Potential of Beauty Leaf Tree
*(Calophyllum inophyllum* L) as a Biodiesel Feedstock

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A thesis submitted in fulfilment of the requirements for the degree of Doctor of Philosophy

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CQ University Rockhampton Australia
29th March 2012
Candidates Declaration

I certify that except where due acknowledgement has been made, the work is that of the author alone; the work has not been submitted previously, in whole or in part, to qualify for any other academic award; the content of the thesis is the result of work which has been carried out since the official commencement date of the approved research program and ethics procedures and guidelines have been followed.

......................................

Subhash Hathurusingha

Dedication

“I wish to dedicate my thesis to my most dearly loved late parents”
Thesis Abstract

Increasing demand for renewable energy entices exploration of potential biodiesel feedstocks. “Beauty Leaf Tree” (*Calophyllum inophyllum* L., Clusiaceae) is a native Australian species which is endowed with numerous positive attributes to be a potential biodiesel feedstock. Beauty Leaf grows on marginal conditions, fruits profusely (3000-10000 seeds tree⁻¹ season⁻¹) and kernels contain up to 65% inedible oil. *Calophyllum* has multiple economic uses; the kernel oil is widely used in pharmaceutical industry, the timber is durable (density ≈600-900 kg m⁻³). Leaf, bark and seed oil extracts contain anti-cancer and anti-HIV compounds. However, existing information on various aspects of this species is scanty. The primary goal of this project was to evaluate the potential of *Calophyllum inophyllum* as a biodiesel feedstock by studying various aspects of biodiesel production. The secondary goal was to determine provenance variations in biological aspects and fatty acid profiles (FAP) of the species in relation to environmental conditions with the intention of determining optimum growth conditions and or selecting suitable cultivars for commercial plantations. Overall research approach constituted the following interrelated components; (i) feedstock parameters (stand variables, yield, seed biology and early growth), (ii) harvesting time (reproductive phenology and variations in FAP during fruit development), (iii) kernel oil (quantity and quality), (iv) oil extraction, conversion of oil into biodiesel, biodiesel characterization and compatibility and (v) engine performance.

Field based studies revealed marked variations in the majority of the tested variables. An algometric relationship (r=0.59**) was found between stem diameter and crown diameter (CD), and fruit yield was found to correlate (r=0.39*) with CD. Significant country to country and provenance variations were found in the majority of seed morphometric characteristics, germination and early growth and were found to be influenced by the maternal environment. *Calophyllum* seeds showed recalcitrant storage behaviour and can be stored in ambient (25-35 °C, 65-75% humidity) up to 9-10 months.

Flowering in both Meegoda Sri Lanka and Yeppoon Australia was found to coincide with monthly rainfall peaks in the respective season. Phenological characteristics were found to vary with the region. Variation in FAP during fruit development was studied using gas liquid chromatography (GLC) to select the optimum maturity stage for biodiesel production.
The oil content and unsaturated fatty acid composition in calophyllum fruits were found to increase with maturity. All maturity stages were found to possess acceptable FAP for biodiesel production.

Provenance and seasonal variations in oil content and FAP were investigated by employing standard solvent extraction methods and ISO 5508 & 5509 analytical methods. Kernels from Anuradhapura, Sri Lanka recorded the highest oil content (~57%) and the extracts from Kurunegala, Sri Lanka had the best FAP for biodiesel production. Autumn seeds were found to have higher oil contents and better FAP than winter seeds.

Screw press extraction gives the best oil yield at medium compression ratio (7.4:1). Steam conditioning significantly (p<0.05) improved the oil recovery and extractability. Kernel drying also increased the oil recovery and showed a sigmoid relationship with drying time.

A new protocol (Australian Patent No 2010902733) was developed to convert highly acidic (22 mg/kg KOH) and viscous (62 cSt) calophyllum oil into methyl esters (biodiesel) and the resultant Calophyllum oil methyl ester (COME) was compared with those derived from the conventional methods for physicochemical properties and metal degradation. COME derived from this novel method was characterised by American Standard for Testing Materials (ASTM 2010) and tested for potential elastomer component degradation.

The patented protocol demonstrated 89% efficiency and the resultant COME had superior physicochemical properties (pH 6.8, density 862 kgm⁻³, cloud point 9.8°C), conformed to ASTM standards and B20 (20% COME+80% mineral diesel) had negligible adverse effects on metal and elastomer components. Engine performance (engine power, specific fuel consumption and thermal efficiency) of COME was tested by MAHA LPS 2000 chassis dynamometer. COME B20, Mobil B20 and diesel were also tested for their effect on engine vibration using SERACCEL V5 tri-axis accelerometer, SIGVIEW32 version 2.2.1 software and analysed by Fast Fourier Analysis. Engine performance of the resultant biodiesel (51.5 kW) was found to be similar that of mineral diesel (50.8 kW). Vibration tests indicated that COME can potentially be used as a diesel additive to reduce engine vibration. Considering its ability to produce approximately 4800 L of biodiesel ha⁻¹ year⁻¹, it was concluded that “Beauty Leaf Tree” has a great potential to be a viable biodiesel feedstock in Australia and other tropical countries.
## Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>APNI</td>
<td>Australian Plant Names Index</td>
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<tr>
<td>ASTM</td>
<td>American Society for Testing Materials</td>
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<tr>
<td>CD</td>
<td>Crown Diameter</td>
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<tr>
<td>CI</td>
<td>Compression Ignition</td>
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<tr>
<td>CFPP</td>
<td>Cold Filter Plugging Point</td>
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<tr>
<td>COME</td>
<td>Calophyllum Oil Methyl Ester</td>
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<tr>
<td>CN</td>
<td>Cetane Number</td>
</tr>
<tr>
<td>CP</td>
<td>Cloud Point</td>
</tr>
<tr>
<td>CSCT</td>
<td>Copper Strip Corrosion Test</td>
</tr>
<tr>
<td>DBHOB</td>
<td>Diameter at Breast Height (Over Bark)</td>
</tr>
<tr>
<td>DPI</td>
<td>Department of Primary Industries and Fisheries</td>
</tr>
<tr>
<td>EN</td>
<td>European Standards</td>
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<tr>
<td>EPA</td>
<td>Environmental Protection Agency</td>
</tr>
<tr>
<td>EU</td>
<td>European Union</td>
</tr>
<tr>
<td>FA</td>
<td>Fatty Acid</td>
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<tr>
<td>FAME</td>
<td>Fatty Acid Methyl Ester</td>
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<tr>
<td>FAP</td>
<td>Fatty Acid Profile</td>
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<tr>
<td>FFA</td>
<td>Free Fatty Acid</td>
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<tr>
<td>FFT</td>
<td>Fast Fourier Transformation</td>
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<tr>
<td>GBHOB</td>
<td>Girth at Breast Height (Over Bark)</td>
</tr>
<tr>
<td>GLC</td>
<td>Gas Liquid Chromatography</td>
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<tr>
<td>HFB</td>
<td>Height to First Branch</td>
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<tr>
<td>HIV</td>
<td>Human Immunodeficiency Virus</td>
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<tr>
<td>HSD</td>
<td>High Speed Diesel</td>
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<tr>
<td>IP</td>
<td>Intellectual Property</td>
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<tr>
<td>IPGRI</td>
<td>International Plant Genetic Resources Institute</td>
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<tr>
<td>ISO</td>
<td>International Standards Organization</td>
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<tr>
<td>ISTA</td>
<td>International Seed Testing Association</td>
</tr>
<tr>
<td>IUCN</td>
<td>International Union for Conserving Nature &amp; Natural resources</td>
</tr>
<tr>
<td>IV</td>
<td>Iodine Value</td>
</tr>
<tr>
<td>KOC</td>
<td>Kernel Oil Content</td>
</tr>
<tr>
<td>KOH</td>
<td>Potassium hydroxide</td>
</tr>
<tr>
<td>MAR</td>
<td>Mean Annual Rainfall</td>
</tr>
<tr>
<td>MC</td>
<td>Moisture Content</td>
</tr>
<tr>
<td>MeOH</td>
<td>Methanol</td>
</tr>
<tr>
<td>MFC</td>
<td>Mean number of fruits/cluster</td>
</tr>
<tr>
<td>MGT</td>
<td>Mean Germination Time</td>
</tr>
<tr>
<td>MSL</td>
<td>Mean Sea Level</td>
</tr>
<tr>
<td>MSp.</td>
<td>Mean Spacing</td>
</tr>
<tr>
<td>NBR</td>
<td>Nitro Butyl Rubber</td>
</tr>
<tr>
<td>NFC</td>
<td>Number of fruit clusters</td>
</tr>
<tr>
<td>NFS</td>
<td>Number of fruiting seasons/year</td>
</tr>
<tr>
<td>NPB</td>
<td>Number of Primary Branches</td>
</tr>
<tr>
<td>QLD</td>
<td>Queensland</td>
</tr>
<tr>
<td>SFC</td>
<td>Specific Fuel Consumption</td>
</tr>
<tr>
<td>SID</td>
<td>Seed Information Database</td>
</tr>
<tr>
<td>SLA</td>
<td>Specific Leaf Area</td>
</tr>
<tr>
<td>SLM</td>
<td>Specific Leaf Mass</td>
</tr>
<tr>
<td>SN</td>
<td>Saponification Number</td>
</tr>
<tr>
<td>TAN</td>
<td>Total Acid Number</td>
</tr>
<tr>
<td>TE</td>
<td>Thermal Efficiency</td>
</tr>
<tr>
<td>TTC</td>
<td>Tetrazolium Chloride</td>
</tr>
<tr>
<td>US</td>
<td>United States of America</td>
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</table>
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Publications

Patents

Australian Patent No 2010902733. Entitled: Biodiesel from Calophyllum inophyllum in the name of Central Queensland University (Chapter 9, Section 9.1).

Journal articles


Symposium papers


Online Articles

- Sustainability take roots in campus
- Seed of hope for motoring fuel from wild native Beauty Leaf Tree
- Native seeds show biodiesel potential
  http://www.abc.net.au/rural/news/content/201006/s2939697.htm
- Australians Evaluate Native Oil-Bearing Tree
  http://advancedbiofuelsusa.info/australians-evaluate-native-oil-bearing-tree
  http://biodieselmagazine.com/articles/3692/australians-evaluate-native-oil-bearing-tree/
- Forget the flux capacitor scientists need time to turn trees into fuel
- ABC Rural Report for Southern Queensland and Capricornia, Tuesday the 12th of June 2012.
  http://www.abc.net.au/rural/regions/content/201206/3522940.htm

Local Television Broadcast

Channel 7 local news “Beauty Leaf Oil” 22/05/2012

Nominations for Awards

- Eni Awards Italy (2012) renewable and non-conventional energy
- Tropical Innovation Award (2012) Cairns Regional Council
  http://www.tropicalinnovationawards.com/2012-applicants
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CHAPTER 1

General Introduction

1.1 Background

1.1.1 Beauty Leaf Tree

*Calophyllum inophyllum* L. (Clusiaceae), commonly known as Alexandrian laurel, beauty leaf calophyllum or Domba (in Sri Lanka) is a littoral tree found in the tropics. Beauty leaf tree commonly occurs above the high-tide mark along the coastal belt of Northern Australia and spreads throughout Southeast Asia and Southern India (Agroforestry Tree Database 2007). The species, regarded as native in Australia, is a medium to large evergreen tree (8–20 m in height) that carries a broad spreading crown having asymmetrical branches (Friday and Ogoshi 2010). The tree bears a dense canopy of glossy, elliptical leaves, fragrant white flowers, and large round nuts (Flora of Australia 2007). Hereafter the word calophyllum is used as the common name for *Calophyllum inophyllum*.

The horizontally expanding root system of *C. inophyllum* has the ability to stabilize coastal dune soils, tolerates salinity and protects other sensitive tree species from salt spray or salty winds (Leaflet 4, EPA QLD 2003; Friday and Okano 2006). It is a good shading tree that can be used in restoring disturbed coastal forests. It is also an attractive ornamental tree, and may be useful in apiculture owing to its crimson coloured young foliage and scented flowers (Agroforestry Tree Database 2007).

With scientifically established medicinal properties (anti-HIV and anti-cancer compounds), *C. inophyllum* can be placed amongst the most important multi-purpose trees of Australia. It is also an important bush medicine to Australian Aborigines. Application of crushed nut on the skins to treat aches and pains is a common practice among northern Queensland Aboriginal communities (Low 1990). *Calophyllum inophyllum* is well known throughout the world for its medicinal value. Almost all parts of the plant (i.e., bark, leaves, and seeds) are used as antiseptics, astringents, expectorants, diuretics and purgatives (Dweck and Meadows 2002). The kernel oil is known to have antimicrobial and antiviral activity (Yimdjo et al. 2004; Itoigawa et al. 2001).
Calophyllum inophyllum is regarded as a multipurpose tree by many indigenous communities throughout the world. Tannins (11.9%) in the bark are used as a fabric dye. Refined seed oil is used as a varnish by Polynesian communities (Friday and Okano 2006).

Kernel oil of C. inophyllum is used by European cosmetic companies to make high quality toilet soap (Dweck and Meadows 2002). Cold pressed oil is sold on the internet for $360 a litre for cosmetic purposes (Friday and Okano 2006). During the eighteenth and nineteenth centuries, calophyllum seed oil which is known as ‘Domba oil’ (Sri Lankan common name) was famous in Europe and might have been first exported from Ceylon (Sri Lanka) when under the governance of British Commonwealth back in 1815-1948 (Drury 1873 cited in Dweck and Meadows 2002).

Calophyllum inophyllum provides a good durable general-purpose timber with density ranging from 560-900 kg m$^{-3}$ at 15% moisture (MTC wood wizard online 2006). The wood is used for light construction, flooring, moulding, joinery, wooden pallets, diving boards, cartwheels and axles, musical instruments and blowpipes (Timber species notes, DPI Queensland 2007). Heartwood of this species is considerably resistant to termite attack (Grace et al. 1996).

A number of authors have reported the existence of active chemical agents (xanthones and coumarins) in Calophyllum extracts that act against HIV 1 (Patil et al. 1993; Taylor et al. 1994; Spino et al. 1998; Powar et al. 2007). Also, there are numerous reports on chemo-preventive and anti-tumour agents found in bark, leaf seed and root extracts of C. inophyllum. Itoigawa et al. (2001) found calocoumarin-A as the most potent inhibitory active agent in a 2-stage mouse skin carcinogenesis test.

1.1.2 Calophyllum as a Potential Biodiesel Feedstock

Calophyllum inophyllum exhibits qualities of a promising biofuel feed stock plant due to its many positive attributes. Calophyllum fruits profusely (6000-1000 tree$^{-1}$) twice a year and kernels contain up to 65% of non-edible oil (Friday and Okano 2006; Hathurusingha and Ashwath 2007) and superior to most of the common oil seed crops; Jatropha- 40%, Pongamia- 30%, oil palm- 60% (Azam et al. 2005). In plantation conditions Calophyllum could yield up to 4680 kg of oil ha$^{-1}$ year$^{-1}$ (Azam et al. 2005).
Calophyllum is native to Australia and is non-aggressive; it does not compete with associated plants for nutrients. This species also demonstrates geo-climatic adaptability/resilience, requires little maintenance and is resistant to most of the common pests (Friday and Okano 2006).

*Calophyllum inophyllum* does not compromise arable lands and since it can be established in degraded and poorly drained soils (Little and Skolmen 1989). Calophyllum attains reproductive maturity in 7 years and can produce fruits up to 200 years (Friday and Okano 2006). Being a perennial tree, it has another advantage over annual biodiesel crops in terms of carbon sequestration in its above ground biomass (Rutz and Janssen 2007).

Based on the fatty acid composition of the kernel oil, some authors have suggested that the Fatty Acid Methyl Esters (FAME) of *C. inophyllum* may conform to American Society for Testing Materials (ASTM) and European Union (EU) biofuel standards (Azam et al. 2005).

1.2 The Issues

1.2.1 Under Recognition and Under Exploitation

*Calophyllum inophyllum* possesses many economic uses and provides a number of ecological services. Currently, there is an appreciable commercial demand for its seed oil. At present, pharmaceutical and cosmetic companies are importing raw materials from the Pacific islands, and South and Southeast Asia (Kilham 2004). Calophyllum has been traditionally renowned for its medicinal value. This exuberant demand indicates its potential as a candidate for value-added plant based industries in Australia. It is unfortunate that a tree of this calibre has not yet been given a proper national recognition and its prospects for commercial use are unknown in Australia (Lullfitz 1978). This situation is also evident in some South Asian countries such as Sri Lanka and India. Bringi (1987) mentioned that in the absence of any systematic survey, there is little agreement on the economic potential of the species in India. The current status of this species in Australia and Sri Lanka is no different to that reported in India.
In Australia little or no effort has been made to exploit the plant’s true economic potential, apart from identifying it as an ornamental tree. According to the latest amendments to the Nature Conservation Act 2006, *Calophyllum inophyllum* is classified as a species of “least concern” based on the lack of information about the species in Australia. No scientific study has been done in Australia, or elsewhere on its growth and habitat variations, or on its medicinal and biodiesel potential.

### 1.2.2 Provenance Variations

Rehfeldt (1995) suggested that there is a strong relationship between performances of plants and habitat variability. Sometimes plants respond to such variations by showing plastic changes (Harper 1977; Boyce 1992). Provenance variations in plants are attributed to environmental and/or genotypic variations.

In Australia *C. inophyllum* is naturally confined to coastal areas. Even though there are subtle variations in climatic and soil conditions, its habitats generally have similar altitudes and soils. Streets (1962) suggested that *C. inophyllum* can be successfully established in inland sites and one herbarium specimen was recorded from an inland site near Roma, Queensland (Australia’s Virtual Herbarium 2010). In Sri Lanka, *C. inophyllum* is dispersed by water (through river systems) and fruit bats (Personal Communication 2008). Small stands can be found in inland areas on river banks and on edges of paddy fields, and occurs in all agro-ecological zones with distinct climatic, altitude and soil conditions (Personal Observation, 2007). Therefore, provenance variations in Sri Lanka may be far more pronounced than in Australia as a result of these geo-climatic variations.

It is quite important to investigate whether there are variations in performance, yield and plant properties (e.g. fatty acid profiles, sapwood density) between provenances of Queensland and Sri Lanka as it may be beneficial for identifying favourable ecophysiological conditions and/or suitable genotypes for commercial cultivation. This information may also expand the plant’s reported biophysical limits and might help in identifying new locations within Northern Australia, where this species can be successfully established.
*Calophyllum inophyllum* is also a potential biodiesel feedstock owing to its favourable characteristics that have been mentioned earlier (section 1.1). Due to its wide geographic distribution, fatty acid composition of kernel oil might also vary from one location to the other. Variations in fatty acid profile (FAP) can directly influence the quality of the resultant biodiesel (Ramos et al. 2009). Currently there are no reports on the provenance or seasonal variations in kernel oil content, fatty acid profiles and the resultant fatty acid methyl ester (biodiesel) quality. Identifying these variations is important to select suitable provenances and or conditions for establishing biodiesel feedstock plantations.

### 1.2.3 Technical Complications in Expelling and Converting *Calophyllum* Oil into Biodiesel

*Calophyllum inophyllum* seeds are resinous (10-20%) and rich in oil (Dweck and Meadows 2002). It is often difficult to expel oil from resinous oil seeds by employing conventional techniques. Cold press extraction of resinous kernels has rarely been reported in scientific literature. The effect of steam conditioning and the method of desiccation on oil yield of resinous kernels have never been studied before.

Highly viscous (62 cSt), highly acidic (22 mg KOH/kg) Calophyllum oil cannot be converted into suitable quality biodiesel by conventional protocols. Specific protocols have been reported by some authors (Sahoo et al., 2006; Venkanna and Reddy, 2009). However, those protocols have a number of fundamental drawbacks according to the reports of Freedman and Pryde (1982), Mittelbach et al. (1992), Liu (1994), and Canakci and Van Gerpan (2001). This necessitated the testing of the effectiveness of those modified protocols. If those protocols happen to be less effective, an improved protocol needs to be developed to convert Calophyllum oil into biodiesel that would comply with industrial standards.

### 1.2.4 Knowledge Gaps

The literature shows contrasting reports about seed storage behaviour of *C. inophyllum*. According to Allen (2002) *C. inophyllum* seeds are recalcitrant but Ng (1992) argues that they are more likely to be intermediate.
According to the database of Kew botanic gardens, the storage behaviour of *C. inophyllum* is inconclusive. Further studies have to be carried out to determine its storage behaviour.

Information on flowering and fruiting phenology and the habitats and biophysical limits of this species is also quite sparse. Studying reproductive phenology is important to understand potential yield and timing of harvest time. Changes in oil content and fatty acid profile during fruit development is another important aspect of an oil crop and no information can be found on the fruit development of *C. inophyllum*.

There are also knowledge gaps in the potential uses of calophyllum kernel oil. Even though there are a few studies on the conversion of calophyllum oil into biodiesel (Sahoo et al. 2007; Venkanna and Reddy 2009), neat calophyllum oil methyl ester (COME-B100) has never been characterised for American Standard for Testing Materials (ASTM) standards and there are no reports on the corrosiveness of COME. Performance of COME has not yet been tested by a chassis dynamometer and no one has demonstrated the real-time mechanical reliability of COME using a vehicle.

The effect of COME on metallic and elastomer component of diesel engines has never been studied before. Studying the above aspects is important to predict outcomes of using COME blends over an extended period of time.

Inherent oxygen molecules in FAME improve combustion characteristics in biodiesel blends. This could be indirectly studied by comparing vibrations of an engine running with mineral diesel and biodiesel blends. This aspect of COME has never been investigated before and the outcomes may indicate the potential of COME as a fuel additive/oxidizer.

### 1.3 Objectives

#### 1.3.1 Primary Objective

- To evaluate the potential of calophyllum tree as a biodiesel feedstock species by studying major aspects of biodiesel production
1.3.4 Secondary Objectives

- To highlight the economic importance of calophyllum through a broad literature review.
- To determine the environmental conditions that favour the growth and yield of *C. inophyllum* by studying provenance variations in stand characteristics and correlating stand parameters with climatic and soil variables.
- To determine the most ideal harvesting time by examining the reproductive phenology, and changes in Fatty Acid Profile (FAP) and oil content during fruit development.
- To investigate the seed storage behaviour and provenance variations in seed morphology, germination and early growth response of *C. inophyllum*.
- To assess provenance variations in kernel oil, fatty acid profiles and kernel oil methyl ester (biodiesel) characteristics of *C. inophyllum* with the view to select the most suitable provenances/cultivars for establishing biodiesel feedstock plantations.
- To develop an improved protocol to convert calophyllum kernel oil into biodiesel that conforms to ASTM standards, and to evaluate the engine performance and mechanical reliability of COME.

1.4 Benefits to CQUniversity

- There is a considerable potential of conceiving intellectual properties.
- This research project focuses on strategic issues and flagship areas of CQUniversity and addresses national issues.
- Creates opportunity to develop collaborative projects with local industries.
- Regional expertise in biodiesel production.
1.5 Research Approach

The Concept

Calophyllum is a viable source to produce biodiesel in Australia.

Claims

*Calophyllum inophyllum* is a profusely fruiting native species and kernels contain high amounts of inedible oil. It can be grown in marginal areas and its kernel oil can be converted into acceptable quality biodiesel and there may be provenance variations in stand characteristics, oil content, and fatty acid profiles (FAP) and the fatty acid methyl ester (FAME-biodiesel) properties. Studying these variations may help to identify suitable provenance/s and/or favourable growth conditions for feedstock plantations in Australia. Testing COME for engine performance will ascertain the feasibility of using it as a commercial transportation fuel.

1.6 Thesis Structure

This study mainly focuses on producing an industrially conforming biofuel from calophyllum oil. The nature of this research and its potential industrial implications directs the thesis into a relatively different structure (Fig 1.1). Studying transportation fuels demands a distinct approach which is similar to its life cycle assessment; “well to wheel” (Fulton 2005). In the current situation the approach becomes “feedstock to engine performance”.

This thesis is horizontally structured to cover major aspects of the biofuel production such as; source variations in feedstock, harvesting times, seed biology and storage behaviour, oil extraction methods, provenance variations in oil chemistry and its bearing on biodiesel characteristics, conversion protocols, characterization and finally engine performance (Fig 1.1). While achieving the said objectives, this approach also unearths some lesser known aspects of this species which could provide basis for future research.
Fig 1.1 Research approach
This chapter presented a generalized introduction to “Beauty Leaf Tree” and its potential economic uses. Chapter 1 also described research issues, the knowledge gap, project justification, objectives, possible outcomes, and thesis structure and research approach.

Chapter 2 details the study area, site selection and general sampling methods.
CHAPTER 2

Study Area & Site Selection

2.1 Defining the Study Area

Field studies were carried out in ten provenances (Fig 2.1, Tables 2.1 and 2.2); six in northern Australia (Townsville, Yeppoon, Cardwell, Bowen, Mackay and Darwin) and four in Sri Lanka (Anuradhapura, Colombo, Kurunegala and Matara), which were selected based on herbarium records and personal contacts. The selected provenances of each country are distinct in terms of their climate (Fig 2.13 and 2.14), topography and soil conditions and they are at least 100 km apart from one another. Information on life histories of the selected stands was obtained from local authorities, botanic gardens and land owners. In this study provenance refers to the maternal environment and its potential influence on phenotypic variations.

Fig 2.1 Selected provenances in Queensland and Northern Territory, Australia (for numbers see table 2.1) (www.anra.gov.au/.../images/veg_type/pre-aus.gif)
Table 2.1: Geo-climatic data of northern Australian provenances

<table>
<thead>
<tr>
<th>Provenance</th>
<th>Coordinates</th>
<th>Altitude from MSL (m)</th>
<th>Mean Annual Rainfall (mm)</th>
<th>Mean Annual Temperature Max/Min(ºC)</th>
<th>Soils*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Townsville</td>
<td>19° 13' 0&quot; S, 146° 48' 0&quot; E</td>
<td>3.5</td>
<td>1124.8</td>
<td>28.9/19.8</td>
<td>Mungol and submature black earths</td>
</tr>
<tr>
<td>2. Yeppoon</td>
<td>23° 07' 42&quot; S, 150° 44' 34&quot; E</td>
<td>5</td>
<td>870.1</td>
<td>25.9/18.5</td>
<td>Rundle, Shallow stony browns, clay loams and sandy clay loams</td>
</tr>
<tr>
<td>3. Cardwell</td>
<td>18° 16' 0&quot; S, 146° 1' 60&quot; E</td>
<td>5</td>
<td>2125.3</td>
<td>28.7/18.9</td>
<td>Solonetz, Planosols, calcareous dune sand</td>
</tr>
<tr>
<td>4. Bowen</td>
<td>20° 01' 0&quot; S, 148° 13' 60&quot; E</td>
<td>3</td>
<td>864</td>
<td>28.6/19.7</td>
<td>Mottled subsoil dune sand</td>
</tr>
<tr>
<td>5. Mackay</td>
<td>21° 08' 36&quot; S, 149° 11' 12&quot; E</td>
<td>3</td>
<td>1522.6</td>
<td>27.0/17.7</td>
<td>Andergrove calcareous variant and sandy loams</td>
</tr>
<tr>
<td>6. Darwin</td>
<td>12° 27' 0&quot; S, 130° 50' 0&quot; E</td>
<td>5</td>
<td>1712.9</td>
<td>32.0/23.2</td>
<td>Regosols, Leptosols and Planosols</td>
</tr>
</tbody>
</table>

*Australian soil resources information system ([http://www.asris.csiro.au](http://www.asris.csiro.au))

Fig 2.2 Selected provenances in Sri Lanka (for numbers see table 2.2) ([www.agridept.gov.lk/HORDI/images/SoilFig1.jpg](http://www.agridept.gov.lk/HORDI/images/SoilFig1.jpg))
Table 2.2: Geo-climatic data of selected provenances in Sri Lanka

<table>
<thead>
<tr>
<th>Provenance</th>
<th>Coordinates</th>
<th>Altitude from MSL (m)</th>
<th>Mean Annual Rainfall (mm)</th>
<th>Mean Annual Temperature Max/Min(ºC)</th>
<th>Soils*</th>
</tr>
</thead>
<tbody>
<tr>
<td>7. Anuradhapura</td>
<td>08° 20' 60&quot; N, 80° 22' 60&quot; E</td>
<td>75</td>
<td>1094</td>
<td>32.1/23.3</td>
<td>Reddish brown earths &amp; immature brown loams</td>
</tr>
<tr>
<td>8. Colombo</td>
<td>6° 54' 0&quot; N, 79° 50' 0&quot; E</td>
<td>59</td>
<td>2500</td>
<td>30.6/24.1</td>
<td>Red-yellow podzolic with soft and hard laterite, dark heavy clay soils.</td>
</tr>
<tr>
<td>9. Kurunegala</td>
<td>7° 45' 0&quot; N, 80° 15' 0&quot; E</td>
<td>55</td>
<td>1500</td>
<td>31.7/22.8</td>
<td>Red yellow podzolic soils with strongly mottled subsoil, low humic gley soils, regosols on red and yellow sands</td>
</tr>
<tr>
<td>10. Matara</td>
<td>5° 56' 55&quot; N, 80° 32' 34&quot; E</td>
<td>40</td>
<td>2150</td>
<td>29.3/24.1</td>
<td>Alluvial soils, red-yellow podzolic with soft and hard laterite</td>
</tr>
</tbody>
</table>

*Soil Science Society of Sri Lanka (http://www.ssssl.org/Bmark.htm)
## 2.1.1 Study Sites in Australia

<table>
<thead>
<tr>
<th>Provenance</th>
<th>Sites</th>
<th>Origin</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Australia</strong></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
| 1. Townsville (19° 13’ 0” S, 146° 48’ 0” E) | Queens garden (3 trees)  
The Strand beach (Fig 2.3, 7 trees) | Inconclusive |
| 2. Yeppoon (23° 07’ 42” S, 150° 44’ 34” E) | Rosslyn bay (Fig 2.4, 3 trees)  
Bell park (1 tree) | Byfield |
| 3. Cardwell (18° 16’ 0” S, 146° 1’ 60” E) | Marine Pde. (beach side 3 trees)  
Cardwell beach (Fig 2.5, 3 trees)  
Victoria St. (beach side 8 trees) | Cardwell |
| 4. Bowen (20° 1’ 0” S, 148° 13’ 60” E) | Queens beach recreational area 1 (5 trees)  
Queens beach recreational area 2 (Fig 2.6, 5 trees) | Bowen |
| 5. Mackay (20° 08’ 36” S, 149° 11’ 12” E) | Seaforth beach (1 tree)  
Campwin (2 trees)  
Sarina beach 3 (Fig 2.7, 1 tree) | Unknown |
| 6. Darwin (12° 27’ 0” S, 130° 50’ 0” E) | 1 km before the entrance of East point nature reserve (3 trees)  
East point nature reserve (8 trees)  
Near Tortfield St. (Fig 2.8, 3 trees) | Indonesia |
| **Sri Lanka** | | |
| 7. Anuradhapura (08° 20’ 60” N, 80° 22’ 60” E) | Thamburthtegama (Fig 2.9, 3 trees)  
Thalawa (3 trees) | Origin - Wariyapola, an inland area of intermediate zone (mean annual rainfall 1500-2500 mm) |
| 8. Colombo (6° 54’ 0” N, 79° 50’ 0” E) | Polkotuwa (3 trees)  
Diyagama (Fig 2.10, 4 trees)  
Meegodda (8 trees) | Origin - Inland areas of Colombo, wet zone (mean annual rainfall ≥2500 mm) |
| 9. Kurunegala (7° 45’ 0” N, 80° 15’ 0” E) | Kokkawila (Fig 2.11, 3 trees)  
Boraluwewa (3 trees) | Origin - Halawatha, coastal area of dry zone (mean annual rainfall < 1500 mm) |
| 10. Matara (5° 56’ 55” N, 80° 32’ 34” E) | Akuressa 1 (Fig 2.12, 4 trees)  
Ruhuna University (3 trees)  
Akuressa 2 (3 trees) | Origin - Matara, coastal area of intermediate zone (mean annual rainfall 1500-2500 mm) |
Fig 2.9 Thambuththegama, Anuradhapura

Fig 2.10 Diyagama, Colombo

Fig 2.11 Kokkawila, Kurunegala

Fig 2.12 Akuressa, Matara
Fig 2.13 Long term climatic data of the selected sites in Australia; 1-Townsville, 2-Yeppoon, 3-Cardwell, 4-Bowen, 5-Mackay, 6-Darwin (source: http://www.bom.gov.au/)

Mean minimal monthly temperature °C  Mean maximum monthly temperature °C  Mean monthly rainfall (mm)
Fig 2.14 Long term climatic data of the selected sites in Sri Lanka; 7-Anuradhapura, 8-Colombo, 9-Kurunegala, 10-Matara (www.meteo.slt.lk/)

Mean minimal monthly temperature °C   Mean maximum monthly temperature °C   Mean monthly rainfall (mm)
2.2 Sampling Method

Sampling was greatly restrained by the small size of the stands found in each provenance. Based on the availability of stands, number of sites selected per provenance varied from 2-3. From each site (2-8) morphologically superior (diseases and defects free and have a healthy form) trees having GBHOB>100 cm were chosen. In general fruit yield in trees increases with its age and once they attain a certain mature age remains steady for a certain period before it declines slowly. While selecting trees GBHOB>100 cm, it was assumed that the selected trees fall under their steady growth phase (average girth of a mature *C. inophyllum* tree (average 70 years) is approximately 116 cm (Soerlanegara and Lemmens 1994).

This chapter outlined the study area; Australian and Sri Lankan provenances and described the site selection criteria, sampling methods and the limitations.

The next chapter (Literature Review) is structured to revitalize the multiple economic uses of *calophyllum* and unveils a number of areas to conduct further research.
CHAPTER 3

Literature Review

3.1 Botanical Description

*Calophyllum inophyllum* L. is a medium-large evergreen tree that grows up to 8–20 m in height. It bears a broad spreading crown of irregular branches (Fig 3.1). The tree carries a thick canopy of glossy, elliptical leaves, fragrant white flowers, and large round nuts (Flora of Australia online 2008).

**Species:** *Calophyllum inophyllum* L.

**Family:** Clusiaceae

**Common names:** Alexandrian laurel, Beauty leaf, Ball nut, Beach mahogany, Domba (Sri Lanka)

![Image of Calophyllum inophyllum tree in Hawaii](Fig 3.1 Calophyllum inophyllum tree in Hawaii (Friday and Okano 2006))
3.1.1 Flowers

Flowers can generally be found in racemes or paniculate inflorescences (Fig 3.2). The inflorescence contains clusters of 4–15 fragrant white flowers (2.5 cm in diameter and 8–14 mm in length) on long, sturdy stalks in leaf axils. Flowers contain 4–8 oblong petals. Stamens are numerous, yellow and grouped in four bundles. Colour of anthers usually changes from deep yellow to brown. Only the hermaphrodite flower has an ovary, a bright pink ball that can be found at the end of the pedicel when the petals drop (Agroforestry Tree database 2007).

![Inflorescence and leaves of *Calophyllum inophyllum*](image)

3.1.2 Leaves

Leaves are opposite, dark green, thick, smooth and polished, ovate, elliptical, obovate or oblong and 8-20 cm long (Fig 3.2). They are rounded to cuneate at the base, rounded, retuse or subacute at the apex with latex canals that are usually less prominent. Stipules are absent. Leaf veins run parallel to each other and perpendicular to the midrib. The scientific name *Calophyllum* comes from the Greek words for “beautiful leaf” (Friday and Okano 2006).
3.1.3 Fruits

The ball-shaped (round) light green fruits (drupe) occur in clusters (Fig 3.3A). Fruits have an average diameter of 2–5 cm. The outer skin turns yellow when ripe and then becomes brown and wrinkled when dry. It consists of a thin pulp, the shell, a corky inner layer, and a single seeded kernel. Fruits are normally borne twice a year from April–June and October–December (Friday and Okano 2006).

