for the selected species under study as well as those applicable for related tropical species ( Chacko, 1981; Chacko *et al.*, 2001; Chacko and Mohanan, 2002; Chacko *et al.*, 2002 a, b; Nair *et al.*, 2002 ) have been consulted apart from popular reference books (ISTA, 1996; Schmidt, 2000; Willan 1985).

Methodology for each of the twelve species is dealt with in detail under subheadings 2.1 to 2.12.

### **2.1. *Albizia lebbek***

*2.1.1. Processing:* Mature fruits (pods) of *A. lebbek* were collected from Kannannor, Palakkad in November 2003. They were sun-dried on cement platform. Under sun drying, most of the pods opened naturally while the intact pods were manually split open to release seeds. The seeds were cleaned of various plant parts by winnowing. The quantities of seeds obtained by natural dehiscence and manual extraction were recorded. A solar seed dryer was designed and fabricated during the course of the study.

*2.1.2. Storage method:* The seeds were dried, sealed in polythene bags and stored under ambient (about 32°C), 16°C, 18°C and 4°C temperature. For each storage temperature there were 3 bags, each containing 2 kg seeds. While packing in polythene bags, air in the bags was replaced by carbon dioxide to increase shelf life by slowing down respiration of seeds. Sample seeds were drawn periodically and sown in vermiculite taken in plastic trays (100 seeds x 3 replications) kept in temperature-controlled germination rooms and the cumulative germination count noted for 45 days.

*2.1.3. Seed viability test:* Seeds were put for germination (100 seeds x 3 replications). Germination percentage at 50 days was compared with results from Topographical Tetrazolium (TTZ) staining test as per ISTA (1998). For TTZ test, the seeds were soaked in water overnight. The seed coat was then removed and the decoated seeds were immersed in 1 % Tetrazolium solution (prepared by dissolving 1 g Tetrazolium salt in 100 ml of distilled water) for 48 hours in dark. The colour of the different parts of the seed was recorded. The staining was classified as light pink, moderately pink and dark pink and also in terms of parts (plumule, embryonic axis, radicle and cotyledons) stained.

*2.1.4. Purity testing:* ISTA (1996) specifies the pure seed fraction to contain (a) Intact seeds of the actual species as well as dead, shrivelled, diseased, immature and pre-germinated seeds, (b) achenes and similar fruits, e.g. samaras, with or without perianth and regardless of whether they contain a true seed, unless it is apparent that no true seed is contained, and (c) fractions of broken seeds, achenes etc., which are more than half of the original size. During purity analysis each 'pure seed' fraction (a, b, c) is separated from the working sample. Purity is expressed as the weight percentage of pure seed fraction over the total weight of the working sample.

$$\text{Purity \%} = \frac{\text{Weight of pure seed (g)} \times 100}{\text{Total weight of working sample (g)}}$$

Five seed samples (1 g to 100 g) were drawn from a lot. Clean seeds were separated on a purity board and weighed. The percentage of pure seeds to total seed quality was calculated using the above given formula. The weight range of sample that gave constant seed purity percentage was taken as appropriate sample size for purity testing.

## ***2.2. Aegle marmelos***

*2.2.1. Processing:* Mature fruits of *A. marmelos*, collected from Vilayannur, Palakkad in May 2005 in cotton bags, were transported to the laboratory within a day. Seeds were extracted by breaking open the fruits by hitting with a wooden mallet and washed in water to remove the mucilage. The seeds were then air-dried on newspaper under fan.

*2.2.2. Storage physiology:* Seeds were taken in plastic trays. They were first air-dried and further dried in desiccator using silica gel to different moisture contents viz., 9.7, 5.3 and 4.8%. The seeds were tested for germination (35 seeds x 3 moisture content x 3 replications) in germination room maintained at 30°C and the cumulative germination recorded for 65 days.

### **2.3. *Artocarpus hirsutus***

*2.3.1. Processing:* The seeds extracted from fresh mature fruits of *A. hirsutus* collected from Vazhakulam, Muvattupuzha in May 2004 and May 2005, were depulped, washed in water and air-dried. Seeds with pulp and without pulp were used for germination studies (100 seeds x 4 replications). Germination count was recorded daily for 60 days.

*2.3.2. Storage physiology:* Depulped seeds were stored under room temperature (about 32°C), 16°C and 4°C. The depulped seeds were first dried to different moisture content (MC %) under ceiling fan and then using silica gel. The seeds were then stored for a month in an open tray under ambient conditions. Samples were drawn from this lot at periodical intervals and put for germination (100 seeds x 4 replications) after determination of moisture content by oven-drying from sub samples.

### **2.4. *Cassia fistula***

*2.4.1. Processing:* Mature pods of *C. fistula* collected from Peechi in March 2004 were sun dried and split open by hitting with a wooden mallet to release the seeds. The advantages of breaking the pods along suture line were studied..

*2.4.2. Seed viability test:* The seeds were first soaked in boiling water for 5 minutes and then in cold water overnight and sown in germination trays that were placed in germination room maintained at 30°C. Simultaneously, representative seed samples were subjected to TTZ, using a 1% solution.

*2.4.3. Seed purity test:* Seed samples 5 g to 100 g from five seed samples were used for purity testing study. The samples were examined under an illuminated purity board and pure seed fractions separated.

## **2.5. *Dysoxylum malabaricum***

*2.5.1. Processing:* Mature fruits of *D. malabaricum* collected from Kummatti, Sholayar in July 2004 were spread on the laboratory floor for two days. During storage, some fruits split open and the seeds were hand picked from them. The fruits that did not split open were cut open vertically along the rind using a sharp knife and the seeds taken out. The seeds were cleaned of the yellow aril and treated with fungicide Captan 50% WP (@4 g/kg) to prevent fungal attack (Nair *et al.*, 2001), which occurs within short time.

*2.5.2. Storage physiology:* The fungicide-treated seeds were stored under different temperature conditions (about 30°C, 16°C and 4°C). The seeds were then put for germination (15 seeds x 3 replications) after 12 days, and thereafter, at an interval of 5 days. For desiccation trials, the treated seeds were taken in plastic trays and air-dried under fan in the processing room. Samples were taken periodically from the air-dried seeds and subjected to germination test (15 seeds x 3 replications). Moisture content of samples was also determined prior to germination.

*2.5.3. Germination test:* The seeds were put for germination in vermiculite. Daily observations were recorded up to 60 days.

## **2.6. *Gluta travancorica***

*2.6.1. Seed collection, transport and processing:* Freshly fallen mature fruits of *G. travancorica* were collected in cloth bags from Rosemala in Shenduruney Wildlife Sanctuary (8°55' N; 77° 9' E) on June 2003 and transported to Peechi within a day.

*2.6.2. Storage physiology:* The fruits were stored in open plastic trays for 163 days in laboratory under ambient condition (mean monthly minimum and maximum temperature of 22°C to 32°C and mean monthly minimum and maximum relative humidity of 68 % to 93%).

For desiccation trials, seeds were allowed to air-dry under laboratory conditions (Anilkumar *et al.*, 2002). Intermittent working of the ceiling fans facilitated air circulation inside the room. The seeds were sown for germination with out any pre-sowing treatment after 5 (T1), 8 (T2), 12 (T3), 19 (T4), 44 (T5), 63 (T6), 103 (T7), 150 (T8) and 163 days (T9) of storage in moist vermiculate, taken in plastic trays. For each set of sowing, 300, 195, 195, 195, 195, 185, 172, 90 and 30 seeds respectively were used in three replicates. The moisture content (fresh weight basis) of the seed sample was determined before sowing (ISTA, 1985). The germination trays were placed in a side-open nursery shed provided with translucent waterproof roof. The germination trays were irrigated manually using shower spray of tap water. Daily germination count was recorded for 170 days after sowing.

*2.6.3. Pre-sowing treatments:* The fruits were subjected to four pre-sowing treatments viz., removal of a small portion of the seed coats just opposite to the pedicel (T<sub>1</sub>), removal of the seed coat at the embryonic axis (T<sub>2</sub>), removal of entire seed coat (T<sub>3</sub>) and seeds with intact seed coat (T<sub>4</sub>). Seeds were then sown in vermiculite in trays kept in germination room maintained at 30°C. There were 10 seeds for each treatment.

