INVESTIGATION OF SEED PROPAGATION METHODS AND SEED BIOLOGY OF THE MEDICINAL LIANA *Coscinium fenestratum* Colebr (Menispermaceae)

By

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DECLARATION

The work described in this thesis was carried out by me at the Department of Botany, Faculty of Science, University of Peradeniya, Peradeniya, under the supervision of Dr. Kushan Tennakoon and Dr. K.K.D.S. Ranaweera. A report on this has not been submitted to any other University for another degree.

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TO MY DEAR PARENTS AND TEACHERS WHO ALWAYS INSPIRED ME IN THE PATH OF LUST FOR KNOWLEDGE.....

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ABSTRACT

Cocculum fenestratum colebr. (Menispermaceae) a dioecious liana, common in disturbed forest of low country wet zone. It is a widely used medicinal liana in Sri Lanka and a potential foreign market, due to the presence of alkaloids such as berberine, jatrorhizine and palmatine in it. While the water extraction of its stem provieds a decoction for treatment of body aches, pains, common colds, tetanus, dressing wounds and ulcers because of its antiseptic properties.

As a result of illegal over exploitation, distructive collection, relatively slow growth rate, seed predation, low seed germination and low percentage of seedling survival, natural populations of this species are decimated and /or are disappearing at an alarming rate. To meet the increasing demand and over come short supply of this species, development and simple cost effective propagation techniques is one of the primary impediments for mass cultivation.

Seeds of this species show relatively long period of dormancy (2-15 months) and under natural conditions seed germination were found to be less than 20 %. This study examined how *C. fenestratum* seeds could be propagated using simple and cost effective methods and attempts were made to develop techniques to overcome seed dormancy. *C. fenestratum* fruits were collected by bagging the mature fruit bunches still intact to the parent liana from disturbed forest fringe in Sinharaja MAB Reserve. Fruits were depulped manually and washed with tap water before the seeds were used for a range of experiments.

The moisture content of fresh seeds was 31± 0.61 % and the subsequent moisture loss of seeds stored under different conditions such as in a humid chamber, polythene sealer bags, paper bags and in open areas were examined. The experimental design was a randomized block design (3 replicates; n = 120). These experiments were carried out under laboratory conditions were the average moisture content was 76±1.39 %. The highest moisture loss was when seeds were stored in open areas and the lowest was found to be when seeds were stored in humid chamber. Seeds stored for 40 days were examined for viability under humid condition and room condition. Viability was 89% under humid conditions and it was 30%

under room condition. Seeds of *C. fenestratum* were categorized under "recalcitrant seed" taking in to consideration of the initial moisture content, moisture losses and viability.

Bioassays carried out using seeds of *Brassica juncea* revealed presence of germination inhibitors in *C. fenestratum* seeds (endosperm & the embryo). *B. juncea* seeds were the germinated on blotting papers soaked in 5% water extracts of endosperm and 5% water extracts of embryo. Germination percentage of *B. juncea* grown on endosperm and embryo extracts were 17% and 25% respectively while 80% and 88% germination percentage was observed in control where the blotting paper was soaked in water.

Seed germination experiments were carried out under plant house conditions (maximum light 800 $\mu mol^{-1}s^{-2}$ and Relative humidity 81±1%). The experimental design was a completely randomized design (3 replicates; n = 20). Seed experiments in order to evaluate the seed germination, seed soaked in gibberllic acid 1500 ppm, 2000 ppm, 2500 ppm and 3000 ppm concentrations, the mechanical treatments included cracking of the seed coat, soaking in tap water and distilled water for 12-24 hours, seed exposure to sunlight followed by soaking in tap water. Another set of seeds (n = 60) collected from forest floor were soaked in gibberllic acid 2000 ppm. Of this experiments *C. fenestratum* seeds collected from the forest floor & subsequently treated with Gibberllic acid 2000 ppm gave the best germination percentage (27%).

Comparison of wood anatomy and morphology in *C. fenestratum* and *Anamirata coccculus*, which is commonly used as a substitute for *C. fenestratum* was carried out by using the cross sections obtained from a range of stem diameter classes (1 cm, 2 cm, 3 cm) of there two species, for study woods stems of both species showed anomalous growth. But in *A. coccculus* anomalous growth was present in secondary wood structure and in *C. fenestratum* it was absent. The bark thickness of *C. fenestratum* was lower than *A. coccculus*. Vessels diameter of *A. coccculus* (20.3 ±1.5 µm) was higher than *C. fenestratum* (19.03 ±2.5 µm).

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CHAPTER 1

GENERAL INTRODUCTION

1. Introduction

The demand for natural herbal remedies and health food products are steadily increasing. More than 9000 plants species are used for medicinal purposes in various parts of the world. Sri Lanka is endowed with ideal natural habitat for a wide variety of medicinal plants.

Early cultures developed its own traditional system of medicine, by trail and error, based on a long process of interaction between man and the habitat. The historical use of plants in the treatment of the ailments resulted in several organized system of traditional medicine. The therapeutic properties of a large number of plants were recognized and documented in "materia medica" and ola leaf manuscripts. These from the basis of much of Sri Lanka's traditional systems of medicine. Although not strictly based on modern science, they are founded on a corpus of documented knowledge.

Today there are two main systems of medicine practiced in Sri Lanka namely the traditional system and the allopathic (western) system. A survey by the WHO (World Health Organization) estimates that 70% of the populations in Sri Lanka still rely on the indigenous system of medicine based mainly on plant formulation (Anon, 1978). The 'Ayurveda', 'Deshiya Chiktsa', 'Siddha' and 'Unani' are four systems widely practiced in the Island.

Ayurveda, which means the "science of life", originated in the Indian subcontinent, but is now prevalent in the South Asia in modified forms. The main purpose of Ayurveda is the restoration and maintenance of metabolic equilibrium and health. The treatment consits of avoiding factors that is responsible for the disease and by administration of medicine, diet and acitivity regimens. As such it a holistic rather than a symtomatic approach. Deshiya Chikitsa is a SriLankan version of Ayurveda but adheres to much of the original Ayurvedic practice. Ayurveda and Deshiya Chikitsa systems use mainly plant and herbal products. The former uses about 2,000 plant species and the latter about 500. Traditional preparations like 'arishta', 'kasaya', Iguli' and 'kudu' contain more than 90% of material derived from plants.

A large number of plants of medicinal value have been published in a compilation called 'Sinhalese materia medica' (Attygala, 1917). Information on the chemistry and the pharmacology of some Sri Lankan and Indian plants (Chandrasena, 1955) and medicinal plants use in Sri Lanka (Jayaweera, 1981-82) are also documented.

As stated in IUCN report on statistics and national demands for medicinal plant, in year 2000 amount of 1,646,685 kg of herbal material were traded in the Ayurvedic retail sales centers in Sri Lanka (Table1). 30% of this amount imported from other countries (mainly from India and China) and local agents supply 68% (table 2).

Table1.1 Herbal material traded in the Ayurvedic retail sales centers in the country and the proportion of the form of herbal material traded (Source; IUCN 2001).

Form	Amount
Row form	102,048 kg
Dry form	1,544,637 kg
Total herbal material traded	1,646,685 kg
Value of herbal material traded	Rs. 210,205,269.46

Table 1.2 National demand for herbal materials and its sources (Source; IUCN 2001).

Source	Quantity (kg)	Value (Rs)	%
Imports	1,509,291	125,991,177.96	32
Locally supply	2,355,559	261,634,461.65	68
Total National demand	3,864,760	387,625,639.61	

Sri Lankan flora comprises around 3,350 species of higher plants, including vascular plants as well as non vascular but half of these are used for medicinal (IUCN, 2001).

The authorities should also constantly take in to account the possible negative social consequences, ecological imbalances and second generation technological problem that may arise by indiscriminate over exploitation of Sri Lanka's potential to involve in the medicinal plants industry.

Due to over exploitation, most of the species of medicinal plants have become rare. A rare, medicinally important the stem of the liana "weniwel" (*Coscinium fenestratum*) are used widely in the traditional system of medicine.

Coscinium fenestratum colebr (Menispermaceae) is known by several names: In Sinhalese as Weniwalgata, Weniwel, Banwel, Banwelgata (Jayaweera, 1982; Muttiah, 1964). In English False-calumba, Tree Turmeric (Muttiah, 1964; Jayaweera, 1982).

Coscinium fenestratum is a liana (woody climber), which is widely used in the Ayurvedic system of medicine mainly as an anti-tetanus measure, & also other therapeutic powers have been attributed to this plant such as in the treatment of certain back-aches, as a blood purifier and for malarial fever. It is also said to possess antiseptic properties and is used for dressing wounds and ulcers. Though this plant is used in the treatment of such a wide spectrum of ailments, its specificity remains to be worked out and this affords an interesting study medicinal research. Because of its wide usage in Ayurveda, increasing attention has been focussed recently on the export of these plants to foreign pharmaceutical firms in countries like Japan and Germany (Muttiah, 1964).

It is of interest to note that this plant is widely used in central and south India where it is well distributed while the related *Berberis* species ("calumba") which is to possess even greater medicinal properties is solely used in North India where this can be readily procured.

No data are available on the total requirement of this plant product per annum in Ceylon but an attest is being made by the Board of Ayurveda to collect the required statistics. In the event of an increased demand, particularly in view of a ready

foreign market, it is very essential to gather more information on the distribution, growth and regeneration of this species to ensure a sustained yield (Muttiah, 1964).

Table 1. 3 The detail of the usage of *Coscinium fenestratum* in Ayurwedic medicine in year 2000 (IUCN 2001).

National demand of *Coscinium fenestratum* (kg)	Total value	Supply from	Total used only dry manufacture in Sri Lanka	Unit price (Rs) weighted average	Uses of dug manufacture as dry or raw forms	
					Raw herbal material	Dry herbal material
34397 kg Rs 24456941	Rs.1,351,015	Locally	32575 (kg) 100%	41.47	2,400 (7.4%)	30,175 (92.6%)

Coscinium fenestratum is a woody dioecious climber easily identified by its yellow colored bark that is shallowly longitudinally fissured. Their young shoots are densely covered with a fine brownish yellow tomentum. It has broadly ovate or roundish (10-20 cm long) simple leaves. Its flowers are very small and creamish-white in colour. Mail and female flowers are borne on separate plants (a dioecious plant). The species flowers from January to March. Fruits set in June and ripen in August to October (Senerath, 1991: Dassanayake *et al*, 1995).

As a result of Illegal over exploitation, destructive collection, relatively slow growth rate, seed predation, low seed germination and low percentage of seedling survival, natural population of this species are decimated and/or are disappearing at an alarming rate. To meet the increasing demand and over come the short supply of this species, its propagation on a large scale is imperative. This would also essentially reduce the pressure on its natural stands.

The overall objective of the study is to investigate how *Coscinium fenestratum* could be propagated. Seed propagation methods are most reliable for *Coscinium fenestratum* (Senerath, 1991). However, seed propagation is most successful method for the vast cultivation than vegetative propagation.

Hence this study was carried out with the objective of improving simple and less expensive propagation method.

1.1 Objectives of the study

The primary objective of this study was to find out the best seed propagation method to raise a large number of plants *Coscinium fenestratum* and to reduce the pressure on the natural stand of *Coscinium fenestratum*.

Specific objectives of the study

1) To identify the best seed treatment methods (GA_3 Socking, Exposure to sunlight, dipped in distilled water, mechanical cracking of the seed coat).
2) To identify the negative factors affecting germination rate of *Coscinium fenestratum* seed.
3) Investigation of seed dormancy and the methods to treated.
4) To find out a simple method to distinguish of *Coscinium fenestratum* from *Anamirata cocculus* which is a common substitute for *C. fenestratum*

CHAPTER 2

LITERATURE REVIEW

2. Literature Review

2.1 Family Menispermaceae

Coscinium fenestratum belongs to the family Menispermaceae which comprises twining dicot shrubs or rarely herbs. The family is characterized by alternate, simple and entire leaves absence of stipules; very small unisexual, diocious flowers with 6 sepals in 2 rows distinct (rarely connate) petals; 6 stamens in 2rows (rarely numerous), opposite to the petals; distinct or monadephous stamens which are usually represented by staminodes in the female flowers; 3-6 (rarely 12 or 1) carpels; each with a solitary ovule; the ripe carpels are in dehiscent, with a lateral or sub-basal or sub terminal style-scar; a thin or hard endocarp of ten deeply excavated on its ventral surface and projected in word; usually more or less remiform or hooked seeds curved round the projection of the endocarps an embryo with flat or narrow cotyledons curved on the axis of even or ruminate endosperm (Dassanayake *et al.*,1995).