3.1.4 Seeds

One large brown seed (2–4 cm in diameter) is found in each fruit. A stone hard seed coat which has a spongy inner layer protects large cotyledons and the radicle which points towards the base of the fruit (Fig 3.3B). Seeds are naturally dispersed by water (Agroforestry Tree Database 2007). In Sri Lanka, calophyllum seeds are mostly dispersed by fruit bats (Personal Observation 2008).
3.2 Ecology and Distribution

3.2.1 Geographic Distribution

*Calophyllum inophyllum* is native to the following countries;

Aruba, **Australia**, Cambodia, Cook Islands, Fiji, French Polynesia, India, Indonesia, Japan, Kiribati, Laos, Madagascar, Malaysia, Marshall Islands, Myanmar, New Caledonia, Norfolk Island, Papua New Guinea, Philippines, Reunion, Samoa, Solomon Islands, **Sri Lanka**, Taiwan, Province of China, Thailand, Tonga, Vanuatu, a Vietnam (Agroforestry Tree Database 2007).

And it is exotic to the following countries;

Djibouti, Eritrea, Ethiopia, Kenya, Nigeria, Somalia, Tanzania, Uganda and United States of America (Agroforestry Tree Database 2007).

3.2.2 Natural Habitat

*Calophyllum inophyllum* naturally occurs above the high-tide level along the coastal line of northern Australia and extends throughout Southeast Asia and southern India (Figs 3.4a and 3.4b). It thrives on sandy beaches and coastal sand dunes. However, it is sometimes found inland on different types of soils i.e. heavy clay bog soils, red yellow podzolic soils and laterite soils (personal observation 2008). It generally grows on the debris carried in rivers and on the sand dunes formed by wind and waves. The top soil layer beneath trees is usually dry on the surface, but the water table can be found a few decimetres below the surface. The tree usually taps brackish water. The tree is light tolerant, requires warm temperatures and the growth in most natural habitats is influenced by the distance to the sea and by the breezes. The sandy soil, exposure to sunlight, the heat from the sand, and salty winds make its habitat xerophytic (Agroforestry Tree Database 2007).
Fig 3.4a *Calophyllum inophyllum*; global distribution map.


Fig 3.4b *Calophyllum inophyllum* distribution map for Australia (source; Australia’s virtual herbarium, http://www.chah.gov.au/avh/avhServlet?task=showMap (viewed in 26/03/2012).
3.2.3 Biophysical Limits

*Calophyllum inophyllum* prefers altitudes ranging from mean sea level to 200 m, and mean annual temperatures between 7-18 °C to 37-48 °C. It can tolerate a drought period up to 4-5 months and rainfall between 750-5000 mm. However, *C. inophyllum* is sensitive to frost and fire. It grows best on well-drained sandy soils in coastal areas but can tolerate clays, calcareous soils and rocky soils and can survive on a range of soil pH levels from 7.4 to 4.0. Calophyllum is considerably salt tolerant (Friday and Okano 2006; Kathiresan and Ramanathan (undated); Beach Protection Authority, EPA Queensland 2003).

3.3 Reproductive Biology

The bisexual flowers are pollinated by insects such as bees. The flowering and fruiting periods vary from region to region. In South Asia, flowers appear in May-June and sometimes again in November. *Calophyllum inophyllum* seeds sometimes show polyembryony which is believed to have resulted from apomixis (Friday and Okano 2006). However, Gupta et al. (2009) reported that calophyllum seed is a true seed. Calophyllum trees often bear fruits throughout the year.

3.3.1 Flowering and Fruiting

The tree flowers twice a year in the northern hemisphere, in the late spring/early summer and late fall. In Sri Lanka, flowers appear in March-April and fruits ripen in May-June, although both flowers and fruits can be found at other times of the year (Dassanayake and Fosberg 1980). In Northern Australia (Southern hemisphere), calophyllum flowers in January and June and fruits mature in April and September (Friday and Okano 2006). Young trees begin flowering after 7 or 8 years in wild.
3.3.2 Propagation Methods

Natural regeneration usually occurs under the mother tree. Woody fruits do not open easily, and the thick shell of the seed acts as a physical barrier which delays its germination. Germination under natural conditions may therefore be delayed for a considerable time until the skin has softened or partially decomposed (Agroforestry Tree Database 2007).

Seeds can be used as a potentially good and convenient planting material, provided the seed is sown soon after ripening. Seeds can be soaked in water to remove any inhibitors and to facilitate complete imbibition of seeds prior to sowing (Liyagel 2005). Calophyllum seeds have a reasonable germination capacity, and experiments conducted in the Philippines have shown that complete removal of the seed shell is very effective, both in reducing the mean germination time (MGT) and in increasing the germination capacity. As a result of this treatment, the germination period was reduced from 57 to 22 days and the germination percentage rose from 63% to 93% (Parras, 1939; Wilkinson and Elevitch, 2004). Seedlings grown in a nursery require shade.

3.3.3 Seed Storage

Ripened fruits can easily be collected from the soil surface under trees. There are two fruiting seasons in most locations. Seed storage behaviour of C. inophyllum is inconclusive due to contrasting reports that are found in literature. Some authors suggest that it is a recalcitrant seed (Allen 2002) while others reported it as an intermediate seed (Ng 1992; Gupta et al. 2009). The seeds are very oily and lose their viability in a short period of time (Dweck and Meadows 2002; Agroforestry Tree Database 2007).

3.3.4 Tree Management

The tree is believed to be brittle and susceptible to wind damage. Seedlings can grow up to 90 cm in the first year after planting, and demonstrate a constant growth rate for about 5 years. After the initial fast growing phase, growth becomes very slow. Frequent weeding is essential until the crop is established.
A number of plantation trials in Indonesia have used (2 m x 3 m) spacing effectively (Friday and Okano 2007). *Calophyllum inophyllum* produces coppices moderately. Selective cutting system and removal of defective trees can be done to enhance regeneration.

### 3.4 Multiple Uses of *Calophyllum inophyllum*

The tree has multiple uses and services. Table 3.1 summarizes some of the uses and services that can be found in the literature.

**Table 3.1: Multiple uses of *Calophyllum inophyllum* based on the cited literature**

<table>
<thead>
<tr>
<th>Use</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pharmaceutical and cosmetic products</td>
<td>Dweck and Meadows 2002;</td>
</tr>
<tr>
<td>Potential source for biodiesel</td>
<td>Azam et al. 2005; Agarwal 2006; Sahoo et al. 2007.</td>
</tr>
<tr>
<td>Stabilizing coastal dunes and guarding against salt spray</td>
<td>Agroforestry tree database 2007; Leaflet 4: Beach Protection Authority EPA Qld. 2003</td>
</tr>
<tr>
<td>Ornamental tree</td>
<td>Agroforestry tree database 2007; Friday and Okano 2006.</td>
</tr>
<tr>
<td>As a natural pesticide</td>
<td>Agrawal and Mall 1984; Palaniswami and Chattopadhyay 2006; Pushpalatha and Muthukrishnan 1999.</td>
</tr>
</tbody>
</table>
3.4.1 Timber Properties of *Calophyllum inophyllum*

The sapwood is yellow-brown with a pink tinge and is well defined from the heartwood, which is deep red, red-brown, pink-brown or orange-brown. Vessels are large to medium in size, solitary, and are arranged in oblique flares. Vessel lines are very prominent on dressed surfaces and darker than the surrounding tissue (Fig 3.5) (Timber Species Notes DPI Queensland 2007).

The timber is generally slightly heavier, stronger and more durable than that of other *Calophyllum* species (Gamble 1972). The wood is often fine textured, and the grain is more interlocked. The density is 560-900 kg/m³ at 15% moisture content (Timber Species Notes DPI Queensland 2007).

![Fig 3.5 Cross section of *Calophyllum inophyllum* wood (A-vessels, B-medullary ray, C-fibres), source: Richter and Dallwitz (2000).](image)

*Calophyllum inophyllum* is a good general-purpose timber (Maiden 1975). Mechanical handling is easy (MTC wood wizard 2006). In several regions, the wood is much sought after for masts, spars, bridgework and scaffolding because of the tall, slender form of the poles. Being close-grained and durable, the wood is used for boat building, railway sleepers, veneer and plywood; being of a rich reddish-brown, it is excellent for cabinet making.
3.4.2 Traditional Uses of Calophyllum inophyllum

The bark usually contains 11-19% tannins, but a certain percentage of tannins are often present in the leaves. Polynesians use these tannins to harden their handcrafted fishnets (Friday and Okano 2006). A decoction of the bark is sometimes used to toughen and dye fishing nets. The seed oil and the latex have occasionally been used in dyeing batik cloth in Java (Dweck and Meadows 2002). The kernels yield 50-73% of a bluish-yellow to dark green viscous oil which is known as ‘Domba’ oil (Sri Lanka), Pinnai oil (South India), or Dilo oil (Fiji) (Drury 1873 cited in Dweck and Meadows 2002). It has a disagreeable taste and odour, as it contains some resinous material that can be conveniently removed by refining (Maiden 1975).

The concentration of resinous substances in the oil varies from 10 to 30% and it makes calophyllum oil an ideal source for making varnish. Calophyllum oil is of excellent quality for soap manufacture. Indian traditional communities have used oil extracted from kernels to light lamps (Lullfitz 1978). The fragrant flowers are used to make bouquets and wreaths and are also worn in the hair by Philippino women. In Hawaii the timber is traditionally famous for boat making (Krauss 1980).

3.4.3 Aboriginal Medicinal Uses of Calophyllum inophyllum

Australian aborigines used to rub crushed nut externally on the skin to treat aches and pains (Low 1990). Primitive tribes of a native community of Papua New Guinea utilize the leaves frequently for different kinds of skin problems. In Manus, the leaves are heated over a fire until soft and then applied to skin ulcers, boils, cuts, sores, and pimples, while on Dobu Island, leaves are boiled and a skin rash is washed periodically with the solution (Dweck and Meadows 2002).

The native people in New Caledonia and in Samoa also utilize these leaves for treating skin inflammations, leg ulcers and wounds. Water in which the macerated leaves have been soaked for some time has been used by them and other tribes to treat haemorrhoids (Quisumbing 1951 cited in Dweck and Meadows 2002).
3.4.4 Medicinal Properties of Calophyllum inophyllum

Calophyllum is well known throughout the world for its medicinal value. Almost all parts of the plant (i.e., bark, leaves and seeds) have been used medically as antiseptics, astringents, expectorants, diuretics, and purgatives. The oil possesses antimicrobial and antiviral activity (Dweck and Meadows 2002).

3.4.4.1 Leaf

Leaves are infused in water, and the oil that rises to the surface is used for sore eyes (Nadkarni and Nadkarni 1999). The leaf infusion is also taken internally for heatstroke and used in combination with an external application of the root decoction.

3.4.4.2 Gum and Resin

The gum extracted from the plant (from the wounded bark) is emetic and purgative but also can be applied to wounds and ulcers. It can also be mixed with strips of bark. The resin is said to be responsible for the colour and the odour of the oil. The resin can be poisonous; it is also said to contain benzoic acids (Quisumbing 1951 cited in Dweck and Meadows 2002). The gum resin is said to be good for old sores and wounds. The resin may be useful for chronic cataract. However, resin is not collected or used in Australia (Maiden, 1975).

3.4.4.3 Bark

The bark is astringent and its juice is purgative (Nadkarni and Nadkarni 1999). It is considered medicinal in Asia. According to some it is an Indo-Chinese medicine for orchitis (Quisumbing 1951 cited in Dweck and Meadows 2002). In Indonesia, it is used after childbirth to reduce vaginal discharge, the passing of blood and also to treat gonorrhoea (Burkill 1994). It is used in decoction for internal haemorrhages and as a wash for indolent ulcers (Nadkarni and Nadkarni 1999). The bark acts as an antiseptic and disinfectant. Ground bark and lime juice paste make a useful application for armpits, groins and feet in bromidrosis.
The bark taken internally acts as an expectorant and is useful in chronic bronchitis and phthisis. The resin is mixed with strips of bark and leaves, steeped in water and the oil which rises to the surface is a household application for sore eyes. The astringent juice of the bark is a purgative and given in the form of a decoction for internal haemorrhages.

3.4.4.4 Root

A decoction of the root is employed for dressing ulcers and also for application in heatstroke. It is taken internally for a stitch (Quisumbing 1951 cited in Dweck and Meadows 2002).

3.4.4.5 Kernel Oil

*Calophyllum inophyllum* oil is in considerable commercial demand (Fig.3.6). The oil is expelled from the seeds (about 60% on dry weight basis). In Europe the *Calophyllum* oil has been proven to be useful in treating rheumatism, pruritus and scabies (Drury 1873 cited in Dweck and Meadows 2002). The oil is also used in cases of gonorrhoea and gleet{s (Nadkarni and Nadkarni 1999).

![Calophyllum oil: A-Cold pressed (Kilham 2004), B-Chemically extracted](http://www.thesage.com/images/prod/300-1361.jpg)
The oil is useful for dermal problems and is an ancient treatment for leprosy (Burkill 1994). Bruised seeds and oils are applied to chronic rheumatism, inflammation of bones and joints and ankylosis (Maiden 1975). The oleoresin is supposed to be beneficial for lung ailments if taken internally and can be applied to chronic ulcers and wounds externally. The oil also bears an ability to heal atonic wounds, physical and chemical burns, radio-dermatitis, anal fissures and post-surgical wounds.

3.4.5 Scientifically Known Medicinal Properties of Calophyllum inophyllum

3.4.5.1 Antibacterial Activity (In vitro)

Yimdjo et al. (2004) studied the antibacterial effect of xanthones isolated from Calophyllum inophyllum. Caloxanthone-A (Fig 3.7), calophylic acid, brasiliensic acid, inophyloidic acid, calophyllolide, and inophyllum C and E were found to inhibit Staphylococcus aureus (Fig 3.8) at a dose of 20 µg per disc in agar under diffusion assay. However, inhibition of Vibrio anguillarium, Escherichia coli, and the yeast Candida tropicalis was found to be far less pronounced.

Fig 3.7 Caloxanthone A¹        Fig 3.8 Streptococcus aureus²

¹Dweck and Meadows, 2002
²http://tymask.files.wordpress.com/2008/06/staphylococcus-aureus.jpg
3.4.5.2 Antiviral Activity (*In vitro*)

Various scientists have examined antiviral activity of various chemical compounds of *C. inophyllum*. They found inophyllums and coumarins that can be used as novel non-nucleoside inhibitors of human immunodeficiency virus type 1 reverse transcriptase. Inophyllum B (Fig 3.9A) was active against HIV-1 (Fig 3.9B) in cell culture with a dose of 1.4 mmol/L. Inophyllum P (soullatrolide) was also found to have potential anti-HIV activity (Patil et al. 1993; Taylor et al. 1994; Spino et al. 1998). Pawar et al. (2007) found anti-HIV dipyranocoumarin expression in callus cultures of *Calophyllum inophyllum*.

3.4.5.3 Anticancer Activity (Animal studies)

Ten natural 4-phenylcoumarins that were isolated from *C. inophyllum* have been found to inhibit (except Inophyllum C and Calocoumarin C) Epstein-Barr virus early antigen in Raji cells. Among them, Calocoumarin-A (Fig 3.9C) exhibited the most potent inhibitory activity in a 2-stage mouse skin carcinogenesis test. It might be used as a potential anti-tumour promoter for melanoma (Fig 3.9D) (Itoigawa et al. 2001).

![Fig 3.9 A- Inophyllum B¹, B- HIV virus*, C- Calocoumarin A³, D- Skin cancer lesion²](http://www.naturalbuy.com/wp-content/uploads/2009/03/hiv.jpg)

¹Laure et al. 2008.
³Itoigawa et al. 2001
3.4.6 Calophyllum inophyllum as a Natural Pesticide

Palaniswami and Chattopadhyay (2006) demonstrated that sweet potato weevil can be successfully controlled by application of \( C. \text{inophyllum} \) mulch at 2 t ha\(^{-1} \). Agarwal and Mall (1984) reported the insecticidal effect of \( \text{Calophyllum inophylum} \) leaf extracts. Some authors have also observed an anti-larval effect of leaf and seed extracts against the larvae of \( \text{Culex quinquefasciatus} \), \( \text{Anopheles stephensi} \) and \( \text{Aedes aegypti} \) (Pushpalatha and Muthukrishnan, 1999).

3.4.7 Calophyllum inophyllum as a Feedstock for Biodiesel Production

\( \text{Calophyllum inophyllum} \) has many attributes which qualifies it as a biodiesel feedstock (raw material). Biodiesel is an alternative transportation fuel which can be used in compression ignition (CI) engines and essentially requires very little or no engine modification because it has properties similar to mineral diesel. Due to its high flash point it can be stored just as mineral diesel and hence does not require separate infrastructure. Biodiesel is usually derived from vegetable oil or animal fat by a chemical process called transesterification where triglycerides of an oil or fat are converted into mono fatty acid (methyl or ethyl) esters (Ma and Hanna 1999).

Transesterification generally reduces the viscosity of oil by approximately one third of its original value (Peterson et al. 1986) thereby making it suitable for normal diesel engines. The use of biodiesel in conventional diesel engines results in substantial reduction in emission of unburned hydrocarbons, carbon monoxide and particulate matter (Agarwal 2006; Sahoo et al. 2007).

In many parts of the world calophyllum has two fruiting seasons. The tree attains its reproductive maturity at 7 years and continues to fruit up to two hundred years. In some places flowering and fruiting can be observed throughout the year (Foxworthy 1927; Agroforestry Tree Database 2007).
*Calophyllum inophyllum* is non-aggressive, non-invasive, yields 6000-10000 fruits tree\(^{-1}\) season\(^{-1}\), kernels contain up to 65% inedible oil and tolerates harsh environmental conditions (acidity, salinity, drought) and requires little maintenance (Little and Skolmen 1989; Hathurusingha and Ashwath 2007).

Azam et al. (2005) reported *Calophyllum inophyllum* oil to be one of the best sources for making biodiesel in India based on its high oil content and conforming fatty acid profile (US, EU and German biodiesel standards). According to Azam et al. (2005), four hundred trees of *C. inophyllum* in one hectare can produce approximately 4680 kg of oil year\(^{-1}\) (approx. 6500 L ha\(^{-1}\) year\(^{-1}\)). The oil can then be converted to approximately 5785 L of biodiesel ha\(^{-1}\) year\(^{-1}\) by a modified 4-stage conversion protocol (at 89% efficiency) (Hathurusingha et al. 2010b).

### 3.4.7.1 Properties of calophyllum Oil

Properties of biodiesel are directly influenced by the physicochemical properties of the feedstock (Knothe 2005; Ramos et al. 2009). Calophyllum oil is dark green in colour at 25 °C and has a disagreeable odour and on saponification yields a bright yellow soap (Maiden 1975). The oil is highly viscous (71.98 cSt) at 40 °C (Sahoo et al. 2007) and acidic (22 mg KOH/g) (Crane et al. 2005). The lipid composition of *C. inophyllum* seed oil is dominated by neutral lipids (92%). The rest is accounted by glycolipids (6.4%) and phospholipids (1.6%) (Hemavathy and Prabhakar 1990).

According to Crane et al. (2005) crude calophyllum oil is dominated by triacylglycerol (76.7%) followed by diacylglycerol (7%) and free fatty acids (5.1%). Calophyllum oil contains a small amount of hydrocarbons and has a gross heat value of 7554 cal/g or 31.62 MJ/kg (Augustus and Seiler 2001). *Calophyllum inophyllum* oil contains a higher amount (70.8%) of unsaturated fatty acids (oleic, linoleic and linolenic) than saturated fatty acids (myristic, palmitic, stearic, behenic and ecosanic) (Ajayi et al. 2008; Azam et al. 2005; Crane et al. 2005; Sahoo et al. 2007).
Presence of saturated fatty acids has both advantages and disadvantages as a biodiesel feedstock. Saturated fatty acid alkyl esters increase the cetane number (CN) and stability of the resultant biodiesel (Knothe et al. 2003; Sahoo et al. 2007). Higher CN implies better ignition characteristics, short ignition delay and low knocking (Knothe 2005). However, higher amounts of saturated fatty acids also increase the cloud point which is an undesirable feature. Unsaturated fatty acids esters help to maintain the resultant biodiesel in liquid form, but excess amounts of poly-unsaturated fatty acid esters can form hard peroxide polymers under heat which can block injectors (Azam et al. 2005).

There are marked variations (Table 3.2) among the fatty acid compositions of calophyllum oil reported by different authors (Ajayi et al. 2008; Crane et al. 2005; Sahoo and Das 2009) which indicate that there could be source-related variability.

Table 3.2: Fatty acid composition of *Calophyllum inophyllum* oil (%)

<table>
<thead>
<tr>
<th>Fatty Acid</th>
<th>Short code</th>
<th>Percentage of fatty acids (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Palmitic Acid</td>
<td>P</td>
<td>12.0 13.7 14.6</td>
</tr>
<tr>
<td>Stearic Acid</td>
<td>St</td>
<td>12.9 14.3 16.5</td>
</tr>
<tr>
<td>Oleic Acid</td>
<td>O</td>
<td>34.1 39.1 39.8</td>
</tr>
<tr>
<td>Linoleic Acid</td>
<td>L</td>
<td>38.3 31.1 27.6</td>
</tr>
<tr>
<td>Linolenic Acid</td>
<td>Ll</td>
<td>0.3   0.3   0.4</td>
</tr>
<tr>
<td>Arachidic Acid</td>
<td>A</td>
<td>-     0.6   -</td>
</tr>
<tr>
<td>Behenic Acid</td>
<td>B</td>
<td>-     0.2   -</td>
</tr>
</tbody>
</table>

Crane et al. (2005) reported the percentages of different forms of triacylglycerides (TAGs) for calophyllum kernel oil. According to them TAGs in *C. inophyllum* oil exists in the following order; OLL (10.8%), POL (10.7%), OOL (10.4%), StOL (9.2%), StOO (8.5%), POO (8.4%) and OOO (7.2%). *Calophyllum inophyllum* contains a lower amount of monounsaturated forms of TAGs (e.g. PPO, StStO, PStO, etc.) and a higher amount in di and tri-unsaturated forms of TAGs (POO, StOO, OOO, etc.). This indicates that triglycerides in calophyllum oil are dominated by poly-unsaturated fatty acids.
Some authors have reported that Fatty Acid Methyl Ester (FAME) of calophyllum oil meets the major specifications of US biodiesel standard (ASTMD 6751-02, ASTMPS 121-99), Germany (DIN V 51606) and European Standard Organization (EN 14214) (Azam et al. 2005).

The following are some of the standard quality values of Calophyllum oil methyl ester (biodiesel) (Azam et al. 2005).

   (i) Saponification Number (SN) 201.4
   (ii) Iodine Value (IV) 71.5
   (iii) Cetane Number (CN) 57.3

Saponification number is usually given as mg KOH and it indirectly gives the average chain length of the fatty acids. Ideally, average chain length of FAME should fall between 12 and 22. If the fatty acid profile of particular oil is dominated by long chain fatty acids, it tends to be dense (Knothe 2005). Iodine value indicates the degree of unsaturation. EN14214 biodiesel standard has specified a lowest maximum value which is 115 (European Standard Organization 2003). The IV (71.5) shows that Calophyllum inophyllum oil has an acceptable percentage of unsaturated fatty acids. Cetane number is the ability of a fuel to ignite under compression (Ramos et al. 2009). The above mentioned cetane number (57.3) shows that Calophyllum inophyllum oil methyl esters are likely to have an acceptable level of ignition properties according to ASTM standards.

There are contrasting reports regarding free fatty acids (FFA) of Calophyllum inophyllum oil. Sahoo et al. (2007) reported that Calophyllum inophyllum oil in India contains 22% FFA, but Crane et al. (2005) found 11% FFA in C. inophyllum kernel oils from Madagascar. This could be an indication of provenance variability in fatty acid profile (FAP) of C. inophyllum oil. Vegetable oils containing high FFA are not suitable for biodiesel production as it can corrode metal components of an engine. If an oil contains large amounts of FFA (>1% w/w), it also complicates the transesterification process by forming soap with an alkaline catalyst. The soap can prevent separation of the biodiesel from the glycerine fraction.
A number of researchers have worked on converting oils that contain a high proportion of free fatty acids (FFA) (Freedman et al. 1984; Mittlebach and Tritthart 1988; Mittlebach et al. 1992; Peterson et al. 1995; Wimmer 1995).

Most of their conversion methods involved alkaline catalysts and FFAs were removed as soap. Higher FFA levels result in feedstock wastage and the soap induces the formation of stable emulsions that prevents phase separation (Canakci and Van Gerpen 2001).

Keim et al. (1945) patented a protocol for converting oils bearing > 50% FFA. However, later it was found that the product from the above method contained a higher acid value (10 mg KOH/g) which is way above the ASTM standard (0.5 mg KOH/g).

Table 3.3: Properties of Calophyllum Oil Methyl Ester blends in comparison with High Speed Diesel (HSD) (Sahoo et al. 2007)

<table>
<thead>
<tr>
<th>Fuel blends</th>
<th>Viscosity (cSt)</th>
<th>Calorific value (MJ/kg)</th>
<th>Flash point (°C)</th>
<th>Cloud point (°C)</th>
<th>Pour point (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HSD</td>
<td>2.87</td>
<td>44.22</td>
<td>76</td>
<td>6.5</td>
<td>-3</td>
</tr>
<tr>
<td>B20</td>
<td>2.98</td>
<td>43.85</td>
<td>86</td>
<td>7.8</td>
<td>2.8</td>
</tr>
<tr>
<td>B40</td>
<td>3.30</td>
<td>42.65</td>
<td>91</td>
<td>8.5</td>
<td>2.8</td>
</tr>
<tr>
<td>B60</td>
<td>3.61</td>
<td>40.98</td>
<td>96</td>
<td>10.6</td>
<td>3.2</td>
</tr>
<tr>
<td>B80</td>
<td>3.72</td>
<td>39.23</td>
<td>111</td>
<td>10.8</td>
<td>3.6</td>
</tr>
<tr>
<td>B100</td>
<td>4.92</td>
<td>38.66</td>
<td>140</td>
<td>13.2</td>
<td>4.3</td>
</tr>
</tbody>
</table>

Specific biodiesel conversion protocols for calophyllum oil have been reported by some authors (Sahoo et al. 2007; Venkanna and Reddy 2009). Those methods had three steps; (a) acid catalysed step (b) base catalysed step (c) purification step.

According to Sahoo et al. (2007), viscosity and acidity of the Calophyllum oil is substantially reduced after transesterification. They reported that certain fuel parameters of lower blends of calophyllum oil methyl ester are similar to that of High Speed Diesel (HSD) (Table 3.3 calophyllum oil methyl ester can be blended in any proportion with mineral diesel to create a biodiesel blend or can be used in its pure form (Venkanna and Reddy 2009).
However, those methods have a number of theoretical drawbacks. Alkaline catalyzed transesterification has been found to be less effective for oils having FFA >1% (Freedman and Pryde 1982; Liu 1994; Mittelbach et al. 1992).

In both the methods, alkaline catalysed methylation had received partially converted oil having FFA >2% which can lead to incomplete conversion and inferior quality biodiesel. This indicates the necessity of developing a new improvised conversion protocol has to be developed in order to produce biodiesel that meets industry standards in Australia.

This chapter strived to unveil some of the lesser known information about calophyllum. The sole purpose of this chapter was to emphasise the strong economic potential of the species. Main sections of this chapter comprised of the biology, ecological distribution, seed storage, propagation methods, indigenous uses, medicinal value, other economic uses and biodiesel production potential of calophyllum.

The next chapter examines the variation in stand characteristics, fruit and predicted oil yield, and their relationship with climatic and soil parameters. Purpose of this study is identifying the conditions that best suits the growth of calophyllum.
CHAPTER 4

Provenance Variations in Stand Characteristics, Fruit Yield, Wood Density and Bark Thickness

4.1 Introduction

*Callocylyum inophyllum* shows a wide geographic distribution and can be found in a range of distinct environments. Geo-climatic variations and soil variations can impart great influence on the performance of any plant species (Koslowski et al. 1991). Sometimes, plants exhibit strong plastic responses to environmental variations (Harper 1977). Some environments favour superior performance while other may cause retarded performance. Rehfeldt (1995) believed that there is a strong relationship between provenance performance and geographic variables. Individuals from different provenances having pronounced environmental variations may exhibit superiority or inferiority in economically important plant characters (e.g. growth rate, fruit yield, seed size etc.) (Langlet 1971). The current literature does not provide any information on stand characteristics of *C. inophyllum* provenances in Australia or elsewhere. Studying variations in stand variables in relation to the habitat environment is beneficial for selecting potential locations (environments) and agronomic conditions that favour the growth performance and fruit yield of *C. inophyllum*.

4.1.1 Variations in Stand Characteristics

Suitability of a given locality (environment) for cultivating a particular species can be identified by the growth responses shown by that species to its habitat environment (Weber et al. 2008). Trees growing in different provenances may vary in height, diameter, branching pattern, specific leaf area, crown diameter and tree architecture. Studying variations in the above mentioned stand characteristics will derive some useful trends that may be useful in selecting areas/localities for commercial cultivation, as well as best cultivars and spacing for either monoculture or mixed cropping.
4.1.2 Variations in Fruit Yield and Oil Yield

Fruit yield and oil yield are two of the most important factors in determining the potential of particular oil seed species to be used as a biodiesel feedstock. Yield is represented by the mean number of fruits that a tree produces in a given fruiting season. *Calophyllum inophyllum* shows profuse fruiting; a tree can bear approximately 6000-10000 fruits in one season (Friday and Okano 2006). On average there can be 100-200 seeds/kg (Jøker 2004). Yield varies from season to season. In commercial perspective, it is also worthwhile to investigate if there is any relationship between environmental variables and fruit and oil yield.

4.1.3 Variations in Bark Thickness

Bark has been well known as a protective shield against adverse environmental conditions for tree species (Starker 1934; Hare 1965; Martin 1963; Vines 1968). Hence it can be assumed that bark thickness is substantially influenced by environmental conditions i.e. temperature, fire and drought. The bark of *Calophyllum inophyllum* is hollowly longitudinally fissured, pale grey and fawn in colour, and the inner bark usually thick, soft, fibrous and laminated, pink to red, darkening to brownish on exposure (Lemmens 2005). *Calophyllum* bark is an Indo-Chinese medicine for orchitis (Quisumbing 1951 cited in Dweck and Meadows 2002). Scientists have found a cytotoxin (calocoumarin A) that can be used to treat skin cancer from bark extracts of *C. inophyllum* (Itoigawa et al. 2001). Bark thickness can be used as a useful raw parameter for selecting suitable provenances for cultivating *C. inophyllum* as a medicinal plant.

4.1.4 Variations in Sapwood Density

*Calophyllum inophyllum* is a good durable general-purpose timber with density ranging from 560-900 kg/m³ at 15% moisture content (MTC wood wizard 2006). Wood properties vary within trees, between trees, and between stands and regions (Zobel and van Buijtenen 1989 cited in Stahl 1998). Sapwood density is a good indicator of the quality of timber. By looking at variations it may be possible to select suitable provenances for timber.
4.2 Materials and Methods

4.2.1 Variations in Stand Characteristics and Fruit Yield of Calophyllum Provenances in Australia and Sri Lanka

Provenances and study sites were selected according to the sampling criteria mentioned in Chapter 2. At each selected site, 2-8 trees were numbered and individual parameters were recorded. Corresponding soil samples were also taken for chemical analysis. Individual tree parameters vary with age. The majority of Calophyllum inophyllum stands in Australia and Sri Lanka have unknown life histories. Before making any comparisons between provenances, samples should be standardized. Based on the literature average girth of a mature C. inophyllum tree (70 years) is approximately 116 cm (Soerlanegara and Lemmens 1994; Friday and Okano 2006). In this study I assumed morphologically mature trees having Girth at Breast Height over Bark (GBHO≥100 cm) to have overcome their rapid growth phase and was at their steady state growth phase.

4.2.1.1 Height (Ht) and Height to the First Branch (HFB)

Tree heights and heights to first branch were measured using a Suunto clinometer.

4.2.1.2 Diameter at Breast Height- Over Bark (DBHOB)

Diameters of the selected trees (at 1.3 m) were measured using DBH tape. Average was taken for those trees that are branched below and at breast height.

4.2.1.3 Crown Diameter (CD)

Two perpendicular measurements were taken for crown diameter using a measuring tape (Fig 4.1).
4.2.1.4 The Number of Primary Branches (NPB) was counted.

The Mean Spacing with Adjoining Trees (MSp.) was measured with distance tape (Fig 4.2).

4.2.1.5 Branching Angle

Branch angle (Fig 4.3) influences the mechanical strength and stability of a particular tree. Sometimes it reflects the kind of environment the tree is dwelling in. Primary branch angles were measured by image analysis. Mean branch angle was determined by the following formula:

\[ \text{Crown diameter} = \frac{(A+B)}{2} \]

\[ \text{MSp.} = \frac{(a + b + c + d)}{4} \]
4.2.1.6 Specific Leaf Area (SLA)

At each selected site, ten fully opened leaves from the lower and mid canopy that were facing the sun were collected from each tree. They were wrapped in moist blotting paper and placed in plastic bags and kept in a cooling box until analysed. Forty eight hours after leaf collection, leaf area was measured using Delta-T scanning software. Then leaves were dried in an oven at 70 °C for 72 hrs and immediately weighed using a chemical balance (Cornelissen et. al. 2003).

Specific leaf area (SLA) was determined by following formula:

\[ \text{SLA (mm}^2\text{mg}^{-1}) = \frac{\text{Area of the leaf (mm}^2)}{\text{Oven dry mass of the leaf (mg)}} \]

4.2.1.7 Fruit Yield:

For each selected tree, the number of fruit bearing clusters was counted and the number of fruits in twenty random fruit clusters was also recorded. Number of fruiting seasons was also noted. Estimated fruit yield per 400 trees/ha was calculated using following formula:

\[ \text{Mean branch angle}^\circ = \frac{\sum (\text{angles of primary branches})}{n} \]
Fruit yield ha\(^{-1}\) year\(^{-1}\) = NFC × MFC × NFS × 400

\(^1\)Oil yield ha\(^{-1}\) year\(^{-1}\) = fruit yield ha\(^{-1}\) year\(^{-1}\) × kernel weight × [KOC\(^1\) (S1+S2)/2]

NFC-number of fruit clusters, MFC-mean number of fruits/cluster, NFS-number of fruiting seasons/year. \(^1\) Kernel oil contents for both seasons (S1+S2) were determined by the method described in Section 7.2.3. There are no distinct seasons in Sri Lanka; hence seasonal variations in Sri Lanka have been ignored.

4.2.1.8 Soil Analysis:

Two sampling locations from each provenance were selected for soil analysis. At each of these two site three morphologically superior trees (from those selected for other parameters) were selected and three cores (0-30 cm) were taken under each tree (3 m radius from the tree trunk) using a 2.5 m soil auger.