## **2.7. *Gmelina arborea***

*2.7.1. Processing:* Mature fruits collected from Kariyammuriyam (Nilambur) in May 2005 and Kottappara, (Malayattur Forest Division) in June 2005 were heaped on cement floor for a week to ferment and enable easy removal of the seeds from the fruits. The seeds were depulped, sun-dried, and used for further studies.

*2.7.2. Storage physiology:* The seeds were stored in ambient conditions (about 32°C). Samples were drawn at periodic intervals and subjected to germination test (40 seeds x 3 replications). Moisture content was determined on separate samples of the same lot. Cumulative germination was recorded daily for 70 days after sowing.

## **2.8. *Neolamarckia cadamba***

*2.8.1. Processing:* Mature fruits of *N. cadamba* were collected from Nellikutha, Nilambur in September 2004 and from Kannimangalam in November 2004. Mature fruits were heaped on the cement floor of the processing shed and allowed to ferment for one week. The fruits were crushed and made into slurry in water. The slurry was further diluted with water. Then the solution was transferred into a plastic bucket from a height to form froth at the top. The process was repeated till all the seeds floated with the froth. The froth was skimmed out into a cotton cloth and the water drained off. Seeds were then dried under sun. This new method of seed separation from the fruit slurry is termed as 'froth method'. The dried seeds were winnowed and sieved using a set of three test sieves of 425, 325 and 210 microns. The seeds that were retained on the 325 micron sieve were used for sowing.

*2.8.2. Storage physiology and germination:* The moisture content of the seeds was determined. The seed were put into germination (100 seeds x 3 replication) on polyurethane foam and daily cumulative germination recorded for 55 days.

*2.8.3. Seed purity test:* Three samples 0.1 to 1g were used for purity testing. Pure seeds were separated under an illuminated purity board. A further confirmation of purity was done under Stereoscopic microscope. The seeds are examined through a Stereoscopic microscope to confirm purity before subjecting to calculations.

## **2.9. *Oroxylum indicum***

*2.9.1. Processing:* Mature pods of *O. indicum* were collected from Orappanpara, near Peechi in March 2004 and Podippara, near Peechi in April 2004. The fruits were sun-dried when they split open to release the winged seeds.

2.9.2. *Storage physiology*: Moisture contents of sun-dried sample seeds were determined. The seeds were subjected to germination test (30 seeds x 4 replications) and cumulative germination was noted for 28 days.

2.9.3. *Seed viability test*: Germination test and TTZ were conducted to determine the seed viability. For tetrazolium staining test the winged seeds were soaked in water overnight. Subsequently, the seed coat was removed and the cotyledons immersed in 1 % Tetrazolium solution. The colour of the embryo and cotyledons was recorded. This was compared with cumulative germination in 30 days.

2.9.4. *Seed purity test*: Seed samples 1 g to 10 g from five seed lots were used for purity testing study.

## 2.10. *Syzygium cumini*

2.10.1. *Processing*: Mature fruits of *S. cumini* were collected from Peechi in May 2004 and in May 2005 from the floor after bringing down the seeds by shaking the branches. The fruits were depulped and the seeds were air dried under ceiling fan.

2.10.2. *Storage physiology*: The seeds dried to different moisture contents [51.34 (fresh), 44.17, 43.59, 35.12, 30.78 and 27.99%] were stored in ventilated plastic bags under 16<sup>0</sup>C. The seeds were subjected to germination test (40 seeds x 3 replications).

2.10.3. *Seed viability test*: Germination test, cutting test and tetrazolium test were carried out to determine the seed viability. For TTZ test, the seeds were soaked in water overnight. After removal of the seed coat the seeds were immersed in 1 % Tetrazolium solution. The colours of the different parts of the embryo were recorded. The staining was classified based on colouring intensity as light pink and moderately pink and colouring parts (plumule and radicle) stained. This data was compared with cumulative germination in 50 days to see whether there was any correlation between the staining pattern and germination.

## **2.11. *Tectona grandis***

*2.11.1. Processing:* Seeds collected in gunny bags were thrashed to remove the thin calyx. The seeds with felty mesocarp, thus obtained, were size-graded using a 9 mm seed grader, specially designed by modifying a rotary sand sieve.

*2.11.2. Seed viability test:* With the object of arriving at a relationship between viability determined through cutting test (30 seeds x 3 replications) and germination test by sowing seeds in vermiculite (100 seeds x 3 replications), both these tests were compared using samples drawn from a number of seed lots. For cutting test, 105 seed samples were drawn from 35 seed lots from 4 locations.

In another study X-ray of seed samples was taken in medical X-ray laboratory to examine the seed filling and correlating with results of cutting test.

## **2.12. *Terminalia bellirica***

*2.12.1. Processing:* Mature fruits of *T. bellirica* were collected from KFRI Campus in December 2004 and Parambikulam in December 2004. Mesocarp of the seeds was removed by hitting with a wooden mallet. The seeds with mesocarp and without mesocarp were sun-dried and used for the study.

*2.12.2. Storage physiology:* The seeds with and without mesocarp were stored under room temperature (about 32°C). Samples were drawn from these at periodic intervals and put for germination after determination of the moisture content

*2.12.3. Seed viability test:* Cutting test and germination test were carried out to determine the seed viability.

2.12.4. *Pre-sowing treatments:* The seeds were subjected to different presowing treatments viz., H<sub>2</sub>SO<sub>4</sub> treatment (T1), soaking seeds with mesocarp in water for seven days (T2), soaking seeds without mesocarp in water for seven days (T3), sun drying of seeds with mesocarp for seven days (T4), sun drying of seeds without mesocarp for seven days (T5), alternate wetting and drying of seeds with mesocarp for seven days (T6) and alternate wetting and drying of seeds without mesocarp for seven days (T7). Each treatment had three replications of 40 seeds from two locations such as KFRI campus, Peechi and Parambikulam.

### 3. RESULTS AND DISCUSSION

Results obtained for various treatments for the 12 species are dealt with under the subsection 3.1 to 3.12.

#### 3.1. *Albizia lebbbeck*

3.1.1. *Processing*: About 71% pods dehisce within 20 days under sun-drying, where as the rest are manually split open to release seeds (Fig.1, Table 1.). Pods yield 22% seeds by weight (Table 1). About 4,000 seeds weigh one kg.



Fig.1. Natural dehiscence of *A. lebbbeck* under sun-drying and manual extraction

Table 1. Yield of *A. lebbbeck* seeds extraction by sun-drying and manual extraction

Method of seed extraction	Sun-dry weight of pods (kg)	Sun-dry weight of seeds (kg)	Percentage yield of seeds from pods (%)
Natural dehiscence	159 (71%)	35.49 (71%)	22.3
Manual opening	65 (29%)	14.50 (29%)	22.3
Total	224 (100%)	49.99(100%)	22.3

\*Values in parenthesis represent percentage of total.

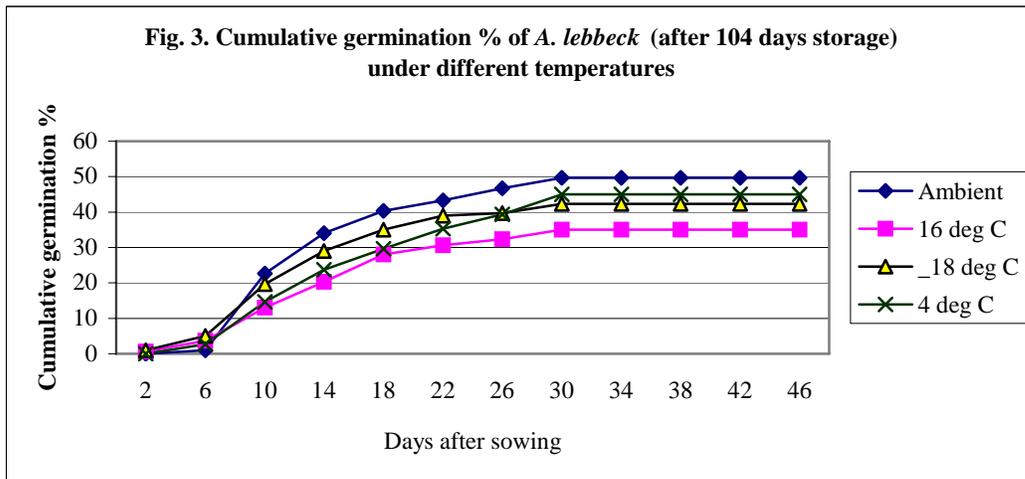
A solar seed dryer with corrugated translucent lid was designed and fabricated to dry *A. lebbbeck* and similar dehiscent fruits (Fig. 2). The dryer is fixed with mesh of

suitable size on which the fruits are dried. The fruits dehisce and seeds pass through the mesh and get collected in the collection bin kept at the bottom.