2.2 Botanical description of *Coscinium fenestratum*

Coscinium fenestratum is a Branchlets brownish tomentose at first, later glabrescent, becoming whitish leaves petioles tomentose at first, 3-16 cm long, often much swollen at both ends, geniculate at base, inserted up to 0.8-2.7 cm from basal margin of lamina; lamina usually broadly ovate, or ovate, sometimes oblong with basal lateral lobes, base broadly rounded, truncate or shallowly cordate, rarely broadly obtuse apex acuminate, upper surface glabrescent, midrib and other main nerves sunken, lower surface often whitish to mentellous with fine reticulation visible, 5-7 basal nerves and usually 2 pairs of lateral nerves, thinly coriaceous.

Inflorescences arising singly or a few together, tomentose or tomentellous; flowers in several-flowered globose heads 6-7 mm diameter. On peduncles 10-30 mm long arranged in a raceme 5-11 cm long. Male flowers sessile or with pedicels up to 1mm long; sepals densely sericeous-pilose externally, glabrous within, broadly elliptic to obviate, inner 3-6 spreading, yellow, 1.5-2 mm long, the outermost

smaller, 1-1.5 mm long, inserted lower; stamens 1 mm long. Female flowers; staminodes claviform, 1 mm long: carpels 2 mm long. Drupes brown to orange or yellowish, 2.8-3 cm diameter; pericarp drying woody, c.1 mm thick, endocarp bony, 2.2-2.5 cm diam. Seed whitish (Dassanayake *et al.*, 1995).

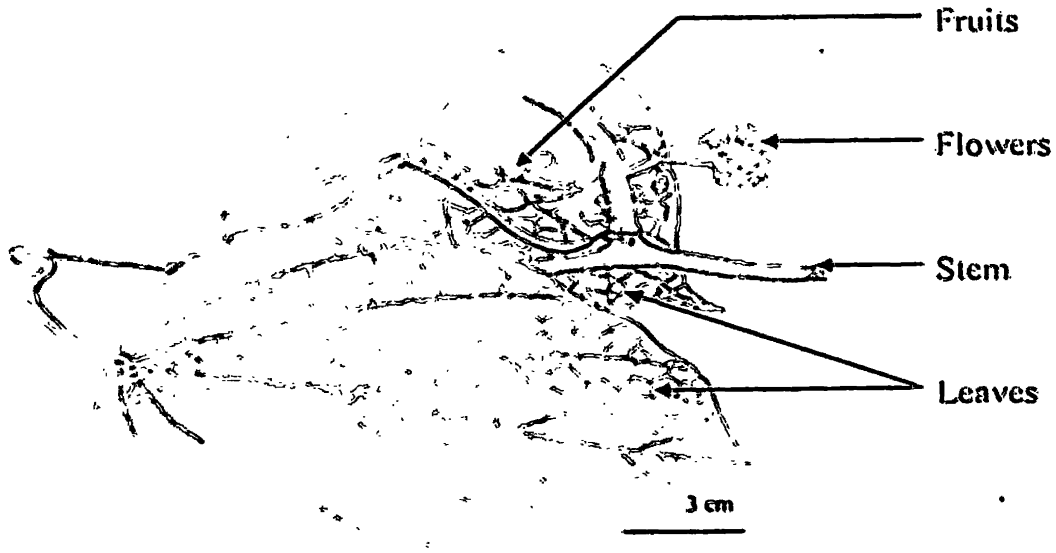

Plate 2.1 Morphology of *Coscinium fenestratum* (Weniwel) liana

Similarly morphological futures were observed in *Anamirata cocculus*

Botanical description of *Anamirata cocculus*

Young stems and petioles usually drying pale greyish straw-coloured. Leaves: petioles 6-18 cm, swollen at both ends, geniculate at base; lamina ovate to broadly ovate, base cordate to truncate, apex usually acuminate, 16-28 x 10-24 cm. 3-5 basal nerves with 4-5 pairs of lateral nerves which are linked together with scalariform veins; lower surface drying pale, upper surface slightly darker, both surfaces glabrous apart from pockets of hairs in the axils of nerves and main veins, thinly coriaceous. Male flowers: sepals white, yellow to pale green, outer 2 sepals scarcely 1mm long, inner sepals broadly elliptic, 2.5-3 x 2 mm; synandrium 1.5-2 mm long (Dassanayake *et al*, 1995).

2.2.1 Distribution of *Coscinium fenestratum*

Coscinium fenestratum is found in Malacca, Singapore, Sumatra, South India and Sri Lanka (Jayaweera, 1982). In Sri Lanka it is commonin the moist low country and intermediate forests. It is found in the areas of Deniyaya, Matara, Kaburupitiya and Akurassa in the southern province, Waga, Agalawatte, Mattugama and Ingiriya in the western province, Kitulgala, Rakwana, Rathnapura, Balangoda, Kegalle and Botele in the Sabaragamuwa province and Kurunegala, Puttalum and Kuliyapitiya in the North-Western province (Jayaweera, 1982; Muttiah, 1964).

2.2.2 Medicinal uses of *Coscinium fenestratum*

Coscinium fenestratum has a remarkable demand in Ayurvedic medicine. According to the Ayurvedic Research Institute of Maharaga the direct stems of *Coscinium fenestratum is* used for preparation of various medicinal prescriptions. In all the them it is the major component. They are (i) Weniwelgata pantaya; (ii) Weniwelgeta surssawaya, (iii) weniwelgeta kvathaya, (iv) Weniwelgeta punarnawasaya, (v) Weniwelgata chandanasawaya and (vi) Weniwelgeta coriander-pathpadegem decoction.

There are also a number of other medicines in which *Coscinium fenestratum* is used in small portions. According to the above Research Institute and the Municipal Ayurvedic Department of Kandy, these *Coscinium fenestratum* mixed prescriptions are used remedies for diseases of the skin, uterus, urinary system, eyes, gums, certain kidney diseases diabetes and accidenton wounds (Senerath, 1991).

The active components of the stem of this plant are belived to act on the blood circulation system and on the skin; it is also used in the treatment of fever including Malarial fever, common colds and tetanus, as a painkiller for body aches and pain, and as a blood purifier (Muttiah, 1964). Consequently the dried stems are sold in almost all the pharmacies of indigenous medicine dotted throughout the country, particularly for the preparation of the Weniwelgeta, coriander-pathpadagam decoction and Weniwelgeta tonic. It is of interest to note that this plant is widely

used in Central and South India where it is well distributed while the related *Berberis* species "Calumba" which is said to possess even greater medicinal properties is solely used in North India where this can be readily procured (Muttiah, 1964).

Table 2.1 Active compound and its uses of *C. fenestratum* (Senerath, 1991)

Species	Uses	Active Compound
Coscinium fenestratum	i) Wood decoction is used to prevent tetanus. ii) Root has antiseptic properties and is used for dressing wounds and ulcers.	i) Alkaloids (e g : berberine Jatrorhizine palmatine).[In addition to those Muttiah (1964) reported cery alcohol, hentriacontane sitosterol, palmitic and oleic sitosterol ,glucoside saponines]

2.2.3 Non Medicinal or Other uses of *Coscinium fenestratum*

In addition to its medicinal properties given above, the local people use *C. fenestratum* in many other ways. The tough fibers in its stem, makes it a suitable substitute for rope. While its slender flexible stems are used to tether cattle, its thicker, larger parts are used for hauling longs by elephants and in the construction of suspension bridges. The stems on boiling with water yield a deep yellow dye used. in the past for dying robes of Buddist monks. In the past the wood has been exported to England as a substitute for calumba root and called "false calumba" or "Ceylon calumba-root" (Muttiah, 1964). When tapping kitul, (*Caryota urens*) people use the thin vine of weniwel to bind the kitul inflorescence. A piece of weniwel bark is used to prevent quick fermentation of sweet toddy of kitul (Senarath, 1991).

2.3 Sexual propagation

With crops that produce seed freely and come true closely enough for the purposes in view, growing from seed usually is the cheapest and most satisfactory method of plant propagation. Many types of seeds may be sown in open ground and, barring

extreme wetness or extreme aridity, germinate well enough for practical purposes. Other kinds, however, are so exacting in their requirements that these are best met in a propagating house where humidity and temperature can be more rigidly controlled. Because of their high oxygen requirement, the medium in which the seeds are sown generally should contain more sand (or other filler or mulch material) than ordinary garden soil does. Greater porosity makes these media more subject to rapid drying, however, and moisture must be carefully monitored. Because many soils harbour fungi destructive to sprouting seed and young seedlings, heat or chemicals sterilize soil that is used for germinating seed commonly. Many diseases of plants are caused by fungi and bacteria carried in or on the seed itself, and treatment of the seed with disinfectants is beneficial (Hudson *et al*, 1997).

Propagation of plants by seeds is *Coscinium fenestratum*, because other ways of plant propagation were not successful. Propagation by stem cuttings and layering methods were unsuccessful (Senerath, 1991). Muttiah (1964) also reported that these methods were not successful for this plant.

2.3.1 Sexual propagation system

Seed propagation is carried out using three basic systems

(a) Field seeding – in the location where the plant is to remain,

(b) Planting in field nurseries and trans planting to a permanent location, and

(c) Planting in protected conditions,

As in a green house, cold frame, or similar structure, and then transplanting to the permanent location. For commercial production of horticultural crops, the most common seed production practices are to produce vegetables from direct field seeding or transplants annual along juvenile phase and may come into first flowering and fruiting bedding plats and herbaceous perennials from transplants; and woody seedlings from field transplant beds to produce bare root liners (Hudson *et al*, 1997).

2.3.2 Importance of sexual propagation

However, sexual reproduction provides the opportunity to generate new genetic combinations for the adaptation of populations to new environmental conditions or biotic challenges. It allows for favorable mutations to spread into different genetic backgrounds. Thus, in the long run, it has generally been a more successful strategy (Raven *et al.*, 1992).

Sexual propagation of plants involves the exchange of genetic material between parents to produce a new generation. Sexual propagation offers the following advantages (Ranlton, 1995).

- It is usually the only method of producing new varieties or cultivars.
- It is often the cheapest and easiest method of producing large numbers of plants.
- It can be a way to avoid certain diseases.
- It may be the only way to propagate some species.

2.4 Seed characteristics

The majority of tropical tree species have very complex life cycles. Many of these species produce recalcitrant seeds. These species have only after 15-20 years of growth or more. In addition, fruiting and seed sets in many tropical species is not an annual occurrence. When it does occur, the seed production period continues for a short period. This is termed as a mass flowering season that normally occur once in 3-7 years. It is also not possible to predict their flowering times owing to their erratic flowering patterns. Consequently, it causes problems to secure large quantities of fruits on a regular basis. Generally, the seeds that are produced do not undergo dormancy, but instead they are metabolically primed for immediate germination as soon as the seeds mature on mother plants. Desiccation tolerance in recalcitrant seeds increases during seed development on the mother plant; however unlike orthodox seeds maturation drying to low moisture contents does not occur (Hong and Ellis, 1990).

Tropical forest seeds can be broadly divided in to three major groups based on their sensitivity to desiccation and to low temperatures as follows.

2.4.1 Recalcitrant seeds

After developing seeds reach physiological maturity, they either proceed to desiccate (orthodox seeds) germinate on the plant (vivipary), or bypass complete desiccation (recalcitrant seeds). By definition a recalcitrant seed loses viability after drying, while orthodox seeds tolerate drying. Germination in recalcitrant seeds must proceed soon after maturity or the seeds must be stored under conditions that prevent drying. This compares to decades or years for many orthodox seeds. Recalcitrant seeds present challenges for propagators and limit germ plasm conservation because of their inability to store. The biological basis for this inability in recalcitrant seeds to tolerate drying is not well understood. It would be suspected that recalcitrant seeds would also show reduced ABA levels or be impaired for the production of lea proteins or same carbohydrates. However most recalcitrant species studied produce these substances at almost normal levels. The true nature of recalcitrance to drying remains to be found for this interesting group of seeds (Hudson *et al.*, 1997).