Cores belonging to the same site were mixed to form a representative composite of the site. Samples were collected in linen bags and brought to the laboratory and transferred to an oven set at 40 °C. For each sample, organic debris and stones were sieved using a 2 mm sieve. One sample (750 g) from each site was analysed for nitrogen, potassium, phosphorus, total carbon, pH and conductivity using ISO 10390, ISO 10694, ISO 11047, and ISO 13878 methods and TPS ion meters.

4.2.1.9 Statistical Analysis:

Data on each tree parameter were subjected to analysis of variance. After testing for normality and homogeneity of error variances, provenance means were compared by the unbalanced Model of ANOVA with GENSTAT ver. 11. Soil chemical parameters and climatic variables (Bureau of Meteorology Australia 2009; Department of Meteorology Sri Lanka 2009) and growth parameters of each provenance were tested for correlations.

Relative provenance distance score for each variable was calculated by dividing the mean value of each provenance by the lowest mean value (Fig 4.8).
4.2.2 Variations in Bark Thickness and Wood Density

Sampling was greatly restrained by the small population size found in each provenance. Based on the availability, 2-4 per provenance were selected. From each site (2-5) morphologically superior (free from diseases and defects and with a healthy form) mature trees were selected having GBH ranging from 100-120 cm. This step was taken to standardize the sample in the comparison of field data and as an effort to address the age effect.

Bark thickness was measured of three trees at each selected site using a bark gauge. Two perpendicular measurements were taken for each tree at 1.3 m height. Two core samples of sapwood (windward and leeward) were taken from 1-3 trees at each selected site using an HAGLÖF increment borer (i.d.5.15 mm). Samples were dried in an oven for 48 hours at 104 °C and then the dry weight of each piece was immediately recorded (Chave 2006).

Density was calculated using the following formula:

\[ \rho_s = \frac{W_d \times 4}{\pi d^2 l} \]

where, \( W_d \) is the dry weight of the core sample, \( \rho_s \) is the sapwood density, \( d \) and \( l \) were diameter and length of core samples.

Statistical Analysis

After testing for normality of the data, differences among provenance means were tested by Tukey simultaneous test using the General Linear Model of ANOVA (GLM) with MINITAB 14.1.
4.3 Results and Discussion

4.3.1 Variation in Height and Diameter at Breast Height Over Bark (DBHOB)

Due to unknown life histories and low sample size, comparison of stand characteristics was limited to simple trend forecasting. *Calophyllum inophyllum* stands from all provenances except Bowen and Yeppoon appeared to have good age and height class distribution and as a result might indicate meaningful trends in character comparisons. Mean heights of *C. inophyllum* provenances ranged between 7-20 m which agrees with the data of Friday and Okano (2006).

Table 4.1: Provenance variations in tree height and DBHOB of *C. inophyllum*

<table>
<thead>
<tr>
<th>Provenance</th>
<th>n</th>
<th>Height (m)</th>
<th>DBHOB (m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Townsville</td>
<td>10</td>
<td>10.6ab</td>
<td>0.75b</td>
</tr>
<tr>
<td>Yeppoon</td>
<td>4*</td>
<td>7.20a</td>
<td>0.29a</td>
</tr>
<tr>
<td>Cardwell</td>
<td>14</td>
<td>11.20b</td>
<td>0.81b</td>
</tr>
<tr>
<td>Bowen</td>
<td>10</td>
<td>7.20a</td>
<td>0.50a</td>
</tr>
<tr>
<td>Mackay</td>
<td>4*</td>
<td>12.80b</td>
<td>0.77b</td>
</tr>
<tr>
<td>Darwin</td>
<td>14</td>
<td>8.70ab</td>
<td>0.66b</td>
</tr>
<tr>
<td>Anuradhapura</td>
<td>6*</td>
<td>17.40c</td>
<td>0.42a</td>
</tr>
<tr>
<td>Colombo</td>
<td>15</td>
<td>13.90bc</td>
<td>0.41a</td>
</tr>
<tr>
<td>Kurunegala</td>
<td>6*</td>
<td>12.60b</td>
<td>0.41a</td>
</tr>
<tr>
<td>Matara</td>
<td>10</td>
<td>22.70d</td>
<td>0.38a</td>
</tr>
</tbody>
</table>

Within a column means that are followed by the same letter are not significantly different (P< 0.05), *number of available trees, n=number of individuals, height data were transformed to log values during analysis and retransformed for meaningful presentation. Sri Lankan provenances are shaded in green.

*Calophyllum inophyllum* provenances in Sri Lanka were found to have taller trees with thinner trunks than Australian provenances (Table 4.1). *Calophyllum inophyllum* trees from Matara recorded the highest mean height and it was significantly higher (P<0.05) than the rest of the provenances (Table 4.1). The second highest mean height was found from trees from Anuradhapura.
In Anuradhapura and Matara, the spacing between \textit{C. inophyllum} trees and adjoining trees was lower (2.56 m and 3.44 m) compared to that of other locations. Trees from Bowen and Yeppoon had the lowest mean height (7.2 m). They also had relatively less variable age (Bowen≈15 year old, Yeppoon≈8 year old) and height distribution (Bowen SE±0.92, Yeppoon SE±0.59). Northern Australian \textit{C. inophyllum} provenances occurring in the coastal belt experience constant strong wind compared to Sri Lankan provenances. It is well known that trees usually reduce their stature to withstand intense winds (Brüchert and Gardiner 2006). \textit{Calophyllum inophyllum} trees in all selected Australian provenances, except Bowen and Yeppoon had significantly (P<0.05) higher mean DBHOB compared to Sri Lankan provenances. The largest diameter trees (mean DBHOB 0.81 m) were found in Cardwell Australia.

\subsection*{4.3.2 Crown Diameter, Height to First Branch & Number of Primary Branches}

Generally all three parameters, crown diameter (CD), height to first branch/first fork (HFB) and number of primary branches (NPB) differed markedly between the two countries. Broadest crowns were observed among Australian provenances, although Yeppoon had crown diameter values similar to that of Sri Lankan provenances (Table 5.2). With the exception of Yeppoon, crown diameters showed a notable country to country variation.

Compared to height to first branch and number of primary branches, crown diameter showed relatively lesser variation within each country. Australian provenances except Yeppoon had CD between 15.6 m-15.9 m and Sri Lankan provenances had CD between 12.4 m-14.1 m. \textit{Calophyllum inophyllum} provenances demonstrated significant (P<0.05) country to country variations in the number of primary branches and height to first branch (Table 4.2).

In Australian provenances \textit{Calophyllum inophyllum} trees had heavy branching patterns and relatively low forking heights (basal forking), whereas those in Sri Lanka had fewer branches and higher forking heights. In contrast, Cornileus et al. (1996) found basal forking in \textit{Alnus acuminate} not to be affected by the provenance.
Table 4.2 Provenance variations in crown diameter, number of primary branches and height to first branch of *C. inophyllum*

<table>
<thead>
<tr>
<th>Provenance</th>
<th>n</th>
<th>Crown diameter (m)</th>
<th>Number of primary branches</th>
<th>Height to first branch (m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Townsville</td>
<td>10</td>
<td>15.70b</td>
<td>11bc</td>
<td>1.59a</td>
</tr>
<tr>
<td>Yeppoon</td>
<td>4*</td>
<td>12.20a</td>
<td>14c</td>
<td>0.74a</td>
</tr>
<tr>
<td>Cardwell</td>
<td>14</td>
<td>15.80b</td>
<td>14c</td>
<td>1.17a</td>
</tr>
<tr>
<td>Bowen</td>
<td>10</td>
<td>15.90b</td>
<td>14c</td>
<td>0.83a</td>
</tr>
<tr>
<td>Mackay</td>
<td>4*</td>
<td>15.60b</td>
<td>11bc</td>
<td>1.71a</td>
</tr>
<tr>
<td>Darwin</td>
<td>14</td>
<td>15.60b</td>
<td>11bc</td>
<td>1.32a</td>
</tr>
<tr>
<td>Anuradhapura</td>
<td>6*</td>
<td>13.50a</td>
<td>6a</td>
<td>4.55b</td>
</tr>
<tr>
<td>Colombo</td>
<td>15</td>
<td>14.10ab</td>
<td>7a</td>
<td>4.68b</td>
</tr>
<tr>
<td>Kurunegala</td>
<td>6*</td>
<td>12.40a</td>
<td>7a</td>
<td>4.51b</td>
</tr>
<tr>
<td>Matara</td>
<td>10</td>
<td>12.60a</td>
<td>7a</td>
<td>6.08c</td>
</tr>
</tbody>
</table>

Within a column means that are followed by the same letter are not significantly different (P< 0.05), n= the number of individuals selected, * the only available trees in that particular site.

4.3.3 Specific Leaf Area (SLA)

*Calophyllum inophyllum* trees in Australian provenances had markedly lower SLA compared to those in Sri Lankan provenances (Table 4.3). Australian provenances were found to have greater leaf mass compared to Sri Lankan provenances. Country to country variation in leaf area was found to be less marked.

SLA showed positive correlations with height to first branch (r=0.35*), altitude (r=0.58*), mean annual rainfall (r=0.52*), soil electrical conductivity (r=0.79**), total soil carbon content (r=0.85**) and mean annual maximum temperature (r=0.46*).
Table 4.3 Provenance variations in specific leaf area (SLA) of *Calophyllum inophyllum*

<table>
<thead>
<tr>
<th>Provenance</th>
<th>Mean leaf area ± SE (cm²)</th>
<th>Mean leaf mass ± SE (g)</th>
<th>SLA (cm²/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Townsville</td>
<td>137.08± 17.45</td>
<td>2.15 ± 0.048</td>
<td>63.6 a</td>
</tr>
<tr>
<td>Yeppoon</td>
<td>152.89± 25.58</td>
<td>1.99 ± 0.031</td>
<td>76.8 a</td>
</tr>
<tr>
<td>Cardwell</td>
<td>139.19± 10.23</td>
<td>1.93 ± 0.033</td>
<td>72.1 a</td>
</tr>
<tr>
<td>Bowen</td>
<td>145.10± 22.43</td>
<td>1.96 ± 0.031</td>
<td>74.1 a</td>
</tr>
<tr>
<td>Mackay</td>
<td>144.31± 21.55</td>
<td>2.30 ± 0.053</td>
<td>62.7 a</td>
</tr>
<tr>
<td>Darwin</td>
<td>111.01± 20.50</td>
<td>1.20 ± 0.030</td>
<td>92.3 b</td>
</tr>
<tr>
<td>Anuradhapura</td>
<td>115.42± 19.65</td>
<td>1.03 ± 0.012</td>
<td>111.5 c</td>
</tr>
<tr>
<td>Colombo</td>
<td>125.87± 25.30</td>
<td>0.81 ± 0.010</td>
<td>154.5 d</td>
</tr>
<tr>
<td>Kurunggala</td>
<td>99.52± 09.76</td>
<td>0.92 ± 0.009</td>
<td>108.4 bc</td>
</tr>
<tr>
<td>Matara</td>
<td>112.65± 12.29</td>
<td>1.04 ± 0.012</td>
<td>108.5 bc</td>
</tr>
</tbody>
</table>

Within a column means that are followed by the same letter are not significantly different (P< 0.05), n=80. Mean values followed by SE are not significantly different at 95% probability.

Fig 4.4 Relationships between SLA and A - Total carbon content, and B - Soil potassium content, r- Pearson correlation coefficient, **P<0.001, *P<0.05, n=80.

Negative correlations were observed between SLA and the number of primary branches (r=-0.39*), mean spacing with adjoining trees (r= -0.32*) , soil nitrogen content (r=-0.33*), soil potassium content (r=-0.65**), pH (r=-0.44*) and mean annual minimum temperature (r=-0.59*).
Out of all correlated variables, total soil carbon content, conductivity, and soil potassium content appear to have the strongest influence on SLA (Fig 4.4A and 4.4B). Calophyllum trees growing in Australian provenances growing in carbon rich soils appear to have higher leaf biomass compared to Sri Lankan provenances. Dowuona and Adjetey (2010) noticed a strong relationship between Leaf Area Index (LAI) of woodland vegetation and soil organic carbon. Calophyllum trees in Australia experience salinity and salty winds. Calophyllum trees growing in Australia had thicker leaves compared to those in Sri Lanka. Jafri and Ahmad (1995) also reported a positive correlation between leaf thickness in cotton and soil salinity. This may be an adaptation to prevent leaf dehydration (Bezona et al. 2001).

### 4.3.4 Branch Angle

In most plants branch growth is controlled by its apical growth (Wilson 2000) and the branching angle defines the mechanical stability of the tree (Niklas 1992).

![Branch angle graph](image)

Fig 4.5 Branch angles of *Calophyllum inophyllum* trees in different provenances. Mean values followed by SE are not significantly different at 95% probability.

Branch angle also reflects the effort of the plant in maximizing light interception efficiency (Pearcy and Yang 1996). *Calophyllum inophyllum* provenances demonstrated modest variation in branch angle, although the grand mean was slightly higher for Australian provenances (61.6 %) compared to Sri Lankan provenances (57.9 %).
Despite having variations in height and diameter, calophyllum trees generally had similar branch angle, which in this case was >55°. Branch angles of provenances Bowen, Mackay and Darwin were somewhat higher than the rest (Fig 4.5). This may be attributed to their wider crown diameter (Table 4.2), and those trees may have adapted to develop wide branch angles to support extensive radial expansion of branches. Branch angle was not found to correlate with any of the environmental or any other stand variables. This shows that branch angle of *C. inophyllum* is more likely to be attributed by genetics rather than the environmental conditions.

### 4.3.5 Estimated Fruit and Oil Yield

Yield estimates were carried out using data from unmanaged, under-maintained *C. inophyllum* stands. Estimated yields are expected to be higher for well-maintained plantation situations. According to the results (Fig 4.6), fruit yield ha\(^{-1}\) year\(^{-1}\) of *C. inophyllum* did not show any statistically significant provenance variation (P>0.05).

This lack of variation was mainly attributed to the high intra-provenance variation (SE>±20000) that was evident among Australian provenances. Apart from Mackay (628000 ± 50866 fruits ha\(^{-1}\) year\(^{-1}\)) and Yeppoon (664000 ± 34120), all other provenances recorded appreciable fruit yields. Fruit yield in *C. inophyllum* trees from Bowen and Cardwell was considerably higher than the rest of the provenances owing to their larger crowns and Bowen recorded the highest estimated fruit yield (1436000 ± 64317 fruits ha\(^{-1}\) year\(^{-1}\)).

Trees from *Anuradhapura* had the highest estimated oil yields (2725.49 ± 348.43 kg ha\(^{-1}\) year\(^{-1}\)) followed by Cardwell (2682.19 ± 672 kg ha\(^{-1}\) year\(^{-1}\)), Townsville (2604.57 ± 626.63 kg ha\(^{-1}\) year\(^{-1}\)) and Bowen (2537.85 ± 615 kg ha\(^{-1}\) year\(^{-1}\)). Mackay and Colombo had relatively poor estimated oil yields (<1100 kg ha\(^{-1}\) year\(^{-1}\)). Kernel oil contents that correspond to the estimated oil yield data and their correlations with environmental variables are discussed in chapter 7.
Fig 4.6 Provenance variations in fruit yield and oil yield ha\(^{-1}\) year\(^{-1}\). Australian provenances are marked in dark green and Sri Lankan provenances are marked in green.

Only two factors; crown diameter (r=0.39\(^*\)) and total soil carbon content (r=-0.25\(^*\)) showed notable positive and negative correlations with fruit yield (Figs 4.7 A and 4.7 B). Wider crowns seem to bear large number of fruits. *C. inophyllum* trees in Sri Lanka had less spacing with adjoining trees (2.5 m-5.5 m), were taller and had smaller crowns compared to those in Australia and as a result had relatively low fruit yield.

It is important to understand that the oil yields estimated in this section may be an underestimation of the actual annual oil yield. This is mainly because the kernel oil content used in the yield calculation was derived from a single extraction (Section 7.2.3). Since this applies to all selected provenances the meaningful comparison is not compromised.
Fig 4.7 Correlation between fruit yield ha\(^{-1}\) yr\(^{-1}\) and, A crown diameter (m) and B total soil carbon (%). r-Pearson’s product and moment correlation coefficient, *P<0.05.

If double extraction was carried out, the kernel oil contents would be slightly higher. As a result estimated oil yields would also have been higher. The majority of the calophyllum stands are unfertilized and under-maintained. If they had been planted as a proper feedstock plantation, the oil yield may have been closer to the oil yield (4680 kg ha\(^{-1}\) year\(^{-1}\)) reported by Azam et al. (2005).

### 4.3.6 Correlations Between Stand Characteristics and Climatic and Soil Parameters

In most plant species, soil nitrogen content has a positive influence on height growth (Abdel-Motagally and Osman 2010). However, the heights of *Calophyllum inophyllum* provenances have shown significant (P<0.001) negative correlations with soil N, P, K and pH values and mean spacing and (Table 4.4). Mitchler et al. (2004) also did not find any positive correlations between soil nutrients and heights of Black Walnut provenances in Kansas USA. However, they did report that Black Walnut shows optimum growth on high pH soils.
*Calophyllum inophyllum* provenances have shown significant (P<0.001) positive correlations (P<0.001) with altitude, mean annual minimum temperature and MAR. In agreement with the findings of this study, Chunyang et al. (2000) noted significant (P<0.01) positive correlation between the height of *Eucalyptus microtheca* and MAR. Graumlich and Brubaker (1986) also found a positive correlation between heights of *Tsuga mertensiana* and *Larix lyallii* and mean annual temperature. *Calophyllum inophyllum* provenances in Sri Lanka receive higher MAR and responded with greater height growth compared to that in Australian provenances.

Above ground competition (MSp.4.07±2.57 m) in Sri Lankan calophyllum provenances was higher than that (MSp.13.02±5.60 m) in Australian provenances and it may have resulted in taller trees i.e. competing for light (Coomes and Allen 2007).

In contrast to height, DBHOB values showed significant (P<0.001) positive correlations with crown diameter, mean spacing, and soil N and K values, and significant (P<0.001) negative correlations with HFB, mean annual minimum temperature and altitude. This suggests that in nutrient rich soils radial growth (DBHOB) in *Calophyllum inophyllum* is promoted over the height growth. In agreement with the findings of this study, Sengloung et al. (2003) also found significant negative correlations (r=-0.64**) between altitude and DBH of *Casuarina junghuhniana*.

The strong relationship between crown diameter and DBHOB has been well documented (Leech 1984). O'Brian et al. (2007) found significant (p=0.002) positive correlations between DBHOB of *Eucalyptus marginata* and mean annual rainfall. Nissen and Midmore (2002) found a strong relationship between stand basal area of four timber species and below ground competition with annual intercrop species. The stand basal area is directly proportional to the stand DBHOB. Lower below ground competition in calophyllum provenances in Australia may have contributed to their relatively high DBHOB values.
Table 4.4: Correlation matrix of stand characteristics vs. climatic and soil parameters of selected provenances

<table>
<thead>
<tr>
<th></th>
<th>DBHOB</th>
<th>CD</th>
<th>NPB</th>
<th>HFB</th>
<th>MSp</th>
<th>MaxT</th>
<th>MinT</th>
<th>Alt</th>
<th>MAR</th>
<th>N</th>
<th>P</th>
<th>K</th>
<th>Con</th>
<th>pH</th>
<th>TC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ht</td>
<td>0.09</td>
<td>0.26</td>
<td>-0.33*</td>
<td>0.70**</td>
<td>-0.52**</td>
<td>0.10</td>
<td>0.40**</td>
<td>0.46**</td>
<td>0.30*</td>
<td>-0.55**</td>
<td>-0.36*</td>
<td>-0.53**</td>
<td>0.21</td>
<td>-0.33*</td>
<td>0.13</td>
</tr>
<tr>
<td>DBHOB</td>
<td>0.59**</td>
<td>0.39**</td>
<td>-0.35*</td>
<td>0.37**</td>
<td>-0.10</td>
<td>-0.39**</td>
<td>-0.46**</td>
<td>0.02</td>
<td>0.53**</td>
<td>0.05</td>
<td>0.43**</td>
<td>-0.35*</td>
<td>0.23</td>
<td>-0.30</td>
<td></td>
</tr>
<tr>
<td>CD</td>
<td>0.18</td>
<td>-0.15</td>
<td>0.10</td>
<td>0.08</td>
<td>-0.09</td>
<td>-0.15</td>
<td>0.09</td>
<td>0.32*</td>
<td>0.11</td>
<td>0.15</td>
<td>0.11</td>
<td>0.11</td>
<td>0.07</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NPB</td>
<td>-0.59**</td>
<td>0.70**</td>
<td>-0.43**</td>
<td>-0.60**</td>
<td>-0.66**</td>
<td>-0.23*</td>
<td>0.55**</td>
<td>0.22</td>
<td>0.64**</td>
<td>-0.52**</td>
<td>0.24</td>
<td>-0.46**</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HFB</td>
<td>-0.63**</td>
<td>0.23</td>
<td>0.64**</td>
<td>0.64**</td>
<td>0.37**</td>
<td>-0.73**</td>
<td>0.36*</td>
<td>-0.76**</td>
<td>0.43**</td>
<td>0.43**</td>
<td>0.31*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Ht-height, DBHOB-diameter at breast height over bark, NPB-number of primary branches, MSp-mean spacing with adjoining trees, MaxT-mean annual maximum temperature, MinT-mean annual minimum temperature, Alt-altitude, MAR-mean annual rainfall, N,P,K-nitrogen, phosphorous, potassium, Con-conductivity, TC-total soil carbon. Values are Pearson’s correlation coefficients r, *P<0.05, **P<0.001
Interestingly, crown diameter of *C. inophyllum* seemed to be uninfluenced by most site variables except soil nitrogen content (*r*=0.32*). This shows that in calophyllum trees, horizontal crown development is promoted under nitrogen rich soil conditions.

Significant positive correlations were found between the number of primary branches and soil nitrogen (*r*=0.55**) and phosphorous (*r*=0.64**) levels. High soil N and K levels seem to strongly induce heavy branching in *C. inophyllum*. Zang (1996) found a highly significant relationship (*F*=170.78**) between soil nutrients and the number of branches in *Cakile edentula*. Bergin et al. (2008) also noticed heavy branching in *Podocarpus totara* found in nutrient rich environments.

The number of primary branches (NPB) in *C. inophyllum* was negatively correlated with mean maximum and minimum temperature, altitude, mean annual rainfall, soil conductivity and total carbon content. Generally branch growth is regulated by apical growth (Wilson 2000). Earlier it was noticed that high MAR, mean annual temperature and altitude can promote height growth in *C. inophyllum*. This promotion of height growth under the above mentioned geo-climatic conditions may have reduced branching in Sri Lankan *C. inophyllum* provenances.

Height to first branch/first fork (HFB) was found have significant (*P*<0.001) negative correlations with mean spacing, soil nitrogen and potassium content. Kubiske et al. (1997) observed elevated crown positioning in *Populus tremuloides* under nitrogen rich soils. Significant positive correlations were found between HFB and climatic parameters (mean minimum temperature, MAR and altitude) and soil phosphorus level, conductivity and pH value. It is clear that this may also have resulted from promotion of height growth.

*Calophyllum inophyllum* provenances were found to vary from one another in terms of their stand characteristics based on the raw mean values (Fig 4.8). It indicates that measured raw mean values of crown diameters and branch angles of *C. inophyllum* provenances were in overlapping range. The most clear country to country variation was observed in height to first branch and to a lesser degree in height, DBHOB, number of primary branches and SLM.
4.3.7 Provenance Variations in Bark Thickness and Wood Density

Results show marked variations in wood density and bark thickness among *Calophyllum inophyllum* provenances in Australia and in Sri Lanka. Significant ($P<0.05$) country to country variations in bark thickness were found between *C. inophyllum* stands in Australia and Sri Lanka (Table 4.5).

Calophyllum trees in Australia are mostly found in coastal areas and are exposed to dry salty air and in contrast, those in Sri Lanka occur in humid inland areas having different soil types (e.g. sandy soils, heavy clay soils) (Personal Observations 2008). Thicker barks found in Australian *C. inophyllum* trees may be due to the exposure to salty winds and low relative humidity. Thicker woody barks may have been developed to prevent dehydration.

---

Table 4.5: Provenance variations in bark thickness

<table>
<thead>
<tr>
<th>Provenance</th>
<th>n</th>
<th>Bark thickness (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Townsville</td>
<td>10</td>
<td>15.4c</td>
</tr>
<tr>
<td>Yeppoon</td>
<td>4*</td>
<td>11.3ab</td>
</tr>
<tr>
<td>Cardwell</td>
<td>14</td>
<td>12.4b</td>
</tr>
<tr>
<td>Bowen</td>
<td>10</td>
<td>11.9b</td>
</tr>
<tr>
<td>Mackay</td>
<td>4*</td>
<td>10.4ab</td>
</tr>
<tr>
<td>Darwin</td>
<td>14</td>
<td>12.2b</td>
</tr>
<tr>
<td>Anuradhapura</td>
<td>6*</td>
<td>8.3a</td>
</tr>
<tr>
<td>Colombo</td>
<td>15</td>
<td>8.8a</td>
</tr>
<tr>
<td>Kurunegala</td>
<td>6*</td>
<td>8.9a</td>
</tr>
<tr>
<td>Matara</td>
<td>10</td>
<td>9.1a</td>
</tr>
</tbody>
</table>

Means that are not followed by the same letter are significantly different (at 0.05 level of probability) *number of available trees, n=number of individuals.

*Calophyllum inophyllum* provenances demonstrated a range of sapwood densities (494-636 kg m⁻³). Sapwood density of *C. inophyllum* showed no pronounced country to country variation (Fig 4.9). Trees from Yeppoon, Mackay and Kurunegala had lower but similar sapwood densities (494-510 kg m⁻³). Similar sapwood densities were also observed among the trees from Anuradhapura and Matara (539, 541 kg m⁻³), and Townsville, Cardwell, Bowen and Darwin (574-590 kg m⁻³).

Highest mean sapwood density value (636 kg m⁻³) was observed among those trees from Colombo and it was found to be significantly different (P<0.05) from the rest of the provenances. *Calophyllum inophyllum* stands in all selected provenances except Colombo were growing on relatively loose sand-mix soils. Most of the trees sampled in Colombo were growing on black heavy clay or compact soils which restrict their root respiration. This induces slow growth and slow growth leads to dense wood formation (Slik et al. 2010).
Bark thickness in *C. inophyllum* was found to be strongly influenced by environmental variables. Negative correlations were found between bark thickness and climatic parameters (Table 4.6). The highest correlation coefficient ($r=-0.72^{**}$) was found between bark thickness and altitude.

A possible cause for the marked difference between bark thickness of Australian provenances and Sri Lankan provenances as mentioned earlier could be the difference in relative humidity (RH) and can be further supported by significant correlations between bark thickness and RH. Among climatic variables, mean annual maximum temperature ($r=-0.31$) and mean annual rainfall ($r=-0.32$) showed the lowest correlations with bark thickness.

Wood densities of *Calophyllum inophyllum* were not found to correlate with climatic variables (Table 4.6). Chave et al. (2006) and Moser et al. (2008) reported that wood density correlates negatively or neutrally with elevation.
Table 4.6: Correlations between bark thickness and wood density, and climatic parameters of selected provenances

<table>
<thead>
<tr>
<th>Wood Character</th>
<th>Mean Annual Maximum Temperature (°C)</th>
<th>Mean Annual Minimum Temperature (°C)</th>
<th>Altitude (m)</th>
<th>Mean Annual Rainfall (mm)</th>
<th>Mean Annual Relative Humidity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bark Thickness (mm)</td>
<td>-0.31 (0.05)</td>
<td>-0.57 (&lt;0.0001)</td>
<td>-0.72 (&lt;0.0001)</td>
<td>-0.32 (&lt;0.05)</td>
<td>-0.57 (&lt;0.001)</td>
</tr>
<tr>
<td>Wood density (kgm⁻³)</td>
<td>0.12 (0.25)</td>
<td>0.06 (0.52)</td>
<td>0.001 (0.98)</td>
<td>-0.14 (0.16)</td>
<td>0.02 (0.84)</td>
</tr>
</tbody>
</table>

Values are Pearson product–moment correlation coefficient, r, P-values are given in parentheses

A number of other authors have also reported that there are no correlations between geoclimatic variables and bark thickness (Warrall 1974; Wright 1987; Bergin et al. 2008). Bark thickness of *Calophyllum inophyllum* was also found to strongly correlate with soil parameters (Table 4.7). Significant positive correlations were found between bark thickness and, soil nitrogen and potassium levels. Bark thickness was negatively correlated with total carbon and conductivity. However, bark thickness was not found to be influenced by either soil phosphorous level or soil pH values.

Table 4.7: Correlations between bark thickness and wood density, and soil parameters of selected provenances

<table>
<thead>
<tr>
<th>Soil Parameters</th>
<th>N</th>
<th>P</th>
<th>K</th>
<th>Total C</th>
<th>pH</th>
<th>Conductivity (µS cm⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bark Thickness (mm)</td>
<td>0.77 (&lt;0.0001)</td>
<td>0.17 (0.13)</td>
<td>0.59 (&lt;0.0001)</td>
<td>-0.49 (&lt;0.0001)</td>
<td>0.03 (0.2)</td>
<td>-0.55 (&lt;0.0001)</td>
</tr>
<tr>
<td>Wood density (kgm⁻³)</td>
<td>0.03 (0.70)</td>
<td>-0.08 (0.41)</td>
<td>-0.08 (0.43)</td>
<td>-0.24 (0.07)</td>
<td>0.18 (0.07)</td>
<td>-0.17 (0.09)</td>
</tr>
</tbody>
</table>

Values are Pearson product–moment correlation coefficient, r, P-values are given in parentheses

*Soil nutrient values were taken as percentage dry weight.

In contrast, wood density was not found to be correlated to any of the measured soil parameters (Table 4.7). The only near significant negative correlation was found between wood density and total carbon %.
Overall, sap wood density of *Calophyllum inophyllum* was not found to be regulated by environmental parameters such as geo-climatic factors or soil parameters. Warrall (1974) also has suggested that heritability of wood characters can be very high. This implies that provenance variations in sapwood density are mainly governed by genotypic variations. In contrast, provenance variations in bark thickness were found to be strongly regulated by environmental variables.

### 4.4 Summary of Outcomes

#### 4.4.1 Stand Variables

Results of this study only derived some justifiable trends due to unknown life histories of the calophyllum stands that were selected (available) for this study. Calophyllum trees were found to grow in variety of soils from sandy to heavy clay bog soils. However sandy soils are preferred for biodiesel feedstock plantations since trees growing in heavy clay soils produces low number of smaller fruits.

Overall, calophyllum trees in Australia are shorter, have wider stems and larger crowns compared to Sri Lankan provenances. Relatively taller trees in Sri Lankan provenances may have been resulted from high above ground competition for light and relatively higher rainfall. The heights of northern Australian calophyllum provenances, being close to coastal environment appears to be influenced by exposure to wind. In general the heights of calophyllum trees seem to be controlled by temperature, MAR and altitude.

The leaf biomass of calophyllum provenances in Australia is significantly higher than that of Sri Lankan provenances. Calophyllum trees growing under N and K rich soils seem to have profuse basal branching, wide stems, large crowns and relatively larger number of fruits. Trees from Bowen, Mackay and Darwin had wider branch angle than the rest of the provenances due to their broader canopy. Closely packed trees from Anuradhapura show superior oil yield and can be grown in sandy soils and dry environment as biodiesel feedstock.
4.4.2 Wood Density and Bark Thickness

*Calophyllum inophyllum* provenances demonstrated marked country to country variations in bark thickness. Variations in bark thickness were found to be influenced by environmental variables. There were significant provenance variations in sap wood density but those variations did not seem to be influenced by environmental variables, suggesting that it could be governed by genotypic variations.

This Chapter revealed variations in stand characteristics, tree form and wood and bark characteristics of calophyllum stands in northern Australia and Sri Lanka. The influence of maternal environment on some of those characteristics was found to be significant.

The next Chapter attempts to determine the most ideal harvesting period for biodiesel production by studying the reproductive phenology and variation in fatty acid composition during fruit development.
CHAPTER 5
Reproductive Phenology and Fruit Development of

Calophyllum inophyllum

5.1 Flowering and Fruiting Phenology of Calophyllum inophyllum in Central Queensland, Australia and Western Province, Sri Lanka¹

5.1.1 Introduction

The subject area of phenology provides an understanding of the pattern of plant growth and development as well as the influence of the environment and selective pressures on flowering and fruiting behaviour. Phenological parameters such as intensity of flowering, duration and overlap are important factors in determining the reproductive effort of a plant (Richards 1986). The flowering of certain plants also signals agronomic time i.e. harvesting time (Richards et al. 1996). It may give an indication about the fruit yield that can be used in yield prediction, which is important in determining the plant’s economic potential. Flowering phenology also reflects the fitness of a stand (de Jong and Klinkhamer 1991; Ashman and Schoen, 1996; Sabat and Ackerman 1996). Information on the reproductive behaviour and development of fruits is sometimes vital in concentrating efforts towards a correct harvesting time.

In most parts of the world Calophyllum inophyllum shows two flowering and fruiting seasons (Little and Skolmen 1989). However, sometimes flowering may occur throughout the year (Foxworthy 1927). In northern Australia C. inophyllum trees flower twice annually, in January and in June (Friday and Okano 2006). In the absence of systematic information, the present study was carried out to investigate the flowering and fruiting cycles of C. inophyllum in northern Australia and in Sri Lanka.

5.1.2 Materials and Methods

Three eight year old trees from Rosslyn bay, Yeppoon, Australia (23° 7' 60 S, 150° 43' 60 E) and three eight year old trees from Meegoda, Sri Lanka (6°18'51"N 80°31'31"E) were selected for the study. Sample size had to be limited due to the limited availability of even aged healthy trees (n = 3) and the poor population sizes (n = 5) in the selected locations. Observations were made during late December 2007 up until late April 2008 in Australia and between May and September 2008 in Sri Lanka.

The number of inflorescences was counted after every six days. A washable ink marker was used to mark every counted inflorescence to avoid double counting. From each tree, five inflorescences containing flower buds were tagged and diameters were recorded after every six days until they became mature fruits. On each tree, the number of flowers and drupes in twenty five inflorescences/clusters was counted. Total number of fruit bearing clusters was also recorded in each tree. The following basic criteria were used to record the phonological observations in calophyllum stands.

Flowering initiation and secession

a) Fruiting initiation and secession
b) Floral life span: period from flower bud to fruit bud (Fig 5.1)
c) Average length of the fruit cycle; time between emergence (fruit bud) and maturity (turn yellow)
d) Mean number of flowers in an inflorescence
e) Mean number of drupes per fruit cluster
f) Quantitative assessment of floral and fruit development (i.e. flower diameter, fruit diameter)
Fig 5.1 Succession of floral development stages (from A-flower bud to F-fruit initiation)
5.1.3 Results and Discussion

5.1.3.1 Flowering Initiation and Secession

Flowering initiation within each location was found to be synchronous and hence partially addressed the issue of the small sample size. The first flowering period of *C. inophyllum* trees in Yeppoon, Australia initiated at the end of December and continued until the end of February. This observation was consistent with those of Friday and Okano (2006). *Calophyllum inophyllum* trees in Yeppoon had a shorter flowering (≈ 40 days) period compared to Meegoda trees (Fig 5.2).

The corresponding flowering season in Meegoda Sri Lanka started at the end of April and partially ceased at late August. Trees in Meegoda had few flowers even after the climax of fruiting. Similar observations to above have also been reported by Foxworthy (1927) in Malaysia. In contrast to Yeppoon, *C. inophyllum* trees in Meegoda Sri Lanka were observed to have a longer (≈ 109 days) flowering pattern (Fig 5.2). Flower bearing in a given observation was higher in trees from Yeppoon than those from Meegoda.