Fig. 2. A solar seed drier for drying dehiscent fruits.

3.1.2. Storage: *A. lebbek* seeds stored well up to 3.5 months under ambient condition (Fig. 3), and cold storage did not have any advantage for such short-term storage. Since the experiment was particularly to compare the effect of low temperature on short-term storage, the result should not be mistaken to convey that *A. lebbek* does not need long-term storage under ambient condition. It is well known that *A. lebbek* can store well for long period if stored under dry and pest-free condition.



3.1.3. *Seed viability test:* The ratio between the number of seeds stained in TTZ test (Fig. 4) and the seeds germinated (4:1) suggest 0.25 as a factor of estimating germination percentage from TTZ testing results. However, this is not a satisfactory result for practice. More studies are required to see the staining pattern that correlates best with germination percentage.

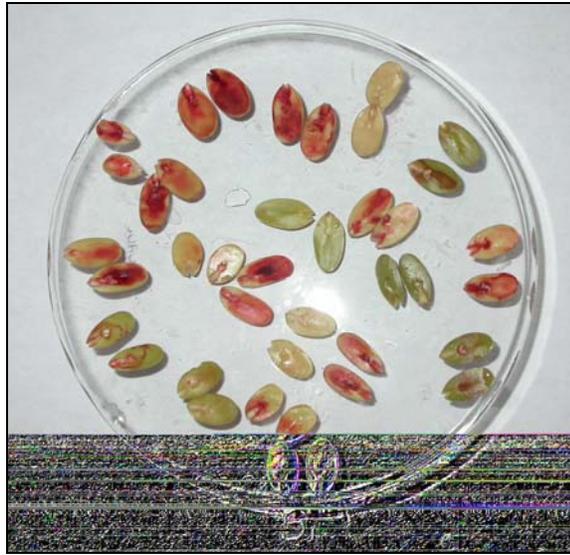
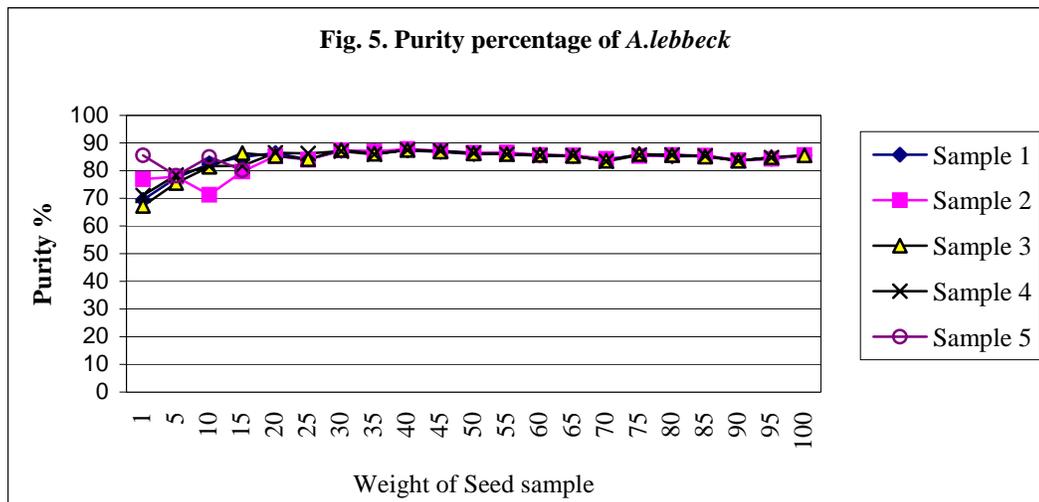


Fig. 4. Seeds after tetrazolium staining.

3.1.4. *Purity test:* Purity testing using seed samples above 30 g gave stable results, (Fig.5) suggesting sample size for purity test as above 30 g. From practical and conservative considerations a sample size is 50 g is suggested.



### 3.2. *Aegle marmelos*

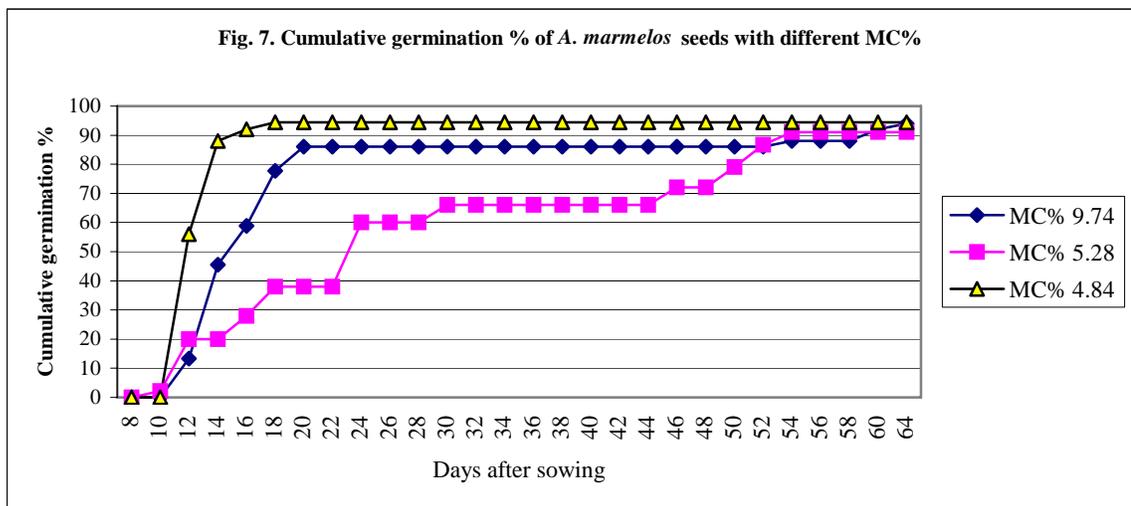
3.2.1. *Processing*: About 20 kg fresh fruits (Fig. 6 a, b) yield 1 kg fresh seeds (6 c) with 21.4% moisture content. About 5000 seeds weigh one kg.



Fig 6. *A. marmelos* fruits and seeds

a. *A. marmelos* fruits      b. Fruit cross section showing seeds in mucilage      c. Seeds

3.2.2. *Storage physiology*: *A. marmelos* seeds show orthodox storage physiology as the seeds dried down to 4.84% moisture content (fresh weight basis) (Fig. 7) give up to 94 % germination. Seeds germinate well without any presowing treatment. Germination commences 10 days after sowing and half of the cumulative germination percentage is obtained within 12-24 days.



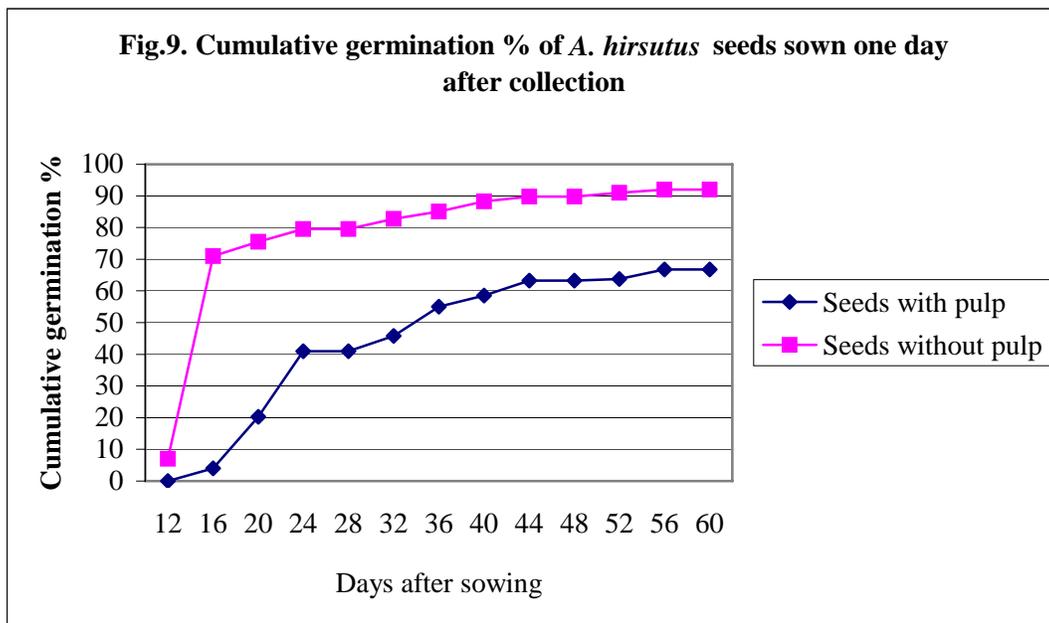
### 3.3. *Artocarpus hirsutus*

3.3.1. *Processing*: From ripe fruits (Fig. 8 a) the rind can be removed to expose the seeds with pulp (8 b) attached to the fruit stalk. These seeds with pulp can be easily removed and depulped by maceration. The yield of depulped seeds (Fig. 8 c) is 12.50% by fresh weight. About 2210 seeds weigh one kg. Depulped seeds germinate better (92%) than seeds with pulp (67%) (Fig. 9). The seeds start germination at 12 days after sowing and reach a maximum in 60 days.