According to Ariyarathna, (2000) *Coscinium fenestratum* may be under recalcitrant seeds, which mean they germinate very short period after detachment of mother plant and also the embryo is very sensitive desiccation.

2.4.2 Intermediate seeds

This is yet another category that has been recently defined. The seeds in this category have storage characters intermediate between orthodox and recalcitrant. Intermediate seeds can be dried to seed moisture levels almost similar to that of orthodox seeds without their viability being affected. However the dry seeds are easily injured when exposed to low temperatures and viability drops rapidly while in storage (Ellis *et al.*, 1990).

2.4.3 Orthodox seeds

Most orthodox seed can be stored safely for at least 1-2 years at moisture content of 8-10% or below. Potential storage period is prolonged by cold storage conditions. For long-term storage at subzero temperatures moisture content 2-4% is desirable. Drying may be carried out by natural sun drying or artificial heating with a dry air current, which is usually supplied by electrical appliances. The relation between moisture content and heat should be recalled; moist seed is less tolerant to heat than dry seed. Once the seeds have been dried to appropriate storage moisture content, they should be stored in air tight containers as soon as possible to avoid re-gain of moisture from the air (Olesen, 2000).

2.5 Seed Identification features

In seed handling the term 'seed' usually refers to the unit extracted from the fruit and handled as a unit during storage, pretreatment and sowing. During seed processing same features such as wings or arils may be deliberately or undeliberately lost or removed (Olesen, 2000).
Some key features in the identification of seeds are enumerated below.

Seed weight: Seed weight is indicated as the number of seeds per unit weight. Seed weight often varies considerably within species both because of genetic and environmental differenees. Seed weight obviously influence for some seed processing events such as dewinging and drying.

Seed size: Seed length, width and thickness including expected variation are usually indicated. Size of seeds varies considerably especially if the seeds have appendices such as wings.

Colour: Most seed are yellowish or brownish when mature; other colours are such as red, black or white are less frequent and usually diagnostic. In addition colours of appendices are important to identify seeds.

Shape: Very few seeds are symetrical globular and seed shape is often one of the main diagnostic characters of seeds. A vast number of botanical terms apply to seed form globose, sub-globose, oblong and orbicular.

Surface: Surface structure or appearances are other important features for seed identification. For very small seeds like *Eucalyptus* it is often the main diagnostic features (Boland et al. 1980). Again a number of Botanical terms apply, like smooth, glabrous, wrinkled, ribbed punctuate, reticulate, pulpy, tomentose and hairy.

Other morphological features: Position and size of the raphe, caruncle and microphyle are often important characters.

Internal structure of seed/ fruit coat and embryo: Thickness and hardness which are often distinct characters. Internal appearance of embryo and endosperm or perisperm and seed coat thickness is important in some species but often specific on genus level only.

2.5.1 Seed viability

Seed longevity and vigor are two other features associated with seed viability. Briyant (1985) described the relationship between these tree terms. If 100 seeds are set to germinate and 99 of them do 50, then the seed batch is obviously a high viability batch; if 50 Germinate the batch may be termed a medium viability batch is non-viable. Seed vigor is related to the speed of germination. Seed of high vigor germinate rapidly; seeds of low vigor germinate more slowly (Briyant, 1985).

As Senerath (1991) stated the loss of viability studied visually by examining the condition of the *Caseinium fenestratum* embryo after cracking open the endocarp in a sample of seeds. The embryos of cracked seeds were compared to that in viable seeds where the embryo was yellowish cream and firm. If the contents of the seed appeared semisolid and/or darker in colour, such seeds were considered as non-viable. If it was comparable with the viable embryo it was considered as viable and treated with TTC solution to confirm its viability. He has used above method tested

loss of viability in terms of days of seeds detaching from mother plant. He concluded that the seeds with 88% initial viability and completely viability loss after 603 days (above seeds his store under room temperature).

2.5.2 Seed Moisture Content

The amount of water present in a seed. It is normally expressed on a weight basis, either as the weight of the water in % of the seeds oven-dry weight (dry-weight basis) or, preferably in the case of seeds and fruits, as a % of the material's fresh weight including water (wet-weight or fresh-weight basis) (Lars, and Dorthe, 2001).

2.5.3 Seed Morphology and Anatomy

The outer appearance of seeds contains structure derived from the fruit, raphe and hilum. This structure use for seed dispersal. The inner structure of the anatomy consists of parts derived from the fertilized oval. The embryo nutritional tissue of varying origin and the seed coat (Olesen, 2000).

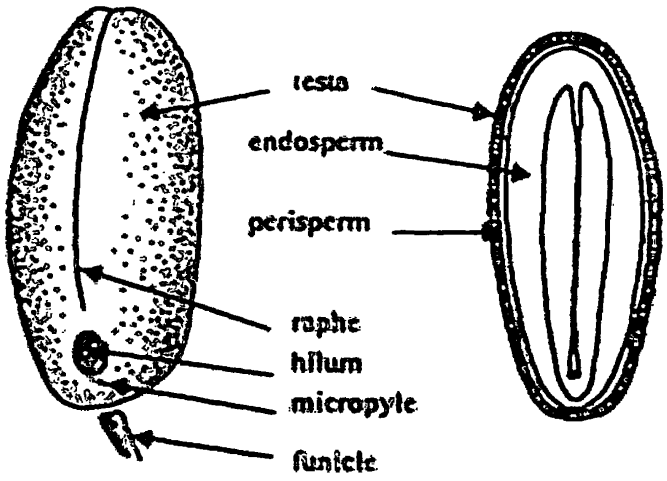

Plate 2.2 Morphology and anatomy of a typical seed

Hilum: Scar on the seed coat left by the funiculus.

Micropyle: A pore sometimes visible on the seed coat derived from the channel between the tip of the integuments. The radical of the embryo always faces the microphyle.

Raphe: A ridge formed on the seed coat if the funiculus is fused with the integument in part of its length in anatropous or compylotropous ovules.
Carunele: Integumentary protuberances near the microphyle.
Perisperm: A layer of nutritional tissue of diploid material origin arisen from the nucellus and often is surrounding the endosperm. It is usually completely absorbed before maturation.
Albumen: A collective term of the nutritional tissue between the embryo and the seed coat, inclusive of perisperm and endosperm.

As Senerath (1991) stated that there were fruits of *Coscinium fenestratum*, which were drupes with an outmost firm relatively, thin exocarp on the outside has very short brownish yellow hairs. The mesocarp is soft, white and fleshy. The seed coat is hard, grayish black and stony. The fruit on the side it is attached to the infructeseence is relatively flat and more or less spherical on its distal side. This shape is quite clearly seen on the seed coat when the fruit is depulped.

The *Coscinium fenestratum* endosperm was located deep within the invaginations of the endosperm. Endospermic tissues were dry and relatively hard. Therefore it may act as a mechanical barrier to the developing embryo (Ariyarathna, 2000).

2.5.4 Embryo morphology

In some seeds the embryo is small or rudimentary in the mature seeds. In others the embryo makes up the majority of the seed lumen. The embryo is an immature plantlet in the seed stage it is often differentiated in to structures that will develop in to the seedling (Olesen, 2000).

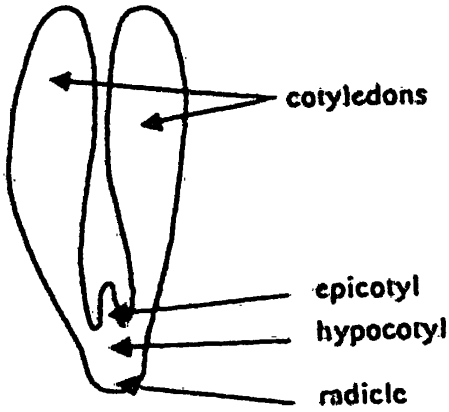

Plate 2.3 Morphology of embryo of Angiosperm

Cotyledons: In monocotyledons one, in dicotyledons two and in conifers often many. During germination the cotyledons may remain underground.
Plumule: The embryonic shoot derived from the epicotyl. In dicotyledons situated between the cotyledons.
Epicotyl: The apical end of the embryo axis above the junction with the cotyledons.
Hypocotyl: The axial part of the embryo between the cotyledons and the radicle.
Radicle: The embryonic root. In seed the radicle is always facing the micropyle.
Suspensor: In gymnosperm seed, a thin threadlike appendix at the radicle end of the embryo.

2.6 Seed collection and handling

For conservation purposes, recalcitrant seeds should be collected from healthy trees with good shape and form. Several techniques of seed collection namely ground collection-shaking seed bearing branches; free climbing or climbing the trees using equipment can be done.
Criteria for collection of good seed should include mature seeds uniformity in colour and size, and healthy. Most recalcitrant seeds respire intensively because of their high moisture content and hence require good ventilation. If large quantities are closely packed, suffocation, physiological breakdown, fungal growth and overheating will occur, resulting in rapid death of the seeds. On the other hand the seeds will also deteriorate rapidly if moisture content is reduced too much or too

rapidly. This likely to happen during transporting in open vehicles due to air movements.

Hessian or jute bags that have been loosely woven are also suitable for transport. Temperature below 16^{0}C or above 32^{0}C should be avoided for such seeds. Seeds should be kept shaded from direct sun at all times during transport to the conservation centers. Humidity content is 80-95% and light (Erica, 1999).

2.6.1 Seed dispersal

The purpose of dispersal is to colonize new ground. As stated in the previous section the morphology of fruits and seeds often reflects the mode of dispersal. Some methods of dispersal are prevalent in some environments than in others and dispersal is closely related to the life cycle of the particular species in its particular environment. Some species have specific method of dispersal. The unit of dispersal is often called diaspore. Major types of seed dispersal are wind, water, animal and mechanical etc (Fahn and Werker 1972, pijl, 1982).

As Senerath (1991) stated that there was no formal method to study seed dispersal of *Coscinium fenestratum* but as surroundings of the mother plants were observed for fruit or seeds. The ripe fruits of *C. fenestratum* are known to be dispersed by fruit bats and pole cats and are foraged by them while they are still attached to the mother plant. On the other hand, it is also reported that ground dwelling small mammals such as rodents and also porcupine feed on the seed once the ripe fruits fall beneath the mother plant.

2.6.2 Determination of optimal harvest time and maturity stage of fruit

The optimal time to harvest is when a large amount of viable, germinable seed can be collected. This is when most fruits and seeds are mature but only few have been lost to predation, dispersal and deterioration. Species can be category mainly three groups due to their seed production rate and time (Olesen, 2000).

1. Trees with more or less continuous reproduction throughout the year but often with one or two peaks.
2. Trees with definite, some times short, seed maturation season and early dispersal, predation and/or short physiological viability.
3. Trees with a definite maturation season but with prolonged persistence on the tree before dispersal.

Structural changers, period and appearance are the important factor to seed collectors. Normally earliest possible collection is when seeds are germinate latest method is before abscission.

As Senerath (1991) stated that there was the linear measurements were made throughout development of *Coscinium fenestratum* fruits from origin to shedding and those at the mature stage were used for comparison. Only ripe fruits that shed were used to measure fruit weight.

2.7 Seed storage

In the past many types of storage methods have been proposed for recalcitrant seeded species but without exception their use has been limited. Thus to conserve recalcitrant seeds availability of seeds should be given priority as this will determine the period of storage as viability is reduced during storage.

2.7.1 Short-term and mid-term storage methods

This method can be applied for recalcitrant and intermediate seed. These can be applied to seeds for which viability is maintained for less than 12 months. The strategy is to desiccate recalcitrant seeds to just above a critical moisture content treat them with an effective fungicides and then store them in semi-sealed packaging which allows adequate gaseous exchange but restricts moisture loss. Several common conventional storage methods include: imbibed storage using sawdust, ground charcoal, pearlite and vermiculate; storage in airtight containers or partial vacuum, regular ventilation and incorporating germination inhibitors in to

the storage system. The lowest safe moisture content of many of the recalcitrant species falls within the range of 20-60% (Hor, 1996; Tompsett, 1992).

However fungicide treatment followed by partial desiccation and storage under ambient or low temperature has yielded some fruitful results for some crop seeds. Hor (1996) found that after treating *Hevea* seeds in 0.3 percent Benlate air drying and storing them loosely packed if perforated polythene bags at 21-24^{0}C temperatures, was able to prolong seed viability from three months to one year with about 50% germinability (Normah, 1987). Although this approach has potential for recalcitrant seed storage (Erica, 1999).