![Flowering periods and number of inflorescence in Yeppoon, Australia and Meegoda, Sri Lanka.](image)

Fig 5.2 Flowering periods and number of inflorescence in Yeppoon, Australia and Meegoda, Sri Lanka, FL-Y-flowering in Yeppoon, FL-M-flowering in Meegoda
The second flowering period in Yeppoon initiates between April-May (Personal observation 2009) and the second flowering in Meegoda usually begins in October (Personal observation 2008). Long term mean monthly rainfall data (Fig 5.3) shows that flowering initiation in both the locations overlap with peak rainfall months. Some authors have also reported gregarious anthesis in Dendrobium crumenatum, and Coffea arabica a few days after rain (Seifriz 1923; Holdsworth 1961).

![Graph showing long term rainfall patterns in Yeppoon, Australia and in Meegoda, Sri Lanka](image)

Fig 5.3 Long term rainfall patterns in Yeppoon, Australia and in Meegoda, Sri Lanka

- Flowering period in Yeppoon
- Flowering period in Meegoda
5.1.3.2 Floral and Fruit Development (Figs 5.4 and Fig 5.5)

*Calophyllum inophyllum* trees at Meegoda, Sri Lanka had a floral lifespan of 28 to 31 days (Fig 5.6) and it was longer than that of *C. inophyllum* trees in Yeppoon, Australia which had a lifespan of 18 to 20 days. Mean diameter of mature flowers of *C. inophyllum* trees in Yeppoon, Australia (10.8 mm) were less than that of Meegoda, Sri Lanka (12.5 mm).

![Fig 5.4 Different stages of floral development (bud to fully developed flower)](image)

Average fruit development cycle of *C. inophyllum* trees at Yeppoon (61 days) was slightly longer than that of Meegoda (56 days) (Fig 5.6). Mature *C. inophyllum* fruits at Yeppoon had greater mean diameter (34.3 mm) than those at Meegoda (23.8 mm).

![Fig 5.5 Different stages of fruit development (immature fruit to mature fruit)](image)
Fig 5.6  Floral and fruit development with time, FL-Y-floral development in Yeppoon, FL-M-floral development in Meegoda, FR-Y-fruit development in Yeppoon, FR-M- fruit development in Meegoda.
Relationships between altitude and seed size has been well documented (Mazer and Wolfe 1998; Lopez et al. 2003; Loha et al. 2006). The altitude of Meegoda is relatively higher than that of Yeppoon, hence, the difference in the average fruit size in calophyllum trees from those locations may be related to their relative difference in altitude.

During maturity green coloured fruits start to get yellow patches and on maturity they become more greenish yellow (Fig 5.5). Early inflorescences of Calophyllum inophyllum trees in Meegoda had significantly (P<0.05) higher number of flower buds compared to that of those found in Yeppoon (Table 5.1). Compared to C. inophyllum trees in Yeppoon, those in Meegoda had higher number of intermediate flowers/inflorescence.

Mean number of mature flowers in a given inflorescence was 4 for C. inophyllum trees in Yeppoon and 5 for those in Meegoda and the difference was not found to be statistically significant (Table 5.1). Both C. inophyllum trees in Yeppoon and Meegoda recorded the same mean number of fruit buds/cluster. The mean number of intermediate fruits in a given cluster was higher in C. inophyllum trees in Yeppoon than those in Meegoda. That trend was similar for mature fruits/cluster as well, but the difference was found to be significant (P<0.05) compared to that for intermediate fruits/cluster. These observations suggest the presence of barren flowers and abscission in both the locations.

The difference in the number of flowers/inflorescence could be due to the difference in orientation and distance between those trees in above locations. Calophyllum trees in Yeppoon occurred in a straight line and ≈ 12 m apart from one another and those in Meegoda were at least 100 m apart from one another and were far more scattered. Due to their proximity from one another and linear orientation, C. inophyllum trees in Yeppoon probably have lesser inter-competition for pollinators; whereas those in Meegoda may have developed more flowers and barren flowers to attract pollinators.
Table 5.1: Variation in number of flowers and fruits at different stages of floral and fruit development

<table>
<thead>
<tr>
<th>Provenance</th>
<th>Flowers per inflorescence (n=3*20)</th>
<th>Fruits per cluster (n=3*20)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Buds</td>
<td>Intermediate</td>
</tr>
<tr>
<td>Yeppoon</td>
<td>7.0a</td>
<td>5.0a</td>
</tr>
<tr>
<td>Meegoda</td>
<td>9.0b</td>
<td>7.0b</td>
</tr>
</tbody>
</table>

Within a column mean values that are followed by different letters are significantly different at P<0.05 level. SE values are used for means that are not significantly different (p<0.05).
5.1.3.2 Floral and Fruit Abscission and Pollination Success

In a given inflorescence of *C. inophyllum* trees at Yeppoon, 57% of flower buds developed into mature flowers. A similar trend was observed among those trees at Meegoda. All fully developed flowers of *C. inophyllum* trees at Yeppoon and 80% of fully developed flowers of *C. inophyllum* trees at Meegoda got pollinated and became fruit buds (Fig 5.7). Mean premature floral abscission in *C. inophyllum* trees at Meegoda (44.4%) was slightly higher than that in *C. inophyllum* trees at Yeppoon (42.8%). *Calophyllum inophyllum* trees at both Meegoda and Yeppoon (Fig 5.8) demonstrated similar mean fruit abscission (25%).

![Graph showing mean number of flowers/fruits over observations](image)

Fig 5.7 Premature floral abscission (A1) and fruit abscission (A2) in selected inflorescences of *Calophyllum inophyllum* trees in Yeppoon (Y), Australia and Meegoda (M), Sri Lanka (P-pollination, Observations (1-1st observation, 2-1 week, 3- 2 weeks, 4-6 weeks, 5-8 weeks, 6-10 weeks, 7-12 weeks, 8-14 weeks) (n=6).
5.1.4 Conclusions

*Calophyllum inophyllum* trees in Australia and Sri Lanka demonstrated considerable variations in flowering and fruiting phenology. Flowering initiation at both locations indicated a hint of dependency on rainfall pattern. *Calophyllum inophyllum* trees in Yeppoon, Australia had relatively shorter and regular flowering periods, shorter floral life span, longer fruit life span, smaller flowers and larger fruits compared to those in Meegoda, Sri Lanka. Even though mean number of flower buds/inflorescence was higher in Meegoda, *C. inophyllum* trees at both the locations had similar mean number of mature fruits/cluster due to the higher floral abscission in *C. inophyllum* trees in Meegoda.
5.2 Fruit Development and Kernel Oil Quality^a

5.2.1 Changes in the Oil Content and Fatty Acid Profiles (FAP) of Various Stages of the Fruit Development

5.2.2.1 Background

In numerous plant species seeds store a significant amount of energy reserves as fatty acids. Fatty acids are favoured as they can maximize energy while utilizing relatively smaller volume (Slack and Browse 1984). Lipid accumulation had been extensively studied in a number of oil seed species by various authors, castor (Ricinus communis) (Canvin 1965), soybean (Glycine max) (Privette et al. 1973), rape (Brassica napus) (Norton and Harris, 1975) and almond (Prunus dulcis) (Hawker and Buttrose 1980). Generally lipid accumulation follows a sigmoidal pattern (Slack and Browse 1984; Yin et al. 2003; Berti and Johnson 2008). Composition of saturated and unsaturated fatty acids varies with maturity stage of the fruit. This information is extremely important if fruits are harvested for extracting oil for biodiesel production as fatty acid composition of the feedstock directly influences the quality of the resultant biodiesel (Ramos et al. 2009). Information on the above provenance may provide vital information on selecting the correct timing for harvesting to improve the quality of the resultant biodiesel.

5.2.2 Materials and Methods

5.2.2.1 Oil extraction

Fruit collection was carried out in Yeppoon, Australia from March-June 2009. Fruits (ten each) were harvested at different stages of maturity (28, 38, 48, 58, 68 and 77 days after anthesis). Kernels were removed by carefully removing the exocarp. Oil of each kernel was individually extracted by n-hexane double extraction at 25 °C using LABORTA efficient 4000 rotary evaporator.

5.2.2.2 Fatty Acid Profiling (FAP)

Oils were methylated by boron trifluoride/methanol followed by KOH/methanol treatment, and analysed by capillary GC with a flame ionisation detector (FID) detection, as described in the AFNOR method (AFNOR NF EN ISO 5509 and NF EN ISO 5508). Methyl ester solution was injected onto a VARION CP 3800 gas chromatograph equipped with a FID.

The capillary column was a 100 m CP-Sil 88 with 0.25 mm i.d., 0.2 µm film thickness. Oven temperatures were programmed from 180 °C for 10 min, with a rise of 5 °C /min to 230 °C for 10 min. The detector temperature was set at 220 °C, and the injector was maintained at 220 °C. The carrier gas used was hydrogen at 30 ml/min and the air flow was 300 ml/min. N₂ makeup flow was 30ml/min. Analyses were performed in duplicates (five samples were run for each provenance) and fatty acid methyl esters (FAME) were identified by comparison of retention times with authentic standards, and quantification was performed by internal normalisation method.

5.2.2.3 Estimation of Biodiesel Parameters

Saponification Number (SN) and Iodine Value (IV) of oils were calculated using fatty acid methyl ester compositions of oil with the help of Eqs. (1) and (2), respectively (Kalayasiri et al. 1996):

\[ \text{SN} = \sum \left( \frac{560 \times A_i}{MW_i} \right) \]

\[ \text{IV} = \sum \left( \frac{254 \times D \times A_i}{MW_i} \right) \]

Where, \( A_i \) is the percentage, \( D \) is the number of double bonds and \( MW_i \) is the molecular mass of each fatty acid. Cetane number (CN) of FAMEs was calculated from Eq. (3) (Krisnangkura 1986)

\[ \text{CN} = 46.3 + \frac{5458}{\text{SN} - 0.225 \times \text{IV}} \]
5.2.3 Results and Discussion

A significant increase in oil content was observed in developing *Calophyllum inophyllum* fruits over time (Fig 5.9). Oil content increased significantly ($P<0.01$) from 26.3% (28 days after anthesis) to 42.7% (68 days after anthesis).

![Graph showing variation in oil content over time](image)

Fig 5.9 Variation in kernel oil content of developing *Calophyllum inophyllum* fruits

According to Gaydou et al. (1987) biosynthesis of triglycerides does not initiate at early stages of fruit development. Many authors have also observed a steady increase in oil content during fruit development which is a known phenomenon in many oil seed species (Kaliangile and Grabe 1988; Wiberg et al. 1997; Rahamatalla et al. 2001; Radic 2006).

Fatty acid composition in *C. inophyllum* kernels also varied significantly with the fruit development (Fig 5.10). These types of variations have been reported by Ozdemir and Topuz (2004) in avocado and Berti and Johnson (2008) in cuphea. Palmitic (16:0) and linoleic acids (18:2) were the most abundant fatty acids in early development stages of calophyllum fruit (Fig 5.10). Similar observations were reported by Robertson et al. (1978) in sunflower seeds and Berti and Johnson (2008) in cuphea seeds.
Palmitic acid (16:0) content has remained steady (≈22%) up until 48 days from anthesis and then fell noticeably to 15.4% by the last observation (78 days after anthesis). Ozdemir and Topuz (2004) also observed a similar decrease in palmitic acid content of developing avocado fruits. Stearic acid (18:0) content was found to increase marginally from 16.8% at 28 days after anthesis to 17.5% at 58 days after anthesis and then declined to 13.7% in the last observation.

Oleic acid (18:1) content steadily increased from 28.5% in the initially stage to 35.7% in the final observation. Fatty acids, mainly palmitic, stearic and oleic acids are important in cell membrane formation in developing seeds (Topfer et al. 1995).

In contrast to other dominant fatty acids, linoleic acid (18:2) content decreased from 30.9% at 28 days after anthesis to 28.2% at 48 days after anthesis and then significantly (P<0.05) increased to 34.3% by 77 days after anthesis. Linoleic acid is also vital to form numerous other cell components (Berti and Johnson 2008).
FAP of calophyllum fruit was least occupied by linolenic (18:3) and eicosanoic (22:0) and their composition remained at the same low levels for all stages of development. Fatty acid profiles determine the quality and end use of a vegetable oil (Gaydou et al. 1987). Fatty acid composition of the source vegetable oil also determines the physicochemical properties of the resultant biodiesel (Knothe 2005). The total saturated fatty acid composition in *C. inophyllum* fruits was found to decrease with the maturity (Fig 5.11). *C. inophyllum* fruits in Australian provenances generally experience cooler temperatures in later stages of development and cooler temperatures favour formation of unsaturated fatty acids (Harris et al. 1978).

High concentrations of saturated fatty acids can increase cloud point (CP) and cold filter plugging points (CFPP) which are undesirable for a liquid fuel (Ramos 2009; Knothe 2005). CP is the temperature at which solutes of a solution are no longer dissolved and CFPP is the temperature at which wax starts to settle and plugs filters and fuel lines (Knothe 2005).

![Saturated and unsaturated fatty acids composition of developing Calophyllum inophyllum fruits](image)

Fig 5.11 Saturated and unsaturated fatty acids composition of developing *Calophyllum inophyllum* fruits

Short to medium chain saturated fatty acids tend to form macro-crystalline structures at low temperature via uniform stacking of the ‘bend’ triglyceride backbone (Salimon and Salih 2009). Such macro-crystals restrict the easy flow of the system due to loss of kinetic energy of individual molecules during self-stacking (Jayadas and Nair 2006).
In contrast to the change in saturated fatty acid composition, the percentage of unsaturated fatty acids in all *C. inophyllum* fruits was found to increase with maturity. The lowest percentage of unsaturated fatty acids was recorded in oil extracted from fruits harvested in 38 days after anthesis. Unsaturated fatty acids help to maintain oil in a liquid form but if the high concentration of polyunsaturated fatty acids can form polymers under heat which can block the fuel system of a vehicle (Azam et al. 2005).

Tested quality parameters of biodiesel derived from oil extracted from all maturity stages were found to comply with industrial standards (Knothe et al. 2005). Saponification number (SN) of resultant biodiesel was found to decrease with fruit maturity (Table 5.2). A similar decrease in SN was observed in developing Macadamia nuts (Jones and Shaw 1943). These authors have also observed an increase in iodine number (IV) in developing macadamia nut, as observed in developing *C. inophyllum* fruits. IV represents the degree of unsaturation.

Table 5.2: Estimated parameters of biodiesel resulting from *C. inophyllum* oil of different fruit maturity (mean ± SE)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Biodiesel</th>
<th>Time (Days after anthesis)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SN</td>
<td>28</td>
</tr>
<tr>
<td>SN</td>
<td>201.4±0.9</td>
<td>201.1±0.8</td>
</tr>
<tr>
<td>IV¹115&lt;</td>
<td>83.2±2.1</td>
<td>83.0±1.8</td>
</tr>
<tr>
<td>CN²47&lt;</td>
<td>54.7±0.8</td>
<td>54.7±0.7</td>
</tr>
</tbody>
</table>

SN-saponification number, IV-iodine value and CN-cetane number.

Cetane number of biodiesel derived from *C. inophyllum* fruits collected at different maturity stages followed a sigmoidal curve. Maximum CN was recorded in biodiesel derived from oil extracted from *Calophyllum* fruits collected at 48 days after anthesis. Cetane number (CN) is widely used as the diesel fuel quality parameter related to the ignition delay time and combustion quality.
Higher cetane numbers give better ignition properties (Meher et al. 2006). An adequate cetane number is required for good engine performance. High cetane number helps ensure good cold start properties and minimizes the formation of smoke.

It is quite common to harvest fruits when they become morphologically mature. Results (Table 5.2) indicated that oil extracted from over-mature calophyllum fruits may result in a slight decrease in the quality of biodiesel. Oil extracted from fruits harvested at 48 days after anthesis had the best FAP for biodiesel (low IV and high CN). However, there was a significant increase (by 6%) in oil content of calophyllum fruits from 48 days after anthesis to 77 days after anthesis. Since FAP of oil extracted from calophyllum fruits harvested in 77 days after anthesis also conformed to ASTM standards, 77 days after anthesis can be proposed as a viable point of harvest.

5.2.4 Conclusions

Oil content in *Calophyllum inophyllum* fruits increase with maturity. Fatty acid composition of early development stages of the fruit is dominated by palmitic, stearic, oleic and linoleic acids. During fruit development, stearic acid content seems to decrease markedly and at the same time oleic acid content increases steadily. Palmitic acid and linoleic acid contents did not follow a linear trend. Palmitic acid content reached its maximum value by 48 days after anthesis and then showed a gradual fall.

In contrast, linoleic acid content felt slightly by 48 days after anthesis and then increased gradually. *Calophyllum inophyllum* fruits had much lower concentrations of linolenic and eicosanoic acids which did not vary with development.

Despite the superior biodiesel parameters found in oils extracted from *Calophyllum inophyllum* fruits harvested in 48 days after anthesis, harvest at maturity (77 days after anthesis) is the generally preferred harvesting time due to the significantly higher oil content than 48 days after anthesis and conformity to ASTM D 6571-01, EN ISO 14214 standards.
This Chapter revealed some important and previously unknown information regarding reproductive phenology of *Calophyllum inophyllum* stands in two different countries. This Chapter also presented unique information on the changes in oil and fatty acid composition in developing fruits.

Next Chapter discusses morphometric variations calophyllum seeds collected different provenances, seed storage behaviour, and provenance variation in germination and early growth.
CHAPTER 6
Seed and Seedling Characteristics

6.1 Provenance Variations in Seed Morphometric Characters of *Calophyllum inophyllum* in Northern Australia and Sri Lanka¹

6.1.1 Introduction

Quality of seeds is an important factor in plantation forestry. Polymorphism has also been found to play a great role in seed germination, survival and seedling growth (Pathak et al. 1980). Source variation tests are crucial to screen the naturally available genetic variation to select suitable planting material for higher productivity (Bhat and Chauhan 2002). Such tests yield valuable information which may be useful to commercial planters.

Calophyllum provenances may also show genotypic variations. Kaushik et al. (2007) and Rao et al. (2008) have noticed such variations and strong heritability in important seed-related characters like oil content among *Jatropha curcas* provenances in India. Searching for favourable variants may be useful in identifying suitable provenances and genotypes for commercial cultivation.

The present investigation was envisaged to evaluate the variations between provenances in seed parameters of *C. inophyllum* in Sri Lanka (northern hemisphere) and in Australia (southern hemisphere).

6.1.2 Materials and Methods

Provenances, corresponding sites and trees were selected as described in Chapter 3. Eight provenances were involved in this study. Seed collection in both countries was carried out from May to August 2008. Heterozygosity within a population is directly influenced by population size (Vergeer et al. 2003).

Hence, narrow population sizes of *Calophyllum inophyllum* provenances imply low heterozygosity within each selected population. Therefore, seeds of the same provenance were mixed together to form a representative seed sample. Variability in seed parameters (length, diameter and exocarp thickness) was investigated by measuring 30 randomly selected seeds from each provenance. Measuring was done with a digital Vernier calliper. For each provenance, kernel weight was determined by individually weighing 30 kernels.

### 6.1.3 Statistical Analysis

Data on each morphological trait investigated were subjected to analysis of variance. Provenance variance ($\sigma^2_{\text{prov}}$) and environment or error variance ($\sigma^2_e$) were estimated, and the proportion of $\sigma^2_{\text{prov}}$ to the total variance ($\sigma^2_T$) calculated in order to compare the magnitude of variation due to provenance (Ibrahim et al. 1997).

After testing for normality and homogeneity of error variances, differences among means were tested by Tukey simultaneous test using the General Linear Model of ANOVA (GLM) with MINITAB 14.1.

### 6.1.4 Results and Discussion

Significant provenance variations in seed-related traits of *C. inophyllum* were evident (Table 6.1). Country to country variation was also evident; Australian and Sri Lankan provenances differed markedly in seed morphometric characters. Several authors have reported similar findings (provenance variations) for other species such as; *Cordia africana* (Loha et al. 2006), *Dalbergia sissoo* (Gera et al. 2000), *Faidherbia albida* (Ibrahim et al. 1997), *Strychnos cocculoides* (Mkonda et al. 2003), and *Tectona grandis* (Jayasankar et al. 1999; Sivakumar et al. 2002).

The country to country variation was significant in all seed-related characters except kernel weight (Table 6.1). All provenances that were compared were found to be significantly different (P<0.05) in their seed length.
Variation in kernel weight was found to be less pronounced compared to that in other seed-related characters. Kernel weights of Anuradhapura differed less significantly (P>0.05) with that of Kurunegala and Yeppoon. Exocarp thickness of *Calophyllum inophyllum* seeds from Australian provenances was found to be greater than that of those from Sri Lankan provenances.

Table 6.1: Provenance means for seed-related traits of *C. inophyllum*

<table>
<thead>
<tr>
<th>Provenance</th>
<th>Seed length (mm)</th>
<th>Seed diameter (mm)</th>
<th>Kernel weight (g)</th>
<th>Exocarp thickness (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Townsville</td>
<td>38.72f</td>
<td>34.02e</td>
<td>5.16c</td>
<td>6.73d</td>
</tr>
<tr>
<td>Yeppoon</td>
<td>36.38e</td>
<td>31.53d</td>
<td>4.51b</td>
<td>5.13c</td>
</tr>
<tr>
<td>Cardwell</td>
<td>40.38g</td>
<td>33.79e</td>
<td>6.45d</td>
<td>5.27c</td>
</tr>
<tr>
<td>Anuradhapura</td>
<td>29.42c</td>
<td>26.11bc</td>
<td>4.71bc</td>
<td>2.93a</td>
</tr>
<tr>
<td>Colombo</td>
<td>27.07b</td>
<td>25.06b</td>
<td>3.63a</td>
<td>3.32b</td>
</tr>
<tr>
<td>Kurunegala</td>
<td>31.26d</td>
<td>26.38c</td>
<td>4.36b</td>
<td>3.34b</td>
</tr>
<tr>
<td>Matara</td>
<td>25.61a</td>
<td>23.07a</td>
<td>3.11a</td>
<td>3.38b</td>
</tr>
</tbody>
</table>

Within a column means that are not followed by the same letter are significantly different (at 0.05 level of probability) as determined by the Turkey simultaneous test.

Table 6.2: Correlations between seed morphometric traits of seven *C. inophyllum* provenances

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Seed diameter</th>
<th>Kernel weight</th>
<th>Exocarp thickness</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seed length</td>
<td>0.98*</td>
<td>0.84*</td>
<td>0.86*</td>
</tr>
<tr>
<td>Seed diameter</td>
<td>0.78</td>
<td>0.93*</td>
<td></td>
</tr>
<tr>
<td>Kernel weight</td>
<td></td>
<td>0.55</td>
<td></td>
</tr>
</tbody>
</table>

Values are Pearson product–moment correlation coefficient, r

*Correlation is significant at the 0.05 level.

Loha et al. (2006) suggested that correlated quantitative traits are extremely important in tree improvement as improving one character may cause simultaneous changes in other characters. These authors have reported a strong correlation between seed length and seed weight. Our data (Table 6.2) show seed length to be strongly correlated with seed diameter, kernel weight and exocarp thickness.
Strong positive correlation was also seen between seed diameter and exocarp thickness. These results are consistent with the finding of Kaura et al. (1998) who observed similar correlations in *Azadirachta indica* (Neem).

Table 6.3: Estimates of provenance variance as determined from measurement of seed-related traits of the *C. inophyllum* provenances investigated in this study

<table>
<thead>
<tr>
<th>Trait</th>
<th>Overall mean</th>
<th>Total variance(σ²T)</th>
<th>Provenance variance (σ²prov)</th>
<th>Error variance (σ²e)</th>
<th>Contribution (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seed length</td>
<td>33.77</td>
<td>0.176</td>
<td>0.0008</td>
<td>98.9</td>
<td></td>
</tr>
<tr>
<td>Seed diameter</td>
<td>29.42</td>
<td>0.132</td>
<td>0.0007</td>
<td>98.6</td>
<td></td>
</tr>
<tr>
<td>Exocarp thickness</td>
<td>4.80</td>
<td>0.563</td>
<td>0.0020</td>
<td>99.6</td>
<td></td>
</tr>
<tr>
<td>Kernel weight</td>
<td>4.45</td>
<td>28.396</td>
<td>0.7017</td>
<td>94.7</td>
<td></td>
</tr>
</tbody>
</table>

* Provenance contribution = (σ²prov)/ (σ²prov + σ²e) x100, Yi ij = µ (Overall mean) + Pi (provenance effect) + ε ij (random error).

Results of this study (Table 6.3) show that variations in seed related characters in *C. inophyllum* were largely due to the provenance contribution. Strong positive provenance contributions indicate the strong influence of genetic variability on these seed related characters (Ibrahim et al. 1997).

The lowest provenance contribution was found for kernel weight (94.7%). Ibrahim et al. (1997) have also observed kernel weight of *Faidherbia albida* to be the least affected by the provenance (provenance contribution 78%).

Mean seed size has been found to strongly correlate with several environmental factors (Hammond and Brown, 1995). This study showed strong correlation between geo-climatic conditions of the provenances and most of the seed-related traits in *C. inophyllum*. Paralleling the findings of Baker (1995) and Loha et al. (2006), our data (Table 6.4) revealed a strong negative correlation between altitude and most of the seed traits (seed length, seed diameter, exocarp thickness).
Table 6.4: Correlations between geo-climatic variables (mean altitude, mean annual rainfall, mean annual temperature) of seed origin and seed morphometric traits of six *C. inophyllum* provenances

<table>
<thead>
<tr>
<th>Seed Trait</th>
<th>Geo-climatic data</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Altitude (m)</td>
<td>Mean Annual Rainfall (mm)</td>
</tr>
<tr>
<td>Seed length (mm)</td>
<td>-0.80*</td>
<td>-0.26</td>
<td>-0.82*</td>
</tr>
<tr>
<td>Seed diameter (mm)</td>
<td>-0.88*</td>
<td>-0.30</td>
<td>-0.85*</td>
</tr>
<tr>
<td>Kernel weight (g)</td>
<td>-0.54</td>
<td>-0.05</td>
<td>-0.48</td>
</tr>
<tr>
<td>Exocarp thickness(mm)</td>
<td>-0.87*</td>
<td>-0.33</td>
<td>-0.77*</td>
</tr>
</tbody>
</table>

Values are Pearson product–moment correlation coefficient, r.
*Correlation is significant at the 0.05 level.

Significant negative correlations were also found between mean annual temperature and the above-mentioned seed traits. Mamo et al. (2006) reported weak correlations between geo-climatic conditions and seed-related traits of *Juniperus procera* populations in Ethiopia. Those observations may have resulted due to the lower degree of distinctiveness between the selected provenances (relatively less significant geo-climatic differences).

6.1.5 Conclusions

*C. inophyllum* provenances have a distinct country to country variation in their seed-related characters. Seed morphometric parameters demonstrate strong correlations between themselves. Most of the seed traits displayed strong correlations with altitude and mean annual temperature. Variations in seed-related characters are largely attributed by the provenance contribution.
6.2 Provenance Variations in Seed Germination and Early Growth of *Calophyllum inophyllum*

6.2.1 Background

Seed germination and early growth performance are important factors which have direct influence on the potential of any biodiesel feedstock species. Early growth traits signify the aptitude of a plant for successful establishment. Identifying stronger and adaptive seed sources is quite useful in order to prevent the planting of poorly adapted cultivars (Hamann et al. 2000).

*Calophyllum inophyllum* is regarded as a tree having a medium growth rate (Friday and Okano 2006). It can reach heights of up to 20-25 m (Flora of Australia 2007). The tree is believed to reach reproductive maturity after 7-8 years (Friday and Okano 2006). Seedlings can attain 1 m in height in their first year (Allen 2002). After five years, the growth rate slows down and becomes stable and reaches 20-25 m in height in approximately 25-30 years (Lamb 1981; Soerlanegara and Lemmens 1994). Two planting trials in Hawaii reported different seedling growth rates. In Opaeula Oahu at an altitude of 380 m, in deep acid soils, *C. inophyllum* seedlings reached 75 cm in height after one year from planting and calophyllum saplings planted in Waiakea Hilo at an altitude of 180 m, in thin acid soil reached 116 cm in height (Friday and Okano 2006). Those reports from Hawaii outline dissimilarities in heights and growth rates owing to differences in climate, altitude and soil type of the locality. According to the literature *C. inophyllum* prefers well drained soils (Agroforestry Tree Database 2007). However, in Sri Lanka trees can be found thriving on heavy clay soils on ridges surrounded by paddy fields (Figs 2.10 and 2.11) and river banks (Personal observation 2007).

The species is widely distributed naturally throughout the coastal areas of northern Australia. However, their populations are not continuous and there are subtle variations in geo-climatic conditions.
Calophyllum inophyllum populations in Sri Lanka are far more scattered and the provenances are relatively distinct in terms of their topography, micro-climate and soil conditions compared to those in Australia (Personal observation 2008). Such geo-climatic isolation can bring about a degree of specificity among provenances (Fleming et al. 1988).

Most plant species show variations in germinability between and within populations (Gera et al. 2000; Thomsen and Kjær 2002; Mkonda et al. 2003). Whilst some of these variations are attributed to genetics; much of it is known to be affected by the maternal environment under which the fruits develop (Mamo et al. 2006). Based on the earlier mentioned environmental variations of the selected calophyllum provenances, it is possible to assume that there could be variations in germination and early growth traits (Kamra and Simak 1968; Basada 1979) due to environmental and genetic differences.

Many authors have reported that the maternal environment is a key determinant of progeny variations (Schaal 1984; Roach and Wulff 1987; Lopez et al. 2003). The most significant influence of the maternal environment applies to early life history traits of progeny such as seed mass, germination and early growth (Schaal 1984; Roach and Wulff 1987; Lopez et al. 2003). Studying early growth variations helps in identifying strong adaptable seed sources for commercial cultivation.

6.2.2 Materials and Methods

6.2.2.1 Germination Test

Six provenances (Table 6.5) were selected which included three provenances each from northern Australia (Townsville, Yeppoon and Cardwell) and Sri Lanka (Anuradhapura, Colombo and Kurunegala). The selected provenances of each country are different in terms of their climate, topography and soil conditions and they are at least 100 km apart from one another.
Table 6.5: Selected Australian and Sri Lankan provenances of *Calophyllum inophyllum*

<table>
<thead>
<tr>
<th>Provenance</th>
<th>Coordinates</th>
<th>Altitude from MSL (m)</th>
<th>Mean Annual Rainfall (mm)</th>
<th>Mean Annual Temperature Max/Min (°C)</th>
<th>Soil types</th>
</tr>
</thead>
<tbody>
<tr>
<td>Australian Provenances</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Townsville</td>
<td>19° 13’ S, 146° 48’ E</td>
<td>3.5</td>
<td>1124</td>
<td>28.9/19.8</td>
<td>Clay loam or sodic clay soils. Dune sands</td>
</tr>
<tr>
<td>2. Yeppoon</td>
<td>23° 07’ 42’ S, 150° 44’ 34’ E</td>
<td>5</td>
<td>870</td>
<td>25.9/18.5</td>
<td>Rundle, shallow stony browns, clay loams and sandy clay loams.</td>
</tr>
<tr>
<td>3. Cardwell</td>
<td>18° 16’ 0 S, 146° 1’ 60’ E</td>
<td>5</td>
<td>2125</td>
<td>28.7/18.9</td>
<td>Solonez, <em>planosols</em>, Planosols, calcareous dune sands</td>
</tr>
<tr>
<td>Sri Lankan Provenances</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7. Anuradhapura</td>
<td>08° 20’ 60’ N, 80° 22’ 60’ E</td>
<td>75</td>
<td>1094</td>
<td>32.1/23.3</td>
<td>Reddish brown earths and immature brown loams</td>
</tr>
<tr>
<td>2 Colombo</td>
<td>6° 54’ N, 79° 50’ E</td>
<td>59</td>
<td>2500</td>
<td>30.6/24.1</td>
<td>Red-yellow podzolic with soft and hard laterite</td>
</tr>
<tr>
<td>3 Kurunegala</td>
<td>7° 45’ N, 80° 15’ E</td>
<td>55</td>
<td>1500</td>
<td>31.7/22.8</td>
<td>Red yellow podzolic soils with strongly mottled subsoil, low humic gley soils, red yellow podzolic soils with soft and hard laterite and regosols on red and yellow sands</td>
</tr>
</tbody>
</table>
Four to ten morphologically superior trees each were selected from 2-3 different locations within each provenance based on the morphometric and qualitative traits i.e. girth at breast height (GBH) ≈100-150 cm, and free from diseases and defects. Sampling was greatly reduced due to low population sizes. Seed collection was carried out from July to August 2008. Seeds from the same provenance were pooled to form a composite sample as the number of parent trees was too low and possibly could have low heterozygosity (Vergeer et al. 2003).

Seeds were stored in ventilated cardboard boxes at ambient conditions (≈25 °C/ 17 °C and RH 70%) until the trial was established (5 days). Ten random seeds from each provenance were used in moisture determination by oven-dry method (ISTA 1999). The germination trial was established at Uva Welllassa University, Badulla (6° 1’ 0 N, 80° 31’ 60 E), Sri Lanka (Fig 6.1) which has an altitude of 500 m, mean annual rainfall of 2250 mm and mean annual temperature of 24.7 °C.

Fig 6.1 Germination trials at Uva Welllassa University, Badulla, Sri Lanka (13/08/08)

Germination trays of Yeppoon seeds

Thirty randomly selected seeds (2 replicates of 15 seeds) from each provenance were planted in plastic trays (60×30 cm²) containing 2:1 sterilised sand to red yellow podzolic soil and were placed according to Randomized Complete Block Design (RCBD) in a partially shaded area (Fig 6.1).
Shells were completely removed before planting to increase the germination capacity and to reduce germination time (Parras 1939; Wilkinson and Elevitch 2004). An automatic misting system (Cloudburst 50) was set to 15 mins in the morning (7.15 am-7.30 am), 15 mins at midday (12.00 pm-12.15 pm) and 15 mins in the evening (7.15 pm-7.30 pm). Mean monthly maximum /minimum temperature at the setup was between 24-26 °C/17-19 °C (Regional weather station Badulla 2008).

After 30 days from initial planting, the number of germinated seeds was counted and the counting was continued for 70 days. No germinants were observed after 70 days. All ungerminated seeds were tested for viability using a tetrazolium chloride (TTC) stain. The germination percentage (GP) and mean germination time (MGT) were calculated using the following equations:

\[
GP(\%) = \left( \frac{N}{S} \right) \times 100\% \quad (1)
\]

where \(N\) is the total number of germinated seeds and \(S\) is the total number of seeds

\[
MGT(d) = \frac{\sum (i \times n_i)}{N} \quad (2)
\]

where \(i\) is the \(i^{th}\) day from sowing, \(n_i\) is the number of germinated seeds at the \(i^{th}\) day from sowing and \(N\) is the total number of germinated seeds (Liu et al. 2005).

After 30 days from germination, length: width ratio of all emergent leaves of germinated seedlings was measured and SPAD chlorophyll meter readings were recorded.

6.2.2.2 Early Growth Trials

Germinated seedlings from the previous experiment (germination trial) were used in this experiment. Eight healthy seedlings (30 days after germination) were selected from each provenance. The trial was established in September 2008 at the same location (same conditions) where the germination test was carried out. A mixture of sterilized sand and red clay loam soil (2:1) was used as the medium. Seedlings were transplanted one each in labelled medium sized pots (20 cm in diameter).
6.2.2.3 Trial Establishment

Five provenances were planted under partial shade in a randomized complete block design comprising of eight replicates (Fig 6.2).