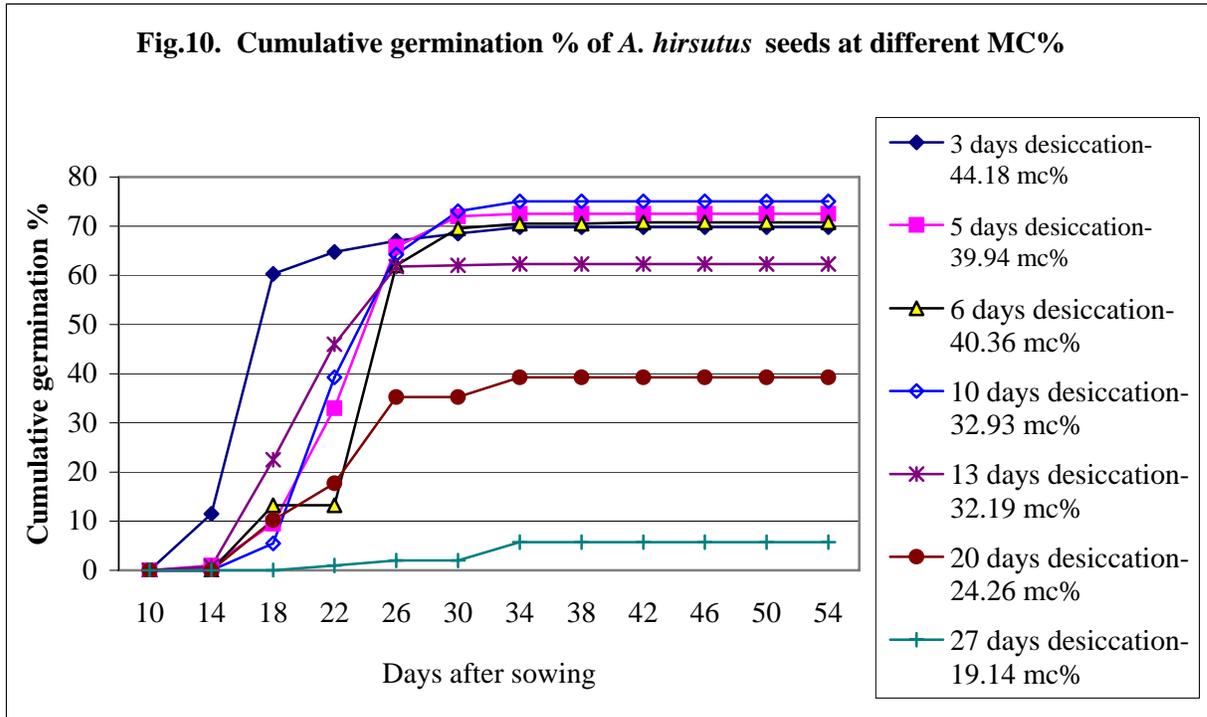


Fig 8 A. *hirsutus* fruits and seeds

Fig. a. *A. hirsutus* Fruit    b. seeds with pulp attached to fruit stalk    c. Depulped seeds



3.3.2. *Storage Physiology*: Seeds are highly recalcitrant; seeds lose viability if moisture content drops below 33% moisture. Under ventilated ambient condition seed drying and viability loss occur within two weeks (Fig.10).



### 3.4. *Cassia fistula*

3.4.1. *Processing*: Breaking the sun-dried pods along the suture line (Fig. 11) using wooden mallet is an efficient method of breaking pods to release the seeds. About 4800 seeds weigh a kilogram.

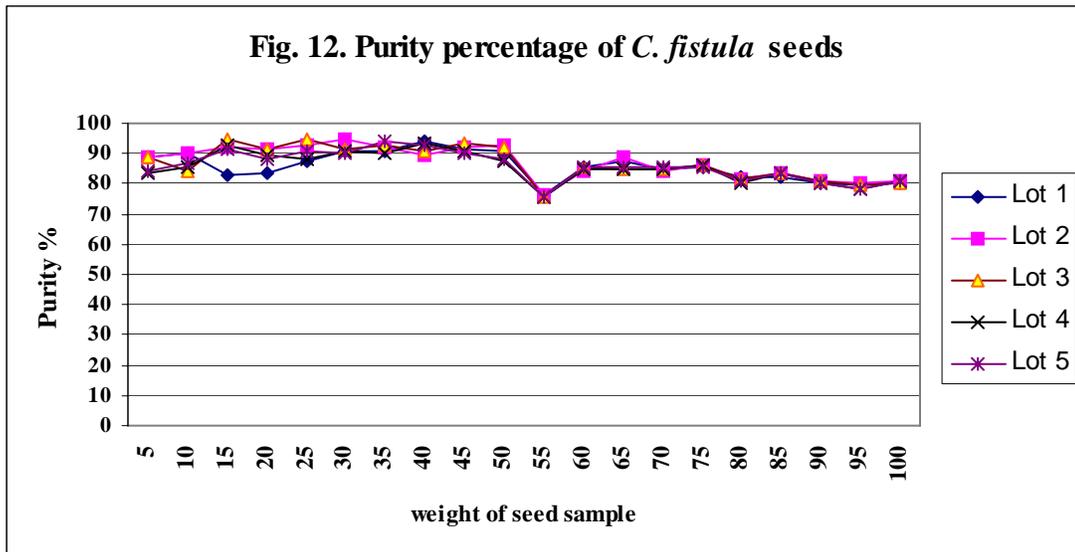


Fig 11. Split open *C. fistula* fruits along suture line and extracted seeds.

a. Fruit-split open along the suture. b. Fruit split open not along the suture c. Seeds

3.4.2. *Seed viability test*: Of the total seeds that showed TTZ staining of both cotyledons and embryonic axis taken together, one third germinated during a period of 45 days after sowing. More studies are required to relate the staining pattern to germination percentage.

3.4.3. *Seed purity test*: The purity studies revealed that the purity percentage remains more or less steady with sample size of 70 g to 100 g. From practical and conservative point of view 100 g is suggested as an optimal sample size for purity testing of *C. fistula* seeds (Fig.12).



### 3.5. *Dysoxylum malabaricum*

3.5.1. *Processing*: Seeds split open while air-drying. Unopened fruits were vertically cut open along the rind to extract seeds. Seeds were cleaned of the yellow aril (Fig. 13) which if retained, would cause heavy fungal infestation. The seeds were further treated with Captan 50% WP fungicide. The yield of seeds from fruit is 40 % by fresh weight. Fresh seeds have 49% moisture content. About 125 fresh seeds weigh a kilogram.

