COMPARATIVE STUDY ON PROPAGATION METHODS OF THE MEDICINAL PLANT *Phyllanthus debilis* Klin ex willd.

By

K.N.S. PERERA.

THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF
BACHELOR OF SCIENCE
IN
NATURAL RESOURCES
OF THE FACULTY OF APPLIED SCIENCES,
SABARAGAMUWA UNIVERSITY OF SRI LANKA,
BUTTALA,
SRI LANKA.

JANUARY 2002

FACULTY OF APPLIED SCIENCES,
SABARAGAMUWA UNIVERSITY OF SRI LANKA,
BUTTALA,
SRI LANKA.

DECLARARION.

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.

.....

K.N.S. Perera.
Date. 19/04/2008

Certified by,

Dr. Kushan Tennakoon,
External Supervisor,
Department of Botany
Faculty of Science,
University of Peradeniya,
Peradeniya,
Sri Lanka.

.....
Date. 12/4/2008

Dr.K.K.D.S. Ranaweera,
Internal Supervisor,
Dean,
Faculty of Applied Sciences,
Sabaragamuwa University of Sri Lanka,
Buttala,
Sri Lanka.

.....
Date. 19/4-2008

AFFECTIONATELY DEDICATED

TO MY EVERLOVING

PARENTS & TEACHERS.

ACKNOWLEDGEMENTS

First and foremost, I wish to express my deepest gratitude to my project supervisors, Dr.K.K.D.S. Ranaweera, Dean, Faculty of Applied Sciences, Sabaragamuwa University of Sri Lanka, Buttala, Sri Lanka and Dr. Kushan Tennakoon, Senior lecturer, Department of Botany, Faculty of Science, University of Peradeniya, Peradeniya, Sri Lanka; for their valuable advises, encouragement and guidance through out this study and sparing their valuable time in bringing this study to a successful completion.

I express my sincere gratitude to Prof. Nimal Gunathilake and Prof. Savithri Gunathilake, Department of Botany, Faculty of Science, University of Peradeniya, Peradeniya, Sri Lanka; for their valuable suggestions and kind encouragement during this study.

Gratitude also extends to Mr. K.P.L Nishantha, course coordinator, degree program in Natural Resources and Mr. C. Rajapaksha, Probationary lecturer, Faculty of Applied Sciences, Sabaragamuwa University of Sri Lanka for giving me kind support and advice during my study.

I would like to extend my thanks Miss. Hashendra and Mr. Rathnasiri (M.phill candidates) department of Botany, Faculty of Science, University of Peradeniya for their helps in numerous ways during my project.

And I wish to express gratitude all the academic and nonacademic staff of the department of Botany, Faculty of Science University of Peradeniya and Faculty of Applied sciences, Sabaragamuwa University of Sri Lanka.

I am deeply indebted to my loving parents and teachers, who have indeed been guidance through out my life.

ABSTRACT

Phyllanthus debilis is an annual herb, which has a high demand in Ayurvedic Medicine. Due to the lack of knowledge in propagation, inexpensive propagation methods cannot be practiced, although there are high technological methods available, which are complex and expensive. Therefore, it is very important to identify a simple, less time-consuming propagation method for *Phyllanthus debilis*.

Establishment of a herbal plantation using simple vegetative propagation (stems) has some limitations such as the late rooting, limited amount of plants and time consuming. But sufficient amount of strong healthy seedlings can be obtained rapidly through the sexual propagation. The experiments of reproductive propagation were carried out in plant house using simple propagation techniques under the conditions of temperature 28 ^{o}C, relative humidity 80 % - 85 % and the maximum instantaneous Light levels of 800 µmol m^{-2} s^{-1} and 300 µmol m^{-2} s^{-1}.

The type of the seed whether orthodox, recalcitrant, intermediate was identified using preliminary experiments (test of seed moisture content, seed viability and seed weight). Results of the preliminary test showed that the moisture content was as 14 %, seed viability varying up to 3 - 4 months, and average seed weight as 0.3 mg. and 178 fruits per gram was precent. Therefore, seeds of *Phyllanthus debilis* were categorized under "intermediate seed" considering their moisture content and viability.

The determination of best potting mixture, best method to raised seedlings and the best maturity stage of fruit for extraction were performed all week study period. The highest growth performance (in terms of number of leaves, height and root collar diameter) of the plants were recorded under the media of loam : compost : sand mixed in a ratio of 1 : 1 : 1, loam :compost 1 : 1 and loamy soil. Seeds obtained from manually split fruits sown in a medium of loam : compost : sand mixed in a ratio of 1 : 1 : 1 respectively was found to be the best method to raise seedlings of *phyllanthus debilis*. Seeds obtained from mature fruits at the blackish green stage and germinated under blotting sheet gave a 91 % success. Hence, it can be concluded that seeds obtained from mature fruit at the blackish green stage by splitting manually and grown in a media of loam : compost : sand mixed in the ratio of 1 : 1 : 1, loam : compost 1 : 1 and loamy soil under the maximum instantaneous light level of 800 µmol m^{-2} s^{-1} give the highest propagation success for *Phyllanthus debilis* .

CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF PLATES

Chapter 1

1 . Introduction

Traditional ailments often have a long tradition of use than western medicine in Sri Lanka. About 35% of the Sri Lankan population primarily depend on Aryurvedic Medicine and traditional of health care. Basically 189 (7.4 %) of 1414 (24 %) herbal plants (flowering plants) are endemic to the island, and distributed in different zones or restricted to the Indian sub continent. Of these fifty are heavily used in Aryurwedic medicine. As recently surveys the annual demand of the herbal raw material was 1500mt and value of the herbal materials were Rs76, 334,895 Out of these 60 % of raw materials were imported and remaining 40 % were collected from natural habitat and small proportion of cultivated land. Decrees the supply problem, regularize the trade, provide certifiable product of uniform quality, and offer a new source of income to poor Peoples in rural aria by farming herbal plants. Conservation through establishment of protected aria, identified ability of agricultural land use, community awareness, training plant researches and documenting the knowledge of traditional uses are the relevant factor for long term sustainability.

1.1 The form, sources and quantity of herbal raw materials used in year 2000 (IUCN, 2002)

Form		
Raw form	644,197 kg	30 %
Dry form	1,573,878 kg	70 %
Total	2,218,075 kg	100 %
Source		
Imported	35,385 kg	1.6 %
Forest	22,222 kg	1.2 %
Cultivated	23,368 kg	1.1 %
Open market	2,133,100 kg	96 %

Atleast 4 billion people in developing country have been used Ayurvedic medicine due to their widespred availlability , a long tradition, and low cost . Rapidly extinct many herbal species within last few decades because of land degradation for agricultural purpeses ,industrialization,increased demand in Ayurvedic medicine etc. as well as high price of western medicine doubled the demand of treditional medicine. it is a reason for increased herbal collection.In Srilanka 70 % of of herbal plants in the wild are threatned with extinction

due to destructive collecting techniques, over harvesting and conservation of habitat for singlecrop use.

Phyllanthus debilis (Ela pitawkka) is a widely used annual, herb in Ayurvedic medicine. However demand cannot be fulfilled at percent due to the limited knowledge of propagation techniques among the growers, and they have little knowledge about the value of medicinal plants. This plant was destroyed from their natural habitat within the past few decades due to industrial, agricultural activities and excessive harvest. Hence it is necessary to study the most acceptable propagation methods to fulfill the demand and increase the awareness of sustainability use among people.

Following table gives the details of the usage of *Phyllanthus debilis* in Aryurwedic medicine in year 2000.

1.2 Usage of *Phyllanthus debilis* in Aryurwedic medicine in year 2000 (IUCN, 2002)

National demand of *Phyllanthus debilis* (kg)	Total value	Supply form	Total used only dry manufacture in Sri Lanka	Unit price (RS) Weighted average	Uses of drug manufactures as dry or row forms	
					Row herbal materials	Dry herbal materials
14582	RS 787804	Locally	3,048 55kg (1.8%)	67.86	574 (18.8%)	2474 (81.2%)

Both vegetative and sexual propagation methods are reliable for *Phyllanthus debilis.* However sexual propagation is most successful method for the vast cultivation than vegetative propagation. Because it was easy to obtained considerable amount of seed lings within the short period due to their specific seed characteristic such as less seed dormant period and medium viability (basically 3 - 4 month).

There are number of advantages having in seed collection and handling process through out the sexual propagation. Those methods should be simple and less time consuming. As far as appropriate propagation techniques were helped to obtain healthy, strong, and uniform seed lings.

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

Chapter 2

2. Literature Review

2.1 Family Euphorbiaceae

Family Euphobiaceae is one of the largest families containing trees, shrubs, lianas or herbs and some with milky or colored latex. Most species in this family are both economic and decorative. Many of these are found in Sri Lanka.

Leaves take spiral, sometimes opposite or whorled, simple or compound and stipules of this family are large represented by small glands or absent. Unisexual (plant monocious or rarely dioecious), usually actinomorphic, cymose inflorescence, flowers and capsule or drupe fruit consist. Seeds often with conspicuous caruncles (Dassanayake and Clayton, 1997).

2.2 Sub family Phyllanthoideae

Phyllanthoideae, a large sub family of Euphorbiaceae. Ovals and seed structures are the basal things to divide this sub family. All species of Phyllanthoideae examined had bitumen ovules with a non-vascularized inner integument and non-pachychalazal, exarilate seeds (Tokuoka and Tobe, 2001).