6.2.2.4 Maintenance

Weeding was done after every two weeks. An automatic misting system (Cloudburst 50) was set to 15 mins in the morning (7.15 am-7.30 am), 15 mins at midday (12.00 pm-12.15 pm) and 15 mins in the evening (7.15 pm-7.30 pm).

Fig 6.2 Provenance trial at Uva Wellassa University, Badulla, Sri Lanka (14/09/2008)

6.2.2.5 Recording Data

Initial heights (30 days after germination) and heights after 4 months and 8 months in the trial were recorded. Mean height increment was calculated by following formula;

\[
MH_i = \sum \frac{H_f - H_i}{n}
\]

where \(MH_i\)=Mean height increment, \(H_f\)=final height of the seedling, \(H_i\)=initial height of the seedling, \(n\)=number of seedlings.

After 8 months from the trial establishment, length and width ratio and SPAD chlorophyll meter readings of 15 random leaves of seedlings from each provenance were recorded.
The seedlings were then carefully removed from the pots and the shoots were cut from the roots-shoot junction. Roots were washed with demineralised water, and shoots and roots were dried at 80°C for 48 h and then sealed in plastic bags containing 20 g of silica gel until weighed (Fleming et al. 1988).

6.2.2.6 Statistical Analysis

After testing for normality and homogeneity of error variances, differences among means were tested by Tukey simultaneous test and the General Linear Model of ANOVA (GLM) using MINITAB 14.1.

6.2.3 Results and Discussion

6.2.3.1 Germination

*Calophyllum inophyllum* seeds from the selected provenances displayed notable variations in their early growth characteristics (Table 6.6) including germination characteristics (Fig 6.3). None of the seeds from Yeppoon germinated after 70 days. Fruits collected from Yeppoon appeared wrinkled and were less hard than those collected from the rest of the provenances. Initial moisture level of seeds from Yeppoon (52%) was higher than that of seeds from other provenances (Townsville 40%, Cardwell 38%, Anuradhapura 36%, Colombo 42%, and Kurunegala 34%).

Higher seed moisture levels and premature harvest can reduce seed germination viability (Radic 2006). De-shelled seeds from Yeppoon appeared dark brown and were different from the rest of the seeds that showed different gradients of yellow colour. Seeds from Colombo, Sri Lanka and Cardwell, Australia demonstrated appreciable germination percentages (>70%).

Seeds from Anuradhapura and Kurunegala recorded relatively similar and low germination percentages; 56.7% and 60% respectively. The environmental conditions under which fruits develop greatly influence the germinability of seeds (Gutterman 2000; Mamo et al. 2006). *Calophyllum inophyllum* provenances in Australia and Sri Lanka have different geo-climatic conditions.
Mean annual rainfall of Cardwell (2125 mm) and Colombo (2500 mm) are relatively similar and higher than the rest of the provenances. This high rainfall in the provenance environment may have positively influenced the germination of seeds from Colombo and Cardwell.

![Germination percentages (GP) and mean germination times (MGT) of Calophyllum inophyllum provenances (n =15×2)](image)

**Fig 6.3** Germination percentages (GP) and mean germination times (MGT) of *Calophyllum inophyllum* provenances (n =15×2)

Seeds from Colombo recorded the lowest mean germination time (MGT) and those from Townsville recorded the highest MGT, followed by Kurunegala. High MGT in Kurunegala resulted from delayed germination of some seeds (SE ± 4.94).

6.2.3.2 Emerging Seedling Colour

There was a remarkable difference in colour of the emerging seedlings between Australian provenances and Sri Lankan provenances. Sri Lankan provenances sprouted red while Australian provenances sprouted green (Figs 6.4A and 6.4B).
After 32 days, seedlings from Sri Lankan provenances also turned greenish. Calophyllum provenances in Sri Lanka show natural regeneration under shady conditions; whereas seedlings in Australian provenances mostly experience open environments (Personal observation 2007-2008). Difference in sprout colour may be related to the difference in light requirement in early development phase.

6.2.3.3 Development of Seedlings

During intermediate stages of development, seedlings from Anuradhapura began to exhibit a peculiar leafing pattern. These leaves were significantly (P<0.05) elongated compared to those of seedlings from other provenances, as indicated by their length: width ratio (Fig 6.6). They remained the most elongated leaves even after 9 months from germination (Fig 6.9). Seedlings from Anuradhapura showed leaves having different colour intensities (Fig 6.6). This peculiar character found in seedlings from Anuradhapura remained until 60 days after germination.
The SPAD-502 chlorophyll meter readings (SCMR) revealed that the darker coloured elongated leaves of Anuradhapura provenance contained higher amounts of chlorophyll compared to other provenances (Fig 6.6). Seedlings from Colombo, Townsville and Cardwell had similar leaf shape (ovate), as indicated by similar length: width ratio (Fig 6.6).

Fig 6.5 Seedlings from Colombo (on the left) and seedlings from Anuradhapura (on the right).

Fig 6.6 SPAD Chlorophyll Meter Reading (SCMR) and length: width ratio of leaves of 30 days old Calophyllum seedlings of five provenances (*values are means of 15 replicates, means followed by the same letter are not significantly different at 0.05 level of probability) Columns with error bars are not statistically significant (P>0.05)
6.2.3.4 Height and Mean Height Increment

The selected *C. inophyllum* provenances showed significant provenance variations in seedling heights after 9 months from germination and in the seedling height increment for 8 months. However, the *C. inophyllum* provenances did not show marked differences (P>0.07) in initial seedling height and seedling height after 5 months from germination (Fig 6.7). Seedlings from Cardwell and Townsville recorded the highest and second highest final height and were best adapted to the conditions of the trial.

The seedlings from all selected provenances, except those from Colombo demonstrated appreciable early growth rates. Seedlings from Kurunegala recorded the highest height increment after 9 months from germination, in spite of having the lowest initial height.

The lowest growth rate was observed among the seedlings from Colombo (3.26 cm/8 months). Initial heights of seedlings from Colombo (15.2 ± 2.5 cm) and Kurunegala (14.2 ± 2.3 cm) were somewhat lower than the rest of the seedlings from other provenances.

Fig 6.7 Height and height increment of *C. inophyllum* seedlings of selected provenances after 9 months from germination. Ht- initial height, Ht5- height after 5 months from germination, Ht9-final height, values are means of 8 replicates, means followed by the same letter are not significantly different at 0.05 level of probability. Columns with error bars do not show statistically significant differences (P>0.05).
The least variation in seedling height was observed after completion of four months in the trial. Seedlings from all the selected provenances except Colombo (18.7 cm) achieved similar heights (23-25 cm) after 9 months from germination. This shows that initial height variation is not a genetic trait.

The parent trees of *C. inophyllum* seedlings from Colombo were growing on heavy clay soils and showed slow growth rate i.e. four trees attained ≈13 m in height at the age of 25 years (Personal communication 2008).

This may be due to poor root respiration as they were growing on heavy clay soils having less number of air-filled pores (Bauma and Bryla 2000). Abundance of air-filled pores is important for plant growth (Wall and Heiskanen 2009).

This slow growth rate in the parent trees of the seedlings from Colombo may have influenced the slow early growth rate in their progeny and those seedlings might overcome slow growth rate as they age further (Schaal 1984; Roach and Wulff 1987; Lopez et al. 2003).

6.2.3.5 Root: Shoot Ratio

After 9 months from germination under partial shade, *C. inophyllum* seedlings recorded significant provenance variations (*P*<0.05) in root: shoot ratio (Fig 6.8).

![Provenance variations in root: shoot ratio of 9 months old *C. inophyllum* seedlings. Values are means of 8 replicates. Means followed by the same letter are not significantly different at 0.05 level of probability](image)
Seedlings originating from Townsville recorded the highest root: shoot ratio (1.5) followed by the seedlings from Kurunegala. The lowest root: shoot ratio was found among the seedlings from Colombo which originated from parent trees occurring in heavy clay soils (Personal observation 2008). Raddad (2007) also reported of seedlings having shorter roots and lower root: shoot ratios associated with clay provenances of *Acacia Senegal*.

This shows that soil characteristics of origin has some bearing on early root growth of calophyllum seedlings. Seedlings from Anuradhapura and Cardwell had similar root: shoot ratios. However, seedlings from Cardwell had relatively higher shoot growth than lower root growth.

6.2.3.6 Leaf Length: Width Ratio after 9 Months from Germination

Seedlings of *Calophyllum inophyllum* provenances maintained their differences in leaf length: width ratio (found after 30 days from germination) for 8 months of the trial (final observation) despite the increase in the leaf dimensions due to growth (Fig 6.9). Distinct elongated leaf shape of the seedlings from Anuradhapura which appeared after 30 days from germination remained unchanged even after 8 months in the trial, and made them the most elongated leaves.

![Leaf length: width ratio of *Calophyllum inophyllum* seedlings after 9 months from germination. Values are means of 8 replicates. Means followed by the same letter are not significantly different at 0.05 level of probability](image)

Fig 6.9 Leaf length: width ratio of *Calophyllum inophyllum* seedlings after 9 months from germination. Values are means of 8 replicates. Means followed by the same letter are not significantly different at 0.05 level of probability
As mentioned earlier, seedlings from Anuradhapura also contained the highest relative chlorophyll levels at 30 days from germination. Seedlings from Cardwell and Townsville had markedly greater leaf size compared to other provenances. Smallest leaves were found in seedlings from Colombo.

Table 6.6: Summary of the provenance variation in early growth traits of *Calophyllum inophyllum*

<table>
<thead>
<tr>
<th>Observation</th>
<th>Anuradhapura</th>
<th>Colombo</th>
<th>Kurunegala</th>
<th>Townsville</th>
<th>Cardwell</th>
</tr>
</thead>
<tbody>
<tr>
<td>Germination (%)</td>
<td>60.00</td>
<td>83.3</td>
<td>56.7</td>
<td>53.3</td>
<td>70.01</td>
</tr>
<tr>
<td>MGT (days)</td>
<td>40.00</td>
<td>34.83</td>
<td>46.61</td>
<td>55.00</td>
<td>41.42</td>
</tr>
<tr>
<td>Sprouting</td>
<td>Red</td>
<td>Red</td>
<td>Red</td>
<td>Green</td>
<td>Green</td>
</tr>
<tr>
<td>SCMR</td>
<td>71.80</td>
<td>61.55</td>
<td>61.52</td>
<td>52.26</td>
<td>60.92</td>
</tr>
<tr>
<td>Leaf Length: Width</td>
<td>2.20</td>
<td>1.61</td>
<td>1.83</td>
<td>1.62</td>
<td>1.59</td>
</tr>
<tr>
<td>Ht</td>
<td>23.24</td>
<td>18.73</td>
<td>23.49</td>
<td>25.16</td>
<td>24.51</td>
</tr>
<tr>
<td>MHI (cm)</td>
<td>7.23</td>
<td>4.26</td>
<td>10.90</td>
<td>6.80</td>
<td>7.34</td>
</tr>
<tr>
<td>Root: Shoot</td>
<td>0.94</td>
<td>0.74</td>
<td>1.18</td>
<td>1.50</td>
<td>0.95</td>
</tr>
</tbody>
</table>

6.2.3.7 Relationship between Maternal Environment and Early Growth

Environment of the seed origin has a strong influence on early growth traits of flowering plants (Alexander and Wulff 1985; Mazer and Gorchov 1996; Andalo et al. 1999). Many authors have described the maternal environment as a key determinant of progeny variations (Schaal 1984; Roach and Wulff 1987; Lopez et al. 2003).

The most significant influence of maternal environment can be found on early life history traits of the progeny such as seed mass, germination and early growth parameters (Schaal 1984; Roach and Wulff 1987; Lopez et al. 2003).

Germination percentage of *C. inophyllum* provenances was found to be strongly correlated (*r = 0.90**) with the mean annual rainfall (MAR) (Fig 6.10) which indicated that germinability of the *C. inophyllum* seeds could possibly be influenced by the maternal environment. Raddad (2007) also reported that seed germination can be greatly influenced by the rainfall of the seed origin.
In this study, the influence of altitude of the source environment on germination of *C. inophyllum* seeds was found to be very weak ($r = 0.18$). In contrast to our findings, Vera (1997) observed high germination percentage in *Erica* sp. collected from higher altitude origins. The author explained that it could be due to chilling induced dormancy alleviation (Probert 1992). Bergin and Kimberley (1992) found significant correlations ($r=0.30^*$) between germination of *Podocarpus totara* and the altitude which they have not included in their discussion.

Later, Bergin et al. (2008) reported that altitude is not correlated to germination of *Podocarpus totara*. In this study, altitudes of the selected provenances ranged between 3.5 -75 m from MSL and consequently had less temperature variations to cause any influence on germination of *C. inophyllum* seeds.

Total soil carbon content and conductivity of the provenances also seemed to significantly correlate with the germination percentage (Table 6.7). Seedling heights of *C. inophyllum* provenances (after 9 months from germination) showed negative correlations with altitude ($r=-0.33^*$), mean annual minimum temperature ($r=-0.42$, soil conductivity ($r=-0.53^{**}$) and total carbon content ($r=-0.54^{**}$) of the selected provenances (Table 6.7).
After 9 months from germination, taller seedlings were evident for provenances that have higher soil potassium and pH levels. Wall and Heiskanen (2009) did not find any correlations between height growth of *Pinus sylvestris* and soil nutrients. Mean height increment of *C. inophyllum* seedlings did not show any correlation with the majority of environmental variables of their seed origins, except pH and total soil carbon content.

Table 6.7: Correlation between germination and early growth parameters (after 9 months from germination) with maternal environmental parameters

<table>
<thead>
<tr>
<th>Parameters</th>
<th>MaxT</th>
<th>MinT</th>
<th>Alt</th>
<th>MAR</th>
<th>N</th>
<th>P</th>
<th>K</th>
<th>Con</th>
<th>pH</th>
<th>TC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Germination (%)</td>
<td>-0.09</td>
<td>0.30</td>
<td>0.18</td>
<td>0.90**</td>
<td>-0.09</td>
<td>-0.30*</td>
<td>-0.24</td>
<td>0.64**</td>
<td>-0.81**</td>
<td>0.77**</td>
</tr>
<tr>
<td>Ht (m)</td>
<td>-0.20</td>
<td>-0.42*</td>
<td>-0.33*</td>
<td>-0.37*</td>
<td>0.22</td>
<td>0.08</td>
<td>0.36*</td>
<td>-0.53**</td>
<td>0.54**</td>
<td>-0.54**</td>
</tr>
<tr>
<td>MHI</td>
<td>-0.03</td>
<td>-0.26</td>
<td>-0.20</td>
<td>0.25</td>
<td>0.04</td>
<td>-0.05</td>
<td>0.13</td>
<td>-0.30</td>
<td>0.46*</td>
<td>-0.48*</td>
</tr>
<tr>
<td>Root: Shoot</td>
<td>-0.30</td>
<td>-0.49*</td>
<td>-0.51*</td>
<td>-0.68**</td>
<td>0.47*</td>
<td>-0.96**</td>
<td>0.29</td>
<td>-0.56**</td>
<td>0.49*</td>
<td>-0.70**</td>
</tr>
<tr>
<td>Survival (%)</td>
<td>-0.13</td>
<td>0.17</td>
<td>0.09</td>
<td>0.96**</td>
<td>-0.10</td>
<td>-0.44*</td>
<td>-0.15</td>
<td>0.58**</td>
<td>-0.73**</td>
<td>0.59**</td>
</tr>
</tbody>
</table>

Ht, MHI- height and mean height increment after 9 months from germination, MaxT-mean maximum annual temperature, MinT-mean minimum annual temperature, Alt-altitude, MAR-mean annual rainfall, N,P,K-nitrogen, phosphorous, potassium, Con-conductivity, TC-total carbon. Values are Pearson’s correlation coefficients r, *P<0.05, **P<0.001, n=40.

Root: shoot ratio of *Calophyllum inophyllum* seedlings showed significant negative correlations with MAR (r=-0.68**), altitude (r=-0.51*), mean annual minimum temperature (r=-0.49*), soil phosphorous level (r=-0.96**), conductivity (r=-0.56**) and total carbon (r=-0.70**) of their origin (Table 6.7).

Natural selection for the development of higher root biomass, relative to above ground mass is likely to be stronger in water limiting environments (Chapin et al. 1993; Schenk and Jackson 2002). Soil nitrogen and pH levels of seedling origins appeared to have a positive influence on root: shoot ratio of the seedlings after 9 months from germination. Fageria (2010) reported greater root growth in rice (*Oryza sativa*) influenced by high soil nitrogen content.
6.2.4 Conclusions

Germination and early growth characters of *Calophyllum inophyllum* appeared to have source related variations. The highest germination percentage was found in seeds from Colombo (84%), followed by seeds from Cardwell (70%). Seedlings from the rest of the provenances recorded weaker germination percentages (≤60%). Germination in *C. inophyllum* seeds has a strong relationship with mean annual rainfall, soil electrical conductivity and total carbon content, and soil pH of the origin. Seeds from Townsville and Kurunegala took slightly longer time to germinate than did other seed sources.

The difference in sprouting colour indicated that Sri Lankan provenances may have developed a shade tolerance and Australian provenances may have adapted to open conditions. Seedlings from Anuradhapura were found to have elongated chlorophyll-rich leaves compared to those from other provenances. Heights of *Calophyllum inophyllum* seedlings (9 months after germination) did not show a significant (P>0.05) variation.

The tallest and the shortest seedlings were found among seedlings from Townsville and Colombo respectively. Early growth rate of seedlings from Kurunegala was found to be significantly (P<0.05) higher than that of the rest of the seedling sources. Seedlings from Townsville and Kurunegala had higher root biomass compared to other provenances.

Overall, maternal environment appeared to have a significant influence on the early growth traits of *Calophyllum inophyllum*. Even though the variations in some of these early growth traits are temporary lived, this study shows the germination rate, adaptability and survival of different provenances which can be used to select suitable cultivars.
6.3 Seed Storage Behaviour of *Calophyllum inophyllum* L.*

6.3.1 Introduction

Seed storage behaviour is an important factor in deciding the economic potential of a crop. It provides vital information for storing seed material for commercial plantation programs. Generally seeds exhibit three types of storage behaviours; orthodox, intermediate and recalcitrant (Song et al. 2003; Tweddle et al. 2003). Seeds that can be dried to moisture contents between 6 and 10% and stored successfully at low temperatures have been described as ‘orthodox’ in storage behaviour, while seeds that cannot be dried to these levels without losing viability have been described as ‘recalcitrant’ (Roberts 1973). Those that fall in between have been categorised as ‘intermediate’ seeds. While these terms have been characterized as less than ideal choices to describe physiological behaviour of seeds (Berjack et al. 1990), they have been widely accepted and are commonly used by most seed scientists today (Bonner 1996).

According to the literature, seed storage behaviour of *Calophyllum inophyllum* is inconclusive. Seed information database (SID 5.0) of the Royal Botanic Gardens, Kew UK also does not provide clear information about the storage behaviour of *C. inophyllum*.

Different authors have given different conclusions; Ng (1992) and Gupta et al. (2009) reported that seed storage behaviour of *C. inophyllum* is intermediate. However, Allen (2002) suggested that *C. inophyllum* seeds are recalcitrant.

Currently, there are no reports with regard to the storage behaviour of *C. inophyllum* in Australia. The following experiments were carried out to investigate the storage behaviour of Australian *C. inophyllum* seeds.
6.3.2 Materials and Methods

6.3.2.1 Desiccation Tolerance Test

Mature seeds (with no exocarp) were collected from three eight year old trees from road side plantings at Rosslyn bay, Yeppoon (23° 7' 60 S, 150° 43' 60 E) on 14th October 2007. One hundred and thirty seeds with no exocarp were used for the experiment. Ten random seeds were used to estimate initial moisture content of the seed sample. They were weighed (W₁) and then oven dried at 105 °C for 17 hours (ISTA 1999) and again weighed (W₂). Initial moisture content was estimated with the following formula:

\[ MC = \frac{W₁ - W₂}{W₁} \times 100 \]

(MC is the moisture content; W₁ is fresh weight of the seeds; and W₂ is dry weight of the seeds)

Seed Drying

Initial moisture content of the collected seed lot was found to be 40%. The International Seed Testing Association (ISTA 1999) method was used in seed drying. Silica gel was used in drying seeds. Seeds (60 each) were kept in two desiccators (6 L capacity) containing silica gel (equal to 75% of the weight of seeds).

Temperature was maintained at 25 °C (Hong et al. 1996) and vacuum pumps were connected to desiccators in order to induce rapid drying. The number of desiccation treatments was reduced to suit the sample size.

A series of moisture contents (38%, 22%, 18%, and 14%) were obtained by removing seeds at different intervals. At each of these moisture levels, 30 seeds were separated from the sample and kept in sealed plastic bags to prevent further drying. Plastic bags were weighed at the time of transfer and reweighed before germination tests were carried out to check for any moisture loss.
Viability Test

A germination test was carried out to test the viability of the seed sample after each treatment. Twelve pots (20 cm in diameter) were filled with sterilized river sand and three pots (replicates) were used for each treatment. Ten seeds from each treatment were planted in each pot. Shells were slightly cracked by way of a mechanical vice before planting to reduce the germination period (Parras 1939). Pots were placed in a shaded and auto irrigated rack (Fig 6.11). Auto sprinkling was set to a 15 mins/twice a day configuration. After 30 days, the number of germinated seeds was counted. Counting was stopped after 65 days. All ungerminated seeds were tested for viability using a tetrazolium chloride (TTC) stain. Seeds were bisected and incubated in 1% (w/v) 2, 3, 5-triphenyltetrazolium chloride at 38 °C for 2 days. Highly viable seeds were uniformly stained red, whereas seeds with reduced vigour remained unchanged. The germination percentage (GP) and mean germination time (MGT) were calculated as per section 6.2.2.

Fig 6.11  Germination trial 55 days after sowing (treatments; moisture levels from left, 38%, 22%, 18%, and 14%)
6.3.2.2 Storage Longevity of *Calophyllum inophyllum* Seeds under Different Storage Environments

**Storage Longevity**

Mature seeds devoid of exocarp (exocarp eaten by fruit bats) were collected from three eight year old trees from roadside plantings at Rosslyn Bay, Yeppoon (23° 7' 60 S, 150° 43' 60 E) during October 2007. Three hundred and seventy (370) mature seeds were used in this experiment. Initial moisture content was estimated as described in the earlier section.

Fresh seeds were dried to 20% moisture content by using Silica gel (equal to approximately 75% of the weight of the seeds) in a sealed plastic container at 25 °C and immediately transferred to three porous sterilized cardboard boxes (30×45×15 cm³). Weights of the boxes were recorded (W1, W2, W3).

Boxes were then placed in different temperature environments; cool room (≈8 °C /RH ≈55-60%), potting shed (≈25-40 °C /RH 50-75%) and ecophysiology lab (≈25 °C /RH ≈50-65%).

RH of each storage environment was measured three times per week using a wet and dry bulb thermometer. After 3, 6 and 8 months of storing seeds in the three storage environments, 30 seeds (3 replicates × 10 seeds) from each box were taken out and germination tests were carried out. Each time the boxes were weighed before taking out seeds.

Seeds from three storage environments were planted in (3 replicates x 3) pots (20 cm in diameter) containing washed river sand (Fig 6.11). Shells were completely removed (without making any injury to the kernel) before planting to reduce the germination time (Parras 1939). Auto sprinklers were set to a 15 mins and twice a day configuration. After 35 days number of germinated seeds was counted and the counting continued for 60 days. Germination percentage and mean germination time were calculated using formulae mentioned in Section 6.2.2.
6.3.2.3 Moisture Loss in Storage

Four seeds each were placed uncovered in the three selected storage environments and individual weights were recorded for 26 days.

**6.3.3 Results and Discussion**

6.3.3.1 Desiccation Tolerance

The highest germination percentage (80%) was found in seeds that contained 38% moisture content. Fig 6.12 shows a significant decline in germination percentage when moisture content falls below 22%. Germination percentage dramatically dropped from 76.7±0.33% to 36.7±0.30% when the moisture content was reduced from 22% to 18%.

Results (Fig 6.12) indicated that viability of *Calophyllum inophyllum* seeds is largely dependent on the moisture content. Sacande et al. (2001) also reported that some seeds of tropical origin are susceptible to dehydration. In dry environments, seeds lose their viability due to the shrinkage, aqueous based degradation or denaturation of storage chemicals caused by dehydration (Pammenter and Berjack 1999).
In this experiment less than 50% of the seeds were found to survive after the moisture content had fallen below 20%. The sharp decline in germination percentage of *C. inophyllum* seeds in response to the reduction of MC from 22 to 18% indicates that *C. inophyllum* seeds have a minimum moisture level to which they can be dried while retaining viability.

This specific moisture content has been described as the CMC “critical moisture content” (King and Roberts 1979) or “lowest safe moisture content” (Tompsett 1984) which is a common characteristic of recalcitrant seeds. Taking into account the presence of CMC (18-22%), *C. inophyllum* seeds can be identified as recalcitrant seeds, supporting similar suggestions reported by Allen (2002).

Fig 6.13 Mean germination time of *Calophyllum inophyllum* seeds with different moisture contents, n=3
Results (Fig 6.13) also show that reduction in seed moisture content can prolong germination. The mean germination time (MGT) increased with the decline of seed moisture content to 18%, but did not increase further in drier seed.

**Storage Longevity**

During the eight months of storage, the majority of *C. inophyllum* seeds retained their viability in the warmer (25-30 °C) conditions (Fig 6.14). In contrast to our findings, Foxworthy (1927) reported that *C. inophyllum* seeds generally do not maintain their viability for long periods, but the exact period was not mentioned in his report.

![Germination percentage of *C. inophyllum* seeds stored under different conditions: Potting shed (≈25-40 °C, RH ≈50-75%), Ecophysiology lab (≈25 °C, RH ≈50-65%) and Cool room (≈8 °C, RH ≈60%). n=3](image)

Gupta et al. (2009) also reported that *C. inophyllum* seeds which were devoid of endocarp have very short storage longevity (less than a fortnight) when kept at ambient temperatures (≈25 °C).
However, the results of this study showed that if stored in warmer and humid environments, more than 70% of *C. inophyllum* seeds are able to maintain their viability for an appreciable period (> 8 months). Seeds of a related species (*Calophyllum calaba*) reportedly are able to maintain viability up to one year if stored in a dry room (Weaver 1990).

Shorter storage longevity of *C. inophyllum* seeds observed by Gupta et al. (2009) may be due to the complete removal of the endocarp. Endocarp prevents seeds from undergoing rapid desiccation. In humid and warmer storage environments, seeds having an intact endocarp could resist rapid desiccation and prevent them from reaching CMC for a somewhat long period (> 8 months).

Slowest moisture loss in seeds was observed in those stored in the ‘potting shed’ followed by those stored in the ‘cool room’ (Fig 6.15). *Calophyllum inophyllum* seeds stored in the potting shed seem to rehydrate at certain intervals while steady reduction in seed weight was observed among those stored in the cool room (Fig 6.16).

![Seed weight reduction in *Calophyllum inophyllum* seeds after 26 days in different storage environments, n=4](image)

Moisture loss in seeds stored in the potting shed (25-40 °C, RH ≈59-75%) was slower than those stored in the ecophysiology lab (≈25 °C, RH ≈50-65%) and the seeds stored in the ecophysiology lab recorded lower germination percentage at the selected intervals compared to those stored in the potting shed.
This indicates the effect of moisture loss in dry environments on seed storage longevity. Despite having a slower seed moisture loss compared to the ecophysiology lab, seeds stored in the cold room recorded the lowest germination percentage.

Fig 6.16 Changes in the weight of four *Calophyllum inophyllum* seeds placed in different environments over time. P-seeds in the potting shed, E-seeds in the ecophysiology lab, C-seeds in the cool room.
In agreement with the findings of this study, Gupta et al. (2009) also reported that calophyllum seeds wrapped in moist paper towels in plastic boxes at 4-10 °C cold storage environments can only retain their viability up to four months. This shows that low temperatures may have a negative influence on seed storage longevity.

Fig 6.17 Calophyllum inophyllum kernels after 8 months of storage; A-cold room, B-ecophysiology lab, C-potting shed

Careful observation of kernels revealed that under cold/dry (≈8 °C /RH ≈55-60%) storage environments the C. inophyllum seeds become darker coloured in the centre (Fig 6.17). This indicates that low viability of cold stored seeds may have been caused by a chilling injury (Hong et al. 1996).

A number of authors also have noted that seeds of some species originating from tropical environments are vulnerable to chilling injury (Corbineau and Come 1988; Tompsett 1994). Those tropical seeds usually get injured when they are stored below 10-15 °C (Sacande et al. 2001), partly due to dysfunction of some enzymes (Lyons et al. 1979; Yoshida et al. 1986) and partly due to leakage of cytoplasmic solutes (Bergevin et al. 1993; Bertin et al. 1996). Darkening of the inner centre parts of the kernels of C. inophyllum stored in cold storage may be due to leakage of cytoplasmic solutes.
After 8 months of storage, seeds stored in the potting shed (25-40 °C, RH ≈59-75%) appeared pale yellow and those stored in the ecophysiology lab (≈25 °C, RH ≈50-65%) were dark yellow. Those seeds stored in the ecophysiology lab had the second highest moisture loss.

Dweck and Meadow (2002) noticed darkening of the colour of calophyllum kernels under desiccation. Kernels of *C. inophyllum* contain high amount (111± 5 mg/kg) of phenolic compounds (Seneviratne and Kotuwegedara 2009). Those compounds are known to cause discolouration in vegetables and fruits (Xu and Diosady 2002). Darker colour in kernels that were stored in the ecophysiology lab for eight months may be due to oxidation of these phenolic compounds. In agreement with our findings, Dweck and Meadows (2002) reported that browning of *C. inophyllum* seeds could also indicate its loss of germinative power.

### 6.3.4 Conclusions

*Calophyllum inophyllum* seeds appear to exhibit “critical moisture content” where germination is reduced by >50% if seed moisture content falls below that point (18-22%). Germination is also delayed if the seed moisture content is low. This indicates that *C. inophyllum* seeds could probably be recalcitrant. *Calophyllum inophyllum* seeds can retain viability for an appreciable period (>8 months) if stored in warmer (≈25-30 °C) and slightly humid (≈70%) environments without removing their endocarp. *Calophyllum inophyllum* seeds appear to be sensitive to low temperatures. More elaborate tests should be carried out to firmly conclude its vulnerability to chilling injury.

This Chapter discussed country to country and provenance variations in seed morphometric characteristics, germination and early growth. Some relatively unknown information on germination and early growth of Sri Lankan seed sources was revealed. Seed storage behaviour of *Calophyllum inophyllum* was found to be recalcitrant.
The next Chapter examines the provenance and seasonal variations in kernel oil content, fatty acids, and fatty acid methyl ester properties.
CHAPTER 7

Kernel Oil, Fatty Acid Profiles and Fatty Acid Methyl Ester Properties

7.1 Provenance Variations in Kernel Oil Content of Calophyllum inophyllum

7.1.1 Background

Chemical composition of plant extracts varies with geographic variations (Canard et al. 1997). Sahoo et al. (2007) suggested that the type and percentage of fatty acids contained in vegetable oils depends on the plant species and the growth conditions. The kernel oil content, fatty acid composition and other organic compounds may also vary with geo-climatic and soil conditions. Marked differences in fatty acid profiles (section 3.4.7), iodine values and saponification numbers reported by different authors (Hemavathy and Prabhakar 1990; Azam et al. 2005; Crane et al. 2005) who used different seed sources, support the above claim (Chapter 3, Table 3.2) on the effects of provenance on the feedstock quality. Properties of biodiesel are largely depending on the fatty acid profile (FAP) of its feedstock (Knothe et al. 2003; Azam et al. 2005; Ramos et al. 2009), and such variations in FAP can directly influence the quality of biodiesel. Understanding these variations is very important, in order to select ideal agronomic conditions, suitable provenance/s and genotypes for biodiesel feedstock plantations.

7.1.2 Materials and Methods

7.1.2.1 Seed Collection

Six provenances (Chapter 6, Table 6.4) were selected which included three provenances each from northern Australia (Yeppoon, Townsville and Cardwell) and Sri Lanka (Anuradhapura, Kurunegala and Colombo). The selected provenances of each country are distinct in terms of their climate, topography and soil conditions and they are located at least 100 km apart from one another.
Seed collection was carried out from May to August 2008. Seeds were collected from 4-8 morphologically superior (no diseases or defects) trees having GBHOB > 100 cm. Small populations indicate less genetic variability (Vergeer et al. 2003). Hence seeds from the same provenance were mixed together to form a representative seed sample. Each sample was separately soaked for 6 hours and excess water was drained. Soaking facilitates the convenient removal of husks.

7.1.2.2 Oil Extraction

Thirty fresh mature kernels from each provenance were randomly obtained by cracking their shells with a mallet, they were oven dried for six days at 40 °C, and crushed oil was extracted with n-hexane single extraction* (sonication 30 min at 35 °C) using LABOROTA 4000 efficient rotary evaporator at 35 °C. Apart from those kernels from Yeppoon all other selected kernels appeared similar in colour (light brown). Ten replications (3 seeds each) were used to represent each provenance. Oil content was expressed as a percentage using the following formula,

\[
\text{Oil content (\%)} = \frac{\text{Weight of the oil fraction (Wo)}}{\text{Weight of the dry kernel used (Wk)}} \times 100
\]

* Dry weight of the kernel used= Weight of the extracted oil + Oven dry weight of the dry matter

7.1.2.3 Fatty Acid Profiling (FAP)

Fatty Acid Profiling (FAP) was done according to AFNOR NF EN ISO 5509 and NF EN ISO 5508 using GLC having a FID as described in Section 5.2.2.

7.1.3 Statistical Analysis

Differences among means were tested by Tukey simultaneous test using the General Linear Model of ANOVA (GLM) with MINITAB 14.1. Provenance means were correlated with selected geo-climatic data. Oil yield ha⁻¹ year⁻¹ was calculated based on the fruit yield data extracted from Chapter 4.
Saponification Number (SN) and Iodine Value (IV) of oils were calculated using fatty acid methyl ester compositions of oil using Equation (1), (2) and (3) mentioned in section 5.2.2.

7.1.4 Results and Discussion

7.1.4.1 Provenance Variation in Oil Content

Oil fractions of different provenances showed remarkable variations in their colour (Fig 7.1) and quantity (Table 7.1). However, it should be noted that due to single extraction and a relatively short desiccation time, oil contents presented in this section are not the maximum amount of oil which can be extracted from those selected kernel lots. However, this does not affect meaningful comparisons of oil contents of the selected provenances.

Fig 7.1 Colour variation in kernel oil extracts of *Calophyllum inophyllum* of different origins; from left Townsville, Cardwell, Yeppoon, Anuradhapura, Kurunegala, and Colombo, *This difference in volume does not represent variations in kernel oil content*
Most of the oils gave different intensities of amber colour except those from Yeppoon which gave a dark green oil fraction. Kernels from Yeppoon appeared brown even before drying. The darker colour of the kernel oil extracts from Yeppoon may be due to over-drying. Oil fractions of Townsville (Australia) and Anuradhapura (Sri Lanka) appeared to have the same colour.