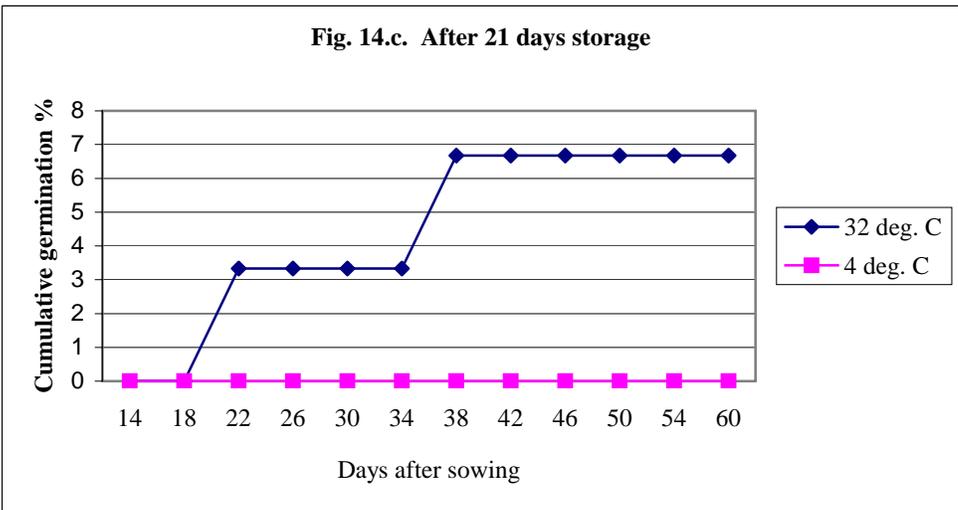
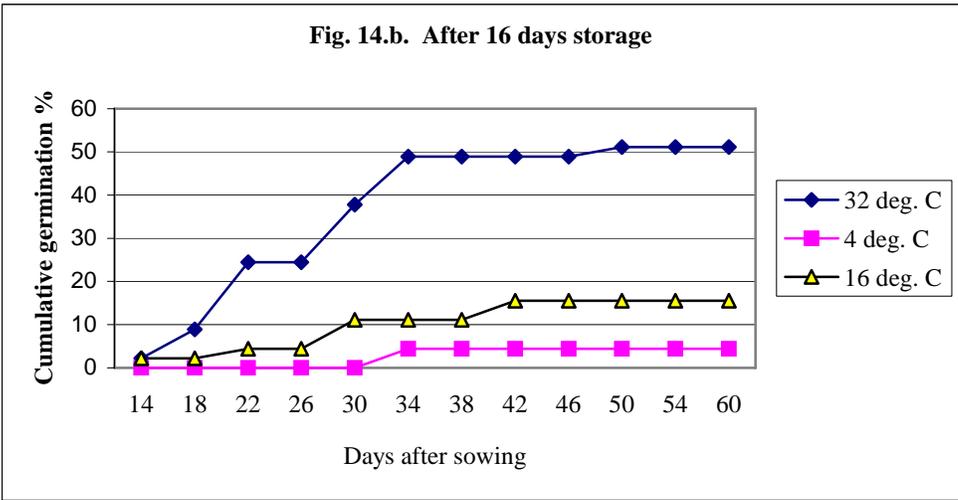
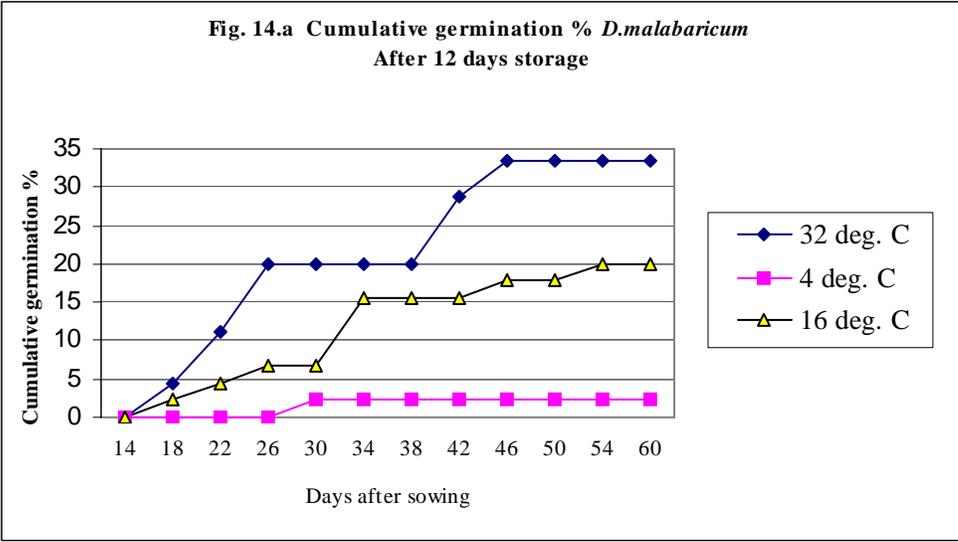


Fig 13. *D. malabaricum* fruits and seeds.

a. Fruits dehiscent during storage      b. Seeds with seed coat      c. Seeds without seed coat

3.5.2. *Storage*: The seeds are recalcitrant and lose viability rapidly under both ambient and low temperature. Seeds stored under ventilated ambient condition (about 30°C) retained viability for about two weeks (Fig.14 a to 14 c), beyond which the seeds rapidly lost viability.

3.5.3. *Seed germination*: Fresh decoated seeds started germinating at 13 days after sowing (14 a & 14 b) and reach a maximum (52.5%) at 60 days. Seeds are desiccation sensitive, and fail to germinate when the seed moisture drops below 30%. Germination drops below 30% when the seed moisture drops below 45%.



### 3.6 *Gluta travancorica*

3.6.1. *Seed characteristics:* Average fruit measured 3.33 cm in diameter and 2.62 cm in length. Largest fruit was 3.9 cm in diameter and 3.1 cm in height. Seeds of smaller size measuring 2.98 cm in diameter and 2.29 cm in height were also collected in 2005. Fresh seeds contained 40.11% moisture (by fresh weight basis) and 58.18 such seeds weighed a kilogram. Seed coat is very hard and has a mean thickness of 1.77 mm and makes up 24.65% of the total seed dry weight.

*Position of embryo:* Unlike many dicot species, it is interesting to note that the embryonic axis is located laterally on the top one third of the seed (Fig.15). Although it is difficult to locate embryo in immature seeds without seed coat removal, in mature fruits the portion of embryo can be easily located by the presence of a dark spot due to resin exudation prior to seed coat rupture. A furrow, which divides the seed into two cotyledons, makes it easy to locate the embryo which sometimes bulges out. However, in small fruits, there is absence of both resin exudation and seed coat rupture even when seeds are mature. In this case, it is fairly difficult to locate the position of the embryo without seed coat removal.