2.7.2 Long-term storage methods

This method can be applied to orthodox seeds. Many orthodox seeds can be stored for long time at ambient temperature provided their moisture content is low. At high moisture content and temperature a major cause of deterioration is mould. Although some fungi may survive low temperature and moisture content their activity rapidly declines below 10% moisture content and temperature is 10 ^{0}C.

Orthodox seeds should be dried down to at least 5-10% moisture content. At that moisture content there is practically no metabolism and little or no fungl activity. Low moisture content should be maintained through out storage; i.e., the seeds should be prevented from re-absorbing moisture. During seed storage seed moisture comes in to equilibrium with humidity of the surrounding air (Thapliyal *et al.* 1991).

2.8 Seed dormancy

All the viable seeds have the capacity to germinate if placed under suitable conditions necessary for germination. While in certain plants such seeds will immediately germinate after harvest in others they fail to germinate for sometime even if placed under such conditions that are ordinarily favorable for germination either due to some internal factors or due to specific requirement for some

environmental factors. During this period the growth of the seed remain suspended and they are said to be in rest stage or dormant stage and this phenomenon is called as dormancy of seed (Jain. 1999).

Since germination of *Coscinium fenestratum* was extreamly poor under natural conditions. The causal factors to unsuccessful germination and possible methods of its improvement were investigated (Senerath, 1991).

There are two kinds of seeds dormancy such as primary and secondary dormancy.

2.8.1 Primary seed dormancy

Primary seed dormancy is a condition where seeds will not germinate even when the environmental conditions such as water, temperature and aeration are permissive for germination. In nature different kinds of primary dormancy such as seed coat dormancy, embryo dormancy, chemical dormancy, physiological dormancy, morphological dormancy etc (Hudson, *et al* 1997).

Seed embryo dormancy

Dormancy occurs in some seeds in which the embryo is not fully developed at the time of seed dissemination. Enlargement of the embryo occurs after the seeds have imbibed water and before germination begins. The process of embryo enlargement is usually favored by a period of warm temperatures (Hudson *et al.*, 1997).

Seed coat dormancy

Modification of seed coverings primarily affects the outer integument layer of the seed, which may become hard, fibrous, or mucilaginous during dehydration and ripening. In addition layers of the fleshy fruit may dry and become part of the seed covering as in cotoneaster or hawthorn. In some drupe fruits as the *Prunus* species, these layers become the hardened endocarp (Hudson *et al.*, 1997).

As Senerath (1991) stated that there was studied *C. fenestratum* seeds inhibition to germination due to the hard stony seed coat, possibly presenting a mechanical barrier to emergence of the developing embryo or presenting an impervious barrier to the imbibition water.

Physiological dormancy

Type of embryo dormancy in which germination is prevented by a physiological inhibiting mechanism, e.g. chemical dormancy or thermo-dormancy (Lars and Dorthe, 2001).

Chemical dormancy

Chemicals that accumulate in fruit and seed covering tissues during development and remain with the seed after harvest can be shown to act as germination inhibitors. Proving third function as germination controls does not necessarily follow, however. Nevertheless, germination can sometimes be improved by prolonged leaching with water, removing the seed covering or both (Hudson *et al.*, 1997).

2.8.2 Secondary dormancy

Secondary dormancy is a further adaptation to prevent germination of an imbibed seed if other environmental conditions are not favorable. These conditions can include unfavorably high temperatures, temperatures too low, prolonged darkness, prolonged white light, prolonged far-red light, water stress and anoxia (Bewley, and Black, 1994).

In nature have two types of secondary dormancy such as Thermo dormancy and Conditional dormancy.

Thermo dormancy

For some species (like *Tectona*) germination at high temperatures (>25^{0}C, 77^{0}F) can include thermo dormancy. This should not be confused with the thermal inhibition most seeds experience when the temperature exceeds the maximum temperature for germination.

Seeds experiencing thermo dormancy will not germinate when the temperature returns to near optimum temperatures, while thermal inhibited seeds will germinate when temperature is lowered (Sharples, 1973).

2.8.3 Seed germination inhibitors

Some times the dormancy of seeds results due to the presence of certain germination inhibitors either in some parts of the seeds such as the juice or pulp of fruit and glumes (Jain. 1997).

Senerath (1991) also reported *Coscinium fenestratum* seed burial has a significant inhibitory effect on germination. Further more, he found out that the pulp of the fruit prevents germination.
However presence of gemination inhibitors pericarp, endocarp and embryo in *Coscinium fenestratum* seeds like amino acid (Ariyarathna, 2000).

2.9 Pretreatments of breaking seed dormancy

Pretreatment is a 'pre-sowing-treatment' carried out in order to enhance rapid and uniform germination of seed sown in the nursery, field or for testing. In some cases pretreatment is a mere acceleration of the natural processes of dormancy release in others it is a simulation of these processes (Olesen, 2000).

2.9.1 Treatments for breaking mechanical dormancy

Acid pretreatment is frequently used where mechanical dormancy is combined with an impermeable seed-coat (double dormancy). For example it has been used successfully to improve germination of *Pterocarpus angolensis* and *Terminalia bellirica*. In the latter case both total germination and germination speed were greatly improvedby an optimal 12 minutes soaking in concentrated sulphuric acid as compared to the control (Bhardwaj and Chakraborty, 1994).

Senerath (1991) also studied scarification of *Coscinium fenestratum* seed coat by using concentrated NH_4OH, con. HCl, and H_2SO_4 samples of fruits or seeds were subjected to these alkali and acids for varying different time periods. He has also pretreated seeds with con. HCl for 15 minute and shown higher germination percentage (18%) than without these treatment.

2.9.2 Pretreatment for breaking physical dormancy

A wide range of methods has been developed to overcome physical dormancy. All methods are derivation of the same principle to pierce the seed coat to an extent that will render it permeable to water so that imbibition can take place.

Unless physical dormancy is combined with mechanical dormancy penetration at one point is sufficient to ensure permeability. Because the outer layer of the coat exerts the impermeability in legumes and the palisade cells absorb water a relatively superficial treatment may over come dormancy in these seeds (Masanga and Maghembe, 1993).

2.9.2.1 Hot water treatment

For small to medium- sized seeds or large quantities of seeds the hot water treatment is more practical than scarification.

For this treatment seeds should be dropped in to about six times their volume of 85 ^{0}C pre-heated water. They should be left to cool and soak in the water for 12 to 24 hours after which they are ready for sowing. An other and more drastic hot water treatment is some times used for corn-especially thick or hard-coated seeds. For this treatment the seeds should be placed in vigorously boiling water for a specific length of time depending on the species then immediately removed from the boiling water and cooled in cold water (Kobmoo and Hellum, 1984).

However Senerath (1991) reported *Casciniun fenestratum* seeds pretreated with hot water (90-92 ^{0}C) for 5-120 minute and shown a germination percentage is 3%.

2.9.2.2 Dry heat treatment

Oven or dry heat is not often recommended and the temperatures required are more suitable to an incubator than a kitchen oven. For this seed coat treatment the seeds should be placed in shallow containers in a preheated incubator or oven.

The specific temperature and duration depend on the species. After the treatment the seeds should be cooled immediately and sown. Where the temperature

suggested is between 180-212^{0}F it is possible that the hot water treatment of the same temperature and for the same length of time would give comparable results (Olesen, 2000).

2.9.2.3 Fire treatment

Seeds of same genera have tough thick seed coats and germinate best when subjected to the heat of fire. For this treatment the seeds should be sown in the fall in a slightly moist medium but not watered. A layer of dry pine needles or excelsior, four to six inches deep, should be placed over the top of the seed bed. A few small pieces of wadded paper will help to ignite the material. One or two strips of aluminum foil placed over the exposed edges of the wood container will prevent it from burning. After the seed bed has cooled following burning it should be thoroughly watered and then treated as any other batch of sown seeds (Olesen, 2000).

2.9.2.4 Biological methods to overcome seed dormancy

Biological methods such as ingestion by large animals or the effect of insects or microbes are rarely used as a managed pretreatment method, but incidents of such action frequently result in improved permeability. Seeds of *Acacia* species extracted from goat faces are often less dormant than non-ingested dry seed (Ahmed and Houri, 1986).

2.9.3 Treatments for breaking chemical dormancy due to inhibitors

Chemical inhibitor may be located in several places in the fruit or seed. The most frequent inhibitors are those occurring in fleshy fruit pulp. Even where seeds are sown immediately after harvest, such seeds usually need extraction and washing to remove inhibitors (Schaefer, 1989).

2.9.4 Treatment for photo dormancy

Photo-dormancy has been most frequently documented from herbal species and tree pioneers. The condition of light requirement in pioneers is simplest sort of photo-dormancy. In some species seeds require specific duration of light-dark cycles for germination to proceed. Under tropical conditions a cycle of 12hour light-12hour dark is prevalent. Photo dormancy seeds normally require only a brief illumination after imbibition to break dormancy. In practice photo-dormancy is not over come by pretreatment but by germinating seeds under appropriate light conditions that will break the dormancy (Boland *et al.* 1980).

2.9.5 Hormonal treatments to breaking physiological seed dormancy

The same type of hormones is for example often involved in both dormancy release and germination processes. Total germination percentage, germination speed and seedling vigour may be promoted by application of germination stimulants. The two main groups of stimulants are growth regulators e.g. Gebberllic acid (GA_3), Benzyl adenine (BA) and nitrogenous compound e.g. potassium nitrate (KNO_3) and thiouria (Hartmann *et al.*, 1997).

2.10 Physiology of seed germination

All the viable seeds which have overcome dormancy either naturally or artificially will readily germinate under suitable environmental conditions necessary for seed germination (e.g. water, O_2, temperature and light. Such seeds, which just wait for suitable environmental conditions to germinate, are said to be 'quiescent'. In most cases these seeds germinate if placed on moist substrate.

The process of seed germination starts with the imbibition of water by seed coats and emergence of growing root tip of embryo. It ends when the embryo has developed into a seedling, which is out of bounds of seed coats and has its own photosynthetic system. Before describing the physiological and biochemical changes accompanying the seed germination, it is better to evaluate the physiological state of seed immediately before germination (Jain,1997).

2.10.1 Main seed germination factors

There are four environmental factors, which affect germination: water, oxygen, light, and temperature.

Water

The first step in the germination process is the imbibition or absorption of water. Even though seeds have great absorbing power due to the nature of the seed coat, the amount of available water in the germination medium affects the uptake of water. An adequate, continuous supply of water is important to ensure germination. Once the germination process has begun, a dry period will cause the death of the embryo (Hudson *et al.*, 1997).

Light

Light is known to stimulate or to inhibit germination of some seed. The light reaction involved here is a complex process. Some crops, which have a requirement for light to assist seed germination, are ageratum, begonia, browallia, impatiens, lettuce, and petunia. Conversely, calendula, centaurea, annual phlox, verbena, and vinca will germinate best in the dark. Other plants are not specific at all. Seed catalogs and seed packets often list germination or cultural tips for individual varieties. When sowing light-requiring seed, do as nature does and leave them on the soil surface. If they are covered at all, cover them lightly with fine peat moss or fine vermiculite. These two materials, if not applied too heavily, will permit some light to reach the seed without limiting germination and will help keep soil uniformly moist. When starting seed in the home, supplemental light can be provided by fluorescent fixtures suspended 6 to 12 inches above the seeds for 16 hours a day (Arora and Gupta, 1996).

Oxygen

Respiration takes place in all viable seed. The respiration in dormant seed is low, but some oxygen is required. The respiration rate increases during germination. Therefore, the medium in which the seeds are placed should be loose and well

aerated. If the oxygen supply during germination is limited or reduced, germination can be severely retarded or inhibited (Hudson *et al.*, 1997).

Temperature

A favorable temperature is another important requirement of germination. It not only affects the germination percentage but also the rate of germination. Some seeds will germinate over a wide range of temperatures, whereas others require a narrow range. Many seed have minimum, maximum, and optimum temperatures at which they germinate. For example, tomato seed has a minimum germination temperature of 50°F and a maximum temperature of 95°F, but an optimum germination temperature of about 80°F. Where germination temperatures are listed, they are usually the optimum temperatures unless otherwise specified. Generally, 65 to 75°F is best for most plants. This often means the germination flats may have to be placed in special chambers or on radiators, heating cables, or heating mats to maintain optimum temperature. The importance of maintaining proper soil and air temperature to achieve maximum germination percentages cannot be over-emphasized.