2.2.1 Botanical Description of *Phyllanthus debilis*

Phyllanthus debilis (Pitawakka) is a glabrous annual herb. It is monoecious and 30 - 60 cm tall. stem often branched at base angular, glabrous. Leaves on main stem reduce to scale. Deciduous branchlets, mostly 4 – 10 cm long (Jayaweera, 1981) with 15 - 35 leaves sharply angled. Leaf tip acute, base wage shaped, margin plane. Flowers in axillary cymules on deciduous branchlets. Proximal 3 - 4 only male flower and distal all solitary female flower. Male flower smaller than female flower. Six sepals and 0.5 - 0.6 mm long. Stamens 3, filament united in to a column 0.25 - 0.3mm high. Anthers 0.2 - 0.25 broad, dehiscing horizontally.

Female flower: pedicle 1 - 1.6 mm long in fruit, sepals 6, 1-1.6 x 0.5-1mm obvate rounded at tip, ovary smooth, fruit: capsular, 2 - 2.2 mm in diameter, oblate, smooth and splitting in to three parts; seeds: three sided, smooth, with fine traverses lines. Yellowish or pale browns 6-7 slender longitudinal ribs on back (Dassanayake and Clayton, 1997).

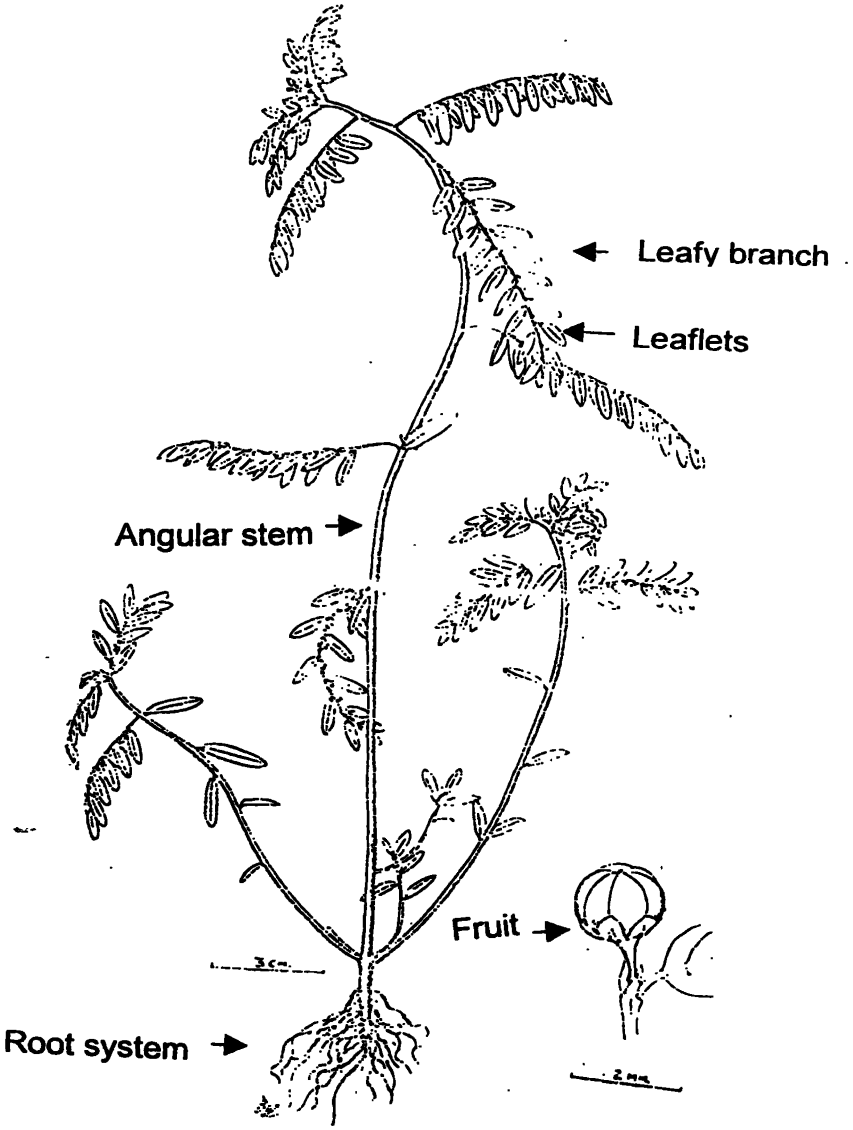

Plate 2.1 Morphology of *Phyllanthus debillis* (Pitawakka) plant

Other common species such as *Phyllanthus umarus, Phyllanthus tenellus, Phyllanthus urinaria* can be easily identified using their fruit, flower, leave, stem and seed differences as follows.

Table 2.1 Botanical description of *Phyllanthus tenellus* Roxb., *Phyllanthus amarus* Schum. and *Phyllanthus urinaria* Linn (Dassanayake and Clayton 1997).

	Phyllanthus tenellus	***Phyllanthus amarus***	***Phyllanthus urinaria***
Leaf	Elliptic/Obviate tip acute/obtuse. Base acute/rounded. Smooth beneath	Tip-Obtuse/rounded often with fine point. Base-obtuse/rounded often unequal sided. Smooth beneath.	Tip- obtuse/acute with fine point. Base-Obtuse sometimes asymmetric finely hairy along margins. Rough along margins beneath.
Cymules	Proximal-2/3 ♂and 1/2♀ Flowers, Distal- all solitary ♀ flower	Mostly one sex proximal 1/2 ♂ Distal with 1 ♂and 1♀ flower	Proximal 5-20 Solitary ♀ Distal all ♂ in group of 5-7
♂ Flower sepals	Five	Five (rarely 6)	Six
Stamens Anther dehiscence	Five, filaments free Horizontal	Three (rarely 2) filaments united into a column Horizontal	3 filaments united into a column. Anthers separate vertical
♀ Flower pedicle	3-8 mm in fruit	1-1.7 (rarely 2) mm	Almost sessile
Sepals	Five ublexed in fruit	5 (rarely 6), obvate, oblong, tip acute	6-reblexed in fruit, liner-oblong
Ovary	Smooth	Smooth	Surface marked with nodules (verrucate)
Seed	3-sided, densely covered with papillae (protuberqnces)	3-sided, smooth 5 or 6 narrow ribs on back	12-15 transverse ridges on back, sides, and often 1-3 circular pits.

2.2.2 Distribution

Phyllanthus debilis distributed throughout the tropics including the Philippine Island, except in Australia. This herb spread out wet, dry, intermediate zone and below 1000 m in Sri Lanka (Jayaweera, 1981).

2.2.3 Uses

This plant contains Phyllathin and a considerable amount of Potash. Therefore much used as a diuretic in dropsical affections, Gonorrhea and to allaygriping in dysentery and in intermittent fevers. The fresh route is said to be and excellent remedy for Jaundice. The milky juice is effective on offensive sores. The fruit is bitter, useful in tubercular ulcers, wounds, sores, bruises, scabies and ringworm. Recently discovered Phyllanthine is most active for Hepatitis B. Sri Lankan peoples expressed juice of the plant is used for diuretic in Gonorrhea and the root along with other drugs for Diarrhea. Ground plant paste is given with cow's milk for jaundice. Th is very useful in inveterate intermittent with infract of the spleen and liver. The infusion of the root and leaves are good tonic. An aqueous extract decoction of the fresh roots, stems, and leaves given internally – bite (Jayaweera, 1981).

2.3 Sexual Propagation

Sexual propagation is the major method by which plants reproduce in nature and one of the most efficient and widely used propagation methods. For crop the plant produced are known as seedlings.

The planting of the seed is the physical beginning of seedling propagation. Seed is the product of growth process within the parent plants. The life cycle of a seed, lings consist of consecutive period of vegetative growth and reproductive development (The formation of flower and fruits). considering their seasonal pattern, growth and development can be category as annual, biennial, perennial, herbaceous perennials and woody perennials.

Annual – these type plants complete their germination, flowering, and production and spread out there seeds within a season and then they die.

Biennial – plant consist two-year cycle. In first season, grow as a low clump or rosette of leaves flowering and seed produced in second year.
Perennial – life cycle tacks more than two years. Herbaceous plants have such dormant period and shoots die within bad climatic season. Continuously increase size and there shoots and roots in woody perennials (Source: Hudson, Dale and Fred, 1993).

2.3.1 Seed characteristics

Seeds producing ancient gymnosperm were developed some three hundred years ago. Most obvious advantage of the seed plants is protective cover. Angiosperm was developed some 100 million years ago and it contains protecting embryo.
Seed is the strict botanical sense. It contains embryo surrounding endosperm or perisperm and protective testa or seed coats (Bold *et al.* 1980).

Seed morphology and anatomy

Rape and Hilum are the outer appearance of seed derived from fruit. These are important for seed dispersal. The inner part anatomy consists from fertilized oval.

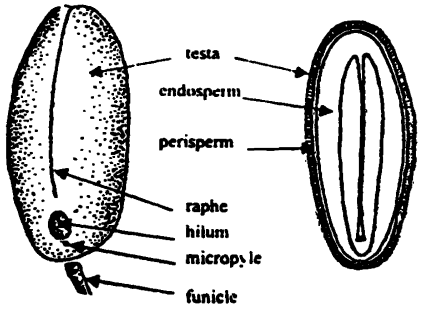

Plate 2.2 Morphology and anatomy of an typical seed

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

Micropyle: A pore sometimes visible on the seed coat derived from the channel between the tip of the integument with the megasporangium.