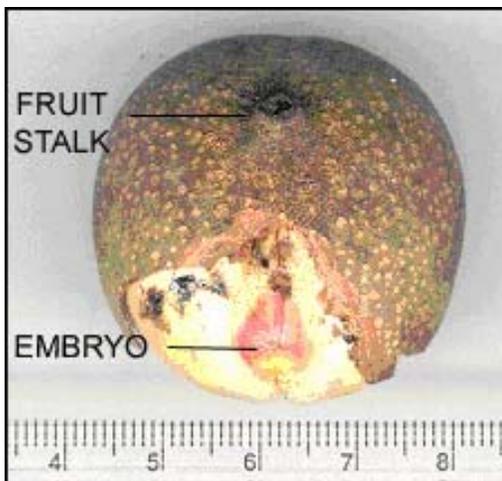


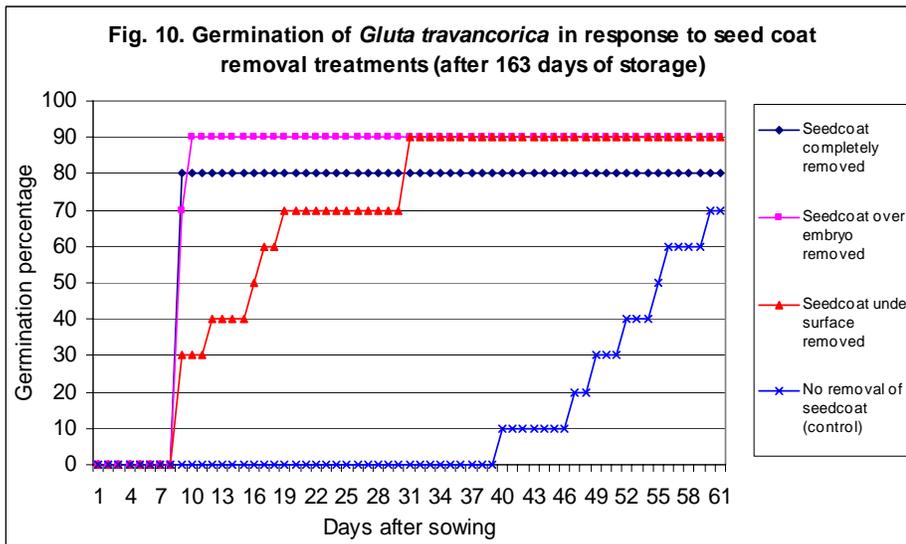
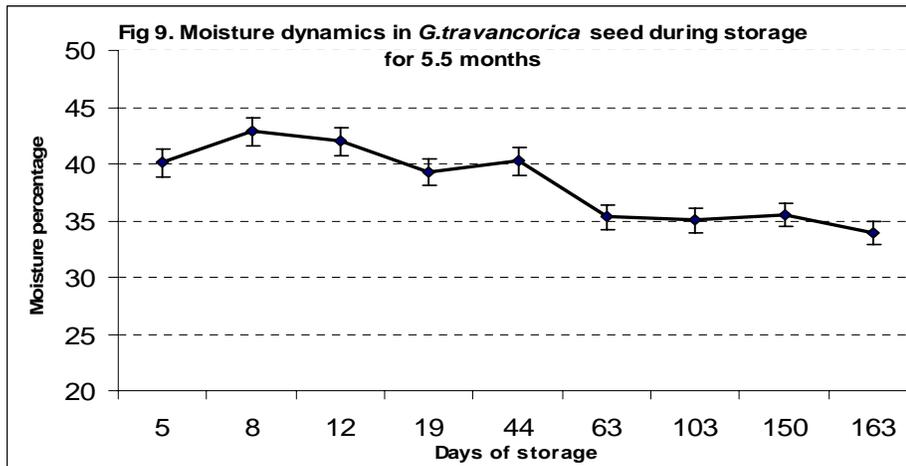
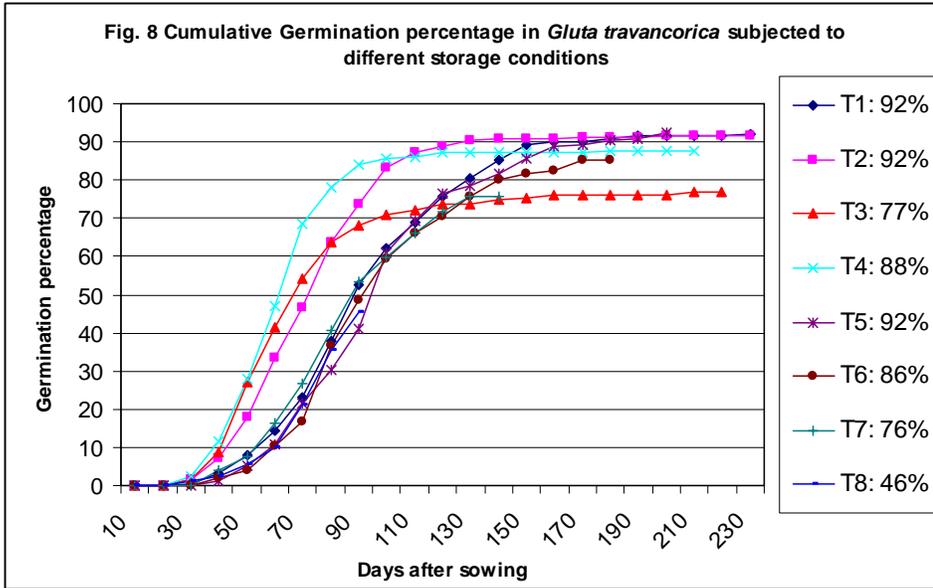
Fig. 15. *G. travancorica* fruit showing the position of embryonic axis.

3.6.2. *Seed storage:* Seeds of *G. travancorica* are reported to be recalcitrant and lose their viability within a month under natural conditions (Jose and Pandurangan, 2003).

Although tropical recalcitrant seeds may be stored only for short periods (Bonner, 1990), present studies revealed that the seeds could be safely stored in ambient laboratory conditions up to 3.5 months without significant losses of viability (Fig.15). Seeds with intact seed coat, germinated within 24 to 38 days after sowing, the germination percentages obtained under different storage durations were 92% (T1,T2, T5), 77% (T3), 88% (T4), 85% (T6), 76% (T7) and 46% (T8) (Fig.15). During the period of storage the drop in moisture content was from 40 % to 34 % (Fig. 16). Seed germination is hypogeal (Fig.18).

*3.6.3. Pre-sowing treatments:* Partial or full seed coat removal enhanced germination as compared to seeds with seed coat intact. Complete seed coat removal gave maximum germination at 8 days after sowing, closely followed by those with removal of seed coat just above embryo and those with seed coat removed at the bottom.

Fig. 18. Hypogeal germination of *G. travancorica*.



### 3.7. *Gmelina arborea*

3.7.1. *Processing*: Yield of seeds from fruits was 10.40% by fresh weight (Fig.19). About 1000 depulped seeds weigh a kilogram.



Fig.19. *G. arborea* fruits and seeds.

a. *G. arborea* fruits

b. Depulped seeds

3.7.2. *Storage physiology*: As the seeds reported very low germination (less than 20 %), the results are not worth presenting.

### 3.8. *Neolamarckia cadamba*

3.8.1. *Processing*: A new ‘froth method’ is recommended for extraction of seeds from fruits (Fig. 20). This involves heaping the fruits to ferment thereby enabling them to be crushed in water to form a slurry. The slurry is further diluted and poured into a bucket. The process of pouring into alternate bucket is repeated several times till good froth is formed at the top and all the seeds get embedded in the froth. The froth is skimmed out, strained through a cloth piece and sun-dried. The seeds are then sieved through a set of 425, 325 and 210 micron sieves. Seeds retained on 325 micron sieve are used for sowing. About 30000 such seeds (10% MC) weigh one gram. The fruits yield 4.34 % seeds by fresh weight.



a. Fruits



b. Cross section showing capsules containing dark seeds



c. Capsule containing seeds.



d. Seeds

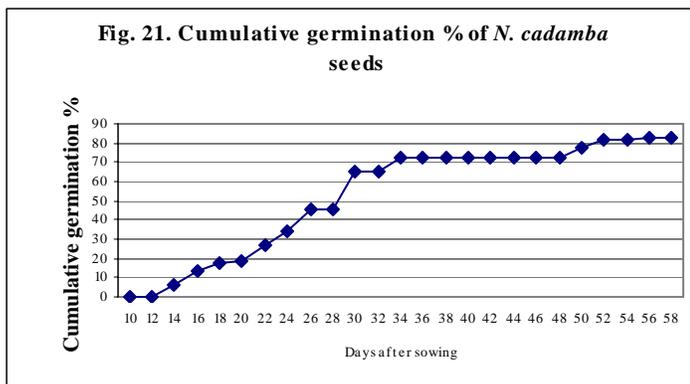


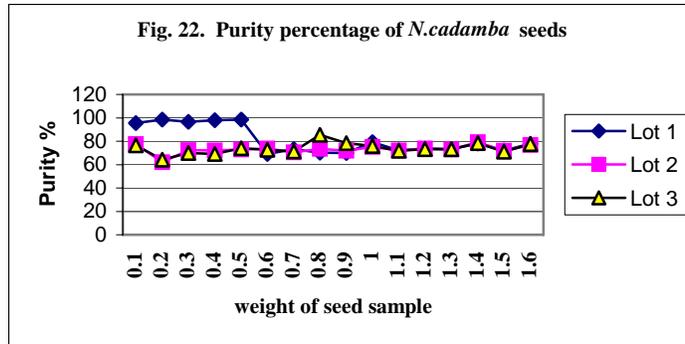
e. Seeds through stereoscopic microscope

Fig. 20. *N. cadamba* fruits and seeds.

3.8.2. *Storage physiology and germination:* *N. cadamba* seeds show orthodox behaviour. Seeds with moisture content of 11.81% register 83% germination percentage as high as (Fig. 21). The seeds start germination 13 days after sowing and give a maximum of 83% of germination in 55 days.

3.8.3. *Seed purity test:* The purity studies revealed that the purity percentage remains more or less steady with sample size of 1.1 g to 1.6 g and therefore, 1.5 g is suggested as an optimal sample size for *N. cadamba* (Fig. 22).





### 3.9. *Oroxylum indicum*

3.9.1. *Processing*: The fruits (Fig. 23 a) yielded 2.48% winged seeds (Fig. 23 b) by fresh weight. About 8333 winged seeds weigh a kilogram.