Germination will begin when certain internal requirements have been met. A seed must have a mature embryo, contain a large enough endosperm to sustain the embryo during germination, and contain sufficient hormones or auxins to initiate the process. Some seeds have a dormancy requirement also (Hudson *et al*., 1997).

2.10.2 Physiological changes accompanying seed germination

Water uptake: Seed germination as mentioned earlier starts with the imbibition of water by dry seed coat, which is purely a physical process. Various hydrophilic groups of proteins, polymeric carbohydrates etc., found in the seed coats attract dipolar water molecules and form hydrated shells around them resulting in the swelling of these substances. Due to imbibition of water the seed coats become (i) more permeable to oxygen and water and ii) less resistant to outward growth of the embryo (Jain, 1997).

Respiration: The uptake is accompanied by rapid increase in respiration rate of embryo. Initially there may be anaerobic respiration but aerobic one due to availability of oxygen soon replaces it. As compared to dry seeds the uptake of O_2 in germination seeds may rise in case of cereals from 0.05µ l/g tissue/hr to 100µ l/g tissue/hr within very short period after germination when water contact has reached about 40% sucrose is probably the respiratory substrate at this stage which is provided by endosperm (Jain, 1997).

Mobilization of reserve materials: As germination progresses there is mobilization of reserve materials to provide (i) building blocks for the development of embryo, (ii) energy for the biosynthetic processes and (iii) nucleic acids for control of protein synthesis and overall embryonic development. Changes in these components during seed germination. Such as nucleic acids, carbohydrates. lipids, proteins and inorganic materials (Jain, 1997).

Emergence of seedling out of the seed coat: All these changes described above gradually result in splitting of seed coat and emergence of the growing seedling. First the radicle comes out and grows downward then plumule comes out and grows upward. Due to continued growth of this seedling the latter comes out of the soil exposed to light and develops its own photosynthetic apparatus.
The spliting of seed coat may take place either (i) imbibitional pressure or (ii) internal pressure created by the growing primary root or (iii) by hydrolytic enzymes, which act on cell wall contents of seed coat, and digests it (Jain, 1997).

2.11 Wood anatomy

The part of the axis of a plant that bears the leaves and reproductive structures and is commonly aerial and ascending is called the stem. Perennial woody plants present apparently simple stem structure. In these an unbroken layer of secondary vascular tissue sheaths a more or less continuous cylinder of primary xylem. Variations in the structure of this cylinder range from cylinders that are unbroken except by leaf and branch gaps to those consisting of discrete bundles often complex in arrangement (Arthur and Laurence, 1993).

2.12 History and Previous work done

An Experiment done in Sri Lanka by Palasutheran *et al.* (1980) showed that an aqueous extract of *Coscinium fenestratum* and its alkaloid berberine hydrochloride have selective inhibitory action on *Clostridium tetani* , and the minimum inhibitory concentration was shown to be significantly lower than that for the other bacteria. A detailed histological and general description of the stem of *Coscinium fenestratum* and *Coscinium wallichianum* was given by Short (1925) who showed that definite histological differences exist between the stems of *Coscinium fenestratum* and *Coscinium wallichianum.*

Additional histological details are also given in the pharmacognosy of Ayurvedic drugs, Central Research Institute, Trivendrum-1953 series No.2 (Muttiah, 1964). He also made a preliminary study of the distribution, growth and propagation of *Coscinium fenestratum.*

In this study he described the distribution of the plant in the Island. The taxonomy and morphology of the plant, flowers and fruits, observation on the periodicity of flowering and fruiting, and its natural regeneration by seeds, which he pointed out to below (18.5%).

In the nursery at Kankaniyamulla he has also pretreated seeds with lime water for 6 hours and shown a higher germination percentage (19.5%) than without any treatments.
However treatments for longer period were shown to decrease germination (This seeds sample collected from forest flow). He had also attempted artificial propagation of this species propagation by cuttings, both shoot and root, had also been tried out but without success. Root cuttings had been a complete failure while stem cuttings had been a near failure. Planting out stumps and seedlings obtained from the natural forest had also not been successful.

Viability test have also been initiated at the nursery. The yield of plants obtained from an acre at different reserves was given by Muttiah (1964) and is reported to be Kankaniyamulla (North-Western province), Beraliya (Southern province) and

Diyadawa (Southern province) 112. 40 and 164 (over 1inch girth at root collars) respectively (Senerath, 1991).

Senarath (1991) in Sri Lanka made Biological Studies on *Coscinium fenestratum* colebr. (Menispermaceae). In this study he described the distribution of the *Coscinium fenestratum* in the Island.

The population biology (Demarcation of population, sex distribution in the population). Intra- population variations (pollination success and fruit set) and phenological studies (flushing, flowering, fruiting and pollination experiments also he has studied in Sinharaja forest.

In the nursery at Sinharaja he has also pretreated seeds normal condition, hot water treatment, alkali treatment, acid treatment, sunlight treatment, soaking in tap water soaking followed by exposure to sunlight, exposure to sunlight followed by soaking and sinking samples exposed to sunlight and shown a higher germination percentage (57%) than without any treatment (Senarath, 1991).

CHAPTER 3

MATERIALS AND METHODS

3. MATERIALS AND METHODS

3.1 Reproductive propagation of *Coscinium fenestratum*

3.1.1 Seed collection and storage

The collection of fruits and seeds of *Coscinium fenestratum* were made during fruiting season September 2002 from the disturbed forest fringe in Sinharaja (Waturawa, near the main entrance, near Seethadola and Doranaella). Above mature ripen fruits were collected by bagging and fallen seeds collected were bagged. Collected fruits and seeds were stored in polythene bags under plant house conditions and these bags were kept in a humid chamber (90-95% humidity and temperature 25^{0}C).

The pericarp of these fruits were removed manually and washed with water before the seeds were used for the experiments

3.1.2 Initial investigation on the seed biology of *C. fenestratum*

3.1.2.1 Investigation of the seeds moisture content of *C. fenestratum*

For seed biological studies, depulped prepared seeds were used. A fully mature fresh seeds sample was divided into three groups each group including 20 seeds. Then the fresh weight of each sample of seeds was recorded and each sample was placed in petridishes and oven dried at 103^{0}C for 17 hours. Before measuring dry weight, the seeds were placed in a desiccation chamber until their dry weight was measured (Table 3.1).

Table 3.1 Initial moisture content of *C. fenestratum* seeds.

Replicates	Weight of container (X)	Weight of fresh seeds + container (Y)	Weigh of dry seeds + container (Z)	Percent moisture content per seed (Y-Z) / (Y-X)x100%
20 seeds	X1	Y1	Z1	M1
"	X1	Y1	Z1	M1
	X1	Y1	Z1	M1

Mean seed moisture content per seed = (M1+M2+M3)/3

3.1.2.2 Determination of moisture loss of *C. fenestratum* seeds stored under different conditions

Studying on seeds moisture losses were carried out under different storage conditions such as, 100% humid condition, in polythene sealer bags, in paper bags and ambient condition.

In order to maintain humid environment, cotton wool sprayed with tap water was used. Three replicates 360 seeds for each conditions were used for seeds moisture losses experiment.

Above experiments were carried out under laboratory conditions (Mean Relative Humidity 76 ±1.39 %). Moisture content of stored seeds were measured with intervals of after 5, 10, 15, 20, 30 and 40 days from the date of initial storage. For this test, three replicates each containg 20 seeds were used.

Data analysis

Data on each variable were analysed by using general linear model procedure of the MINITAB Version 12.1. Differences among the seeds under storage conditions were tested. Means were compared using the Tukey Pairwise Comparisons Test at $P \leq 0.05$ level.

3.1.2.3 Seed viability test

For initial viability test, 3 replicates of 60 seeds were used. Initial viability test was determined by carrying out a 2, 3, 5 triphenyl tetrazolium chloride (TTC) test (See Appendix. I).

TTC is based upon the reduction in germinability of seeds, due to a gradual dying off of the embryo. Embryos of fresh seeds were carefully excised by using a sharp-bladed dissecting needle, washed several time with distilled water and placed in small glass bottles with a freshly prepared 1% TTC solution.

The embryo sank in this TTC solution and therefore, there was no need to ensure that they were fully submerged. The bottles were then kept in the dark. The observations were made after 12-24 hours (plate 3.1). The TTC test was used to examine the viability of embryos in seeds stored under different conditions (Humid chamber, polythene bags, paper bags and Ambient condition) during 40 day period of storage.

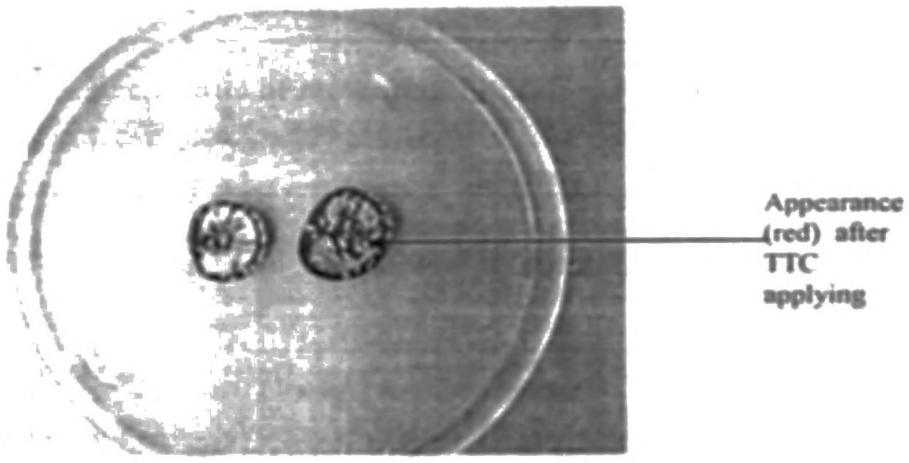

Plate 3.1 Application of TTC solutions on *C. fenestratum* seeds

3.1.2.4. Investigation of seed germination inhibitors

Bioassays carried out using seeds of *Brassica juncea*. *B. juncea* seed sample was divided in to three groups each containg 25 seeds. These *B. juncea* seeds were surface sterilized with 1% sodium hypochlorite. The *C. fenestratum* seed coat cracked by using pair of pliers and endocarp and embryo removed simultaneously in seed coat by using forsep. and the embryo from the endocarp was removed carefully by using forsep and blade.

Removed embryo and endocarp weighted to 1.5g separately and crashed with 30ml of distilled water were prepared 5% extractions of endocarp and embryo separately. Seventy-five of *B. juncea* seeds were soaked in the 5% of water extracts obtained from the embryos and endocarp of *Coscinium fenestratum* and sterilized distilled water was used as the control. These *B. juncea* seeds were germinated on blotting papers soaked in relevant extractions.

These treatments with petridishes were incubated in humid chambers for 96 hours at room temperature (25 ^{0}C). Each treatment was replicated. Results were analyzed by a simple T-test.

3.2. Investigation of best pretreatment method in breaking down the seed dormancy of *Coscinium fenestratum*

To overcome the mechanical barriers of hard seed coat and accelerate seed germination, samples of seeds were subjected to different physico-chemical treatments. The pericarps of all fruits were removed before the seeds were subjected to various experiments. All seed treatments were carried out under plant house conditions (maximum instantaneous light intensity, 800 μmolm^{-2}s^{-1} and mean daily humidity 81±1 %).

3.2.1 Ambient environment

Seeds were depulped and planted without any treatment. These seeds were planted in seed trays filled with a mixture of sand and top soil mixed in 1:1 ratio. Germination tests for 3 replicates of 60 seeds for this experiment were carried out.

3.2.2 Sunlight treatments

The seeds were exposed to direct sunlight for 2,4 and 6 hours to split the seed coat, which would then possibly permit germination of *C. fenestratum* seeds subject to these different light exposures. These seeds planted in seed trays filled with a mixture of sand and top soil in equal proportions. Above germination tests with 3 replicates of 60 seeds for each treatment were used.