Clearly distinguishable intra-specific variation \((P<0.05)\) was found among selected *Calophyllum inophyllum* provenances. Highest (57%) and lowest (30.8%) oil contents were recorded from the kernels of Anuradhapura Sri Lanka and Cardwell Australia, respectively. Colour differences in oil fractions indicated that there may be variations in their chemical properties.

Overall, seeds from Sri Lankan provenances contained higher kernel oil contents (43.7%) as compared to Australian provenances (39.5%) (Table 7.1). Even though this difference is only \(\sim 4\%\), it is quite significant value when selecting the best provenance for biodiesel production.

Table 7.1: Seed oil content of kernels from different provenances

<table>
<thead>
<tr>
<th>Australian Provenance</th>
<th>Kernel oil content (%)</th>
<th>Sri Lankan Provenances</th>
<th>Kernel oil content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Townsville</td>
<td>42.9 b</td>
<td>7. Anuradhapura</td>
<td>56.3 d</td>
</tr>
<tr>
<td>2. Yeppoon</td>
<td>44.6 c</td>
<td>8. Colombo</td>
<td>31.5 a</td>
</tr>
<tr>
<td>3. Cardwell</td>
<td>30.8 a</td>
<td>9. Kurunegala</td>
<td>43.3 bc</td>
</tr>
<tr>
<td>Grand Mean</td>
<td>39.5 x</td>
<td>Grand Mean</td>
<td>43.7 y</td>
</tr>
</tbody>
</table>

Means that are not followed by the same letter are significantly different (at 0.05 level of probability) as determined by the Turkey simultaneous test \((n=10)\)

However, seeds from Cardwell, Australia and Colombo Sri Lanka recorded similar oil contents; 30.8% and 31.5% respectively. Interestingly, both provenances share similar mean annual rainfall \( (> 2000 \text{ mm})\).
In contrast, seeds from Anuradhapura, Sri Lanka and Yeppoon, Australia recorded the highest oil contents, despite having the lowest mean annual rainfall in their respective countries (Chapter 6, Table 6.4). This suggests that regular drought periods could promote oil accumulation in *C. inophyllum* seeds. Moles and Westoby (2004) have also observed high accumulation of reserves in the seeds originating from stressful environments. Rose (1988) reported a positive correlation between water deficiency stress and the oil content in soybean. This suggests that drier maternal environment may induce lipid accumulation in *Calophyllum inophyllum* seeds.

According to results (Table 7.2), geo-climatic factors had considerable influence on kernel oil content and oil yield ha⁻¹ year⁻¹ of *C. inophyllum* seed sources. Influence of mean annual temperature on kernel oil content was found to be relatively low compared to that of altitude and mean annual rainfall.

Table 7.2: Correlations between kernel oil content (%) and yield, and environmental variables of selected provenances

<table>
<thead>
<tr>
<th>Climatic and Soil Variables</th>
<th>MaxT</th>
<th>MinT</th>
<th>Alt</th>
<th>MAR</th>
<th>N</th>
<th>P</th>
<th>K</th>
<th>TC</th>
<th>CD</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kernel Oil (%)</td>
<td>0.26*</td>
<td>0.29*</td>
<td>0.43**</td>
<td>-0.78**</td>
<td>-0.47**</td>
<td>0.69**</td>
<td>-0.17</td>
<td>-0.09</td>
<td>-0.16</td>
<td>0.51*</td>
</tr>
<tr>
<td>Oil Yield¹ (kg ha⁻¹ year⁻¹)</td>
<td>0.14</td>
<td>0.04</td>
<td>0.07</td>
<td>-0.46**</td>
<td>0.04</td>
<td>0.30**</td>
<td>-0.04</td>
<td>-0.30**</td>
<td>-0.20**</td>
<td>-0.30**</td>
</tr>
</tbody>
</table>

¹ Oil yields were extracted from chapter 5, MaxT- Mean Maximum Temperature (°C), MinT –Mean Minimum Temperature (°C), Alt-altitude (m), MAR- Mean Annual Rainfall (mm), N,P,K-Nitrogen, Phosphorus, Potassium (%), TC-Total Carbon (%), CD-Conductivity (µS cm⁻¹). *P<0.05, **P<0.01.

A significant negative correlation (r=-0.78**) was found between kernel oil content (Table 7.2) and the MAR and a similar relationship was found between oil yield ha⁻¹ year⁻¹ and MAR (r=-0.46**).

Significant positive correlations were found between kernel oil contents and soil phosphorous (r = 0.69**) and pH (r = 0.51*) and a significant negative correlation (r = -0.47**) was found between kernel oil contents and soil nitrogen levels (Table 7.2). Anderson et al. (1996) also observed a negative correlation between oil content of winter rape seed and soil nitrogen content.
Seeds with very high oil content are sometimes dormant (Dweck and Meadows 2002). Calophyllum seeds originating from dry provenances had low germination rates (Section 6.2). This high kernel oil content and dormancy found in calophyllum seeds originating from dry localities having low soil nitrogen content could be an adaptation which could improve the survival rate of germinated seedlings by decreasing competition for water and nutrients.

7.1.4.2 Provenance Variation in Fatty Acid Composition

Kernel oil extracts of different origin were found to have different fatty acid compositions (Table 7.3). Australian provenances showed higher percentages of unsaturated fatty acids compared to Sri Lankan provenances.

Table 7.3: Fatty acid composition of kernels from different C. inophyllum provenances

<table>
<thead>
<tr>
<th>Fatty Acid</th>
<th>Short Formula</th>
<th>Australian Provenances</th>
<th>Sri Lankan Provenances</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Townsville</td>
<td>Yeppoon</td>
</tr>
<tr>
<td>Palmitic (%)</td>
<td>16:00</td>
<td>14.2b</td>
<td>14.4bc</td>
</tr>
<tr>
<td>Stearic (%)</td>
<td>18:00</td>
<td>12.9a</td>
<td>14.1b</td>
</tr>
<tr>
<td>Oleic (%)</td>
<td>18:01</td>
<td>41.3f</td>
<td>33.4a</td>
</tr>
<tr>
<td>Linoleic (%)</td>
<td>18:02</td>
<td>30.3c</td>
<td>36.5e</td>
</tr>
<tr>
<td>Linolenic (%)</td>
<td>18:03</td>
<td>0.4a</td>
<td>0.5a</td>
</tr>
<tr>
<td>Eicosanoic (%)</td>
<td>20:00</td>
<td>0.7a</td>
<td>0.9a</td>
</tr>
<tr>
<td>Behenic (%)</td>
<td>22:00</td>
<td>0.2a</td>
<td>0.2a</td>
</tr>
<tr>
<td>Unsaturated</td>
<td>NA</td>
<td>72.0c</td>
<td>70.4c</td>
</tr>
<tr>
<td>Saturated</td>
<td>NA</td>
<td>28.0a</td>
<td>29.6a</td>
</tr>
</tbody>
</table>

Within a row means that are not followed by the same letter are significantly different (P< 0.05)
NA-not applicable
Percentages of stearic acid and linoleic acid seemed to differentiate the two countries. Significant differences (P<0.001) in unsaturated fatty acid content was observed between Sri Lankan (66.2%) and Australian (71.4%) provenances.

Chain lengths of the dominant fatty acids (FA) in kernel oil extracted from both the countries ranged from 16 to 22 which tallies with the ASTM Standard. Oil fractions of Sri Lankan provenances contained a higher amount of stearic acid (17.8-18.5%) as compared to Australian provenances (12.9-14.1%). Azam et al. (2005) found 18.5% stearic acid in Indian provenances which is exactly similar to that in Sri Lankan provenances. In contrast, Australian provenances recorded a higher amount of linoleic acid (30.3-36.5%) compared to Sri Lankan provenances (25.5-31.3%). Fatty acid profiles of Australian provenances were similar to those reported by Crane et al. (2005) who analysed calophyllum kernel oil from Madagascar.

7.1.4.3 Provenance Variations in Biodiesel Parameters

The strong relationship between fatty acid composition and the resultant biodiesel quality has been well established (Knothe et al. 2003; Knothe 2005; Ramos et al. 2009). This relationship justifies the viability of using estimated biodiesel parameters to describe the quality of the resultant biodiesel. According to the results of the current study, estimated quality parameters of FAME of all provenances were in accordance with the ASTM and EU biodiesel standards (Table 7.4) and were closely in line with the saponification number (SN), iodine value (IV) and the cetane number (CN) estimated from fatty acid profiles of calophyllum oil reported by Crane et al. (2005).

Saponification number (SN) corresponds to the amount of potassium hydroxide or sodium hydroxide (milligrams) required to saponify 1 g of fat. It indicates the average molecular weight (or chain length) of all the existing fatty acids. As most of the mass of a fat/tri-ester is represented by three fatty acids, it permits a comparison of the average chain length of fatty acids (Azam et al. 2005).
The long chain fatty acids found in fats generally have a low saponification number since they have a relatively fewer carboxylic functional groups per unit mass of the fat as compared to short chain fatty acids (Azam et al. 2005). Long chain unsaturated fatty acids are undesirable as they give higher cold filter plugging points (CFPP) (Knothe 2006).

CFPP is the temperature at which a specific volume of fuel fails to flow through a standard filtration device in a given time when cooled under certain conditions (Ramos et al. 2009).

Table 7.4: Provenance variation in estimated biodiesel quality parameters (calc. by eq. 1, 2 and 3 of section 5.2.2) of *C. inophyllum* oil in comparison to the ASTM

<table>
<thead>
<tr>
<th>Parameter</th>
<th>ASTM</th>
<th>Townsville</th>
<th>Yeppoon</th>
<th>Cardwell</th>
<th>Anuradhapura</th>
<th>Colombo</th>
<th>Kurunegala</th>
</tr>
</thead>
<tbody>
<tr>
<td>SN</td>
<td>N/A</td>
<td>198.6 a</td>
<td>199.2 b</td>
<td>198.9 b</td>
<td>198.8 b</td>
<td>199.2 b</td>
<td>198.4 a</td>
</tr>
<tr>
<td>IV</td>
<td>115</td>
<td>92.0 d</td>
<td>96.6 e</td>
<td>97.8 f</td>
<td>84.7 b</td>
<td>87.4 c</td>
<td>82.5 a</td>
</tr>
<tr>
<td>CN</td>
<td>47</td>
<td>53.1 c</td>
<td>51.9 b</td>
<td>51.7 a</td>
<td>54.7 e</td>
<td>54.0 d</td>
<td>55.2 f</td>
</tr>
</tbody>
</table>

Within a row means that are followed by the same letter are not significant (P< 0.05 probability) (ASTM- American standard for testing materials, SN-Saponification number, IV- Iodine value (*Max), CN-cetane number (*Min), NA-not applicable)

Cetane Number (CN) is commonly used as a quality parameter for diesel fuel. It indicates ignition properties and combustion quality of a fuel. Higher cetane numbers give better ignition properties (Meher et al. 2006). Engine performance of a fuel is greatly dependent on its cetane number. High cetane numbers result in good cold start properties and reduce smoke.

Iodine value is a measure of total unsaturation within a mixture of fatty acid. It is expressed in grams of iodine which react with 100 g of the respective sample when formally adding iodine to the double bonds (Knothe et al. 2005). Unsaturated fatty acids are important as they help in maintaining the oil and the resultant biodiesel in liquid form (Ramos et al. 2009).
Oil extracts from all selected *Calophyllum inophyllum* provenances had similar SN values. Unsaturated fatty acid compositions of all selected samples were dominated by C18 fatty acids. According to the results (Table 7.4) significant (P< 0.05) variations in CN and IV were found among *C. inophyllum* provenances. FAME of Kurunegala was found to have the highest CN (55) and the lowest IV (82).

In agreement with the findings of this study, Azam et al. (2005) found similar estimated SN (201) and CN (57.3) values for oil extracted from *C. inophyllum* kernels collected from South Indian provenances. However, the estimated IV (71.5) reported by Azam et al. (2005) was considerably lower than the calculated IVs (82.5-97.7) of the current study.

Although Anuradhapura (Sri Lanka) provenance recorded the highest oil content, kernel oil extracts from Kurunegala (Sri Lanka) were found to possess the most ideal FAP for producing biodiesel (CN-55.2 and IV-82.5). FAME of Sri Lankan provenances appears to have superior biodiesel properties than Australian seed oil sources. Among Australian provenances, kernel oil extracts from Townsville were found be the best source for biodiesel production (CN 53.1, IV 92).

According to the results (Figs. 7.2A and 7.2B) only two acids showed relatively stronger correlations with CN. Stearic acid (18:0) recorded a strong positive correlation (r = 0.75**) while linoleic acid (18:2) gave strong negative correlation (r = -0.90**).
Van Gerpan (1996) has made similar observations. Knothe et al. (2003) have also observed higher cetane numbers (CN) for palmitic and stearic acids. The superior quality of Sri Lankan calophyllum oil sources may be influenced by the relatively high percentage of stearic acid (18:0) and low percentage of linoleic acid (18:2).

7.1.5 Conclusions

A significant variation in oil content and colour was observed among Calophyllum inophyllum provenances in Australia and Sri Lanka. Oil content in calophyllum kernels seems to be strongly influenced by soil phosphorous level, pH, periodic drought and soil nitrogen deficiency. Strong relationships were found between the estimated oil yield and MAR and soil phosphorous level.

There are marked country to country variations in fatty acid profiles of C. inophyllum provenances from Australia and Sri Lanka. Kernel oil extracts from all selected provenances are suitable and can be used as raw materials for biodiesel production although those from Sri Lanka had superior chemical properties owing to their higher stearic and lower linoleic acid contents.
7.2 Seasonal Variation in Kernel Oil Content, FAP and FAME Properties of *Calophyllum inophyllum* Provenances in Northern Australia

### 7.2.1 Background

Properties of biodiesel are largely dependent on the fatty acid profile (FAP) of its feedstock (Ramos et al. 2009). Hence, variations in FAP can directly influence the quality of biodiesel. In most parts of the world *Calophyllum inophyllum* shows two flowering and fruiting seasons (Little and Skolmen 1989). However, sometimes flowering may occur throughout the year (Foxworthy 1927). In northern Australia *C. inophyllum* trees flower twice, in January and in June (Friday and Okano 2006). However, in northern Queensland the second flowering season starts in May (Personal observation 2009). Mature fruits are available to harvest in two periods, between April-May and July-September (Personal observation 2008).

The occurrence of two fruiting periods suggests a possible variability in content and chemical properties of kernel oils from different harvesting periods. Identifying periodic variability in oil content and chemical properties is quite important in determining the viability of *C. inophyllum* as a biodiesel feedstock. Even a small difference in concentrations of a few fatty acids can cause a major difference in the resultant biodiesel (Ramos et al. 2009). The aim of this study was to determine the seasonal variability in kernel oil properties and to estimate its effects on biodiesel properties.
7.2.2 Materials and Methods

Seeds were collected from 4-8 numbered trees (GBHOB>100 cm) in three Northern Queensland locations namely; Cardwell (18° 16' 0 S, 146° 1' 60 E), Townsville (19° 13' S, 146° 48' E) and Yeppoon (19° 52' 60 S, 140° 54' 0 E) during season 2 (July –August 2008) and season 1 (April-May 2009). Thirty seeds from each location were selected randomly. They were then deshelled, bisected and dried at 35 °C for 16 days.

The oil of thirty kernels from each provenance was individually extracted using standard n-hexane double extraction (Section 7.1.2). Fatty acid profiling was carried out using gas-liquid chromatography (AFNOR NF EN ISO 5509 and NF EN ISO 5508 methods) as described in Section 5.2.2.

7.2.3 Statistical Analysis

After testing for normality and homogeneity of error variances, the data were subjected to analysis of variance by ANOVA using GENSTAT ed. 11.1.

Saponification Numbers (SN) and Iodine Values (IV) of oils and CN of the resultant biodiesel were calculated using the equations (1), (2) and (3) described in section 5.2.2.

7.2.4 Results and Discussion

Results revealed a marked periodic variation (P<0.05) in kernel oil content (Table 7.5). Variation due to provenance seemed to be less pronounced compared to seasonal variation.

Table 7.5: Kernel oil content of Queensland provenances in two fruiting periods

<table>
<thead>
<tr>
<th>Period of Collection</th>
<th>Season</th>
<th>Oil Content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Cardwell</td>
</tr>
<tr>
<td>Jul-Aug 2008</td>
<td>Winter</td>
<td>29.86a</td>
</tr>
<tr>
<td>Apr-May 2009</td>
<td>Autumn</td>
<td>42.82c</td>
</tr>
</tbody>
</table>

Means that are followed by the same letter are not significant at P<0.05 level of probability
Seeds from Cardwell showed the highest increase (13%) in kernel oil content due to the seasonal effect. Kernel oil contents in northern Queensland seed sources (provenances) did not differ significantly (P>0.05) in season 1 (April-May) of 2009.

In two of the three locations, winters (July-September) in both years were drier than autumns (March-May); the only exception was Townsville in 2008 (Table 7.6). According to the results, seasonal rainfall during fruit development appear to influence oil content and this trend was evident in all locations and in both years (2008, 2009). The only exception to this trend was observed in Townsville.

Table 7.6: Mean rainfall, mean maximum temperatures in flowering and fruiting seasons and the number of dry months in the selected northern Queensland provenances (2008-2009)

<table>
<thead>
<tr>
<th>Location</th>
<th>Year</th>
<th>Rainfall (mm)</th>
<th>Mean Max Temp(°C)</th>
<th>Number of dry months (&lt;20 mm) before the harvest</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>S1 Autumn</td>
<td>S2 Winter</td>
<td>S1 Autumn</td>
</tr>
<tr>
<td>Cardwell</td>
<td>2008</td>
<td>676.2</td>
<td>74.7</td>
<td>31.0</td>
</tr>
<tr>
<td></td>
<td>2009</td>
<td>328.2</td>
<td>15.2</td>
<td>29.4</td>
</tr>
<tr>
<td>Yeppoon</td>
<td>2008</td>
<td>144.8</td>
<td>143.6</td>
<td>27.6</td>
</tr>
<tr>
<td></td>
<td>2009</td>
<td>203.0</td>
<td>11.4</td>
<td>26.5</td>
</tr>
<tr>
<td>Townsville</td>
<td>2008</td>
<td>5.2</td>
<td>100.0</td>
<td>31.0</td>
</tr>
<tr>
<td></td>
<td>2009</td>
<td>76.6</td>
<td>5.4</td>
<td>30.0</td>
</tr>
</tbody>
</table>

* S1-March to May, S2-July to September. Season of harvest is highlighted

However, the above evidence is inadequate to draw a relationship with periodic precipitation and lipid accumulation in developing seeds. On the other hand, the relationship between drought and kernel oil content has been well documented in the literature. This relationship was also illustrated in the previous study (Section 7.1.) In harsher conditions, trees produce slightly smaller oil-rich seeds (Pallardy 1981).
Rose (1988) found a positive correlation between oil content of soybean and the length of drought period. However, according to the results (Table 7.6), it is difficult to relate the dry period before the harvest in 2008 and 2009 to the kernel oil contents of *C. inophyllum* seed sources in northern Queensland. This suggests that temporary drought has little or no contribution to the seasonal variation in kernel oil content.

Even though there is no apparent influence of temporary drought on kernel oil content, variations in mean annual rainfall (MAR) and the longterm average drought period can bring about variation in kernel oil content. These data showed that kernel oil contents of *C. inophyllum* seeds corresponded well with the MAR of the selected provenances; Yeppoon (Oil 36%, MAR 870 mm) > Townsville (Oil 35 %, MAR 1125 mm) > Cardwell (Oil 30%, MAR 2125 mm).

By plotting mean monthly maximum temperatures in 2008 and 2009 with mean kernel oil content of the three provenences, a certain relationship can be observed between temperature and kernel oil yield (Fig 7.3).

Figure 8.7 shows that higher temperatures during fruit development found in the autumn harvest may have induced more lipid accumulation compared to the lower temperatures during fruit development found in the winter harvest.

Similar observations have been made by Wolf et al. (1981) in soybean and Ahmad and Hassan (2000) in sunflower. Low temperatures can reduce oil accumulation in seeds by hindering moisture loss which is essential in triglycerides synthesis (Morozov 1973 cited in Radic 2006).
Fig 7.3 Mean monthly temperatures °C (lines) and kernel oil contents (% by dry weight) in two seasons (Columns) of *Calophyllum inophyllum* provenances in northern Australia, C1-Cardwell in S1, C2-Cardwell in S2, T1-Townsville in S1, T2-Townsville in S2, Y1-Yeppoon in S1, and Y2-Yeppoon in S2.

Fatty acid profiles (FAP) of *C. inophyllum* seed sources showed significant (P<0.05) provenance and seasonal variations (Table 7.7). The majority of saturated fatty acids (palmitic, stearic, eicosanoic) and oleic acid in oils of most seed sources recorded higher concentrations in autumn 2009 than in winter 2008.

Hassan and Ahmad (2003) reported high concentration of oleic acid (18:1) (unsaturated) in Sunflower seeds during summer. Temperature variations during seed development can directly influence fatty acid composition (Slack and Browse 1984). As discussed previously in section 6.2, high temperatures during fruit development generally favour the biosynthesis of saturated fatty acids.
Table 7.7: Seasonal variations in fatty acid profiles (FAP) of *Calophyllum inophyllum* provenances in northern Australia

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Palmitic</td>
<td>16:00</td>
<td>Cardwell</td>
<td>13.6a</td>
<td>14.4b</td>
<td>14.3ab</td>
<td>14.6b</td>
<td>14.4b</td>
<td>15.4c</td>
</tr>
<tr>
<td>Stearic</td>
<td>18:00</td>
<td>Cardwell</td>
<td>13.2ab</td>
<td>16.5c</td>
<td>12.8a</td>
<td>14.3b</td>
<td>14.0b</td>
<td>13.7b</td>
</tr>
<tr>
<td>Oleic</td>
<td>18:01</td>
<td>Townsville</td>
<td>35.2a</td>
<td>47.6c</td>
<td>41.2b</td>
<td>40.0b</td>
<td>33.5a</td>
<td>35.5a</td>
</tr>
<tr>
<td>Linoleic</td>
<td>18:02</td>
<td>Townsville</td>
<td>36.3cd</td>
<td>20.7a</td>
<td>30.3b</td>
<td>29.8b</td>
<td>36.5d</td>
<td>34.3c</td>
</tr>
<tr>
<td>Linolenic</td>
<td>18:03</td>
<td>Yeppoon</td>
<td>0.5b</td>
<td>0.3a</td>
<td>0.4ab</td>
<td>0.4ab</td>
<td>0.5b</td>
<td>0.2a</td>
</tr>
<tr>
<td>Eicosanolic</td>
<td>20:00</td>
<td>Yeppoon</td>
<td>0.7a</td>
<td>0.9b</td>
<td>0.7a</td>
<td>0.8ab</td>
<td>0.8ab</td>
<td>0.7a</td>
</tr>
<tr>
<td>Behenic</td>
<td>22:00</td>
<td>Yeppoon</td>
<td>0.5b</td>
<td>0.3ab</td>
<td>0.2a</td>
<td>0.2a</td>
<td>0.4b</td>
<td>0.2a</td>
</tr>
<tr>
<td>Unsaturated</td>
<td>N/A</td>
<td>Yeppoon</td>
<td>72.0</td>
<td>68.5</td>
<td>71.8</td>
<td>70.2</td>
<td>70.5</td>
<td>70.0</td>
</tr>
<tr>
<td>Saturated</td>
<td>N/A</td>
<td>Yeppoon</td>
<td>28.0</td>
<td>32.0</td>
<td>28.0</td>
<td>29.5</td>
<td>29.5</td>
<td>30.1</td>
</tr>
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</table>

Within a row means that are followed by the same letter are not significant at P<0.05 level of probability

Demurin et al. (2000) reported that each 1 °C increase in temperature can lead to about 2% increase in oleic acid. Data for oleic acid revealed a similar positive correlation (r=0.78**) with temperature at fruit development (1.6% increase in oil content for 1 °C increase in temperature (Fig 7.4).

Fig 7.4 Relationship between mean temperature at the fruiting season and oleic acid content, Pearson coefficient r, **P<0.001
Higher concentrations of saturated fatty acids can increase cloud point (CP) and cold filter plugging points (CFPP) which are undesirable for a liquid fuel (Ramos et al. 2009; Knothe 2005). CP is the temperature at which solutes of a solution are no longer dissolved and CFPP is the temperature at which wax starts to settle and plugs filters and fuel lines (Knothe 2005). Despite their negative effects, saturated fatty acids play vital role in improving the ignition properties of the resultant biodiesel.

In contrast, the percentages of polyunsaturated fatty acids in all seed sources were found to be higher in winter 2008 than in autumn 2009. This shows that lower temperatures favour the formation of unsaturated fatty acids in *Calophyllum inophyllum* seeds. Similar observations have been reported by a number of authors (Hilditch and Williams 1964; Canvin 1965; Wolf et al. 1981; Weselake and Taylor 1999; Werteker et al. 2009).

Overall, oils from Cardwell seed sources recorded the highest seasonal variation in FAP. This large variation has been mainly attributed by the variations in oleic (18:1) and linoleic acid (18:2) contents and this trend is consistent with the findings of above mentioned authors.

Harris et al. (1978) suggested that higher temperatures can inhibit desaturase enzymes and thereby reduce unsaturated fatty acids in developing fruits and Richards et al. (2008) reported that lower temperatures can stimulate enzymatic desaturation. Variations in FAP of kernel oils from Cardwell do not appear to have been caused by the variation in periodic temperature.

Despite having the highest periodic temperature variation, fatty acid composition of kernel oils from Yeppoon exhibited a lesser degree of periodic variation compared to that of kernel oils from Cardwell. Cardwell had the highest periodic variation in rainfall. It suggests that the above marked variations in FAP may have been due to the variation in rainfall.

Unsaturated fatty acids help to maintain oil in liquid form, but if the concentration of polyunsaturated fatty acids exceeds a certain limit FAME derived from them can form polymers under heat which can block the fuel system of a vehicle (Azam et al. 2005).
Biodiesel properties of calophyllum oil methyl ester obtained from empirical equations (Table 7.8) showed a relatively low seasonal and provenance variations compared to those variations in FAP (Table 7.8). Methyl esters of most oil sources except those from Townsville had higher cetane numbers and lower Iodine values in autumn 2009 compared to winter 2008.

Kernel oil extracts from Townsville showed relatively less significant periodic variations in their fuel properties. This may be due to regular intervention (i.e. regular watering) from Townsville City Council. The highest seasonal variation in cetane number (CN) and Iodine value (IV) was observed in methyl esters derived from Cardwell kernel oils. These variations corresponded directly to the variations in FAP. Higher concentration of palmitic acid and stearic acid lead to higher cetane number and lower concentrations of polyunsaturated fatty acids (linoleic and linolenic) can cause lower cetane numbers (Knothe et al. 2003).

Table 7.8: Seasonal variations in calculated biodiesel parameters of calophyllum oil methyl esters extracted from northern Queensland seed sources

<table>
<thead>
<tr>
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<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>SN</td>
<td>N/A</td>
<td>200.9</td>
<td>202.0</td>
<td>200.7</td>
<td>201.2</td>
<td>201.1</td>
<td>201.5</td>
</tr>
<tr>
<td>IV</td>
<td>¹115</td>
<td>98.7 c</td>
<td>81.0 a</td>
<td>92.8 b</td>
<td>91.1 b</td>
<td>97.6 c</td>
<td>94.6 b</td>
</tr>
<tr>
<td>CN</td>
<td>²47</td>
<td>51.2 a</td>
<td>55.1 c</td>
<td>52.6 b</td>
<td>52.9 b</td>
<td>51.5 a</td>
<td>52.1 ab</td>
</tr>
</tbody>
</table>

Within a row means that are followed by the same letter are not significant at P<0.05 level of probability. ASTM-American Standard for Testing Materials, SN-saponification number, IV-Iodine value, ¹max, CN-cetane number, ²min

Cetane number refers to the ability to ignite. Fuels that possess high cetane numbers have better ignition properties (Azam et al. 2005; Ramos et al. 2009). Iodine value is a measure of total unsaturation within a mixture of fatty acids. It is expressed in grams of iodine which react with 100 g of the respective sample when formally adding iodine to the double bonds (Knothe et al. 2004).
Unsaturated fatty acids are important as they help to maintain the oil in liquid form (Azam et al. 2005; Ramos et al. 2009). The above mentioned seasonal variation in IVs suggests that kernel oils extracted in winter 2008 may give lower CP and CFPP compared to those extracted in autumn 2009.

According to the calculated values, there was no significant provenance or seasonal variation in saponification number among *Calophyllum inophyllum* kernel oil sources. Saponification number (SN) indicates the average molecular weight (or chain length) of all the existing fatty acids.

The long chain fatty acids found in fats generally have a low saponification value since they have a relatively smaller number of carboxylic functional groups per unit mass of the fat, as compared to short chain fatty acids. But long chain unsaturated fatty acids are undesirable as they give higher cold filter plugging points (CFPP) (Knothe et al. 2004). CFPP is the temperature at which a given volume of fuel fails to pass through a standardized filtration device in a specified time when cooled under certain conditions.

Among all seed sources autumn kernel oils seem to have FAPs that lead to superior biodiesel quality compared to winter kernel oils owing to their higher cetane numbers and lower iodine values. However oils extracted in both seasons had FAPs that can form biodiesel that conforms to ASTM standards.

**7.2.5 Conclusions**

*Calophyllum inophyllum* provenances demonstrate a significant seasonal variation in oil content and fatty acid profile, and as a result alter the quality of fatty acid methyl esters (biodiesel). Higher temperatures at fruit development seem to increase the oil content and the amount of saturated fatty acids.

In contrast, higher mean annual rainfall and lower seasonal temperatures seem to have induced lower oil contents and higher unsaturated fatty acids causing lower CPs and CFPPs.
Kernel oil extracted from autumn harvest have the most ideal FAP for biodiesel production, but kernel oils extracted from winter harvest can also be converted to biodiesel that complies with ASTM standards.

Chapter 7 revealed noticeable provenance and seasonal variations in kernel oil contents, fatty acid profiles and resultant biodiesel quality. The influence of short term and long-term climatic conditions on the kernel oil contents, fatty acid profiles and the resultant biodiesel quality was illustrated.

The next chapter details different aspects of kernel oil extraction and the technical complications in mechanical oil extraction.
CHAPTER 8

Oil Extraction

8.1 Oil Extraction¹

8.1.1 Background

The technology of processing oil seeds goes as far back as thousands of years yet it is still being developed. There are three common practises in seed oil extraction. They are hydraulic pressing, expeller pressing and solvent extraction (Newkirk 2010). These methods are frequently used as combinations. Hydraulic pressing is a batch process and hence time consuming. Cold press (expeller pressing under 120 °C) was selected as the best method for extracting oil for biodiesel despite the comparatively higher oil yield of the solvent extraction method. Cold press extraction is less expensive (Goss 1946; Newkirk 2010) and the process is continuous. Solvent extraction (n-hexane, petroleum ether, diethyl ether) sometimes results in inclusion of undesirable non-polar compounds that can interfere with transesterification reactions. Solvent extraction also requires trained individuals for its operation (Goss 1946; Newkirk 2010). Another advantage of cold pressing is that the cake can be used as a fertilizer/soil improver or insect repellent.

Calophyllum kernels contain <20% resin (Dweck and Meadows 2002) and as a result it is often difficult to expel oil using conventional techniques. The resin is mostly composed of oleoresin which appears greenish yellow in colour (Fig 8.4). Cold press extraction of resinous kernels has rarely been reported in the scientific literature. In this study, a satisfactory level of oil extraction efficiency was achieved by introducing modifications to the machinery and the extraction process.

8.1.1 Materials and Methods

8.1.1.1 Seed Collection and Preparation

Seed collection was carried out in Sri Lanka between June and August 2008 from the following locations; Matara (5° 56' 55N, 80° 32' 34E), Colombo (6° 54'N, 79° 50'E), Anuradhapura (08° 20' 60'' N, 80° 22' 60'') and Kurunegala (7°45′N, 80°15'E). Seeds (75 kg) were deshelled and kernels (Fig 8.1a) were dried for 18 days in a solar kiln (Fig 8.1b). Temperature was maintained at 45-55°C using exhaust fans.

8.1.1.2 Construction of the Oil Press

A screw press (expeller) should be able to generate adequate pressure (4-35 MPa) to compress kernels and rupture cell walls to extrude oil through the slits provided along the barrel length (Ward 1976).

\[\text{Compression Ratio } (R) = \frac{DB - DF}{DB - DE}\]

Where DB-diameter of the barrel, DF-root diameter of the shaft at the feeding end, DE-root diameter of the shaft at the start of the plug section.
Based on the above rules (Fig 8.2), an expeller was designed and developed at Uva Wellassa University, Sri Lanka. It was designed and constructed on a trial and error basis. The first model (Fig 8.3) was developed in July 2008. It consisted of a motor (A), a gear box (B), hopper (C), barrel 1 (D) and barrel 2 (E).

![Fig 8.3 the first oil press (July 2008)](image1)

![Fig 8.4 Oleoresins oozing from a kernel](image2)
It was designed to give a fixed compression ratio of 12:1. The shaft had two different diameter sections. Root diameter of the feed section of the screw was 50 mm and at the plug section it was 25 mm.

The first model performed poorly in its initial milling trials. The expeller barrel reached very high temperatures (150-180 °C) due to friction. This resulted in increased load on the motor. Due to unexpected excessive pressures that developed in the feeding and ram sections, a creamy meal was found to be escaping through spacings of the barrel instead of moving forward to the plug section where oil is collected.

Fig 8.5 the second oil expeller and the screw design ‘X’ (November 2008)

To overcome the above complications a second expeller (Fig 8.5) was designed in October 2008.

Drive train: Motor-> Belt drive->Gear box-> Screw

The tapering end of the screw was expected to provide adjustable compression ratios by changing the clearance. This was achieved by moving the shaft back and forth. The specification of the second expeller is given in Table 8.1.
Table 8.1: Components of the screw press

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Dimensions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Motor</td>
<td>5.6 kW/3 Ph/ 1440 rpm</td>
</tr>
<tr>
<td>Belt drive ratio</td>
<td>1:4</td>
</tr>
<tr>
<td>Gear box ratio</td>
<td>1:3</td>
</tr>
<tr>
<td>Total ratio</td>
<td>1:12</td>
</tr>
<tr>
<td>Outer diameter of the barrel</td>
<td>85 mm</td>
</tr>
<tr>
<td>Inner diameter of the barrel</td>
<td>52 mm</td>
</tr>
<tr>
<td>Shaft length</td>
<td>250 mm</td>
</tr>
<tr>
<td>Outer diameter of the shaft</td>
<td>50 mm</td>
</tr>
<tr>
<td>Depth of the cut</td>
<td>7 mm</td>
</tr>
<tr>
<td>Helix angle</td>
<td>60°</td>
</tr>
<tr>
<td>Pitch</td>
<td>25 mm</td>
</tr>
<tr>
<td>End taper</td>
<td>10%</td>
</tr>
</tbody>
</table>

8.1.1.2 Extraction Trials

The compression ratio was optimized by using 2 kg of dry copra and 2 kg of kernels. Three kilograms of dried kernels (moisture content 8%) were fed to the expeller at a constant rate while measuring the temperature (Metris TN400L infrared type thermometer, accuracy ± 2 °C) and the oil was collected and separated at every rise of 10 °C. The cake was again fed in two cycles to achieve the maximum oil recovery and the efficiency was calculated by using weights of the kernels (W1) used and weight of the cake after three cycles (W2).

Oil extracted from the second screw press was filtered using a cheese cloth and the creamy residue was fed to a hydraulic oil press to examine the possibility of extracting more oil.