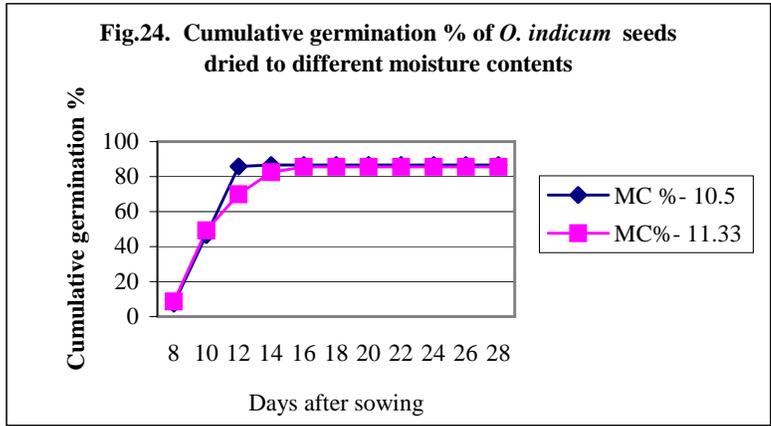


Fig 23. Fruits of *O. indicum*

a. Fruits

b. Fruit split open

3.9.2. *Storage physiology*: *O. indicum* seeds are orthodox in nature as seeds dried to 10.5 % moisture content retained viability. Seeds started germination 7 days after sowing and reach a maximum of 87% in 30 days (Fig. 24).



3.9.3. *Seed viability*: Untreated seeds germinated within 7 to 30 days after sowing. The ratio between germination test and Tetrazolium staining of embryonic axis (Fig. 25) is 1:1 (86.5% and 85.9% respectively) indicating the high prediction power of tetrazolium test for estimating cumulative germination percentage.

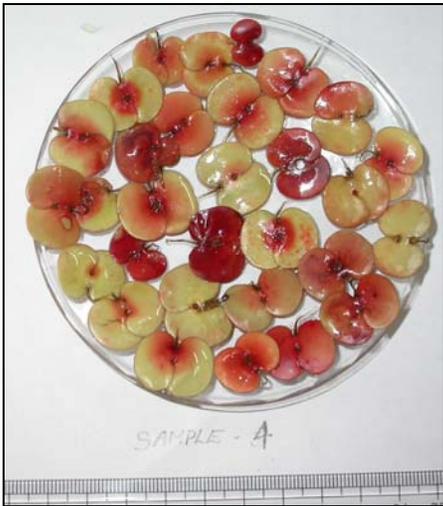
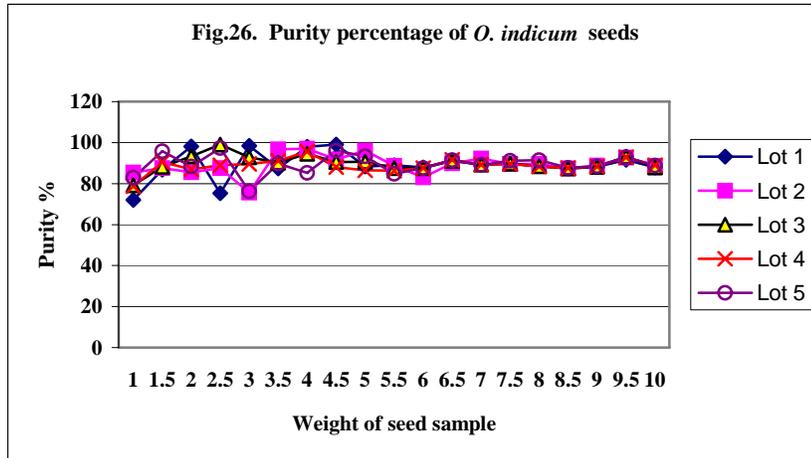


Fig. 25. Seeds after tetrazolium staining.

3.9.4. *Seed purity test*: Purity studies revealed that the purity percentage remains more or less steady with sample size of 8 g to 10 g and therefore 10 g is suggested as an optimal sample size for purity test of *O. indicum* (Fig. 26).



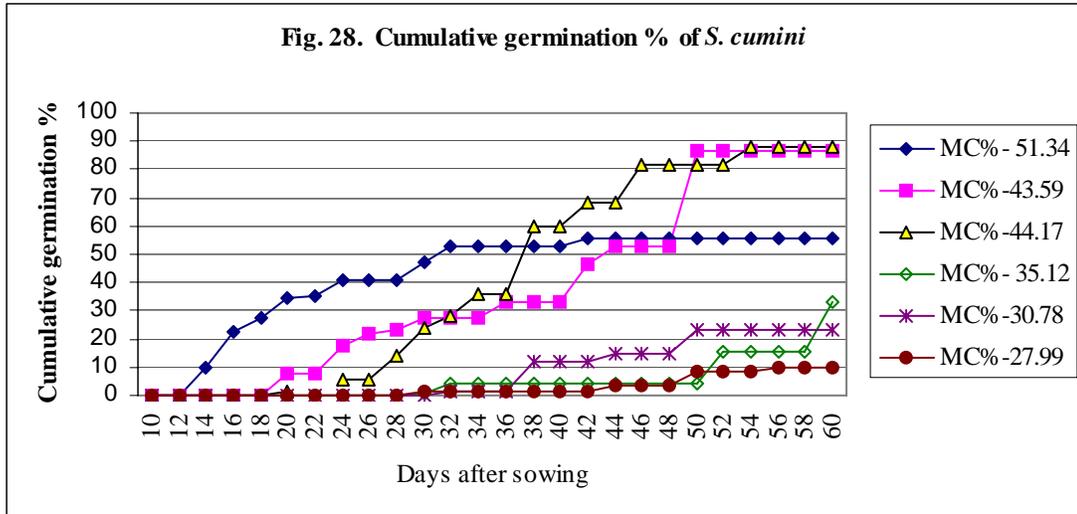
### 3.10. *Syzygium cumini*

3.10.1. *Processing*: Yield of depulped seeds from fruits was 34 % by fresh weight (Fig. 27). About 730 seeds with 54.69 % moisture content weigh a kilogram.



Fig. 27. Fruits and seeds of *S. cumini*

3.10.2. *Storage physiology*: Seeds are highly recalcitrant as germination percentage drastically drops from 88% to 10% when the moisture content drops from 51% to 28-35% (Fig. 28).



3.10.3. *Seed viability*: The percentage of viable seeds through cutting test (Fig. 29.), tetrazolium test (Fig. 30) and germination test (in the ratio 96: 100: 90) suggest the possibilities of reasonably predicting the germinability of seeds using cutting test and tetrazolium test.



Fig. 29. A cross-cut seed to show the embryonic axis.



Fig. 30. Seeds after tetrazolium staining

### ***3.11. Tectona grandis***

*3.11.1. Processing:* A specially designed seed grader which can be used to separate seeds above 9 mm size (Fig. 31) is a practical outcome of the project. This has been since used in the Kerala Forest Seed Centre for commercial use. The grader costed Rs. 6500/- for fabrication in 2004. It may now cost around Rs. 10,000 for fabrication of a similar one. There is 50 % saving in labour cost when the grader replaces by manual grading using sieves. The slope of the rotary sieve is 9° to allow rolling down of the seeds above 9 mm while permitting those below 9 mm to pass through while rolling on the entire length (1.2 m) of the rotary sieve.



Fig. 31. A hand-operated size-grader for teak seeds

*3.11.2. Seed viability:* A relation between cutting test and cumulative germination at different days after sowing has been established using 105 samples from 35 seed lots from Nilambur, Olavakkod, Mananthavady and Kulathupuzha. The equation is:

$\text{Log}(R+2) = b \text{Log } D + c (\text{log } D)^2$  where R is G/V where G is the cumulative germination percentage and V is the viability percentage as determined by cutting test and D is the days after sowing.

G can be predicted using the formula:

$$G\% = [\exp(b \text{Log } D + c (\text{Log } D)^2) - 2] * V\%$$

This can be easily worked out on an Excel spread sheet.

A specially made heavy-duty seed cutter and used for cutting test is shown in Fig.32.



Fig. 32. A heavy duty seed cutter used for cutting test of teak seeds.

The X rays of seeds (Fig. 33) taken in commercial medical X- ray unit provide an opportunity to examine seed filling. However seed tester's ability through experience to identify the well-filled and the ill-filled seeds may bring in subjectivity.