Raphe: A ridge formed on the seed coat if the funicular is fused with the integument in part of its length in anatropous or campylotropous ovules.

Caruncle: integumentary protuberance near microphile.

Perisperm: A layer of nutritional tissue of diploid maternal origin arisen from the nucleus 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 perisperm and endosperm (Source; Olesen, 2000).

Seed identification features

Seeds are divers in different shape and size. Specific features of the seeds such as seed weight, seed size, color, internal structure of seed/fruit coat and embryo are most important in seeds handling process (Olesen, 2000).

Seed weight: Seed weight indicates no of seeds per unit weight. Genetic and environmental factors respond to the seed weight within the species. Seed weight obviously influence for some seed processing events such as dewinging and drying.

Seed size: seed length, width and thickness are the good indicators to identified seed. Some times size of the seed depends upon appendices.

Color: most of the seeds get yellowish or brownish color in maturity stage. Other colors such as red, black, or white are less frequent and usually diagnostic. In addition, colors of appendices are important to identify seeds.

Shape: shape is one of the important characters to separate the seeds. There are no of shapes such as globes, sub globes, oblong and orbicular.

Surface: surface structure or appearance is the other features in seed identification. For very small seeds like *Eucaliptus* species, it is often the main diagnostic features (Olesen, 2000). Smooth, glabrous, wrinkled, ribbed, punctuate, reticulate, pulpy are the some types of surface appearance in seeds (Boland *et al.* 1980).

Other morphological features: position and size of the raphe, carucle, and micropyle are the often important in seed identification.

Internal structure of seed/fruit coat and embryo: hardness, thickness of the protective layers is the often-distinct character. Internal appearance of embryo and endosperm or perisperm and seed coat thickness is important in some species but often-specific genus level only (Olesen, 2000).Considering above characters *Phyllanthus debilis* fruits often under depressed, globose, quit smooth, and seeds trigonous coci, yellowish or purl brown.

Table 2.2 Common fruit types and their seed characters (Olesen, 2000).

Fruit	Seed
Berries	Angular and small packed neatly in a fleshy surround.
Capsules	Very fine dust as slender, fleshy, winged, sticky.
Drupes and fleshy fruit	Woody case of various thickness surrounding the seed. Some woody case acts as a waterproof. Some drupaceous fruits have thin coat.
Cycads and palm	Have a tough woody case.
Follicles	Some one have winged seeds
Legumes	Most of the shapes in legumes are kidney shape, and have a white fleshy ring around stalk or Funicular which connect the seed to ovary
Nut	Contain one or few seed and contain very thin testa

2.4 Physiology of fruits and seed maturation

2.4.1 Fruit maturation

The late event of the fruit development change due to fruit type. Sugar substance in the fruit pulp like fleshy animal dispersal fruits, podocarpus, juniperus and tornus, are allocated with increasing moisture content (Sedgley and Griffin, 1989). The fruit change its color from green to usually bright and conspicuous red, orange or yellow, when it becomes soft.
Loss of water with desiccation occurs late in the development of dry fruits. Disintegration chlorophyll when the fruit dehydration and change there color green to yellow, brown or black.

2.4.2 Seed maturation

The late event of seed maturation process consists with biochemical formation and dehydration. The seed dehydration similar to fruit dehydration and increase their osmotic pressure due to increasing sugar formation in fruit pulp (Olesen, 2000).
Change color of *Phyllanthus debilis* fruit from light green to brownish yellow during the maturation and change color of the seeds yellow to dark brown.

2.4.3 Dehiscence

Dehydration leads dehiscent of dry fruits and spread their seeds. Some fruit have hygroscopic properties to release seeds (Boland, 1980).

2.4.4 Seed dispersal

Seed dispersal is important to colonize new ground. Types of the dispersal depend on fruit and seed morphology. Some dispersal types are more prevent in some environment than others do. Dispersal closely related to the life cycle of particular species in particular environment. Some species have specific mode of dispersal. The unit of dispersal is called diaspore (Fahn and Welker 1972; Pijl 1982) main types of seed dispersal are wind, water, animal, mechanical etc. *Phyllanthus debilis* fruit dehiscence and dispersal their seeds within wet than dry conditions. Splitting and wind help to seed dispersal of *Phyllanthus debilis* seeds.

2.5 Seed production and handling

Seed production and handling are the most important steps to get high quality seeds. Following methods are useful to best handling of seeds.

- Which species to be collected (species selection)
- How much seed to be collected (quality)
- From where to collect (seed sources, seed trees)
- When to collect (harvest time)
- How to collect (collection method)

Above last three questions based on knowledge of the biology of species and current observation. Prediction quantity and quality of seed crop with planing suitable harvest time method are essential for efficient allocation of resources for seed collection. The best seed produced in most years, high flowering, efficient pollination and few predators. Also best time to seed collection is before lost predators and dispersal. Most plants consist certain period for fruiting and flowering according to the climatic factor such as rainfall and temperature. Many plants fruiting and flowering in dry season. High air humidity, temperature and windy conditions give considerable effects for harvest period. High temperature negatively effects to the efficiency of fruit or seed collection and high windy conditions could be loss fine seeds and fruits. Fruits and flowering assessments are the important factors to get an idea about the seed plant. Bud and antithesis stages help to preliminary fruits and flowering assessments. These assessment methods vary from species to species and among season (Source; Olesen, 2000).

2.5.1 Determination the optimal harvest time and maturity stages of fruits

Most of the seeds fully mature and few of these damage by pests, dispersal or deterioration. Species can be category mainly three groups due to there seed production rate and time period (Olesen, 2000).

1. Trees with more or less continuous reproduction through out the year but often one or two peaks
2. Trees with definite, some time short, seed maturation season and early dispersal, redaction and -or short physiological viability.
3. Trees with definite maturation season and it take continuos long time 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.

Species, which have very short dispersal period and small seeds that, are easy to loss e.g. *Phyllanthus debilis* therefore early picked helps to decrease seed wastage. If the fruit will open upon desiccation, there mature and ready to be collected, if not should be postponed harvest (for. Com 1994). Following tables give the details about the maturity event and methods of examination.

Table 2.3 Fruit mature events and methods of examination (Olesen, 2000)

Maturity event	Methods of examinations
Color change: Dry fruit; green to yellow, brown, black Fleshy fruit; green to conspious red	Visual
Dehydration (dry fruits)	Visual, touching or "weighing" in the hand measurement of specific gravity
Dehiscence and abscission	Observation of fruit fall or opening of dehiscent fruit shaking or beating fruit-bearing branches beating or manual splitting of dehiscent fruit Breaking off fruit stalks
Hydration (fleshy fruits) softening of fruit flesh	Squeezing
Loosening of fruit pulp (fleshy fruit)	Squeezing, rubbing, or other separation of fleshy part from seed or endocarp
Accumulation of sugar substance (Fleshy)	Taste (some fleshy fruits are poisonous to human body) observation of visiting frugivores
Endosperm or embryo development of seed	Cutting of seeds

2.5.2 Handling of fruits and Seeds

Best handling procedure helps to reduce seed pathogens and decease. The redundant materials promote unfavorable moisture content and pathogenic activities in the fruit sample. However, aeration can be increased by mixing small amount of twig materials for small type of fruits (For.com.1994; ATSC 1995). seeds in dehiscent fruit easy to extract under dry conditions and little additional racking or shaking. Empty fruits and debris can be removed manually or raking.

2.5.3 Seed storage

Main advantage of seed storage is supply good quality seed for planting program whenever want.

Seed storage time depend upon the species. Seed viability changing due to 1. Factor of production and methods of handling during the harvest 2. Kind of seeds, environmental condition (temperature, humidity) (Priestley, 1986).

Seeds can be divided in to recalcitrant and orthodox considering their longevity (Roberts, 1973). Recalcitrant seeds have short longevity period (month, maximally year) and it contains relatively high moisture content, typically around 25 % - 30 %. Adding cool temperature under certain moisture contents can increase longevity. Most of the dry zone species and humid zone pioneers are orthodox. the viability of orthodox seeds can be maintain 2-3 years and perhaps 15 years, providing low humidity and low temperature. Most of the orthodox seed safety stored fewer than 4 - 6 % moisture content (Crocker and Barton, 1953). The most probable moisture content is 2 - 4 % for long-term storage in sub zero temperature. Moisture content in orthodox seeds becomes 5 - 10 % during the maturation period. The various kind of storage problem could be increased due to increasing moisture content. Example-more of harmful insects activate within 8 - 9 % or more moisture content, above 12 - 14% increased fungal decease, germination will start at 40-60%moisture content. If moisture content less than 1 - 2 % it damage to seed viability And decrease germination percentage Seed longevity gets higher value if the relative humidity maintains within 20 - 25 %. Each 1 % decreases the seed moisture between 5 - 14 % double the life of the seed. ("Horington rules") seed longevity (lengthens) increased due to decreasing temperature. As Harrington rules each, decrease of 5 °C (9 °F), between 0 - 44.5 °C in storage temperature, also double the seed storage life. Sub freezing temperature at 18 °C (0 °F), will increase longevity of most kind of seeds equilibrium with 70 % relative humidity.

Many long live seeds consist hard seed coat and that are impermeable to water. Undamaged hard coat seeds viable at least 15-20 years. The maximum viability of orthodox seeds takes 75-100year (source; Hudson, Dale and Fred, 1990).

There are several types of storage such as open storage (with out moisture, or temperature control), sealed containers, conditional storage, and moist, cool storage. Seeds pack in moisture proof containers when the refrigerator storage.