8.1.2 Results and Discussion

At maximum compression ratio (9:1) creamy extrusion was observed to flow between the slits of the barrel at minimum compression ratio (2.8:1) under crushed kernels were collected with the cake and the best compression ratio was found to be 7.4:1. However the viscous dark greenish oil contained considerable amounts of residue. Adjustable 1 mm muslin cover was fixed to the barrel in order to reduce the creamy residue. The ideal operating temperature was found to be 65 °C (Fig 8.6).
The barrel temperature of the expeller increased with the addition of kernels and remained constant after reaching 72 °C. Maximum oil yield was obtained at 65 °C (Fig 8.6).

Fig 8.6 Oil yield after first cycle at different barrel temperatures

Oil content declined after 65 minutes due to the pressure reduction in the barrel caused by the decrease of load in ram section. Later (after 105 mins) the oil yield increased gradually after gaining the optimum load. At the compression ratio of 7.4:1 and operating temperature of 65-72 °C the second modified expeller had 65±3.2% efficiency.

Fig 8.7 the hydraulic press used in extraction trials

The use of hydraulic press (Fig 8.7) is an optional step. It recovered approximately 2% more oil than the screw press alone.
8.1.3 Conclusion

The extraction of calophyllum oil is difficult to accomplish with a conventional screw press. Modifications that were made to the expeller and the extraction process resulted in appreciable efficiency of 65%. The extraction process can be made continuous by using medium compression ratios (7.4:1) and the optimum operating temperature was found to be 65 °C.

8.2 Kernel Desiccation Period for Two Drying Methods (Oven Drying and Air Drying Under Sunlight)

8.2.1 Background

Traditionally *C. inophyllum* kernels are air dried for 30-40 days on ventilated racks before expelling oil (Kilham 2004; Agroforestry Tree Database 2007). Fresh kernels do not appear to contain much oil, and during desiccation kernels turn brown, develop an aromatic odour and increase their oil content (Dweck and Meadows 2002). Drying might induce enzymatic activity and increase the oil extractability in calophyllum kernels. This experiment was carried out to estimate the optimum desiccation time for two different drying methods to achieve satisfactory oil yield from the kernel.

8.2.2 Materials and Methods

Seed collection was carried out in various parts of northern Australia and Sri Lanka during May to August 2008. Due to the availability and superior appearance, only seeds collected from Kurunegala, Sri Lanka were used in this experiment. Three hundred seeds were dehulled and 140 fresh (light yellow) morphologically superior kernels were selected for the experiment. They were separated into two lots of 70 kernels each. One) was kept in air drying racks (temperature 24-30 °C, RH ≈60-70%) (Fig 8.8A). The other lot was kept in an oven at 40 °C (Fig 8.8B). The oven was equipped with a blower and an exhaust duct to control its humidity.
Fig 8.8 Different drying methods A-Air drying racks, B-Laboratory oven

Ten (5 replicates×2) seeds each from the oven and air drying were removed at different drying periods (72 hrs, 7 days, 17 days, 23 days, 30 days, 41 days and 46 days) and oils were extracted from standard n-hexane (BP-64.7 °C) extraction (sonication-30 min x 2) using LABOROTA 4000 efficient rotary evaporator at 25 °C. Oil content was expressed as:

\[
\text{Oil Content (\%)} = \frac{\text{Weight of extracted oil (g)}}{\text{Dry weight of the kernels used (g)}} \times 100
\]

* Dry weight of the kernel used= Weight of the extracted oil+ Oven dry weight of the dry matter

8.2.3 Results and Discussion

A notable increase in kernel oil content was observed over time under both drying regimes (Fig 8.9). Some authors have noticed a small increase (≈2%) in oil content by desiccating sunflower seeds to 22-36% moisture (Smirnova and Malayhin 1974 cited in Radic 2006)

Throughout the world traditional extraction techniques for medicinal purposes involve air drying for approximately one to two months (Kilham 2004). The reason for prolonged drying is unknown; it may be to increase the oil content (as shown in Fig 8.9) or to improve the quality.
Fig 8.9 Changes in the kernel oil content of calophyllum kernels over time under different drying regimes, bars represent SE (n=5)

Slow and low temperature drying may be a useful technique to preserve volatile aromatic compounds. Drying denatures protein structure in cells due to the simultaneous influence of heat and loss of water. As a result, fat drops lose their phosphoprotein envelopes and consolidate into larger globules (Fornal et al. 1994). This may improve the extractability of oil in kernels.

In general different authors have recommended different optimum kernel desiccation times, expressed in terms of seed moisture contents; 25% Palmer and Sanderson (1976), 30-35% Kosovak and Sudimac (1980), 40% Gumanuic et al. (1980) cited in Miklic et al. (2001) and 45% Gubbels and Dedio (1985).

Oven dried calophyllum kernels reached their maximum oil content (53.3%) earlier than in air drying. With air drying, increase in oil content was relatively slow and irregular. This may have been due to variations in relative humidity. The drying oven had a blower and an exhaust duct to control its humidity. After reaching a peak value the oil content fell gradually.
This decline was slightly steeper with oven drying compared to air drying. The final oil content (after 48 days) of air dried kernels was higher than that of oven dried kernels. Kachel-Jakubowska and Szpryngiel (2008) reported similar degree of difference in oil contents of air and oven dried rape seeds. There is a possibility that escape of volatile aromatic compounds (Dweck and Meadows 2002) was greater and more instantaneous in oven drying than air drying.

8.2.4 Conclusion

Both air drying and oven drying cause a significant improvement in oil recovery and extractability in *Calophyllum inophyllum* kernels. Optimum desiccation time for oven drying is shorter than air drying. However, for medicinal purposes air drying is far more suitable than oven drying method due to the possibility of escaping volatile aromatic fatty acids at high temperatures.

8.3 Effect of Steam Conditioning on the Oil Recovery of *C. inophyllum*

8.3.1 Background

Oil extraction requires a number of pre-treatments; de-hulling, splitting, cracking, grinding, flaking and cooking or steam conditioning (Galloway 1976). Pre-treatments break the seed walls and release the oil for extraction (Ward 1976). In Sri Lanka, traditional oil extraction methods involve cooking or steaming. Heat can induce enzymatic hydrolysis which causes biodegradation of cell walls, and breaking of lipoproteins and lipopolysaccharides (Shanker et al. 1997). Steam conditioning has been reported as an effective pre-treatment in increasing oil recovery (Norris 1964; Galloway 1976). Shankar et al. (1979) also reported that enzymatic hydrolysis in conjunction with steam treatment can induce the extractability and expellability. The current study was carried out to investigate the effect of steam treatment on the oil yield of *Calophyllum inophyllum* kernels.
8.3.2 Materials and Methods

One hundred desiccated kernels collected from Kurunegala Sri Lanka were dried at 40 °C for six days in a drying cabinet. Dried kernels were then divided into 20 samples containing 5 kernels each. Ten samples were treated separately with steam using a Labotec Ecosteam steam bath for 30 mins while the other ten samples were kept as the control (untreated). Then oils of each sample were extracted by standard n-hexane double extraction using the LABOROTA 4000 efficient rotary evaporator (Section 7.1.2).

8.3.3 Statistical Analysis

After testing for normality and homogeneity of error variances, mean oil contents of the treatment and the control were compared by Tukey simultaneous test using the General Linear Model of ANOVA (GLM) with MINITAB 14.1.

8.3.4 Results and Discussion

A significant difference (P<0.05) was found between the oil content of steam treated kernels (55.1± 2.9%) and the control (52.5± 2.6%) (Fig 8.10). Apart from two replicates, oil contents in the steam treated kernels were higher than the untreated kernels. Even though the increase was small (2.6%) in this experiment, when considering large scale production it can be economically significant.
Fig 8.10 Effect of seed conditioning on the kernel oil content of *C. inophyllum*, mean values followed by different letters are significantly different (P<0.05), n=10

It indicates a positive influence of seed conditioning on the oil content. Sing et al. (2002) also found significantly higher (P<0.01) oil recovery in cooked oil than uncooked oil and the increase ranged between 3.6-7%.

Shankar et al. (1997) also noted the positive effect of steam conditioning while testing the effect of steam conditioning and enzymatic hydrolysis on kernel oil content of soybeans. They reported 4.3% increase in oil content of soybean after steam and enzyme treatment.

### 8.3.5 Conclusion

Steam conditioning can improve the oil recovery and extractability of *Calophyllum inophyllum* kernels. Even though the increase in oil recovery is small; it can be commercially significant in large-scale oil production.

This Chapter discussed various aspects of calophyllum kernel oil extraction. Chapter 9 also described the technical complications in dealing with oleoresins and modifications that were made to the expeller to overcome those issues. The influence of desiccation time and steam conditioning were also presented.

Chapter 10 examines the efficiency of existing biodiesel conversion protocols and compares it with a newly developed protocol which was developed by the author/s. It also provides information regarding various physicochemical properties of the resultant biodiesel.
CHAPTER 9
Biodiesel Conversion, FAME Characterisation

9.1 Fatty Acid Methyl Ester (FAME) Conversion: Evaluation of Different Biodiesel Conversion Protocols

9.1.1 Background

During World War II, a number of countries shifted their focus in the search for alternative fuels. Some scientists looked at the possibility of using straight vegetable oil directly in compression ignition (CI) engines. Vegetable oils have some unfavourable properties that make them less viable as an alternative transportation fuel (Peterson 1986). Vegetable oils have viscosities 10-20 times higher than mineral diesel (Peterson et al. 1986). Viscosity is a critical quality parameter of a transportation fuel as it affects the fuel atomization, spray patterns and deposit formation (Nwafor and Rice 1996; Ma and Hanna 1999). Viscosity of straight vegetable oil can be significantly reduced by converting it into methyl esters. Zhang (1988) has shown that the methyl esters of high erucic acid rapeseed oil perform similarly to diesel in both short and long term engine tests.

The common chemical process of converting vegetable oil into fatty acid methyl ester (biodiesel) is known as *transesterification* (Van Gerpan 2005). Transesterification (Fig 9.1) is the process of a triglyceride reacting with an alcohol in the presence of a catalyst to produce glycerol and fatty acid esters that have physicochemical properties similar to mineral diesel (Kusy 1982). The process is a sequence of three reversible reactions, in which the triglyceride molecule is converted step by step into diglyceride, monoglyceride and glycerol (Mittelbach and Remschmidt 2004).

The mechanism for acid-catalysed transesterification of a monoglyceride is shown in Fig 9.1. It also can be formulated for di- and triglycerides. The carbonyl group of the ester undergoes protonation which leads to the carbocation. The nucleophilic attack of the alcohol produces the tetrahedral intermediate, which excludes glycerol to form the product (alkyl ester), and regenerates the catalyst H⁺ (Schuchard et al. 1998).
Acid Catalysed Transesterification

Alkaline Catalysed Transesterification

Fig 9.1 Transesterification of vegetable oils, R′-Carbon chain of the fatty acid, R- alkyl group of the alcohol, B - KOH/NaOH (Schuchard et al. 1998)

The mechanism of the alkaline-catalyzed transesterification is shown in Fig 9.1. The first step involves the reaction of the base with the alcohol which produces an alkoxide and the protonated catalyst.
The nucleophilic attack of the alkoxide at the carbonyl group of the triglyceride forms a tetrahedral intermediate which gets converted into the alkyl ester and the corresponding anion of the diglyceride. The diglyceride deprotonates the catalyst and the regenerated catalyst reacts with a second molecule of the alcohol to initiate another catalytic cycle (Schuchard et al. 1998).

Most of the edible oils (e.g. palm oil, rape seed oil, and canola oil) can be conveniently converted to biodiesel by employing simple transesterification protocols. However, Sahoo et al. (2007) reported that highly viscous (72 cSt) and highly acidic (22 mg/KOH) calophyllum oil cannot be converted to suitable quality biodiesel by conventional methods.

A number of researchers have worked on converting oils having high amount of free fatty acids (FFA) i.e. brown grease, high FFA tallow (Freedman et al. 1984; Mittlebach and Tritthart 1988; Mittlebach et al. 1992; Peterson et al. 1995; Wimmer 1995). Most of their conversion methods involved alkaline catalysts and FFAs were removed as soap. Higher FFA levels result in feedstock wastage and the soap induces the formation of stable emulsions that prevent phase separation (Canakci and Van Gerpen 2001).

Keim et al. (1945) patented a protocol for converting oils bearing > 50% FFA. However later it was found that the product from their method had contained a higher acid value (10 mg KOH) which is well above the ASTM standard (0.5 mg KOH). Sahoo et al. (2007) have proposed a 3-stage transesterification protocol which claims to be capable of producing clear FAME product. The 3-stage method has a number of theoretical drawbacks and results in inferior quality FAME. During our experiments, an improved protocol was developed to convert dark coloured viscous (62 cSt), high FFA (>11%) calophyllum oil into clear particle free amber coloured FAME.
9.1.2 Materials and Methods

9.1.2.1 Comparison of Transesterification Protocols

The following methods were used to convert 100 ml (3 replicates) of cold pressed filtered calophyllum oil into FAME and FAME were assessed for selected physicochemical properties using ASTM methods D 2500 (CP-cloud point), D 97(PP-pour point) and D1298 (density). They were also observed under a microscope for the presence of suspended particles.

Method 1: Base catalysed transesterification (Mittelbach et al. 1992)

One hundred millilitres of cold pressed calophyllum oil was preheated at 70 °C for 30 minutes to remove the excess water. Then 20 ml of methanol (MeOH) was separately mixed with 0.65 g of potassium hydroxide and added to the oil (in an air-tight reaction flask) with constant string (550 rpm). Before adding the potassium hydroxide and methanol mixture, the oil was cooled to 55 °C. Temperature was then gradually increased and maintained at 60 °C for 180 minutes. The solution was allowed to cool and settle for 15 hrs. After attaining phase separation the upper layer was separated out using a separation funnel.

Method 2: Acid catalysed transesterification (Canakci and Van Gerpen 2001)

One hundred millilitres of cold pressed calophyllum oil was preheated as above protocol. Then 0.68 ml of concentrated sulphuric acid was mixed with 25 ml of methanol separately while allowing oil to cool to 55 °C. The methanol-acid mix was gradually added to the oil (in an air-tight reaction flask) while maintaining the temperature at 55 °C. Temperature was gradually increased and maintained at 60 °C. The solution was stirred at 200 rpm for 180 mins. The solution was then allowed to cool for 14 hours. After observing phase separation, the upper layer (biodiesel) was separated out.
Method 3: Three stage transesterification (Sahoo et al. 2007)

One hundred millilitres of cold pressed calophyllum oil was mixed with 35 ml of methanol and 0.5 ml of toluene and 0.5 ml orthophosphoric acid as reagents. The mixture was stirred in the air closed reaction flask for 2 h at 65 °C. The heating was set at just above the boiling point of the alcohol i.e. 65 °C to accomplish the reaction. The speed of the stirrer was kept the same as for all test runs. The reactions were carried out with continuous stirring with a magnetic stirrer with stirring speed of 500 rpm. The product from the first stage was allowed to settle for 1 h and complete phase separation was visualized. The upper layer which consisted of a methanol–water fraction, organic matter toluene and other impurities was separated from the lower layer.

Anhydrous (98.4%) sulphuric acid (0.65 ml) was used in the acid catalyzed transesterification. The duration of the reaction was 4 h. The product of the second step was used as the raw material for the final stage where 0.9 g of potassium hydroxide was added and allowed to react for 4 h. After the reaction was completed the products were allowed to separate in to two layers. The lower layer contained impurities and glycerol. The upper ester layer was separated and purified by using warm water. After washing, the final product was heated to 70 °C for 15 min and dried under vacuum condition.

Method 4: Modified protocol for converting calophyllum oil into biodiesel (Australian Patent: No.2010902733)

One hundred millilitres of C. inophyllum oil was pre-heated as per first method and was allowed to cool to 60 °C. Then 0.5 ml of toluene was added to the solution while mechanically stirring the solution (450 rpm). After reacting for 45 minutes, the impurities were precipitated at the bottom (Fig 9.2 A).

The solution was allowed to settle for an hour. The upper layer was separated and filtered. Then 25 ml of methanol was mixed separately with 1.25 g of KOH pellets. After reducing the temperature of the filtrate to 60 °C, the methanol/ KOH solution was gradually added with constant stirring (500 rpm).
They were allowed to react for 35 minutes. Immediately after that, stirring was stopped and 10 ml of methanol and 0.65 ml of 98% H$_2$SO$_4$ were directly added to the above solution (Fig 9.2 B).

Stirring was gradually increased to 550 rpm after adding 5 ml of Acetone. Then the solution was allowed to react for 4 hrs and the resultant solution was allowed to settle for 18 hrs. After the reaction was completed the products were allowed to settle (phase separation). The lower layer contained impurities and glycerol (Fig 9.2 C). The final product (upper layer) was separated and heated up to 70 °C for 15 minutes and dried under vacuum condition and clear amber coloured biodiesel solution was achieved (Fig 9.2 D).

9.1.2.2 Presence of Polyunsaturated Free Fatty Acids

Cold pressed calophyllium oil (20 ml) was allowed to react with 4 ml of methanol and cobalt chloride at 65 °C for 2 hrs with constant stirring (500 rpm) and then the temperature was raised to 100 °C and left to react for 1 h without stirring. The product was visually observed for the presence of peroxide polymers.
9.1.3 Results and Discussion

Figure 9.3 shows that the colour and the clarity of the biodiesel vary with the methods used. Method 4 (the modified transesterification protocol) yielded the clearest biodiesel which resembled the colour of mineral diesel. Under visual observation, the transparency of biodiesel decreased in the following order method 4 > method 3 > method 2 > method 1.

As evident in Table 9.1, FAME derived from different protocols differed significantly (P<0.05) in pH, conversion %, cloud point, pour point and the amount of by-product. Density of neat calophyllum oil was found to be 960 kg m$^{-3}$. It dropped significantly after conversion. However, densities of resultant FAME did not differ significantly (P>0.05).

Methods 1 and 2 had the lowest conversion rate. Calophyllum oil contains a high amount of free fatty acids. The lower conversion rate from the alkaline catalyzed method could be due to the poor esterification of free fatty acids (FFA).
With that method, FFA becomes saponified and removed with by-products which decrease the conversion rate (Canakci and Van Gerpen 2001). For this reason, alkaline catalyzed transesterification is not suitable for oils having FFA >1% (Freedman and Pryde 1982; Liu 1994; Mittelbach et al. 1992).

Table 9.1: Comparison between four transesterification protocols and the quality of the resultant FAME; Method 1- base catalysed, Method 2- acid catalysed, Method 3- method described by Sahoo et al. (2007), Method 4- the new modified protocol..

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Method 1</th>
<th>Method 2</th>
<th>Method 3</th>
<th>Method 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calophyllum oil (ml)</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Methanol (ml)</td>
<td>25</td>
<td>25</td>
<td>35</td>
<td>35</td>
</tr>
<tr>
<td>Ortho phosphoric acid (ml)</td>
<td>0</td>
<td>0</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Toluene (ml)</td>
<td>0</td>
<td>0</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Sulphuric acid (ml)</td>
<td>0</td>
<td>0.65</td>
<td>0.65</td>
<td>0.65</td>
</tr>
<tr>
<td>Propanone (ml)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.5</td>
</tr>
<tr>
<td>Potassium hydroxide (g)</td>
<td>0.65</td>
<td>0</td>
<td>0.9</td>
<td>1.25</td>
</tr>
<tr>
<td>Reaction temperature (ºC)</td>
<td>60</td>
<td>68</td>
<td>66</td>
<td>64</td>
</tr>
<tr>
<td>Stirring speed (rpm)</td>
<td>550</td>
<td>200</td>
<td>500</td>
<td>550</td>
</tr>
<tr>
<td>Reaction time (min)</td>
<td>180</td>
<td>180</td>
<td>660</td>
<td>350</td>
</tr>
<tr>
<td>Settling time (h)</td>
<td>15</td>
<td>14</td>
<td>17</td>
<td>18</td>
</tr>
<tr>
<td>Total Product (ml)</td>
<td>111.9 a</td>
<td>110.5 b</td>
<td>129 c</td>
<td>126.3 d</td>
</tr>
<tr>
<td>FAME yield (ml)</td>
<td>77.0 a</td>
<td>83.0 b</td>
<td>86.3 c</td>
<td>88.2 c</td>
</tr>
<tr>
<td>By product +(ml)</td>
<td>34.3 a</td>
<td>27.3 b</td>
<td>42.6 c</td>
<td>38.3 d</td>
</tr>
<tr>
<td>Conversion (%)</td>
<td>77.0 a</td>
<td>83.0 b</td>
<td>86.0 c</td>
<td>88.0 c</td>
</tr>
<tr>
<td>pH of FAME</td>
<td>7.6 a</td>
<td>6.2 b</td>
<td>6.6 c</td>
<td>6.8 d</td>
</tr>
<tr>
<td>Density (kg m⁻³)</td>
<td>874 a</td>
<td>869 a</td>
<td>866 a</td>
<td>862 a</td>
</tr>
<tr>
<td>Cloud Point (ºC)</td>
<td>11.4 a</td>
<td>10.5 b</td>
<td>10.8 c</td>
<td>9.8 d</td>
</tr>
<tr>
<td>Pour Point (ºC)</td>
<td>6.2 a</td>
<td>4.5 b</td>
<td>4.3 b</td>
<td>3.9 c</td>
</tr>
</tbody>
</table>

Physicochemical properties of the products from different methods are highlighted. Within a row means sharing the same letter do not differ significantly (P<0.05)

FAME conversion in the acid catalyzed method may have been reduced by the interference caused by the formation of water as a result of FFA reacting with the H₃O⁺ and methanol (Canakci and Van Gerpen 2001).
Methods 3 and 4 had the highest conversion rate. In methods 3 and 4, organic impurities that inhibit conversion reactions were removed using solvents. Effectiveness of method 3 may also be due to the considerable reduction in free fatty acids (2-4 %) in step 2 which simplifies the alkaline transesterification. In method 4, saponification of FFA was prevented by immediate addition of $\text{H}_2\text{O}^+ + \text{CH}_3\text{OH}$ and by adding acetone. Acetone dehydrates the solution (Palomo et al. 2008), inhibits saponification and at the same time due to its high intermolecular vibrations (Nagai et al. 2005) induces the transesterification reactions.

Cloud point (9.8 ºC), pour point (3.9 ºC) and pH (6.8) of the FAME derived from method 4 were found to correspond more closely with the industrial standards compared to the resultant FAME of other methods. These cold flow properties are ideal for Australian conditions.

9.1.3.1 Microscopic Observations

According to microscopic images of the FAMEs of the four methods, method 4 was found to be superior in terms of the completeness of conversion and the exclusion of suspended particles (Fig 9.4). Suspended particles in biodiesel are a major concern for its mechanical reliability. Some authors have evaluated the quality of biodiesel by quantifying hard deposits on the tip of the injector pump (Sem 2004a) and piston rings (Sem 2004b) while running long term engine performance studies using neat biodiesel (B100).

Those deposits can create complications in fuel injection. According to microscopic observations (Fig 9.4), particle and oil globule free FAME derived from method 4 has the possibility of causing the least injector problems.
9.1.3.2 Presence of Polyunsaturated Free Fatty Acids

Dark polymer formation was observed over yellowish layer of FAME in the second experiment (Fig 9.5). FFAs of calophyllum oil do not completely react with methanol in the absence of catalysts. The dark polymers mentioned above may have resulted from the oxidation of un-reacted FFA at > 65 °C. Polyunsaturated free fatty acids in vegetable oil get oxidized at higher temperatures and forms peroxide polymers (Azam et al. 2005).

This observation suggests that FFA composition in calophyllum oil is dominated by unsaturated fatty acids. Hemavathy and Prabhakar (1990) likewise reported that FFAs of calophyllum oil are dominated by unsaturated fatty acids (oleic (48.3%) and linoleic (29.8%) acids.)
Fig 9.5 Formation of peroxide polymers

This also suggests that un-reacted oil globules found in FAME of methods 1, 2 and 3 may have contained unsaturated fatty acids. In that respect, those methods are not suitable for converting calophyllum oil into biodiesel as under heat polymerized un-reacted unsaturated FFA can form hard deposits on injector tips and piston tips. The new method is thus far superior in terms of its ability to produce quality biodiesel.

9.1.4 Conclusion

Existing transesterification protocols are not effective in converting highly viscous highly acidic calophyllum oil into biodiesel that meets industrial standards. The newly developed patented 4-stage conversion protocol was found to be superior in terms of conversion rate and the physicochemical properties of the resultant biodiesel. The current conversion protocols have a strong possibility of deriving biodiesel that contains un-reacted free fatty acids mainly composed of unsaturated fatty acids. These are known to form peroxide polymers under heat and hence are more likely to cause injection problems, injector coking and hard deposit formation on piston rings. These issues can be minimized by the use of biodiesel from the new protocol. Cold flow properties of the biodiesel derived from the new protocol are ideal for Australian conditions.
9.2 The Biodiesel Reactor

9.2.1 Basic Biodiesel Reactor

A basic model reactor was constructed at Uva Wellassa University, Badulla, Sri Lanka in May 2008. Purpose of this was to explore the feasibility of producing biodiesel from calophyllum oil at regional areas e.g. farmers.

The major operational components were fixed to a single chamber which included a mechanical stirrer, a 500 W heat element and a thermostat. Potassium hydroxide and methanol were separately mixed and pumped into the chamber. Recovery of excess methanol was done separately using a condenser.

Palm oil was used to test the efficiency of the reactor. One litre of filtered palm oil was allowed to react with 225 ml of methanol and 6.5 g of potassium hydroxide at 60 °C at a stirring speed of 450 rpm for 45 minutes. Then the product was manually transferred to a separation funnel. A significant colour change from yellow to reddish orange was observed. Phase separation was seen after 50 minutes with a dark layer at the bottom and lighter layer at the top.

9.2.2 Complete Basic Biodiesel Reactor

After testing the functionality of the first model with palm oil, the reactor was modified by adding a transparent settling vessel. Modifications were done in mid May 2008 (Fig 9.6). All operations were controlled manually and reaction temperatures and the stirring were difficult to control.

The temperature inside the reaction vessel was controlled by manually turning the heat element on and off. The A/C motor attached to the stirrer only had two adjustable speeds (450 and 550 rpm). Changes in the reaction vessel were not visible and it was difficult to intervene with the reactions.
9.2.3 **Automated Biodiesel Converter**

In order to overcome the above mentioned difficulties with manual operations, the reactor was further upgraded to a programmable bioreactor at Uva Wellassa University, Badulla, Sri Lanka in March 2009. The reactor (Fig 9.7) was made up of high density polyethylene (HDPE) and consisted of three main chambers; viz. a reaction chamber, a mixing chamber and a settling chamber. In the reaction chamber heat was generated by a 2000 W heat element, and the temperature was regulated by a temperature sensor (Digi-Key J type thermo couple).
Other components included a methanol condenser, a small AC motor (0.47 W / 1 Ph/ 2831 rpm), two pumps, control panel with LCD display and a microcontroller PIC16F877. The microcontroller regulated the temperature, stirring speed and the reaction time.

### 9.3.4 Conclusion

Biodiesel conversion process can be successfully automated. The automated biodiesel reactor can be used to test the effect of reaction temperature, mixing rate and stirring speeds on conversion rates (ester yield %).
9.4 Effect of Calophyllum Oil Methyl Ester (COME) on Metal and Components and Fuel lines of Diesel Engines

9.4.4 Corrodibility of FAMES from Different Transesterification Protocols

9.3.1.1 Background

Inferior quality biodiesel can contain considerable amounts of free fatty acids (FFA) and sulphur compounds which can create acidity. Acids and sulphur-containing compounds have the potential to cause corrosion in an engine system. The Copper Strip Corrosion Test (CSCT) indicates the potential of a particular transportation fuel to affect copper and brass fuel system parts. Polished copper strips are immersed in the biodiesel sample and placed in a sample tube in a heated (100 °C) bath for three hours (Stuart 2004). The sample test strip is then compared to a standard test strip to determine the effect of the biodiesel on the copper. ASTM D 6751 has specified the maximum copper strip corrosion grade which is 3 (Biodiesel Guidelines 2009). This approach is subjective and does not consider corrosion caused by long term exposure to FFA and sulphur compounds.

9.3.1.2 Materials and Methods

Filtered cold pressed oil was converted into FAME by four transesterification methods as mentioned in the previous experiment (section 9.1.2). The resultant FAMEs were separately transferred to 12 enclosable tubes making 3 x 10 ml replicates to represent each resultant FAME.

Twelve 0.5 mm (gauge 24) * 15 mm copper wire strips were weighed using a digital scale and dipped into the 12 tubes containing FAME and capped (Fig 9.8). After every two weeks strips were taken out, wiped with acetone and weighed individually over a 10 week period using a chemical balance (accuracy ±0.0001g).
9.3.1.3 Statistical Analysis

Data were subjected to analysis of variance. After testing for normality and homogeneity of error variances means were compared with ANOVA GLM using GENSTAT ver. 14.1.

Fig 9.8 Tubes carrying copper strips in FAME derived from four different methods

9.3.1.4 Results and Discussion

Results (Fig 9.9) revealed a significant difference (P<0.05) between the corrosion caused by conventional methods (acid and alkaline catalysed methods) and modified methods (Sahoo et al. (2006) method and the new method). The highest and the lowest copper strip weight loss were observed in the alkaline catalysed method and the new method respectively.

In Table 9.1, a significant variation in pH values was observed among calophyllum oil methyl esters derived from the different transesterification methods. Method 1 (alkaline catalysed) and method 2 (acid catalysed) yielded methyl esters having the highest and the lowest pH respectfully. Both the products resulted in heavy copper strip corrosion. Method 3 (Sahoo et al. 2007) and method 4 had pH values closer to 7. Low corrosibility in the products of methods 3 and 4 may be partially due to their close to neutral pH values (6.6 and 6.8).
Fig 9.9 Corrosion of FAMEs derived from different methods: Method 1-base catalyzed Method 2- acid catalyzed, Method 3-method of Sahoo et al. (2006) and Method 4- the new method (Hathurusingha et al., 2010b). Columns labelled by different letters are significantly different at P<0.05 level of probability, lsd=346.3

Furthermore, conversion rates of methods 1 and 2 were significantly lower than methods 3 and 4 (Table 9.1) and the FAMEs might contain un-reacted free fatty acids (FFA). FFA in biodiesel can induce metal component degradation in an engine.

9.3.1.5 Conclusion

Modified conversion protocols produce less corrosive biodiesel from calophyllum oil than conventional methods. The newly developed method produced the least corrosive biodiesel while the products of conventional methods tend to be more corrosive.
9.4.5 Effect of COME on Fuel Lines

9.3.2.1 Background

Many vehicle manufacturers and experts believe that neat biodiesel (B100) can soften and degrade certain types of elastomers and rubber compounds used in fuel lines under prolonged exposure (National Biodiesel Board 2007). Elastomers can be a complex mixture of polar and non-polar substances such as polymers, plasticizers, fillers, curling agents, antioxidants, oil, anti-zonants and processing agents (Sircar 1991). Vehicle manufacturers are reluctant to recommend biodiesel blends over B20 to come into contact with natural or synthetic rubbers (elastomers). This has been identified as one of the major issues related to material incompatibility in biodiesel (Thomas et al. 2007; Maru et al. 2009). Studying the effect of biodiesel blends on elastomers used in fuel pumps is important in guaranteeing its suitability for long term usage in unmodified engines.

9.3.2.2 Materials and Methods

Diesel fuel hose (i.d. 8 mm - Acrylonitrile-butadiene) was used in this experiment (Fig 9.10). The hose was cut into twelve pieces, equal in length (2 cm). They were immersed in 100 ml beakers filled with a series of COME blends derived from the new method (Australian Patent: No.2010902733) and neat diesel at room temperature (25 °C) and at 50 °C for 500 h (ASTM D471; Haseeb et al. 2010).

Fig.9.10 SYTEC IH001 fuel hose
The blending range was B0-neat diesel, B20, B40, B50, B60 and B100. To measure swelling hose sections were taken out gently blotted with filter paper and the swelling was measured by a Vernier calliper and the weight was recorded using a chemical balance (accuracy ± 0.0001 g).

9.3.2.3 Results and Discussion

A notable increase in mass was observed in acrylonitrile-butadiene rubber (NBR) hose sections (Fig 9.11). A marked ascent in mass increase was noted with the increase of biodiesel concentration. The magnitude of increase in mass increased with temperature.

![Fig 9.11 Changes in weight of NBR hose sections after immersion at two temperatures for 500 h](image)

Similarly, a sharp increase in volume was observed in NBR sections dipped in blends with increasing biodiesel concentration (Fig 9.12). Intensity of volume increase was higher at 50 °C, although the rate of increase with increasing biodiesel concentration was similar at both temperatures.

The difference between percentage mass increases between temperatures was higher than that for percentage volume increase. In both mass and volume change there was a steep rise from B60 to B100. Difference between swelling of NBR hose section under COME-B20 and neat diesel was found to be less pronounced compared to that with higher biodiesel concentration blends.
Elastomers appear to swell and/or degrade in biodiesel due to reactions with the polymer backbone and cross linking system, or by reactions with the filler system (Haseeb et al. 2010). Swelling of elastomers in biodiesel is mainly regulated by the solubility and absorbing capabilities of certain components of either polymer backbone or the filler. Polar substances are more prone to dissolve in polar solvents and non-polar substances are more likely to dissolve in non-polar solvents (Zhang and Cloud 2007).

Polar solvents have molecules with positively and negatively charged ends and they can attract oppositely charged polar solute molecules by dipole-dipole interactions. If those interactions are stronger than polymer-polymer interactions then a visible polymer swelling can be observed (Pekcan and Ugur 2002).

Dipole-dipole interactions between biodiesel and an elastomer molecule can be higher than that of diesel due to possible differences in charge which comes as a result of the increased polarity of esters (Hendricks et al. 2008). Besides the dipole-dipole interactions, all molecules have weak intermolecular forces called ‘London dispersion forces’ (Haseeb et al. 2010). Positive nuclei of solute molecular atoms attract negative electrons of the solvent molecular atoms by these weak intermolecular forces which enable absorption of solvent.
In this case it could be assumed that the increase in mass and volume in NBR tubes may possibly be due to higher solvent (COME) absorption by polymers rather than to the extraction of soluble components from elastomers to COME.

Mass and volume increase in NBR sections immersed in COME-B100 at 25 °C were found to be 7 and 14% respectively. In contrast, Haseeb et al. (2010) found 14% increase in mass and 20% increase in volume for NBR immersed in palm oil methyl ester (B100) at 25 °C. The difference in elastomer swelling may be due to the lesser abundance of negatively charged molecules in COME esters which lowers solvent absorption. Palm oil methyl esters have high concentration of saturated fatty acids (Saad et al. 2007) than COME (Chapter 7).

The presence of high acrylonitrile content in NBR results in more profound cross linkage and thereby reduces swelling in the presence of biodiesel (Hofman 2001). But it could also increase the polarity of the elastomer components (Cho et al. 2007). Relatively higher mass and volume increases observed at 50 °C can be explained by the higher diffusion rate in NBR at elevated temperatures.

9.3.2.4 Conclusion

Neat COME (B100) at higher operating temperatures can cause serious swelling in NBR hoses. However, the degree of swelling was considerably lower than reported values for palm oil methyl ester. COME B20 demonstrated the lowest swelling in NBR hoses and hence can be recommended for use in unmodified engines.
9.5 Characterization of Calophyllum Oil Methyl Ester (COME) Derived from the New Modified Transesterification Protocol

9.5.4 Background

Transportation fuels are required to meet particular industrial standards. The most common are American standards for testing materials (ASTM) and European (EN ISO) standards. Currently, there are no reports on characterization of calophyllum oil methyl ester (COME). Hence, the product of the newly developed method had to be evaluated for its preliminary compliance to ASTM D 6751-01 and EN 14214 biodiesel standards.