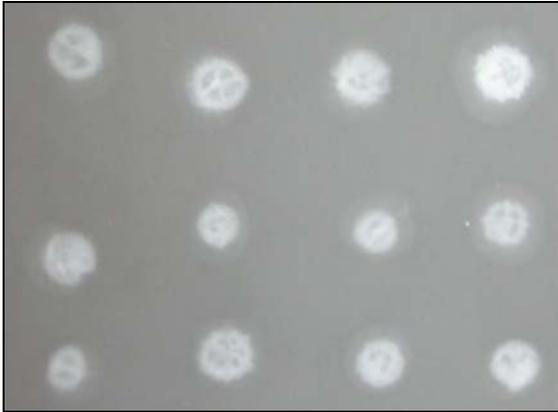


Fig. 33. X- ray of teak seeds

*3.11.3. Seed Pre-treatments:* Use of mesh bottomed trays (Fig. 34 a) for mesocarp removal of seeds using termite was introduced. The trays are of 100 cm x 100 cm x 10 cm and fitted with legs of 15 cm height. A tray can contain about 30 kg seeds. The trays are placed on a termite-infected area in such a way that termites can enter the trays and feed on the seeds. It is already reported that termite- aided mesocarp removal enhances germination while reducing seed bulk and weight (Chacko 1998). The trays can also be made in such a way to stack one above the other; as many as five trays can be stacked this way (Fig. 34 b).



Fig. 34. Method of pre-treating the *T. grandis* seeds by promoting termite feeding.

a. Termite infected seeds in single tray

b. Five trays stacked one above the other

### 3.12. *Terminalia bellirica*

3.12.1. *Processing*: Removal of mesocarp by hitting with wooden mallet and further subjecting to water wetting and sun drying for one week gives germination up to 100%. About 89 seeds with mesocarp weigh a kilogram (Fig. 35).

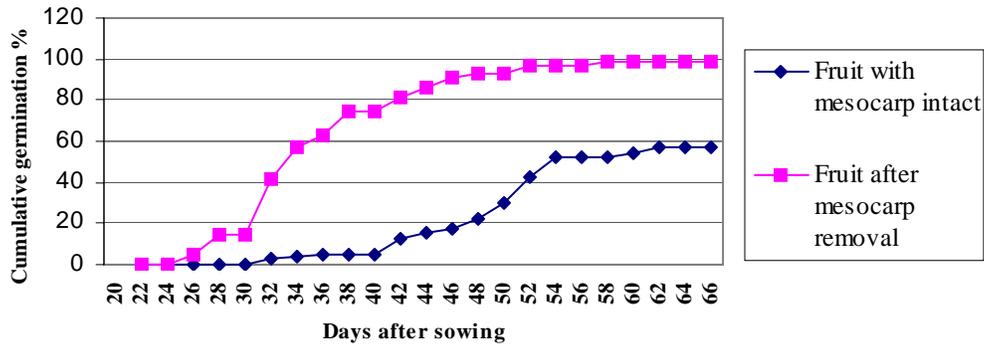


Fig. 35. *T. bellirica* fruits and seeds

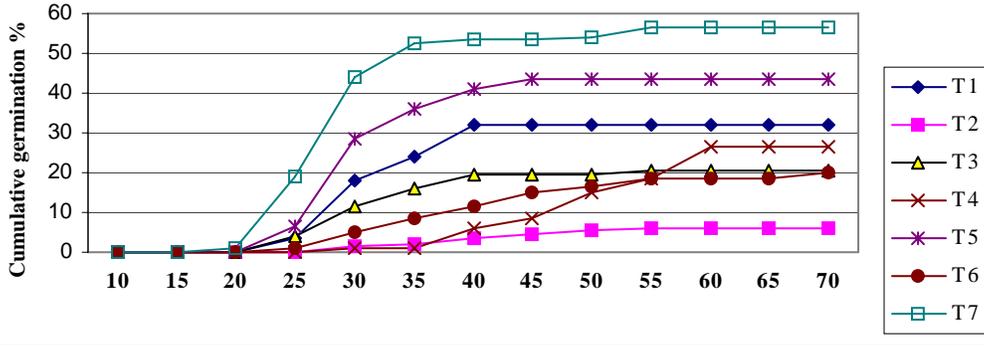
3.12.2. *Storage physiology*: *T. bellirica* seeds are orthodox as the seeds dried to 9.06% moisture content gave 96.67% germination.

3.12.3. *Pre-sowing treatments and seed germination*: Seed germination starts 15 days after sowing and reaches a maximum in 66 days. About 50% of the germination is obtained in half the time. i.e., about 30 days. Seeds without mesocarp germinate better (100%) as compared to those with mesocarp (57 %) of the pre-sowing treatment (alternate wetting and drying for seven days without mesocarp) (Fig. 36). Seed separation using concentrated sulphuric acid is an efficient pre-sowing treatment (Fig. 37 & 38).

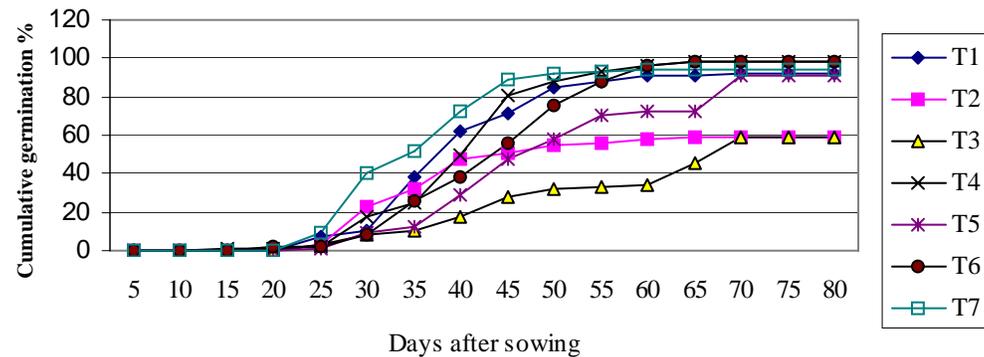
**Fig. 36. Cumulative germination % of *T. bellirica* seeds with and without mesocarp**



**Fig.37. Cumulative germination % of *T. bellirica* (Different presowing treatments) -Seed source 1:- KFRI campus**



**Fig. 38. Cumulative germination % of *T. bellirica* (Different presowing treatments) Seed source 2:-Parambikulam**



#### 4. CONCLUSIONS

The following are the major conclusions from the study on the twelve species.

*Albizia lebbek*: Although the species is reported to store well for long periods under low temperature and humidity, short-term storage for about 3.5 months can be done without loss of viability under ambient temperature. For purity test, 50 g sample is optimum. A solar seed drier designed for drying *A. lebbek* pods for seed extraction even on rainy days can be used for other dehiscent fruits also.

*Aegle marmelos*: This species, earlier suspected to be recalcitrant, shows orthodox behavior as seeds dried up to 4.8% moisture content give up to 94% germination.

*Artocarpus hirsutus*: Seed viability is short-lived. They store well only upto two weeks.

*Cassia fistula*: For efficient splitting of pods and easy extraction of seeds, the pods may be hit along suture line with wooden mallet. For short-term storage, cold storage has no added advantage. For purity test 100 g is the optimal sample size.

*Dysoxylum malabaricum*: The seeds are recalcitrant and gradually lose viability after two weeks under well- ventilated ambient conditions.

*Gluta travancorica*: Seeds store well for three months under ambient conditions without significant loss of viability. Partial or complete removal of seed coat ensures good germination.

*Gmelina arborea*: Preliminary results suggest intermediate storage behaviour of seeds.

*Neolamarckia cadamba*: The newly developed 'froth method' is an easy and efficient method of extraction of seeds from fruits. Seeds show orthodox behaviour. For purity test 1.5 g is the optimal sample size. For purity test 1.5 g is the optimal sample size.

*Oroxylum indicum*: The seeds show orthodox storage behaviour. Good correlation exists between Tetrazolium staining and germination tests. The optimal sample size for the purity test is 10 g.

*Syzygium cumini*: This highly recalcitrant species loses viability drastically on drying below 35% moisture content.

*Tectona grandis*: A seed grader for separating seeds above 9 mm size is designed, fabricated and put to use in Kerala Forest Seed Centre. The seed grader, which saves 50% labour, costs about Rs. 10,000/- for fabrication. Strong correlation exists between cutting and germination test. An equation is suggested for predicting germination from results of cutting test. Special trays have been designed and tested for pre-treating the seeds by the already reported termite-aided mesocarp removal of seeds to enhance germination.

*Terminalia bellirica*: Seeds show orthodox storage behavior. Germination of seeds is improved by removal of mesocarp and weathering of seeds (wetting with water and drying under sun) for about a week.

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