2.5.4 Seed germination process

The activation of the metabolic machinery of the embryo leading to the emergence of a new seedling is known as germination. There are three conditions should fulfilled to seed germination. Such as viable seed, proper environmental conditions (water, temperature, oxygen, light) and primary dormancy (Temay,1972 ; Jann and Amen, 1977).

2.5.5 Main germination steps

Imbition water:

The main process in this step is absorbing water by dry seeds and rapidly increases moisture content. Then water softens the seed coat and hydration protoplasm. Finally seed swell and coat will break.

Synthesis of enzymes:

In this step reactivated the stored enzyme and development of embryo. Then synthesis new enzymes at the germination period. After become cell elongation and emergence of radicle.

Digestion and translocation:

Digested fats, proteins and carbohydrates transport to the growing parts and activate existing cells and synthesis enzymes, hormones etc. finally increased cell division on two ends of embryo and formed seedling.

High air humidity, temperature and windy conditions give considerable effects for harvesting fruit. high temperature negatively effects to the efficiency of fruit or seed collection and high windy conditions could be loss fine seeds and fruits. Fruits and flowering assessment are the important factor to get an idea about the seed plant. Bud and Anthesis stages help to preliminary fruit and flowering assessment. The assessment methods vary from species to species and among season (Source: Olesen, 2000).

2.6 Seed dormancy

Seed dormancy is viable seed fail to germinate when provided normally favorable condition to fail germinate. There are two kinds of dormancy such as primary dormancy and secondary dormancy (Hudson, Dale and Fred, 1993).

2.6.1 Primary seed dormancy

Seeds unable to immediate geminate as it removed from tree. Time, place, seed conditions are the regulate factors of primary dormancy. Different kinds of primary dormancy lead to survival of seed in nature. Example: seed coat dormancy, chemical dormancy, morphological dormancy, physiological dormancy, intermediate dormancy, embryo dormancy, epicotyl dormancy (Olesen, 2000).

Seed coat dormancy:

This leads hard seed coat and it is act as a waterproof. These types of seeds can easy to preserve for long time. Seed coat dormancy can be removed by softens and scarifies seed coat. Physical dormancy is a genetic characteristic of certain plant families including Leguminoceae, Malvacea, Convolvulaceae, and herbaceous legumes etc. seed coat hardness increased condition during the maturation and storage period (Hudson, Dale and Fred, 1993).

Chemical dormancy:

Some types of chemicals accumulated around the seed covering tissue during development and remain with seed after harvest (Evenari, 1949). The accumulated inhibitors can be removed by well soaking (Toit, Jacobs, and Strydom 1979).

Morphological dormancy:

Incompletely developed embryo promotes morphological dormancy.

Physiological dormancy:

Physiological dormancy occurs in more of fleshy harvested seeds in herbaceous species. It is often transitory and tends to disappear during dry storage so that it is gone before germination (Assoc. off. Seed Anal. 1975).

Intermediate dormancy: These types of seeds sensitive to chilling. However, do not have absolute requirement. Eg.Conifer species (Nikolaeva, 1977).

2.6.2 Secondary dormancy

Secondary dormancy increased due to unfavorable environmental conditions like high temperature prolonged red and white light, very low temperature, water stress and anoxia (Bewlex and Black, 1985).

2.7 Pretreatment for seed dormancy

Pretreatment helps to obtain rapid and uniform seed germination. As well as it is accelerate process of dormant release, in others it is a simulation of this process.

2.7.1 Treatment for mechanical dormancy

Moist pretreatment helps to overcome thermo dormancy also mechanical dormancy (Boland, 1980). duration of the treatment depend upon species, degree of dormancy, temperature. But typically ranges between three and five weeks.

Acid and hot water treatments are sensitive for physically dormant seeds. However, purely mechanical dormant seeds resist to this method. Some times chemical and hot water treatments damage to embryo. Because of some seeds permeable to liquid chemicals.

2.7.2 Pretreatment for physical dormancy

Methods for over coming physical dormancy with out damaging embryo. In addition, nicking, filling, piercing can be used as manual scarification methods to remove physical dormancy. However, those are labor-intensive methods. Some seeds consist strong aril. It creates a resistance to germinate seed (Msanga and Maghembe, 1993).

2.7.3 Hot water treatment

This method overcomes the physical dormancy by tension and cracking the cell layer (Brant, *et al.* 1971). In this treatment seeds should not heated together with water. Quick dip is better to decrease heat damage of embryo. However, hard coat species submerge in hot water at least much than one minute. Time of the submerge changing due to the species. The most common procedure is pure the seeds in boiling water and then leave them to the cool water 12-24 hours (Olesen, 2000).

2.7.4 Heating or burning

This method same to the hot water treatment. It creates tension on outer cell layer of the seed and then cracking. Pour the seeds in cool water, as expose to heat is not good. Because of it damage to inner part of the seed. Dry heat treatment is often less effective than boiling water treatment (Olesen, 2000).

2.7.5 Biological methods for overcoming dormancy

Large animals, insects, microbes give considerable effect for overcoming dormancy. Because of the organism can be increased permeability of the seed by damaging seed coat. Example: seeds of *Acacia* species extracted from goat dung are less dormant than non-injected dry seed (Ahmed, 1986).

2.7.6 Treatment for chemical inhibitors

Chemical inhibitors are mostly frequent in fruit pulp. Therefore, this type of fruit washes out immediately after harvest (Olesen, 2000).

2.7.7 Remove photo dormancy

Photo dormancy can be seen in some kind of herbal species and tree pioneers. Example: *Cecropia obtusifolia* and some Latin American *Ficus* species (Vasques-Yanes, 1996) consist very low germination under dark or far red light. It can be easy to breakdown by supplying appropriate light condition.

2.7.8 Treatment for thermo dormancy

This covered widest range in seed dormancy. Seed dormancy increases or decreases by fluctuating temperature. Some types of seeds decrease their dormant period because of exposed fire. Low temperature thermodormancy experienced in most temperate species. Example: *Pines*, *Fagus*. Some seeds need to expose cold moist for breakdown the dormancy (Olesen, 2000).

2.7.9 Hormonal treatments

Some type of hormones interacts with physiological mechanism of seed dormancy. Total germination percentage, germination speed and seed ling vigor increased by germination stimulant. Some compounds promote individual metabolic process in germination without being directly link to the dormancy. Example: Giberalic acid (GA3), benzyl adenine (BA), and nitrogenous compounds (KNO_3, Thiouria) (ISTA, 1996).

2.8 Main seed germination Factors

The optimal levels of germination factors differ from one species to another and carefully balance each of them.

Water

The first step of the germination process is ambition water. The two main factors effect for water up take by seeds.

1. Seed covering 2. Amount of water available in its surround. Temperature is another factor to help water imbition. The rates of imbition increase at the higher temperature level. Supplying adequate moisture can increase germination percentage and germination rate. The germination percentage tends to be equal over most of the available soil moisture from field capacity (FC) to permanent wilting point (P.W.P) (Ayers, 1952).

Excess soluble salts in the medium can decrease germination rate and reduce the seedling' quality. Soluble salts originate from soil, irrigated water; fertilizer etc. salinity gets higher value in low irrigated land and higher irrigated soil become low aeration (Ayers, 1952).

Temperature and moisture content can rapidly change in upper part of the medium due to seed germination. This problem is greater in shallow planting of the small seeds. Careful watering and the application of the mulch are way to maintain uniform moisture and temperature.

Temperature

Favorable temperature is the second requirement for seed germination. Plants can be category as three groups considering their temperature requirement.

1.those seeds germinate relatively low temperature.

2.those seeds germinate relatively high temperature.

3.those seeds germinate rang between lower to cool.

Temperature is a reasonable factor to adapt particular species for particular environment. Example: Alpine seeds well germinate under low temperature and most tropical seeds

sensitive for higher temperature. Some kinds of annual herbs like *Phyllanthus debilis* increase their seed dispersal rate in dry season.

There are three levels of temperature conditions such as minimum, maximum, and optimum considering their germination requirement (Edward, 1932). These levels differ from species to species. Example: in cool season temperature become 40°F (4.5°C) or less. The minimum temperature requirements of the tropical seeds are 50°F – 60°F (10°C - 15°C). If the temperature below this level germination get fail. Survival of the most vegetable seeds between 86°F – 104°F (30°C – 40°C) in upper level of the soil. If seeds placed under 113°F (45°C) viability become zero within 24 hours. Optimum temperature for the seed germination is 80°F - 90°F (26°C – 30°C). Germination is much better if the seeds are subjected to daily alternating temperatures rather than constant temperature. Commonly used alteration are 59 °F (15°C) or 68°F (20°C) for 18 hours and 86°F (30°C) for 6 hours. As well, temperature helps to increase the activity of pathogenic organism (source: Arora and Gupta, 1956).

Oxygen

Oxygen is the most consuming factor in respiration. Therefor highly amount of oxygen up take by seeds within the germination process. Oxygen demand of the seed differs from one species to another. Example: rice can germinate under low oxygen concentration. and some type of seeds like Cattalis (*Typha latifolia*) can be seen poor or non germination under higher o_2 condition.

Light and germination

Light plays a big role in seed germination and seed ling growth. Visible light can stimulate or inhibit seed germination in some species such as *Phacelia, Allium, and Amaranthus. Viscum album, Ficus aurea* and all Epiphytes are the good examples for great light demand plants. Others are medium light demanding species. Example: Tobacco, grass (Ball, ed. 1985).