3.2.3 Water treatments

Seeds soaked in distilled water for 12 and 24 hours imbibed the seeds water. These germination test with 3 replicates of 60 seeds for each treatments were used and planted in seed trays filled a mixture of sand and soil 1:1 ratio.

3.2.4 Mechanical cracking treatment

Seed coat was mechanically cracked by using a pair of pliers, thus mechanically splitting them and removing the mechanical barrier due to seed coat. This treatment was replicated three times. Each replicate included 20 seeds and seeds were placed seed trays containing sand and top soil in equal proportions.

3.2.5 Gibberellic acid treatments (GA_3)

Seeds soaked in different concentrations of Gibberllic acid (1500, 2000, 2500 and 3000 ppm) were tested for the germination success. Fresh seeds were soaked in the

above concentrations of Gibberllic acid for 12 hours and transferred to a medium of sand and top soil mixed in equal proportions. As the control a similar sample of seed were placed in tap water for 12 hours and planted seed trays above medium. Seeds were also treated with Gebberllic acid 2000ppm for 12 hours and these seeds were collected from forest floor of Sinharaja 1-2 months after the seed fall. Each treatment was replicated 3 times and each replicates include 20 seeds.

3.2.6 Seeds treatments for *C. fenestratum* were carried out under field conditions (without plant house conditions)

Two samples of 500 seeds were exposed to direct sunlight for ½, 1 hours then planted in nursery with a sand and top soil medium mixed in the ratio 1:1. These germination test with 5 replicates of 100 seeds for each replicates were used

3.2.7 Sunlight and soaking treatment

Three samples of 100 seeds were exposed to direct sunlight for 2 hours then soaked in tap water for 1 hour and planted in Meewatura nursery with a mixture of sand and top soil in equal proportions. Each treatment was replicated. These treatments were carried out under maximum instantaneous light level of 850 $\mu molm^{-2}S^{-1}$.

Watering was done regularly for all seeds treatments.

Statistical analysis

Analysis of variance (ANOVA) was performed on transformed data to evaluate the differences in the percentage germination of seeds above treatments.

3.3 Comparison of wood morphology and anatomy of *Coscinium fenestratum* and other related species

The anatomy of the stems of two selected liana *Coscinium fenestratum* and *Anamirata cocculus* were examined by light microscope. The objective of this is to develop a method that could be used to identify the species easily from each other. First transverse sections of the stems were cut to a thickness of 15-20 µm by using a microtome and treated with 70% alcohol followed by toludeane blue. They were allowed to air dry and drying subsequently slides were prepared by adding 'egg albumin'. Stems with three different diameters (1 cm, 2 cm and 3 cm) were examined in this study. For each diameter class three replicates were investigated comparison of morphology were based primarily on cell types, growth anomalies and bark characters.

Vessel diameters were measured using 'eyepiece graticule' and the stage micrometer to compare with differences of both species.

CHAPTER 4

RESULTS AND DISCUSSION

4. RESULTS AND DISCUSSION

4.1 Reproductive propagation of *C. fenestratum*

The preliminary studies of seeds including seed initial moisture content, seed moisture losses, seed viability and seed germination inhibitors carried out during the experiment period helped to identify the seed type and biology of *C. fenestratum*.

4.1.1 Investigation of the moisture content of *C. fenestratum* seeds

Table 4.1 Moisture content percentage in each replicates of the seeds

Seeds samples	Weight of container (g) (X)	Weight of 20 fresh seeds + Container (g) (Y)	Weight of 20 dry seeds + container (g) (Z)	percent moisture content per seed (Y-Z)/(Y-X)x100%
1	31.55	81.56	65.85	31.41
2	"	81.88	67.05	29.46
3	"	79.73	64.75	31.09

Mean seed moisture content = (31.41+29.46+31.09) /3 = 30.65%

The initial moisture content in *Coscinium fenestratum* seeds was 31±0.61% (Table 4.1). This is a specific feature of recalcitrant seeds as most of the recalcitrant seed moisture contain more than 30% (Erica, 1999). Therefore, *C. fenestratum* seeds are very sensitive to desiccation.

Hence this type of seeds should be stored to avoid moisture loss. They should not be collected at mature stage and sowed. immediately before they die (Erica, 1999). The period between collection and sowing of *C. fenestratum* seeds was very short. If they need to be stored for a longer time, they should be kept under high moisture condition (100%). Usually recalcitrant seeds should be collected and sown as soon as they reach the maturity stage.

4.1.2 Determination of moisture loss of *C. fenestratum* seeds stored under different conditions

According to the present study, clear variations were observed in terms of the moisture content of *C. fenestratum* seeds at different storage conditions. The highest moisture loss of *C. fenestratum* seeds were recorded when seeds were stored under ambient conditions. The least moisture loss was observed when they were stored under 100% humid levels (moisture chamber).

These results suggest that the instantaneous moisture content of *C. fenestratum* seeds closely associated with storage conditions. If may be due to the recalcitrant nature of seeds. Seeds stored in paper bags showed higher moisture losses than these in polythene bags. On the other hand, the moisture loss of seeds stored in polythene bags was found to be high compared with the moisture loss of seeds kept under high humid conditions.

Statistical analysis of the data showed a significant difference ($p<0.05$) in the moisture content humid and ambient seeds storage conditions. According to mean comparison of Tukey Pair wise Comparisons Test, seeds storage under humid (100%) conditions were better than others conditions (Figure 4.1). The viability of recalcitrant seeds is based on their moisture content. Hence the prevention of moisture loss is very important in maintaing the viability of *C. fenestratum* seeds.

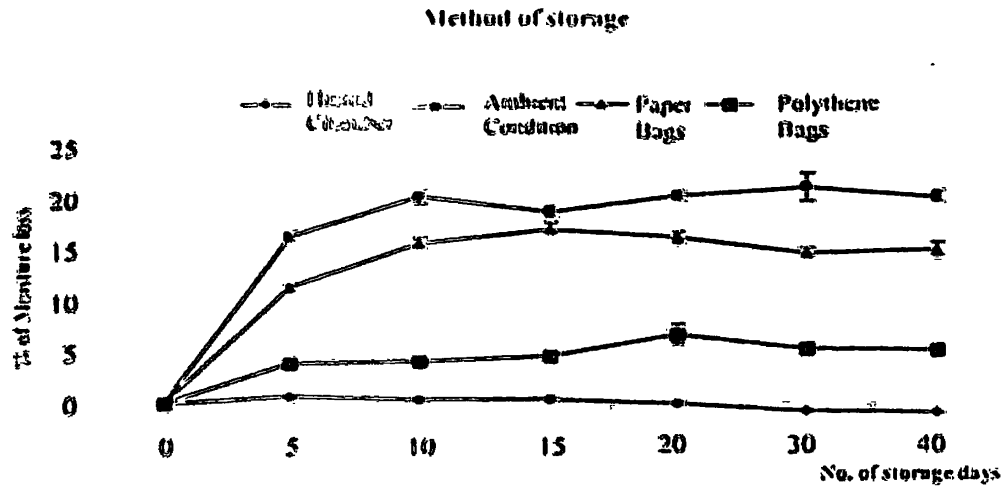

Figure 4.1 Moisture loss of *C. fenestratum* seeds when stored in different methods

4.1.3 Seed viability

The TTC (2, 3, 5-triphenyl-tetrazolium-chloride) test was carried out to examine the viability of embryos of seeds stored under humid chamber, polythene bags, paper bags and ambient conditions. According to the seeds viability test on seeds stored for 40 days embryos were observed in red colour and the colour variation was detected in 12-24 hours after immersion of seeds in the TTC solution are in sequent keeping them in the dark.

The highest viability (89%) was observed in seeds stored under humidity conditions after duration of 40 days while the least viability (30%) was observed in seeds stored for 40 days in ambient condition. Out of the other two conditions, higher viability was observed in seeds stored in polythene bags against seeds stored in paper bags.

4.1.4 Investigation of seed germination inhibitors

Bioassays carried out using seeds of *Brassica juncea* revealed presence of germination inhibitors in *Crotalaria fenestratum* seeds endosperm and the embryo Germination percentage of *B. juncea* grown on endosperm and embryo extracts

were 17% and 25% respectively while 80% and 88% germination (Figure 4.2) percentage was observed in control where the blotting paper was soaked in water. However; the amount of inhibitors does not effect on dormancy. The factors that affect the dormancy of *C. fenestratum* seeds may be due to the germination inhibitors.

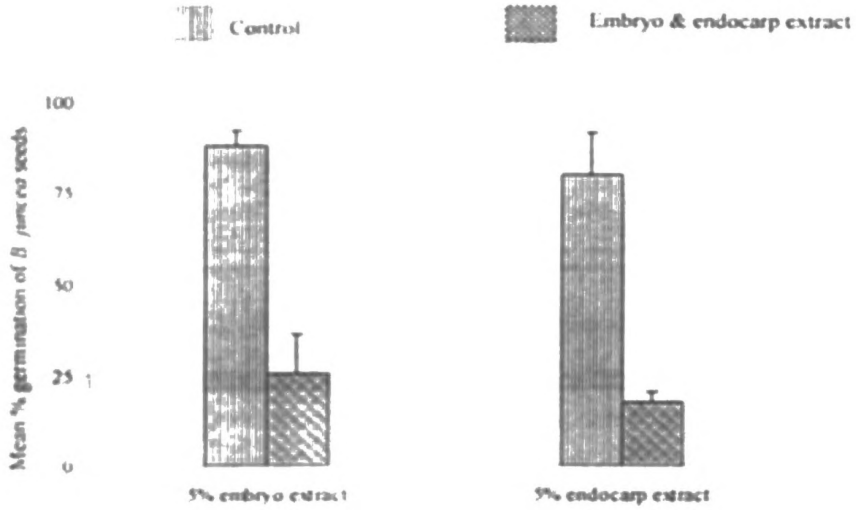

Figure 4.2 Presence of germination inhibitors in embryo and endocarp of *C. fenestratum* seed.

4.2 Investigation of best pretreatment method to break the seed dormancy of *C. fenestratum*

4.2.1 Result of ambient environment

Seeds depulped and then planted in a medium showed no germination with the program of time. *C. fenestratum* seeds under normal conditions undergo a dormancy period of 2-15 months (Senerath, 1991). The germination of *C. fenestratum* seeds were highly affected by seed dormancy and germination inhibitors.

4.2.2 Result of sunlight treatments

Exposure of seeds to direct sunlight for different time periods of 2, 4 and 6 hours showed no germination. The failure of seed germination in the this treatment may be due to the dehydration of the embryo due to the low humidity level (in contrast to forest floor) prevailing at the plant house.Germination of *C. fenestratum* requires higher humidity (100%) levels and low temperature.

However, in nursery conditions sunlight treatments for ½ to 1 hours showed germination only 5 seeds (Figure 4.4) out of five hundredth (500) within the 2-5 months. Seeds exposed to direct sunlight were observed revealed a narrow longitudinal split along the line on the flat side of the hard seed coat. Thus the mechanical barrier to the emergence of the developing embryo present on the seed coat may be overcome.

4.2.3 Sunlight and soaking treatments

Exposure to direct sunlight followed by soaking did not show a higher germination or reduced dormancy of *C. fenestratum* seeds. When seeds were exposed to direct sunlight, the seed coat splited and endosperm was seen. When exposure of seeds to direct sunlight for 2 hours and then soaked in tap water for 1 hour resulted in germination of 16 % (Figure 4.4). These experiments were carried out in Meewatura nursery and planted medium of sand and soil ratio was 1:1. (Mean light intensity 850 $\mu mol^{-1}s^{-2}$).

4.2.4 Mechanical cracking treatment

Only 2% of seeds subjected to cracking was germinated (Figure 4.4), possibly due to damage of the embryo or infection of embryos by soil organisms consequent to damages.

4.2.5 Soaking in distilled water treatment

Seeds soaked in distilled water for 12 and 24 hours did not germinate within the 2-5 months. But seeds soaked in tap water for 12 hours were observed to germinate with a percent of 3%. This study may be that soaking results in rehydration, which is vital to permit seed germination. Soaking in tap water also reduced dormancy but no significant increased germination were recorded. Early studies have shown that the dormancy period of *C. fenestratum* seeds can vary from 2-15 months (Senerath, 1991).