Germination control photochemically reversible reaction involving the response of a pigment known as Phytocrome. When seed expose to the red light it can increase germination but inhibit under far red light. Light penetration into the soil depends upon the length of rays.

Red-light penetrates to a depth of about an inch in sandy soil. Most of the lights demanding seeds are small. Therefore fine seeds sowed in top of the medium.

2.9 Importance in sexual propagation:

Sexual propagation is the principle mode in silviculture. There are number of specific characters and importance in sexual propagation (Olesen, 2000).

- The specific genetic materials resulting from mixing parent genetic materials. These genetic formations increased the adaptability to nature.
- Plants produce large number of seeds each year and readily available.
- Seeds can be stored long time under cool dry condition.
- Seed act as a nutrient pack and it helps to resist environmental stress than vegetative propagles. However, there are few limiting factors in sexual propagation. Such as difficulties in collection, few life time, seed storage problem, problems in extraction, seed dormancy and genetic variations.

Chapter 3

3. Materials and Methods

3.1 Reproductive propagation of *Phyllanthus debilis*

3.1.1 Fruit and Seed collection

Seeds of *Phyllanthus debilis* were collected from Meewatura farm and around the University grounds. The harvested fruits were stored in brown paper bags at room temperature 27 °C. after 7 days these fruit had split open and dispersed the seed in to the bag. These seeds were separated from segments of the fruit wall and stored under a humidity level 20 – 25 % using sealer polytene bags.

3.1.2 Initial investigations on the seeds of *Phyllanthus debilis*

3.1.2.1 Determination of the number of fruits and seeds per gram of fresh weight

A sample of fully mature fleshy fruits was divided in to four sets, each containing 25 fruits. Each set was weighed immediately after harvest (assume that the weight of each set in grams is A1, A2, A3, A4) and then calculated the mean fruit weight each set in grams (assume that the mean weight (g) of each fruit in a given set is B1, B2, B3, B4).
Therefore meant weight of a single fruit of *Phyllanthus debilis*

$$(B1+B2+B3+B4)/4 = (C)g$$

Number of Fruits/g = 1g/C
A *Phyllanthus debilis* fruit contains 6 seeds.
Therefore, number of seed in a fruit (6) x No. of fruits/g = Amount of seeds extracted from one gram of fruit.

3.1.2.2 Preliminary experiment to determine Seed moisture content

A fully mature fresh seed sample was collected and divided in to four sets each With100 seeds. Then the fresh weight of each set of fruits was recorded. Thereafter each set of fruits was placed in paper bag and oven dried at 103°C for 17 h. After drying the seeds were placed in a desiccation chamber until their dry weight was measured. Readings were taken as given in (table 3.1)

Table 3.1 Determination of seed moisture content

Replicate	(A) Weight of container	(B) Weight of fresh seeds+container	(C) Weight of dry seed+container	percent Moisture content per seed (B-C)/(B-A) x 100%
100 seeds	A1	B1	C1	D1
,,	A2	B2	C2	D2
,,	A3	B3	C3	D3
,,	A4	B4	C4	D4
Mean seed moisture content per seed =(D1+D2+D3+D4)/4				

3.1.2.3 Seed viability test

A seed sample containing more than five hundred of fully mature fresh seeds was selected for this experiment. The sample was divided in to sub samples, s each comprising 50 seeds. These sub samples were stored under Room condition using polytene sealer bags. Then a blotting sheet (5 cm x 10 cm) was lined on a sterilized plastic tray and one of the prepared seed sub samples (50 seeds) was placed evenly on the wet blotting sheet as ten sets of seeds (10 seeds x 5 lines). Then the plastic tray covered by a transparent cover. The ventilation in the tray was maintained by keeping a small space between the cover and the container. These were then placed under light level, where the intensity was 300 µmol m^{-2} s^{-1}. The containers were exposed daily to a maximum light level of 800 µmol m^{-2} s^{-1} in the Plant house. The Moisture content was maintained 90 %. (but there was no water film around the seed). The germinated seed lings (plate 3.1) were counted after 7 days and there percent germinated was calculated using on of the sub samples of seeds stored at the commencement of the experiment. The above procedure to test seed viability was carried out at 7 day intervals using on of the sub samples of seeds stored at the commencement of the experiment until the seed viability become near or equal to zero. This experiment was carried out for nine weeks.

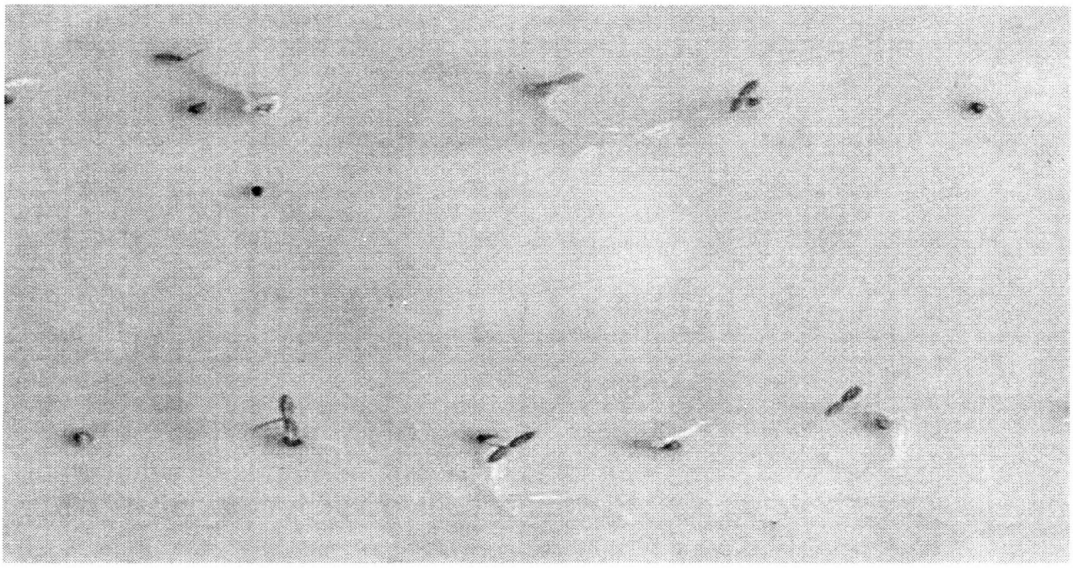

Plate 3.1 Germinated seedlings on the blotting sheet after a period of seven days.

3.1.3 Determination of the best stages in Fruits development to obtain high quality seeds

Fresh fruit samples at four different stages of development were collected in to brown paper bags. These developmental stages corresponded with their Fruit colors, such as Light green, dark green, Blackish yellow and Brownish yellow (plate 3.2). Ones they were collected, they were stored room temperature (27°C) until they dehisced. Then 120 seeds were separated from each stage and sowed in two different media (coirdust : sand mixed in a ratio of 1:1 and blotting sheet), each with three replicates. Each replicate consisted of 20 seeds (Table 3.2). After placing the seeds in Containers covered with transparent polythene to maintained the moisture content at 90 %.

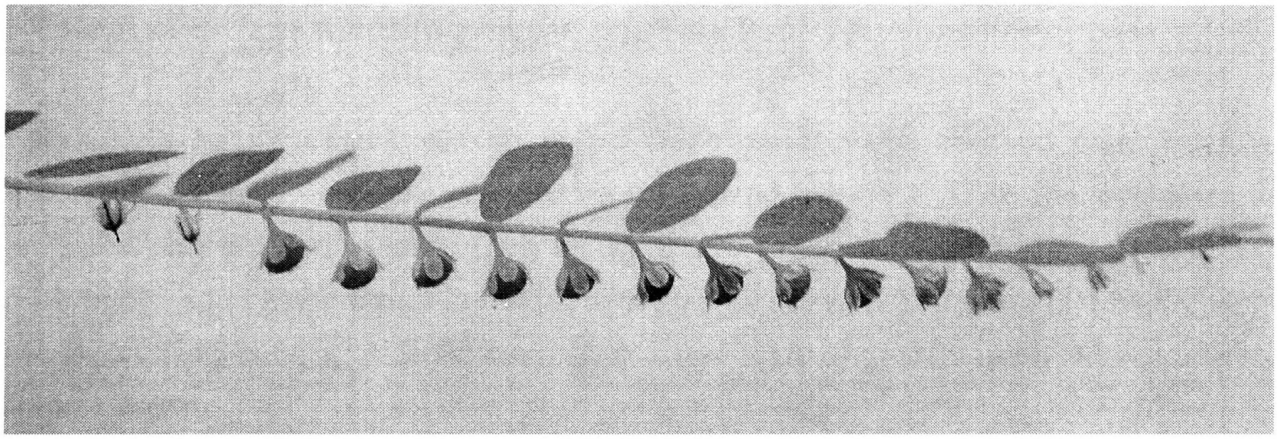

Plate 3.2 Developmental stages of the fruits of as seen from the *Phyllanthus debilis*, at the ventral side of leafy branch

Table 3.2 experimental details for determination of percent germination of *Phyllanthus debilis* seeds at different stages of fruit development and in different germination media.

Fruit Developmental stages	MEDIUM					
	Coir dust: Sand (1: 1)			Blotting sheet		
	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3
Stage 1 (light green)	-	-	-	-	-	-
Stage 2 (green)	20 seed	20 seed	20 seed	20 seed	20 seed	20 seed
Stage 3 (blackish green)	,,	,,	,,	,,	,,	,,
Stage 4 (brownish yellow)	,,	,,	,,	,,	,,	,,

Data analysis

Differences among treatments each variable were identified using general linear model procedure of the Statistical Analysis System (SAS). Analyses were tested for differences among the interactions between the germination medium and fruit maturity stages. Means were compared using Duncan's Multiple Range Test (DMRT) at $p<0.05$ level.

3.2 Effect of different potting media on growth of *Phyllanthus debilis*

The separated fully mature brownish yellow fresh seeds were sown in seed trays and covered with a thin layer of potting mixture (one seed per cell). Then the container was placed in full sunlight and watered Daily using sprayer. Nine different potting mixtures were used as treatments. Each treatment was replicated twice, with 10 individuals per replicate. (10seeds per replicate x 2 replicates). The experimental design used was completely randomize design.

Evaluation of Growth performance

The growth performances of each plant were recorded at weekly intervals. Measurements height, number of leaves, root collar diameters of seedlings were taken for period of two months after germination.

Data Analysis

Data on each variable were analyzed using general linear model procedure of the Statistical Analysis System (SAS) version 6.12. Differences among the potting mixtures were tested. Means were compared using the Duncan's Multiple Range Test (DMRT) at $p<0.05$ level.

3.3 Determination of the ability of soil seed banks to raise seedlings.

Three soil seed banks were collected from different places. Each places consisted with different soil and light conditions (loamy soil with shade place, loamy soil with open places, clay soil with shade place).

The seed banks which were less damage and same volume (1' x 1' x 2") collected into the plastic trays. These were then placed in plant house, where the light intensity was 800 µmol m^{-2} s^{-1} and watered daily using shower or sprayer. Germinated seedlings were counted using weekly intervals during the period of two months.

3.4 Determination of the best method to raise seedlings within a short period

1. Seeds of *Phyllanthus debilis* naturally split out and sown under control condition

Three *Phyllanthus debilis* plants in same age level (two month old) were grown under three different potting mixtures such as loam : Compost : Sand (1:1:1), Clay : Compost : Sand (1:1:1) and Coirdust : Sand (1:1) using Styrofoam boxes (40cmx35cmx17cm). The containers were then placed in plant house where the light level was 800 µmol m^{-2} s^{-1} and watered daily. Then germinated seedlings were counted using weekly intervals during the period of one month.

2. Seeds of *phyllanthus debilis* manually split out and sown under control condition

In other part of the experiment was carried out using three potting mixtures such as loam: compost : sand mix in the ratio of 1:1:1, clay : compost : sand 1:1:1 and coirdust : sand 1:1 and Twenty seeds which obtained from manually split out were sown in each media. Then the container was placed under the light level of 800 µmol m^{-2} s^{-1}. Daily supplied considerable amount of water using shower or sprayer. Numbers of germinated seedlings were recorded at weekly intervals within the period of two months.

3.5 Pests and controlling methods

Mealy bugs, caterpillars of some moths, butterflies and Coleopterans were the harmful pests in *Phyllanthus debilis*. Mealy bugs sapped the guise from stems or leaves. Many caterpillar stages damaged to the immature seeds and leaves.

Insect damage spread rapidly during the rainy season. Adding Insecticides at low concentration (example. Dymethoate-0.25ml/Liter-quarter of the recommended amount) minimized the insect damages.

Chapter 4

4. Results and discussion

4.1 Result of reproductive propagation

The preliminary seed studies such as seed weight, seed moisture content, and seed viability were examined before seed germination, because they help to identify whether the type of seed in *Phyllanthus debilis* is recalcitrant, intermediate or orthodox. This information provides valuable clues as to the seed treatments that could be tested for a given species. treatments for the seeds and basic propagation techniques.

4.1.1 Number of fruits and seeds /g

There were four replicates and each comprised with 25 fruits. Weights of the each sample and mean fruit weight are given in following table (4.1). Number of fruits and seeds/ g was calculated as follows.

Table 4.1 Calculated Mean fruit weight of each fruit samples.

Number of fruits per Replicate	Weight of the sample of 25 fruits (A)g (10^{-1})	Mean weight of a single fruit (B)g (10^{-3})
25 seeds	152	6
25 seeds	139	55
25 seeds	142	56
25 seeds	128	51

C: Mean fruit weight = (B1+B2+B3+B4)/4 = 0.0056g ± 0.00018g

D: Fruits /g = 1g/C = 178

Number of seeds/g of fruit:

Number of seeds in a *P.debilis* fruit (6) x Number of fruits /g =

6 x 178= 1068 seeds

Using fresh and less damage fruits could increase the accuracy of the result. It prevents desiccation and minimized the weighing error.

4.1.2 Seed moisture content.

Table 4.2 Moisture content percentage in each set of the seeds

Replicate	Weight of container (g) (A)	Weight of 100 fresh seeds+container (g) (B)	Weight of 100 dry seeds+container(g) (C)	Percent Moisture content (D)
1.100seed	23.362	23.39	23.38	16.7
2. ,,	,,	23.39	23.38	10.7
3. ,,	,,	23.30	23.37	16.6
4. ,,	,,	23.38	23.38	13.8

Seed Moisture content = (D1+D2+D3+D4)/4= 14.45%

Moisture content of *Phyllanthus debilis* seeds varied rapidly with atmospheric humidity due to its high surface area. Another reason could be the lack of light for some seeds. Because seeds are small. Therefore, fluctuation of moisture content had to be controlled before seed testing. This effect was minimized using waterproof material such as desiccation chamber. In the above experiment the dried seeds were placed in a desiccation chamber because it prevented re absorption of moisture from atmosphere.

4.1.3 Seed viability

Seed viability was observed using a direct germination test under plant house conditions. A higher percentage of germination (82%) was observed during the first week. Thereafter, seed viability decreased over a period of nine weeks. (See Figure 4.1)

This experiment was conducted in a moisture chamber to minimize desiccation and under supplied favorable light condition. Seed viability was rapidly lost under low moisture conditions. During the germination period most seeds of herbal plants are highly light demanding. The containers used for germination were definitely sterilized prior to their use to avoid microbial contamination.

4.2 Best stage to harvest fruits of *Phyllanthus debilis*

The propagation of seeds that germinated from fruits that were harvested at different stages of developed gave the following results. Seeds from fruits that were blackish green in color gave a higher percentage (91 %) of germination. Percent germination of seed from dark green and those from brownish yellow fruits were less, *viz.*, 26 % and 65 % in blotting sheet and 23 % and 11 % in coirdust : sand (1 : 1) respectively compared with blackish green stage. This may due to the decrease in moisture content when the fruit matures. On the other hand the dark green fruits may not be fully mature. The levels of germination in the coirdust : sand mixture in the ratio of 1:1 was poor compared with that on blotting paper. This may due to the low pH in the coirdust : sand mixed in a ratio of 1 :1. Could it also be due to lower moisture in the mixture compared to moist blotting sheet. Figure (4.2) shows the variability of percent germination at different seed maturity stages.

Table 4.3 Number of germinated seedlings in four maturity stages under two potting Mixtures

Blotting sheet			
Stages	Number of seeds	Germinated seed lings	percentage
Stage I	-	-	-
Stage II	60	16	26
Stage III	60	55	91
Stage IV	60	39	65
coir dust : Sand (1:1)			
Stage I	-	-	-
Stage II	60	14	23
Stage III	60	34	56
Stage IV	60	07	11

According to the above data analyses showed that, percent germination was significantly different among the interaction between medium substrates with different seed maturity stages. As the Duncan grouping test the best interaction was stage III with blotting sheet.

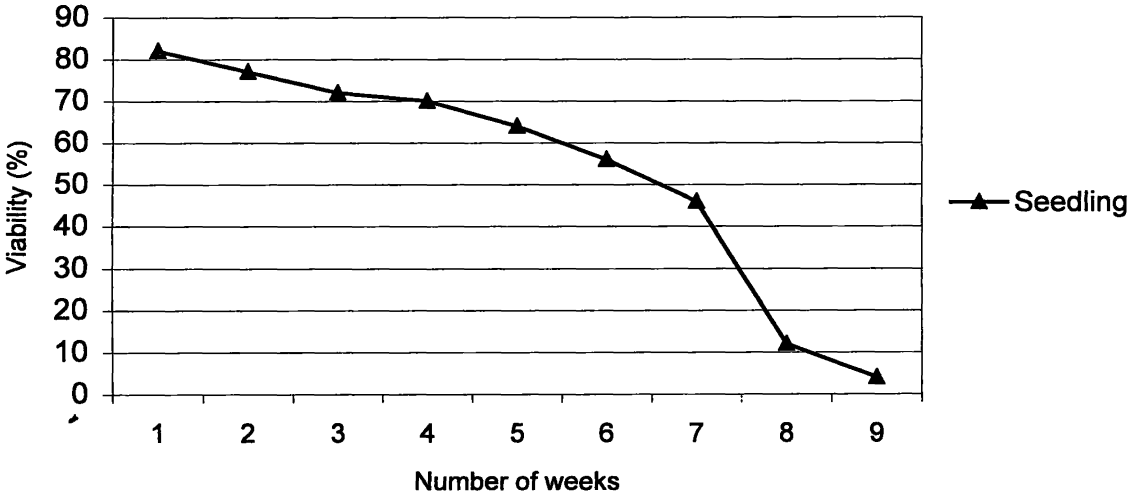

Figure 4.1 Viability variation of *Phyllanthus debilis* seeds for the peroid of nine weeks