4.2.6 Results of Gibberllic acid (GA_3) treatments

In this treatment the effect of different concentration of Gibberllic acid were observed. Seeds treated with 2000 ppm GA_3 started to germinate after 45 days they planted. After five months 2% germination was observed. Treatment of seeds with three concentrations namely Gibberllic acid (GA_3) 1500 ppm, 2500 ppm and 3000 ppm, showed germination after five months of planting at. 2%. 3% . 3% respectively. Control experiment also recorded 3% germination (Figure 4.3).

Naturally fallen seeds collected after 1-2 months of seed fall from the forest gave 27% germination (Plate 4.1) when treated with 2000ppm GA_3. This method can be used for successful germination.

Therefore even though GA_3 is known to be a dormancy breaking hormone in many species, it did not increase significantly the percentage germination in *C. fenestratum*. But germination was higher than in other treatments.

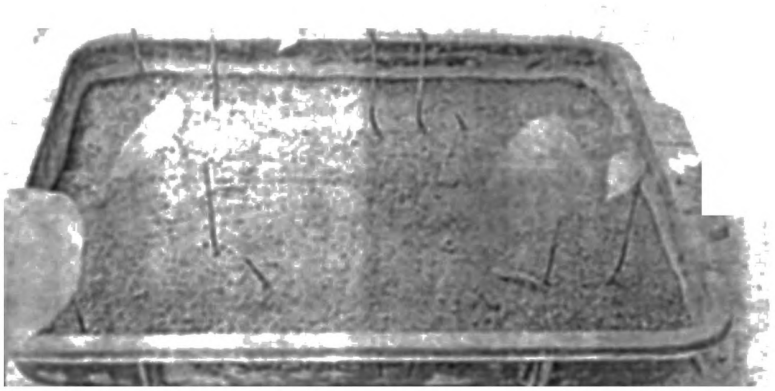

Plate 4.1 Best germination treatment of *C. fenestratum* seeds treated with gibberllic acid 2000 ppm for 12 hours.

Damage of the hard seed coat by mechanical, Physico-chemical or other means did not increase the percentage germination significantly. Seeds pretreatment methods carried out by Senerath (1991) revealed that *C. fenestratum* seeds have a long dormancy period (2-15 months). Muttiah (1964) also reported that the *C. fenestratum* seeds germination were not successful.

Table 4.2 Seeds treatments and germination % of *C. fenestratum* seeds

Seed Treatment		Germination % (within the 5 months)
1) GA_3 Treatments (12 hours)	· 1500ppm	2%
	· 2000ppm	2%
	· 2500ppm	3%
	· 3000ppm	3%
control	· tap water	3%
2) Sunlight treatments	· 1/2 hours	1%
	· 1 ..	1%
	· 2 ..	no germination
	· 4 ..	..
	· 6 ..	..
3) Sunlight and soaking treatment- exposure to sunlight for 2 hour & soaked in tap water for 1hour		16%
4) water treatment	· 12 hour	no germination
	· 24 hour	..
5) Mechanical cracking		2%
6) Fallen after 2-3 months seeds treated with GA_3 2000ppm (12 hours)		27%

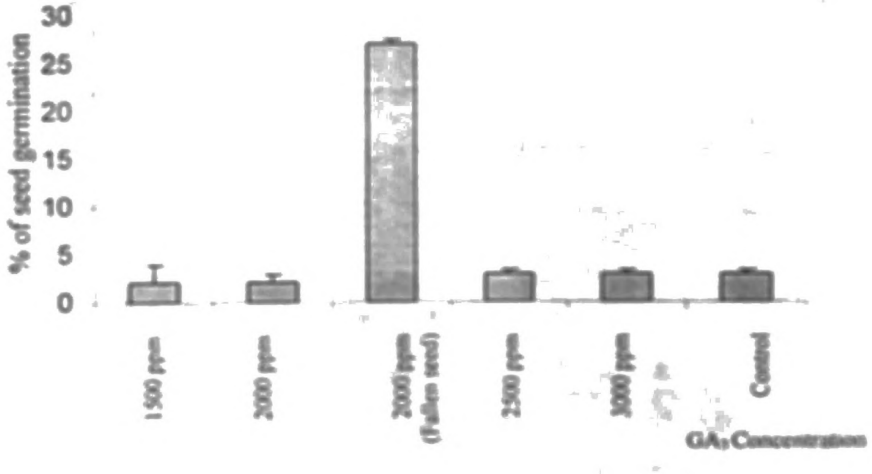

Figure 4.3 Seeds germination % of *C. fenestratum* subjected to different GA_3 concentrations (% germination was calculated 5 months after the treatment)

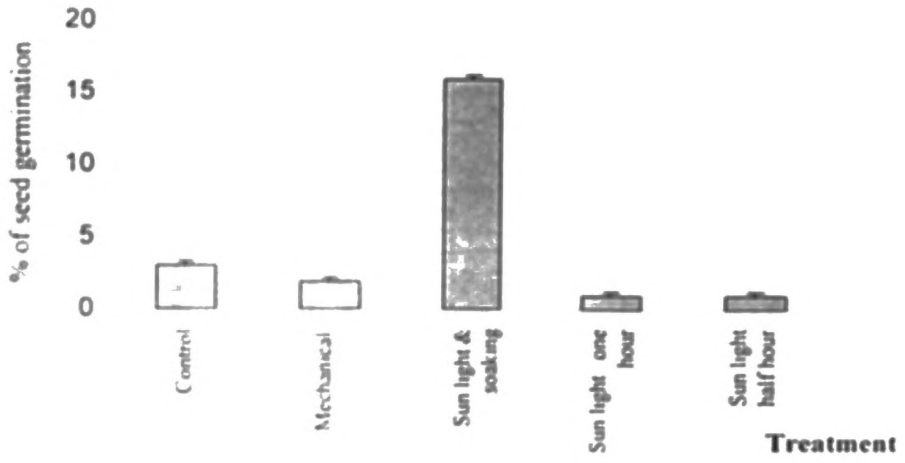

Figure 4.4 *C. fenestratum* seeds germination with different treatments

4.3 Comparison of wood morphology and anatomy of *C. fenestratum* and other related species

Coscinium fenestratum and *Anamirta cocculus* showed to have anomalous growth structure but *C. fenestratum* has a primary anomalous growth and *A. cocculus* was anomalous secondary growth (Plate 4.2). *C. fenestratum* bark is relatively thin (1.93±0.12 mm) and grayish whitin older stems due to the heavy growth of lichens which impart a smoothness to bark. It is lightly and longitudinally furrowed and when scraped or blazed reveals the dark yellow wood. *A. cocculus* bark was much thicker (2.73±0.08 mm) than *C. fenestratum* and dark brawn in older stems.

A cross section of the *C. fenestratum* stem show a diffuse porous. Wood with large porous intercalated by prominent broad bands of medullary rays radiating from the central pith (Plate 4.3) to outer cortex. *A. cocculus* woody structure was observed 'Multisereate rays' and cambium forms centrifugally. The "bundules" formed in this way may be arranged in definite 'concentric rings' (Plate 4.4). *C. fenestratum* stem structure was observed cambium forms very little paranchyma and 'bundules' formed arranged irregularly.

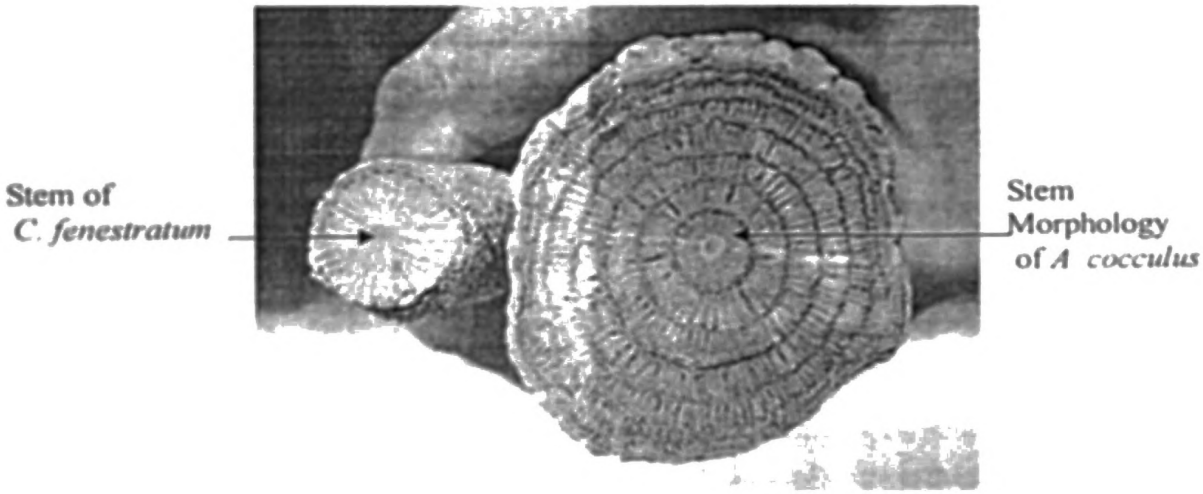

Plate 4.2 Stem morphology of *C. fenestratum* and *A. cocculus*

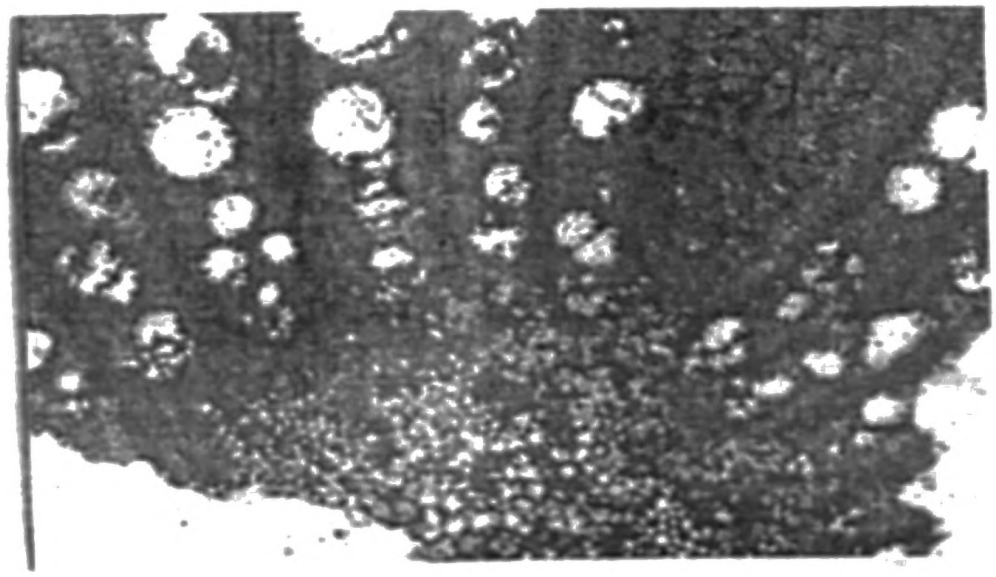

Plate 4.3 Transverse section of *C. fenestratum* under light microscope (10 x 10)

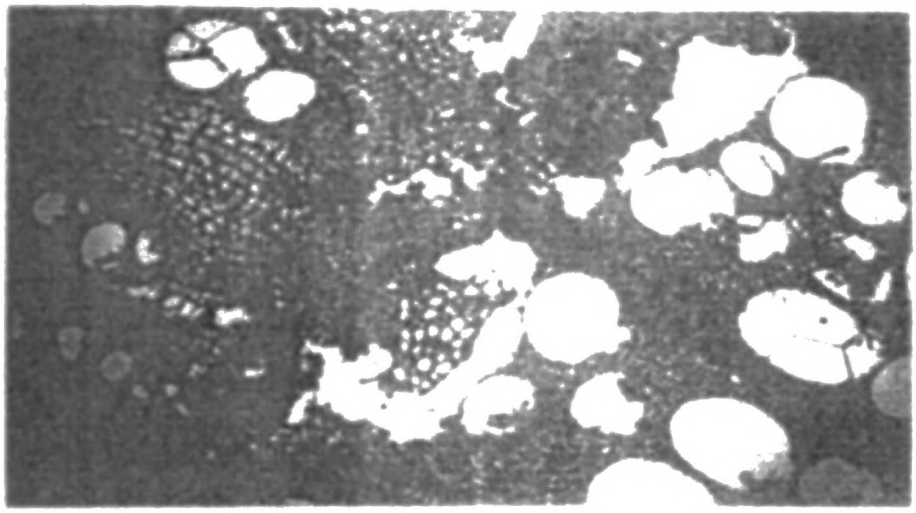

Plate 4.4 Transverse section of *A. coccidus* under light microscope (10 x 10)

Vessels diameters were examined by using 'eyepiece graticule' and stage micrometer revealed variations of vessels diameter in different stem diameter sizes (1 cm, 2 cm and 3 cm) of both species. The size of average vessels diameters observed in *A. coccidus* was higher (20.3±1.5 µm) than the in *C. fenestratum* (19.03±2.5 µm) but there is no significunt difference in vessels diameter of two species as shown in figure 4.5. Therefore the "Vessels diameter" can not be used in identification purposes of two species.