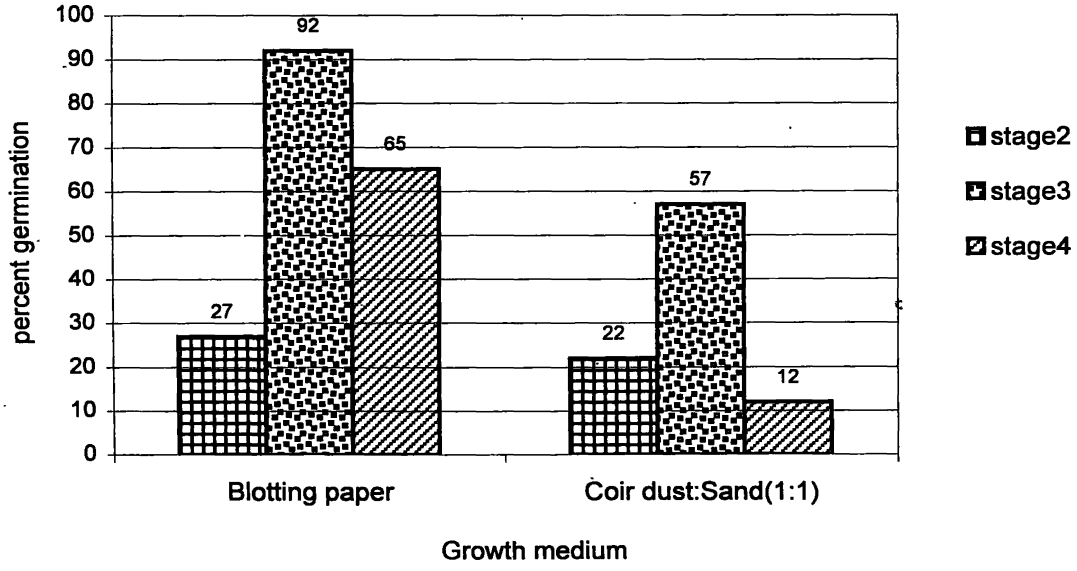

Figure 4.2 Germination persentage variation of three seed matuarity stages of *Phyllanthus debilis* seeds under two growth medium

4.3 Best Medium substrate

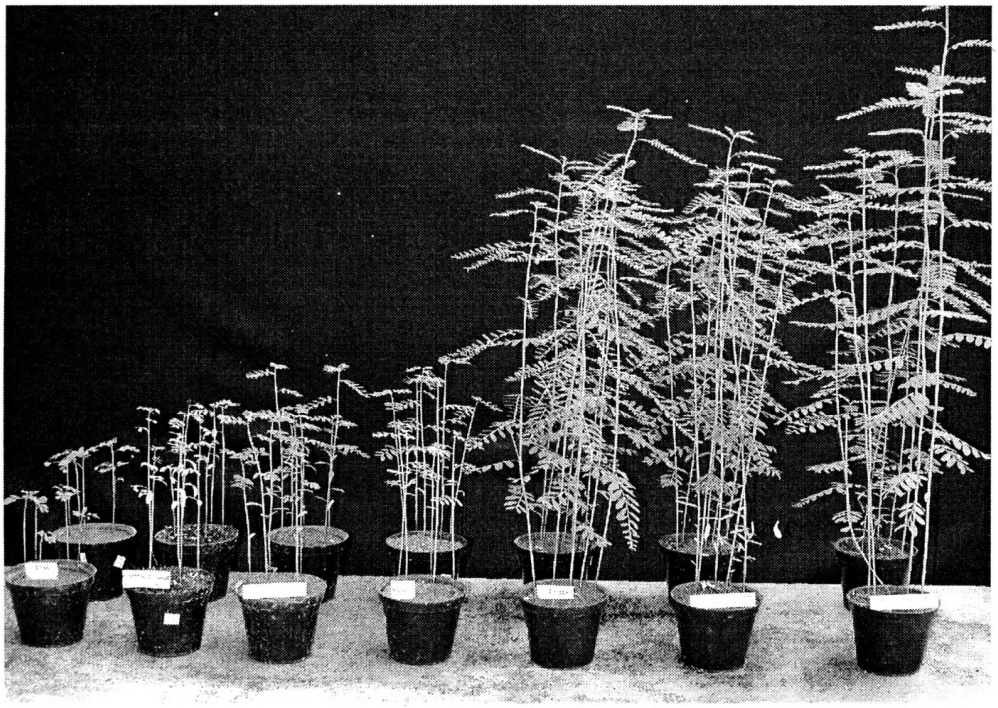

Plate 4.1 Variation in growth performance of *Phylanthus debilis* plants grown in different potting mixture for period of 6 weeks under light intensity of 800 μ mol $m^{-2} s^{-1}$.

Comparing the performance of seedlings 10 days After seeds were sown higher number of (20/20) seedlings were observed in sand : loam : compost (1:1:1) medium. Fewer no of seeds germinated in the sand only (8/20) and in coir dust : sand mix in the ratio of 1:1(9/20). This may be attributed to the low pH oH coir : sand mixture and low moisture content in the sandy medium.

The mean plant height, mean root collar diameter per plant and mean number of leaves per plant were recorded over a period of 6 weeks at weekly intervals, to compare the growth performance of seedling in the different plant media.

Statistical analyses of the data showed a significant difference among the potting mixtures ($p<0.05$). According to the Duncan's Multiple Range Test three potting mixtures *viz.*, Loam : Compost (1 : 1), Loam :Compost :Sand (1:1:1) and loam were better than the others mixtures (Figure 4.3)

The mean height per plant, number of leaves per plant, and mean root collar diameter per plant were also highest in the potting mixture sand : loam : compost at the ratio of (1:1:1) and loam : compost at the ratio of (1:1) compared to the plant performance in the other media. Early flowering and fruiting in the plants was also observed in the better potting mixtures.

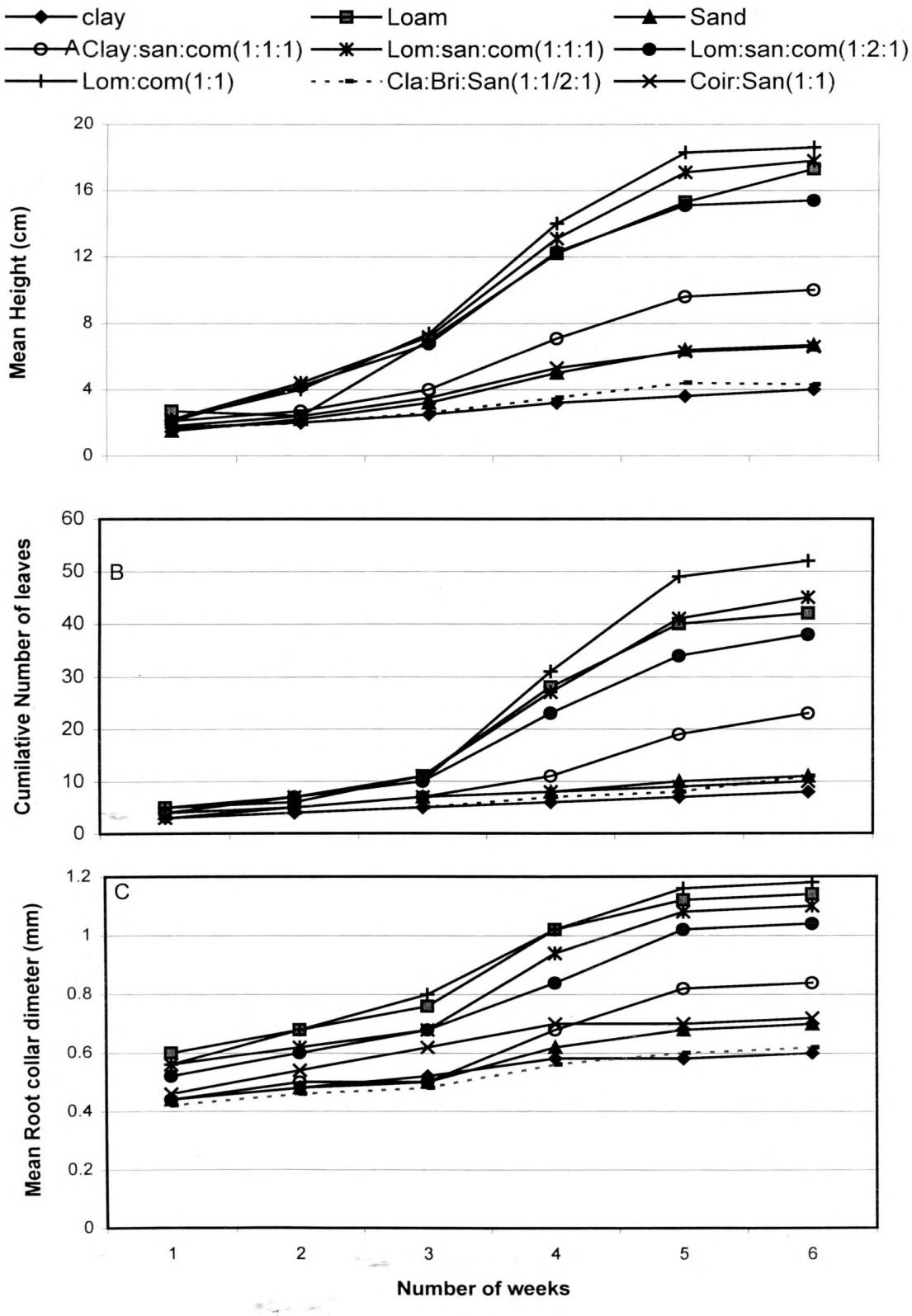

Figure 4.3 Growth performance of *Phyllanthus debilis* plants, based on mean height per plant (A), mean number of leaves per plant (B) and mean root collar diameter (C) grown under nine different potting mixtures for a period of six weeks.

4.4 Result of seed bank test

Seedlings from seed banks that were loamy soil with open place gave a maximum number of seedling (42). Number of seedlings from clay soil with shade place were much less, *viz.*, 15 seedling. Seed bank, which collected from loamy soil with shade place was, gave 38 seedlings.

These variations of the result may due to the light condition of the place and their soil conditions. Because *Phyllanthus debilis* is a highly light demand plant (most of the herbal plants are highly light demand). Therefore low light failed the germination rate of the seed also unfavorable germination conditions in the media (example: clay soil consisting with low soil air compare to the loamy soil. As well as compactness of the soil particles are higher than loamy soil. It caused to low penetration of red light (red light is most important factor in seed germination). It may be reason to the low germination rate of clay soil compared with loamy soil.

4.5 Result of two different seedling obtaining method

Maximum number of seedlinges were observed under Method (I) within the period of two month. But the seeds which mannually split out and sown under meadia with loam: compost: sand mix in the ratio of 1:1:1 were given strong seedlings within a period of seven days compared with to other method. This may due to the favourable moisture content of the seeds in maturity stage of blackish green and favorable germination conditionsof the media. But the other method (seeds naturally split out and sown under contaol condition) was time consuming and it gave 56 seedlings withing the period of two months. Because the seeds of *Phyllanthus debilis* were split out at fully mature stage. The moisture content of this stage (fruit color brownish yellow) was fewer compared with blackish green stage.

Importances of method two

1.Less time consuming

2.seedlings in same age levels

3.Easy to obtain maximum number seedlings within short period.

Chapter 5

5. Conclusions and recommendations: *Phyllanthus debilis*

Phyllanthus debilis seeds can be categorized under the group "Intermediate seeds" as its viability period was 3 - 4 months and their moisture content was about 14%.

According to the statistical analysis, the best potting media were loam: compost (1:1), loam: compost: sand (1:1:1) and loamy-soil. Possibly these media provided considerable amounts of soil air and moisture, and a suitable temperature for seed germination.

Seeds obtained from manually split fruits sown in a medium of loam, compost and sand mixed in a ratio of 1:1:1 respectively was found to be the best method to raised seedlings of *Phyllanthus debilis*.

A mature fruit at the blackish green stage was best to obtain seeds that are suitable for germination. This may be because the seeds at this stage contain higher moisture content, compared to those that are over mature and brownish yellow in color.

The experiments were carried out under two instantaneous light levels. The accuracy of the results could be increased using several light levels.

REFERENCES

Abeywardana, N. and Hettiarachi, L. K. 2001. Statistic on the national demand for medicinal plants, IUCN - The World Conservation Union, Sri Lanka.

Arora, D. K. and Gupta, S. 1996. Advance in Plant Physiology, Vol. 10. Anmol Pvt. Ltd.

Ayers, A. D. 1952. Seed germination as affected by soil moisture and salinity. Agrron. Jour. 44: 82-84.

Ball, V. 1985. Ball red book: Greenhouse growing (14th ed.). Reston, Va: Reston pub. Co.

Bewly, J. D., and Black, M. 1985. Seed physiology of development and germination. New York: Plenum press.

Bold, H. C., Alexopoulus, C. and Delevoras, T. 1980. Morphology of plant and Fungi. Hgrper and Row pub., N.Y.

Boland, D. J., Brooker, M. I. H. and Turnbull, J. W. 1980. Eucalyptus seed.CSIRO, Australia.

Brant, R. E., Mckee,G. W. and Cleveland, R. W. 1971. Effect of chemical treatment on hard seed on pengrift crownvetch. Cropscience 11:1, 1-6.

Ching, T. M. 1972. Metabolism of germinating seeds. *seed biology*, vol. 2, T.T. Kozlowski, ed. New York, Academic press.

Crocker, W. and Barton, L. V. 1953. Physiology of seed. Waltham, Mass.: chronica Botanica.

Dassanayake, M. D. 1997. A Revised Handbook to the Flora of Ceylon. Vol. 11. Gulab Primalani, Oxford and IBN Publishing Co.Pvt.Ltd.

Edwards, T. J. 1932. Temperature relations of seed germination. Quart. Rev. Biol. 7: 428-43.

Evenari, M. 1949. Germination inhibitors. Bot. Rev. 15: 153-94.

Fahn, A. and Welker, E. 1972. Anatomical mechanisms of seed dispersal. *Seed Biology*. (Kozlowski, T.T., ed.). 1: 152-222. Academic press, New York and London.

For. Com. 1994. Seed manual, procedures for seed collection, handling, storage, purchase and accounting. Forestry commission, Hobart, Tasmania.

Hereman, S. 1980. Paxton's Botanical Dictionary, International book Distributors, India.

Hudson, T. H., Dale, E. K. and Fred, T. D. 1993. Plant propagation principles and practices, Prentice-Hall of India Pvt. Ltd., New Delhi.

ISTA 1996. International rules for seed testing. *Seed science and Technol*, vol. 24, supplement. International seed testing Association, Zurich.

Jann, R. C. and Amen, R. D. 1977. What is germination, In the biochemistry of seed dormancy and germination, Khan, A.A, ed. Amstradam, North Holland publishing co., p.7-8.

Jayaweera, D. M. A. 1981. Medicinal plant (indigenous and exotic) use in Ceylon, the national Science council of Sri Lanka .

Kaluthota, C. D. 2000. Vegetative propagation of *Dipterocarpus zeylanicus* , B.Sc. thesis Sabaragamuwa University,Buttala.

Kirtikar, K. R., Basu, B. D. and An, I. C. S. 1994. Indian Medicinal Plants, Vol. III, India.

Kerby, J., Elliott, S., Maxwell, J. F. and Blakesley, D. 2000. Tree seeds and seedling for restoring forest in Northern Thailand, Biology Department, Science Faculty, Chiang Mai University, Thailand.

Kumar, S., Singh, J. and Sharma, A. 1999. Asian region inventory of medicinal and aromatic plants and polyherbal formulations, Department of Biotechnology, Government of India, New Delhi.

Mesanga, H. P., and Maghembe, J. A. 1993. Germination of woodland Mahogany(*Trichilia emetica*) following manual seed-coat scarification and Potassium nitrate treatment. Jour. Trop. For.Sci. 5: 4, 518-527.

Nikolaeva, M. G. 1977. Factor affecting the seed dormancy pattern. *Physiology and biochemistry of seed dormancy and germination*, Khan, A. A, ed. Amstradam, Noth-Holland publishing Co., P. 51-76.

Olesen, K. 2000. Guide to handling of tropical and Sub tropical forest seed, Danida Forest Seed Center, Humlebake, Denmark.

Phyllanthus species sources of new antiviral compound.htm

Priestley, D. A. 1986. Seed aging. Ithaca, N.Y. : Cornell univ. press

Roberts, E. H. 1973. Predicting storage life of seed. Seed sci. and Technol. 1: 499-514.

Schopmeyer, C. S., ed. 1974. Seed of woody plants in the United States. U.S. Dept. Agr. Hand book 450. Wshington, D. c.: U.S. Govt. printing office.

Sedgly, M. and Griffin, A.R. 1989. Sexual propagation of seed crops. Academic press.

Thomsen, K. 2000. Handling of Desication and Temperature Sensitive Tree Seeds, DFSC series of Technical notes. Danida Forest Seed Center, Humlebaek, Denmark.

Thomsen, K. and Diklev, S. 2000. Laboratory manual for basic tree seed studies. DFSC series of technical notes. TN 57. Danida Forest Seed Center, Humblebake, Denmark.

Toit, H. J., Cobs, G. and Strydom, D. K. 1979. Role of the various seed part in peach seed dormancy and initial seedling growth. Jour. Amer. Soc. Hort. *Sci.* 104(4): 490-92.

Tokoka, T. and Tobe, H. 2001. Ovules and Sees in Sub family Phillanthoidae (Euphorbiaceace) : structure and systematic implication. *Journal of plant research.* 114(1113): 75-93.

Warrier, R. K., Nambiar, V. P. K. and Ramankutty, C. 1995, Indian medicinal plants: a compendium of 500 species, Vol. 4, Orient Longman Ltd.

National Digitization Project

National Science Foundation

Institute : Sabaragamuwa University of Sri Lanka

1. Place of Scanning : Sabaragamuwa University of Sri Lanka, Belihuloya

2. Date Scanned : ..2017-09-20.....

3. Name of Digitizing Company : Sanje (Private) Ltd, No 435/16, Kottawa Rd, Hokandara North, Arangala, Hokandara

4. Scanning Officer

Name : ...S.A.C. Sadaruwan.....

Signature : .....

Certification of Scanning

I hereby certify that the scanning of this document was carried out under my supervision, according to the norms and standards of digital scanning accurately, also keeping with the originality of the original document to be accepted in a court of law.

Certifying Officer

Designation : .....Librarian.....

Name : .....T. N. Neighsoorei.....

Signature : .....

Date : ..2017-09-20.....

"This document/publication was digitized under National Digitization Project of the National Science Foundation, Sri Lanka"