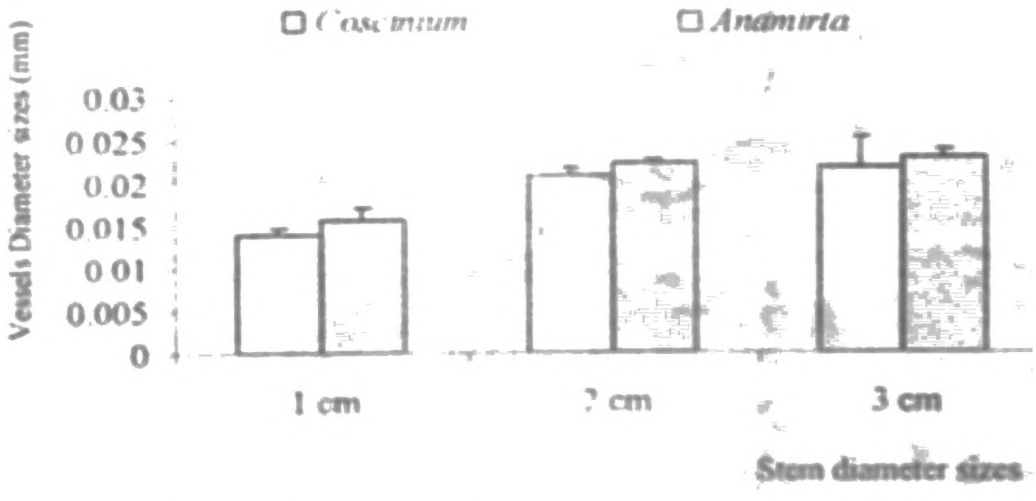

Figure 4.5 Relationship between stem diameters and vessels diameters (in *C. fenestratum* and *A. coccidus*)

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

5. CONCLUSIONS AND RECOMMENDATIONS:

Mean moisture content of fresh *C. fenestratum* seeds was 31%. Therefore *C. fenestratum* seeds can be categorized under the group of "Recalcitrant seeds".

According to statistical analysis, the best conditions seed storage was inside a humid chamber for 40 days.

The presence of germination inhibitors were higher in extracts obtained from the endosperm than these extracts obtained from the embryo.

The best seed material of *C. fenestratum* for satisfactory germination are those seeds collected from the forest floor, which for 1-2 months after the seed fall.

The best treatment of these seeds for better germination was 2000ppm of GA_3. The seeds were germinated 6 days after harvesting from the mother plant.

The major factors that affect the dormancy of *C. fenestratum* seeds may be due to the hard seed coat and the germination inhibitors.

Further studies are recommended to conduct on germination of *C. fenestratum* seeds harvested from mother plant and kept under 100% humidity for 1-2 months.

The stem of *C. fenestratum* shows anomalous growth structure while *A. excelsus* shows secondary anomalous growth structure.

References

Abeywardana, N. and Hettiarachi, L.K. (2001). *Statistics on the national demand for medicinal plants*, IUCN-The World Conservation Union, Sri Lanka, 27-41 pp.

Ahmed, A. and Houri, (1986). Some aspects of dry land afforestation in the Sudan with special reference to Acacia tortilis (Forsk) Hayne, *A. senegal* wild. and *Prosopis chilensis* (Modina) stuntz. Forest Ecology and Management 16:209-221.

Anon (1978). The Promotion and Development of Traditional Medicine. World Health Organization Technical Report. Geneva, 622, 10.

Ariyarathna, H.A.C.K. (2000). A Preliminary Investigation on seed dormancy in *Cocculus fenestratum* (Colebr) (Menispermaceae). B.S.c. thesis, Department of Botany, University of Peradeniya, Peradeniya, Sri Lanka.

Arora, D.K. and Gupta, S. (1996). *Advances in Plant Physiology*, vol.8. Physiology of Photosynthesis. Anomol Publications PVT/LTD, New Delhi.

Arthur, J.E. and Laurence, H.D. (1993). *An Introduction to Plant Anatomy*. Tata McGraw-Hill Publishing Company Limited, New Delhi 293-314 pp.

Attygalla, J. (1917). *Sinhalese Materia Medica*, Gunesena & Co., Colombo.

Balasubramanian, S.D. and Chakraborty, A.K. (1994). Studies on time of seed Collection, Sowing and Presowing treatments to Terminalia bellirica Roxb and Terminalia chebula Retz. *The Indian Forester*, 120:5, 459-459.

Baland, D. J., Brooker, M.I.H., and Turnbull, J.W. (1980). *Eucalyptus* seed. CSIRO, Australia.

Bewley, J.D. and Black, M. (1994). *Seeds Physiology of development and Germination*. Plenum Press, New Yourk.

Bryant, J.A. (1985). *Seed Physiology*. Institute of Biology's studies in Biology No. 165. Edward Arnold (publishers) Ltd., Britain 16-71pp.

Chandrasena, J.P.C. (1955). *The Chemistry and Pharmacology of Ceylon and Indian Plants*. H & C Press, Colombo.

Dassanayake, M. D., Fosberg, F. R., and Clayton, W.D., (1995). *A Reversed Hand Book to the Flora of Ceylon*. Publishing Co. Ltd., New Delhi. Vol. IX.

Diane, R. and Ball, E. (2001). *Plant Propagation from Seed*. Virginia Teah.

Ellis, R.H., Hong, T.D. and Roberts, E.H., (1990). *An intermediate category of seed behaviour*. I. Coffee, J. Exp. Bot., 41, 1167-1174pp.

Erica, E. Benson, (1999). *Plant Conservation Biotechnology*. Taylor and Francis Ltd. 11 New Fetter Lane, London EC 4P 4EE.

Fahn, A. and Werker,E. (1972). *Anatomical Mechanisms of seed dispersal*. In: Seed Biology, Vol.I, 152-221. Academic press, New York and London.

Hartmann,H.T., Kester,D.E., Davies, F.T. Jr., Geneve, R L.(1997). *Plant Propagation Principles and Practices* Prentice Hall International,INC.

Hong, T.D. and Ellis, R.H., (1990). *A comparison of maturation drying, germination and desiccation tolerance between developing seeds of Acer pseudoplatanus L. and Acer platanoides L.*, New Phytologist. 116. 589-596.

Her,Y.L.(1996). *Storage of tropical tree seeds: On Recent Advances in Tropical Tree Seeds Technology and Planting Stock Production*, 12-14 June 1995, AFTSC Thailand.. 63-69 pp.

Hudson, R.C., Dale, E.K. and Fred, T. D. (1997). *Plant Propagation Principles and Practices*. Prentice-Hall of India Pvt. Ltd., New Delhi.

Hyde, E.O.C. (1954). The function of the hilum in some Papillionaceae in relation to ripening of the seed and the Permeability of the testa. Ann. Bot., 18:241-256.

Jain, V.K. (1996). *Fundamentals of Plant Physiology*. S.Chand and Company LTD, Ram Nagar, New Delhi 417-427pp.

Jayaweera, D.M.A. (1981). *Medicinal plants (indigenous and exotic) used in Ceylon*. Parts 1. The National Science Council of Sri Lanka. 162-163pp.

Jayaweera, D.M.A. (1982). *Medicinal Plants (indigenous and exotic) used in Ceylon*. Parts 4. The National Science Council of Sri Lanka. 71p.

Kebreab, E. and Hellum,A.K. (1984). Hot water and acid improve the germination of *Leucaena esculenta*, Brim seeds. The Embryo. 1-1. 27-33

Lars, S. and Donbe, J. (2001). *Glossary of Seed Biology and Technology* DFSC Series of Technical Notes. TN59. Danida Forest Seed Center. Humlebaek, Denmark 6-17pp.

Malcolm and Wilkins, B (1987). *Advanced Plant Physiology*. 1st edn Longman Singapore Publishers (pte) Ltd., Singapore 70-99 pp

Masango, H.P. and Maghembe, J.A. (1993). Germination of Woodland Mahogany (*Trichilia emetica*) following manual Seed-Coat Scarification and Potasium nitrate treatment. *Jour Trop For. Sci* 5.4. 518-527.

Midgley, S. (1996). Seed collection strategies in a changing world. In : IUFRO: In novations in tropical tree seed technology. *Proceedings of the IUFRO Symposium of the project Group*. Tanzania.

Mumbi, S. (1964). *A preliminary study on the distribution, growth, and Propagation of* [illegible] (*Coscinium fenestratum*). 113-120 pp.

Napier, I.A. and Robbins, A.M.J. (1988). *Seed storage in the nursery* (*Prunus, Alnus and Pinus spp*). Banko Janakari, vol.1:1. 13-20pp.

Nermath, M.N., (1987). Effects of temperature on rubber (Hevea brasiliensis) Seed Storage. Unpublished PhD thesis. University Pertanian Malayasia.

Olesen, K. (2000). *Guide to handling of tropical and sub tropical forest seed. Danida Forest Seed Center*, Humlebake, Denmark.

Pakasuntheram,C., Iyer, K.S., de Silva,L.B., de Silva, T. (1982). Amibacterial activity of *Coscinium fenestratum* Colebr. Against *Clostridium tetani*. Indian J. Med. Res. 76 (December, suppl.), 71-76pp.

Pijl, L., Vander, (1982). *Principles of dispersal in higher plants*. Springer verlag, Berlin, Heidelberg, New York.

Ramton, L.A. (1995). *Seed Technology*, Oxford and IBH Publishing Co.pvt. Ltd. New Delhi

Raven, P.H., Evert, R.F. and Eichhorn, S.E. (1992). *Biology of Plants* New York: Worth.

Schaefer.C. (1989). Seed testing research on species indigenous to Kenya. In : *Tropical tree seed Research, ACIAR Proceedings* No.28, 132-139 pp.

Senarath, D.M.B.D. (1991). Biological studies on *Coscinium fenestratum* Colebr. (Menispermaceae). M. Phil. thesis. University of Peradeniya, Sri Lanka.

Sharples, G.C. (1973). Stimulation of lettuce seed germination at high temperatures by ethe. Phon and Kinetin. *Jour. Amer.Soc.Hort Sci.* 98(2): 107-9.

Shon, G.R.A. (1925). The Structure of *Coscinium*. *Pharm. J.* 115: 156-162pp.

Thapliyal, R.C., Sood, O.P. and Rawat, M.M.S. (1991). Effect of Moisture Content and Storage temperature on the Viability of *Bambusa tulda* seed. *The International Tree Crops Journal*, 7:67-75pp.

Topsen, P.B., (1992). A review of the literature on storage of dipterocarp seed. *seed Sci Tech*., 20(2), 251-267 pp.

Trimen, H. (1931). *Handbook to the Flora of Ceylon*, Dulau & Co., London.

Appendix. I Viability test for *C. fenestratum* seeds were carriedout using TTC (2, 3, 5 triphenyl tetrazolium chloride) test (Olesen, 2000).

For *C. fenestratum* seeds 1% TTC solution was used. This concentration is achieved by dissolving 1g 2. 3. 5 triphenyl tetrazolium chloride in 1 litre of water. The practical steps of the TTC test are as follows:

1. Prepare hard seed coat for imbibition by scarification.
2. Pre-moisten seeds by soaking 25 ^{0}C for 12-24 hours drain off water.
3. Immerse seeds in the TTC solution. The seeds should be completely covered.
4. Incubate seeds in the TTC solution in darkness at 30-35 ^{0}C for 10-12 hours.
5. Wash seeds in distilled water and place them on moist filter paper until evaluation.
6. Evaluate staining.

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