9.5.5 Materials and Methods

Mature fresh fallen seeds were collected from Cardwell, Queensland, Australia in May 2009. Seeds were deshelled and kernels were dried at 40 °C for 15 days. Oil was extracted using a Desmet Rosedowns Mini 40 (United Kingdom) screw press at 65 °C and converted to methyl esters (biodiesel) by the new protocol (Hathurusingha et al. 2010b). Quality parameters of resultant biodiesel were measured using standard ASTM and EN ISO methods at T & S laboratory in Sydney. Overwhelming laboratory costs and material scarcity limited the number of samples to two 500 ml bottles.

9.5.6 Results and Discussion

Test results (Table 9.2) revealed that calophyllum oil methyl ester (COME) satisfies all major international biodiesel specifications. High efficiency of the conversion process was indicated by the high ester content (95.6 %). A few minor non-conformities were found in the total acid number, total glycerol content and in the amount of carbon residue.

Total acid number (TAN) of COME was found to be 0.82 mg KOH/g which is 0.32 units higher than ASTM D 6751 specification. However, it was in accordance with that of Australian bio-fuel standard which is 0.8 mg KOH/g [Australian Biodiesel Quality Standards 2012].
Acid number is a measure of acids in the fuel (Knothe 2006). These acids emanate from three sources: (i) free fatty acids in the oil, (ii) acids used during production (iii) by-products from biodiesel oxidation. If TAN exceeds a certain limit it could corrode injection systems and other metallic components.

Table 9.2: Characterization of COME B100 (neat biodiesel) derived from the new conversion protocol

<table>
<thead>
<tr>
<th>TEST</th>
<th>RESULT</th>
<th>ACCEPTABLE</th>
<th>UNITS</th>
<th>METHOD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Acid Number</td>
<td>0.82</td>
<td>0.5 max</td>
<td>Mg KOH/g</td>
<td>ASTM D664</td>
</tr>
<tr>
<td>Total contamination</td>
<td>20.2</td>
<td>24 max</td>
<td>mg/kg</td>
<td>EN 12662</td>
</tr>
<tr>
<td>Density @15 °C</td>
<td>0.86</td>
<td>0.86-0.90</td>
<td>kg/l</td>
<td>ASTM D1298</td>
</tr>
<tr>
<td>Water</td>
<td>0.026</td>
<td>0.03 max</td>
<td>%</td>
<td>ASTM D6304</td>
</tr>
<tr>
<td>Flash point</td>
<td>150.0</td>
<td>100.0 min</td>
<td>°C</td>
<td>ASTM D93</td>
</tr>
<tr>
<td>Cetane Number</td>
<td>55</td>
<td>47 min</td>
<td>N/A</td>
<td>ASTM D613</td>
</tr>
<tr>
<td>Sulphur</td>
<td>&lt;10</td>
<td>10 max</td>
<td>mg/l</td>
<td>ASTM D4951</td>
</tr>
<tr>
<td>Methanol content</td>
<td>0.2</td>
<td>0.2 max</td>
<td>%</td>
<td>EN14110</td>
</tr>
<tr>
<td>Free glycerol</td>
<td>&lt;0.005</td>
<td>0.02 max</td>
<td>%</td>
<td>ASTM D6584</td>
</tr>
<tr>
<td>Total glycerol</td>
<td>0.29</td>
<td>0.25 max</td>
<td>%</td>
<td>ASTM D6584</td>
</tr>
<tr>
<td>Oxidation stability</td>
<td>9</td>
<td>6 min</td>
<td>H</td>
<td>EN14112</td>
</tr>
<tr>
<td>Ester content (C17 corr)</td>
<td>95.6</td>
<td>N/A</td>
<td>%</td>
<td>EN14103</td>
</tr>
<tr>
<td>Viscosity @40 °C</td>
<td>3.65</td>
<td>1.9-6</td>
<td>mm²/s</td>
<td>ASTM D445</td>
</tr>
<tr>
<td>Cloud point</td>
<td>9.8</td>
<td>Report</td>
<td>°C</td>
<td>ASTM D2500</td>
</tr>
<tr>
<td>Pour point</td>
<td>3.6</td>
<td>Report</td>
<td>°C</td>
<td>ASTM D97</td>
</tr>
<tr>
<td>Copper Corrosion</td>
<td>1B</td>
<td>3 max</td>
<td>-</td>
<td>ASTM D130</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>&lt;10</td>
<td>4 max</td>
<td>mg/kg</td>
<td>EN 14107</td>
</tr>
<tr>
<td>Carbon Residue (10% res)</td>
<td>0.067</td>
<td>0.05 max</td>
<td>%</td>
<td>ASTM D4530</td>
</tr>
<tr>
<td>Sulphated Ash</td>
<td>0.004</td>
<td>0.005 max</td>
<td>%</td>
<td>ASTM D874</td>
</tr>
<tr>
<td>Calcium</td>
<td>&lt;1</td>
<td>5 max</td>
<td>mg/kg</td>
<td>EN14538</td>
</tr>
<tr>
<td>Sodium</td>
<td>3</td>
<td>5 max</td>
<td>mg/kg</td>
<td>EN14538</td>
</tr>
<tr>
<td>Potassium</td>
<td>3</td>
<td>5 max</td>
<td>mg/kg</td>
<td>EN14538</td>
</tr>
<tr>
<td>Magnesium</td>
<td>&lt;1</td>
<td>5 max</td>
<td>mg/kg</td>
<td>EN14538</td>
</tr>
<tr>
<td>Distillation temp @90% rec</td>
<td>339</td>
<td>360 max</td>
<td>°C</td>
<td>ASTM D1160</td>
</tr>
</tbody>
</table>
COME met the EN14110 standard requirement in regard to the level of methanol. Generally excess amount of alcohol is used to induce the forward reaction in transesterification of vegetable oil into biodiesel (Schuchardt et al. 1998).

If the reaction is incomplete these alcohols can remain in biodiesel and can lower their lubricity and flash point. Lower lubricity leads to engine wear and lower flash point creates problems in handling and storing. Higher flash point (150°C) observed in COME also indicates its lesser degree of methanol contamination.

Density of biodiesel is normally higher than that of mineral diesel (Peterson et al. 1992) and the value depends on the fatty acid composition of the feedstock and on the purity of the product. It is usually reported to the buyer. Density of diesel usually falls between 0.83 to 0.84 kg/l (Wirawan et al. 2008) and the density of COME was 0.86 kg/l and is comparable to commercially available biodiesel. Kinematic viscosity of COME (3.65 mm² s⁻¹) was found to be in the acceptable range (2-5 mm² s⁻¹).

Viscosity of a fuel directly affects injector lubrication and fuel atomization. Higher viscosity leads to larger droplets and causes poor combustion which results in increased exhaust smoke (Biodiesel Guidelines 2009). Cold flow properties of fuels are important for temperate countries. Cloud points and pour points of biodiesel have to be reported to the customer. COME had relatively low cloud point (9.8 ºC) and pour point (3.6 ºC). These features are ideal for Australian conditions.

Cetane number (CN) indicates ignition and combustion characteristics of a fuel. Higher cetane number implies better fuel quality. The CN of COME (55) was in accordance with ASTM biodiesel standard (ASTM D6751).

COME recorded low water content and contamination. Water can accelerate oxidation and corrodirability and enhances microbial growth in the biodiesel. Contaminants may form as a result of esterification and purification or as compounds in the feedstock. In order to reduce emissions, and fuel consumption and to increase the performance, latest engines have been designed to operate at higher injection pressures.
This has been achieved by reducing orifice sizes and component clearance of injectors. Those engines cannot tolerate particles larger than 5 µm (Biodiesel Guidelines 2009). According to the current results COME had low particle contamination.

Total glycerol content of COME (0.04 %) was higher than the accepted level. If the level of glycerol is high, in long term usage they may settle at the bottom of the fuel tank and attract polar compounds (e.g. water) and form soap which may cause injector coking. However, this difference is not significant enough to cause any damage to the vehicle and fuel tanks of modern cars are now modified to withstand this problem.

Carbon residue of COME was also 0.02% higher than the specified level. Carbon residue forms due to combustion of particulate matter in the biodiesel. If this value is high the fuel can block injectors. This difference is too small to raise any issues and can be easily overcome by an efficient filtration system.

Alkali and alkaline metal levels of COME were also found to comply with the standards. Likewise, sulphur sulphated ash and phosphorus levels were found to be in accordance with the ASTM and EN ISO specifications. According to the copper strip corrosion test corrosiveness of COME was found to be negligible.

9.5.7 Conclusion

Calophyllum Oil Methyl Ester (COME) conforms to the majority of ASTM D 6751-01 and EN 14214 biodiesel standards. The only three non-conforming values (total glycerol, TAN and carbon residue) comply with Australian biodiesel standards and are not significant (0.02-0.3%) enough to cause any long term damage to the engine.

This Chapter revealed the incompetency of the existing transesterification protocols, and superiority of the newly developed protocol in converting calophyllum oil into biodiesel which meets the industry standards.

The next Chapter looks at the engine performance of the COME derived from the modified method.
CHAPTER 10

Engine Performance

10.1 Engine Performance of COME®

10.1.1 Background

Biodiesel is rapidly becoming popular as an alternative fuel, yet certain consumer concerns must be addressed before equipment owners, manufacturers and the general public completely accept it (Schwartz et al. 2005). Mechanical reliability is the most important factor that determines the consumer acceptance of biodiesel. High lubricity of biodiesel reduces the overall engine wear and it can be estimated via the “pin on disk” test (ASTM G99-95) (Knothe and Steidley 2005). Commercial types of biodiesel essentially require quality assurance and most commonly they can be found in two blends, B20 and B5.

Laboratory quality assurance can indicate to some extent the possible performance of a biofuel. However such values may not entirely reflect its absolute reliability. The most common biodiesel certification methods involve engine performance tests. They employ parameters such as engine power, torque, ignition delay, crank angle, specific fuel consumption, brake thermal efficiency etc. (Sahoo et al. 2007).

Fig 10.1 A Chassis Dynamometer
These tests can be performed either on an engine dynamometer or a chassis dynamometer (Fig. 10.1). Engine dynamometer testing of calophyllum oil methyl ester (COME) has been done on two previous occasions (Sahoo et al. 2007; Sahoo et al. 2009).

They have found COME B20 to perform marginally better than diesel in terms of better thermal efficiency and specific fuel consumption, and emissions reduction. However, those authors have used biodiesel derived from an inferior method (Section 9.1) and up to now the performance of COME has never been tested with a chassis dynamometer.

Where an engine dynamometer measures power directly from the engine, a chassis dynamometer measures engine output or more accurately, drivetrain output at a vehicle’s drive wheels. In its basic form, a chassis dyno consists of a platform with a pair of drums or rollers, a braking or power absorption system, and software to calculate power output. The chassis dynamometer has the ability to measure power at the drive wheels the “real world” performance of a vehicle. Aim of this study is to evaluate the real on road engine performance of COME which has more practical implications than engine dynamometer test.

10.1.2 Materials and Methods

Seven litres of filtered cold pressed calophyllum oil were converted into FAME using the new transesterification protocol (Hathurusingha et al. 2010b) and the resultant 6 L of filtered and vacuum dried calophyllum oil methyl ester (COME) was blended with 24 L of High Speed Diesel (HSD) to form COME B20.

In this experiment, engine performance of COME B20 was compared with HSD. B20 was selected due to material shortage and since it has the maximum globally accepted biodiesel to mineral diesel ratio. The test vehicle was a 2.5L Nissan Caravan utility (Fig 10.2). After making minor modifications to the fuel delivery system, each fuel was pumped through 2000 PULS PRO 1 LT fuel consumption measuring equipment (Fig 10.3).
The MAHA LPS 2000 chassis dynamometer was used to measure engine power and fuel consumption (Figs 10.4 and 10.5). It has software that converts wheel power from rollers to engine power (EP). EP (kW) and fuel consumption (FC) (L h⁻¹) were measured under full throttle. Three runs were carried out at constant speed mode for each parameter and for each fuel and the engine temperature was maintained at 80 °C. The chassis dynamometer had the following specifications (Table 10.1).

Table 10.1: Specifications of the MAHA LPS 2000 chassis dynamometer

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximum speed</td>
<td>260 km/h</td>
</tr>
<tr>
<td>Wheel base</td>
<td>2000-3000 mm</td>
</tr>
<tr>
<td>Maximum power</td>
<td>Front 260 kW Rear 520 kW</td>
</tr>
<tr>
<td>Inertia that could be tested</td>
<td>454-2752 kg</td>
</tr>
<tr>
<td>Cooling Fan (Fig 11.3)</td>
<td>26500 m³/h corresponds to approx. 98 km/h</td>
</tr>
<tr>
<td>Accuracy</td>
<td>±1.5 %</td>
</tr>
</tbody>
</table>
10.1.3 Analysis

The MAHA LPS 2000 dynamometer has its own data acquisition system. Output data were engine power (EP) and fuel consumption (FC). EP and FC values were used to calculate specific fuel consumption (SFC) and thermal efficiency (TE).
Data were subjected to ANOVA using GENSTAT ver.11.1 after testing their normality and homogeneity of error variance. The following are the theoretical equations that apply to the current data:

\[
\text{Engine Power (kW)} = \text{Torque (Nm)} \times \text{Engine Speed (rpm)} \tag{1}
\]

\[
\text{Torque} = \text{Force (N)} \times \text{Distance (m)} \tag{2}
\]

\[
\text{Fuel Consumption} = \frac{\text{Volume of Fuel Used (L)}}{\text{Time (h)}} \tag{3}
\]

\[
\text{SFC} = \frac{\text{Fuel Consumption (L h}^{-1})}{\text{Engine Power (kW)}} \tag{4}
\]

\[
\text{Thermal Efficiency (}\eta_{\text{Th}}) = \frac{\text{Engine Power (kW)}}{\text{Fuel Power (kW)}} \tag{5}
\]

### 10.1.4 Results and Discussion

A very slight reduction in engine power was observed when changing fuel type from mineral diesel to B20 (Fig 10.6).

---

**Fig 10.6** Wheel power and engine power vs. speed of the test vehicle while running with B20 and high speed diesel (HSD), P-wheel = wheel power, P-eng = engine power
Biodiesel generally has lower calorific value than diesel due to the presence of oxygen (Sahoo et al. 2009). The difference in engine power was found to be statistically non-significant (P>0.05). Sahoo et al. (2009) found that reduction in engine power for all COME blends were lower at full throttle and at engine speeds between 1200 and 1400 rpm.

As shown in Fig 10.6, both the fuels attained their respective peak power at the same speed of 63 km/h. According to results, engine power produced by HSD (51.5 kW) was marginally higher than that of B20 (50.8 kW). After reaching the peak, wheel power and engine power values gradually declined.

As the speed of the vehicle passed 65 km/h, the engine power corresponding to B20 was somewhat higher than that for HSD. Stalin and Prabhu (2007) have also observed a similar trend against load. Some authors have reported that lower engine power associated with biodiesel can be due to its comparatively lower calorific value and higher viscosity (Knothe et al. 2004).

![Fig 10.7 Fuel consumption of COME B20 and high speed diesel (HSD)](image)

Fuel consumption of B20 was found to be lower than that of HSD (Fig 10.7) and similar observations were reported by Sahoo et al. (2009) for COME blends and Wirawan et al. (2008) for palm oil methyl ester blends.
As expected fuel consumption in both HSD and B20 was found to increase with speed. However, the difference between fuel consumption of B20 and HSD was found to decrease with speed. Some authors argue that this may have been caused by the relatively lower pumping rate of B20 owing to its higher viscosity (Wirawan et al. 2008).

The test vehicle, Nissan Caravan 2.5 L engine was found to consume more B20 than HSD when it was generating relatively lower power (≈35-36 kW). The opposite trend was observed (Fig 10.8) when the engine was generating relatively more power (≈45-46 kW). This trend may have resulted from the comparatively inferior flow properties of B20 at the start.

As fuels heat up, their flow properties are improved and injection becomes regular. Biodiesel generally has better ignition properties due to their higher cetane numbers (Knothe 2005). B20 may have lesser ignition delay and complete combustion compared to diesel and could generate power more efficiently at higher engine speeds (heat up).

![Fig 10.8 Relationship between fuel consumption and wheel power](image)

Differences in physicochemical properties of HSD and B20 were marginal (Table 10.2). However the magnitude of influence caused by those differences on engine performance could be vital. Apart from calorific value, HSD was found to have lower values for all other properties.
Table 10.2: Physicochemical properties of mineral diesel and COME B20 derived from different conversion protocols and high speed diesel

<table>
<thead>
<tr>
<th>Fuel Type/Blend</th>
<th>Cetane Number</th>
<th>Density kgm⁻³</th>
<th>Calorific Value kJ/kg</th>
<th>Flash Point °C</th>
<th>Viscosity cSt</th>
<th>Cloud Point °C</th>
<th>Pour Point °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>High Speed Diesel</td>
<td>47</td>
<td>848</td>
<td>44000</td>
<td>76</td>
<td>2.87</td>
<td>6.5</td>
<td>-3.1</td>
</tr>
<tr>
<td>B20 (Sahoo et al. 2007)</td>
<td>NA</td>
<td>852</td>
<td>43850</td>
<td>86</td>
<td>2.98</td>
<td>7.8</td>
<td>2.8</td>
</tr>
<tr>
<td>B20 (Patent No. 2010902733)</td>
<td>55.2</td>
<td>850</td>
<td>43880</td>
<td>84</td>
<td>2.96</td>
<td>8.2</td>
<td>3.1</td>
</tr>
</tbody>
</table>

Thermal efficiency for both the fuels was found to increase with speed (Fig 10.9). Raheman and Phadatare (2004) observed a similar trend for brake thermal efficiency (BTE) against load when using Karanja (*Pongamia pinnata*) methyl ester in an engine dynamometer test.

![Graph showing thermal efficiency and specific fuel consumption for HSD and COME B20 at different speeds](image-url)

**Fig 10.9** Thermal efficiency (TE-columns) and specific fuel consumption (SFC-curves) for HSD and COME B20 at different speeds.
Thermal efficiency of COME B20 was slightly but not significantly lower than that of HSD at 40 km/h and as the speed was increased, thermal efficiency of COME B20 started to improve over that of HSD. This may have been caused by the differences in kinematic viscosities of the test fuels (Table 10.2). As the engine heats up injection of COME B20 becomes better and due to the addition of 20% COME, improves the ignition properties of the other 80% diesel by raising its cetane number. As a result COME B20 undergoes complete combustion and generates more power and TE compared to HSD.

Specific fuel consumption for both the tested fuels was found to decrease with the speed. Baitiang et al. (2008) observed a similar trend between fuel consumption and engine rpm using Jatropha oil methyl esters. Specific fuel consumption for COME B20 was lower than that for HSD at lower speeds (∼40 km/h). With the increase of engine speed the SFC for COME B20 became lower than that for HSD. This may also be explained as a result of the improvement in flow properties by rising temperature and better ignition and combustion properties of COME B20.

10.1.5 Conclusion

Blending 20% calophyllum Oil Methyl Ester (COME) made from the new transesterification protocol improves the ignition and combustion characteristics of diesel. COME B20 produces fractionally less engine power (0.5 kW less), has better thermal efficiency and lower specific fuel consumption compared to those of HSD. Considering this performance of COME B20, it can be recommended as an alternative transportation fuel in Australia and other tropical countries.
10.2 The Effect of Calophyllum Oil Methyl Ester (COME) on Diesel (CI) Engine Knock and Vibration

10.2.1 Background

10.2.1.2 Diesel Knock

Combustion knock in compression ignition (CI) engines is generally caused by a sharp increase in pressure which forms a shock wave that generates heavy vibrations and a knocking sound. Excessive knock can overheat the piston and the cylinder head, damage bearings and possibly seize the piston (Gupta 2006).

Knocking in diesel engines is inescapable to a greater or lesser extent, since fuel is injected into highly compressed air towards the end of the compression stroke. One of the major causes of the rapid pressure rise is prolonged injection delay. If the delay period is longer than a certain level, combustion of the first few droplets is delayed, and therefore an excessive amount of fuel droplets accumulate in the chamber. When the combustion commences, the excess fuel may cause pressure explosion resulting in shock and heavy vibration (Davis 1951).

Another major reason for diesel knock may be irregular or incomplete combustion (Van Zanten 1985). Engine knock or pinging resulting from irregular combustion appears when some of the unburnt gases ahead of the flame impulsively ignite. The unburnt gas over the flame is compressed and as the flame propagates and the pressure in the combustion chamber increases. Spontaneous ignition occurs as a result of the high pressure and corresponding high temperature of unburnt reactants. This pressure creates a shock wave to traverse from the end gas region and an expansion wave to traverse into the end gas region. The two waves reflect off the walls of the combustion chamber and interrelate to generate high amplitude standing waves (Gupta 2006). Apart from the undesirable mechanical effects of diesel knock, the noise it creates also leads to sound pollution and for this reason knocking should be avoided or reduced as much as possible.
One of the methods of overcoming diesel engine knock caused by unusual combustion is to prevent ignition delay by increase its Cetane number (Clothier et al. 1993). CN reflects the ignition properties of a fuel (Azam et al. 2005). Biodiesels generally have higher Cetane number (CN) compared to diesel (Ma and Hanna 1999; Watkins et al. 2004; Joshi and Pegg 2007) and hence can be added to diesel to reduce knocking.

10.2.1.3 Vibration in Diesel Engines

Extensive engine vibration is harmful as it wears engine components and causes irritating noise. Determination of vibration magnitude in an engine depends on the excitations and the propagation path. Vibration in a vehicle is caused by both external and internal factors. The main internal sources that induce the engine vibration response are impacts due to piston clearances (piston slaps), fuel injection pressure, rapid rise of pressure during combustion and the impacts of admission and exhaust valves (Ftoutou et al. 2008). This experiment was designed to compare vibration induced by combustion forces as a result of changing fuel types while other internal sources were the same for all trials.

In the combustion phase, a considerable rapid increase of pressure in the engine cylinders occurs. The combustion force causes the pistons to descend and promotes rotation of the crankshaft. Sometimes the combustion force is compared to a hammer stroke and it relies on the power demand of the engine. Excitations caused by combustion forces are intervallic and can extend to high frequencies (Lecelere cited in Ftoutou et al. 2008).

The shock prompted by the combustion pressure directly causes excitation on the engine head (1 in Fig 10.10). This makes up the first path of propagation (Favre et al. cited in Ftoutou et al. 2008). Conversely, the shock caused by the spontaneous pressure rise excites the dynamic components (piston-rod-crankshaft) in two ways (Fig 10.10):

1. The transverse excitation (2 in Fig 10.10) which is transmitted through the piston to the cylinders while conjugating with the piston-slap.
2. The vertical excitation (3 in the Fig 10.10) which propagates to the crankcase bearings.

![Fig 10.10 Combustion forces (source: Ftoutou et al. 2008)](image)

Biodiesel has lower calorific value than mineral diesel due to the higher oxygen content (Ghadge and Raheman 2005; Sahoo et al. 2009). However, the presence of oxygen promotes complete combustion of biodiesel (Sahoo et al. 2009). While conducting engine performance tests (Section 10.1.2.), a significant reduction in the vehicle body vibration and noise was observed after changing fuel type from diesel to COME B20.

In order to quantify this reduction in vibration, a comparative vibration analysis was carried out. If the reduction proves to be significant, it will indicate the fuel oxidizing ability of COME. Eventually it will evaluate the potential of COME as a fuel additive.
10.2.2 Materials and Methods

Seven litres of filtered cold pressed calophyllum oil were converted into COME by the new transesterification protocol (Hathurusingha et al. 2010b). The resultant FAME (2 L) was mixed with 8 L of mineral diesel to form 10 L of B20.

In short term engine trials (5 min x 3 reps), vibration using COME-B20 and commercially available B20 (Mobil) were compared with that of high speed diesel (CALTEX) in a 2.0 L Land Rover Free-Lander engine (2L TCIE) at idle speed 850 rpm and rated speed 1500 rpm using the following set up (Fig 10.11).

![Vibration measuring setup](Coolest Point Engine Analysis Display Data Acquisition System Accelerometer)

10.2.2.1 The Three Axis Accelerometer

The SerAccel v5 is a 3 axis enclosed serial accelerometer which can measure up to +/−6g acceleration in all three axis (x, y and z) having a simple serial (Fig 10.12B). It has many new improvements including variable baud rate, a factory reset command, and a complete triple axis measurement system based on the newly released MMA7260Q sensor from free scale (Ocean Control Electronics Adelaide 2010).

Power is gained from any RS232 port (including USB-to-RS232 converters) so no external power supply is needed. The on board PIC (16LF88) runs at 10MHz and outputs three different types of outputs including calculated, binary, and raw outputs. The SerAccel v5 has software configurable settings to select between four sensing ranges (± 1.5, 2, 4, and 6 g), as well as a software selectable measurement frequency (0-590Hz). Port capturing was carried out by RS232 firmware.
10.2.2.2 Vibration Testing Setup

The SerAccel V5 accelerometer was mounted on a point having low temperature fluctuations (Fig 10.12).

Fig 10.12 Vibration testing setup: A-test vehicle, B-SerAccel V5 accelerometer, C-changing diesel filter, D-recording temperature, E- mounting accelerometer
Vibration data were recorded at operating temperature of 78 °C. Engine temperature was recorded using a J type thermocouple and an infrared type thermometer. The diesel filter was changed before changing each test fuel. The first data series was obtained for diesel at idle rpm (850) and rated rpm (1500). The same procedures were followed for B20 from MOBIL and COME B20. For every fuel 2 min stabilization period was given before recording data.

The accelerometer was configured to gravity mode and ± 1.5 g sensitivity. The data logging frequency was set to 5 Hz and the accelerometer was oriented according to Fig 10.13.

The data acquisition software provided by Ocean Controls Pty. Ltd. was used to directly record calculated acceleration values (g) as CSV files. Output frequency was set to 5 Hz. Displacement was calculated through the following formula where D is displacement in cm, A is acceleration in g and CPM is cycles per minute in Hertz.

![Fig 10.13 Orientation of the accelerometer](image)
Acceleration and displacement data were transformed from time domain to frequency domain by Fast Fourier Transformation (FFT) (Azzoni et al. 1995).

Fast Fourier Transformation (FFT)

An FFT computes the Discrete Fourier Transformation (DFT) and produces exactly the same result as evaluating the DFT definition directly; the only difference is that an FFT is much faster. In the presence of round-off error, many FFT algorithms are also much more accurate than evaluating the DFT definition directly, as discussed below.

Let $x_0, \ldots, x_{N-1}$ be complex numbers. The DFT is defined by the following formula:

$$X_k = \sum_{n=0}^{N-1} x_n e^{-\frac{2\pi i k n}{N}} \quad k = 0, \ldots, N - 1.$$  

(Source: Ftoutou et al. 2008)

In the time domain, a visual analysis usually derives a limited amount of information (Ftoutou et al. 2008). FFT was performed using SIGVIEW32 version 2.2.1 in order to determine vibration amplitude.

10.2.3 Results and Discussion

The test vehicle (Land Rover Free-Lander 2L TCIE) is a turbo charged, electronic fuel injection (EFI) engine which has the capability of adapting to different fuels. It is designed to minimize knocking caused by injection faults. In such a new engine (if no mechanical faults), vibration due to internal forces other than combustion forces are minimal. This situation applies equally to all trials and made way for a clear assessment of vibration caused by combustion forces.
There was no visible difference in the vibration of different fuel types. It is difficult to observe visible variation in vibration due to auto adjustments made by the test engine and due to the presence of anti-knocking agents in Australian diesel fuels. CALTEX Australia uses 0-10% methyl esters of known lipid sources as additives in their diesel (MSDS Diesel Fuel CALTEX, 2007). Biodiesel (B20) sold at MOBIL is made out of tallow. These anti-knocking agents raise the Cetane number (CN) of mineral diesel. CN reflects the ignition properties of a fuel (Azam et al. 2005).

Increasing CN results in reducing the ignition delay and thereby control the possibility of sudden pressure increase. This also controls engine vibration. The EFI engine of the test vehicle also controls the amount of fuel injected to the piston and reduces the chance of accumulation of unburnt fuel droplets (Mathur and Sharma 1993).

Acceleration data revealed that vibration waves that resulted from calophyllum oil methyl ester (COME B20) were far smoother than those resulted from B20 obtained from MOBIL and diesel obtained from CALTEX Rockhampton (Fig 10.14). Each vibration wave represents a combustion cycle. The data indicate that combustion of calophyllum oil methyl ester is far more smoother compared to B20 obtained from MOBIL and diesel obtained from CALTEX. It can be assumed that combustion knock resulting from COME is significantly lower than for other tested fuels and those inherent oxygen molecules in COME may have improved ignition characteristics of diesel. Knocking has a tendency to cause vibration in engines (Gupta 2006).
Fig 10.14 Acceleration (g) vs. time for diesel, COME B20 and Mobil B20 at 1500 rpm with time.
Fig 10.15 Displacement and acceleration magnitudes of engine vibration for different fuel types on three axes (X, Y and Z) by Fast Fourier Transformation (FFT)
Vibration magnitudes caused by COME B20, MOBIL B20 and diesel are shown in Fig 10.15. Both acceleration and displacement magnitudes were found to be more pronounced at idle (850 rpm) than rated (1500 rpm) engine speeds. This difference may be due to the difference in air: fuel ratio. Vibration displacement caused by diesel and B20 from MOBIL was significantly higher than that of COME B20. The vibration magnitude for COME B20 was lower than diesel and MOBIL B20 in all three axes for both engine speeds, except the low vibration magnitude found through X axis resulted from MOBIL B20 at 1500 rpm.

At 850 rpm, the displacement magnitude through the Y axis was found to be significantly higher than through other axes for all fuel types. At 1500 rpm, displacement magnitude through the Z axes was found to be higher than through other axes when operating with diesel and MOBIL B20. Vibration through the X axis was lower than through Y and X axes for all three fuels at both rpms. Acceleration values followed the same trend with the exception of acceleration value through X axis for MOBIL B20 at 1500 rpm.

The Y axis represents the direction of crank shaft rotation and the Z axis represents the movement of pistons. Acceleration data in time domain (Fig 10.14) indicate that there is an irregularity in forces created by pistons when operating with diesel and MOBIL B20 compared to that of COME B20.

Table 10.3: Performance parameters of the selected fuel types

<table>
<thead>
<tr>
<th>Fuel type</th>
<th>Calorific value (MJ/kg)</th>
<th>Engine power (kW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diesel</td>
<td>44.12</td>
<td>51.5</td>
</tr>
<tr>
<td>B20 from MOBIL</td>
<td>43.90</td>
<td>50.9</td>
</tr>
<tr>
<td>COME B20</td>
<td>43.88</td>
<td>50.8</td>
</tr>
</tbody>
</table>
One might argue that the reduction in vibration that resulted from COME B20 may be caused by its lower engine power. Biodiesel generally has lower power (low calorific value) compared to diesel due to the presence of oxygen (Sahoo et al. 2009). But if one compares the difference in engine power values at full throttle (Section 10.1.4) and the difference in calorific values of the selected fuel types (Table 10.3) with the difference in the magnitude of vibration (Fig 10.14), it is clear that vibration reduction in COME B20 is solely due to the presence of calophyllum oil methyl ester than due to its relatively low power. The differences between engine power and calorific value of test fuels were negligible when compared with the differences in vibration magnitude.

Another important fact is that diesel from CALTEX already comes with additives and MOBIL B20 is an industrially accepted commercial biodiesel blend. Despite having additives and cetane boosters in the tested commercial fuels, the 2L TCIE engine driven by COME B20 still had the lowest vibration. This demonstrates its possible application as a fuel additive or oxidizer. However, further work has to be done to confirm the oxidizing and anti-knocking properties of calophyllum oil methyl ester.

10.2.4 Conclusions

Addition of calophyllum oil methyl ester can significantly reduce vibration, and knock, in CI engines. Further laboratory work has to be done to confirm the oxidizing and anti-knocking properties of calophyllum oil methyl ester.

Previous Chapters examined the major aspects of biodiesel feedstock evaluation. This Chapter demonstrated the mechanical reliability and anti-knocking properties of COME B20.

The next Chapter outlines the implication of the present series of studies.
CHAPTER 11

Potential of Beauty Leaf Tree as a Biodiesel Feedstock in Australia: Implications of the Project

11.1 Current Situation of the Biodiesel Industry in Australia

The necessity of developing sustainable fuels has become inevitable with the depletion of fossil fuels. Many countries have focussed on developing their own sustainable fuel sources. The Australian biodiesel industry is still considered to be substandard compared to European countries and United States that have progressed exceedingly well in the past few decades. Biodiesel production in Australia has increased from 21.2 million litres (ML) to 76.3 ML in 2006/2007 (APEC 2008). Currently, the existing biodiesel plants have the production capacity of 286 ML and this is expected to be doubled in 2015 (Energy Business News 2010). In the face of stern international competition especially from the United States (backed by government incentives), Australian biodiesel producers are experiencing threats to their market territory (The Sydney Morning Herald 2010). More than 20 ML of biodiesel has been imported from the US so far. After operating only for a year, Australia’s largest biodiesel plant in Darwin discontinued their operations in October 2007 due to poor production economies (ASIA CLEANTECH 2008).

The majority of the biodiesel plants in Australia are using tallow or imported vegetable oils as feedstock. With the increased demand, the price of the Australian tallow has jumped from $450 to $550 in 2005 (Whittington 2006). After 2007, the industry’s focus has shifted towards the potential of two common Indian feedstock plant species Jatropha curcas (Whittington 2006) and Pongamia pinnata.

Both the species have been subjected to extensive research for nearly a decade. Few large-scale plantations have been established to strengthen the supply of feedstock to high capacity biodiesel plants. Currently, Australia has an oilseed crushing capacity of 2-3 million tonnes of seeds per annum (Australian Oilseeds Federation Strategic Plan 2010) which requires a large improvement to maintain a secure oil supply.
The best solution for a viable biodiesel production would be regionalizing the biodiesel production where different biodiesel plants use different feedstock based on their regional specificity. Beauty leaf (*Calophyllum inophyllum*) is such a source for Queensland.

11.2 Why “Beauty Leaf Tree” *Calophyllum inophyllum* is Suitable for Biodiesel Production in Australia

Beauty Leaf Tree *Calophyllum inophyllum* is a native Australian species which grows naturally along the coastal belt of Northern Australia (Friday and Okano 2006). In this study it was discovered that calophyllum trees have the ability to grow well on a variety of soils, and at different temperature and rainfall conditions. Calophyllum seeds can be conveniently established from seeds. Calophyllum seeds showed high germination percentage (>80%) at 38% moisture content (section 6.3.2). The non-aggressive nature of growth and competition shown by calophyllum (Agroforestry Tree Database 2007) allows it to be intercropped with other plantations e.g. *Azadirachta indica*, *Pongamia pinnata*, *Ziziphus mauritiana*. Calophyllum seeds were found to retain their viability up to more than eight months under warmer humid storage (section 6.3.2).

Calophyllum trees from Australian and Sri Lankan provenances recorded appreciable fruit yield (overall mean 184756 ± 27146 fruits ha⁻¹ year⁻¹) in under-maintained conditions (Section 4.2.2).

Table 11.1: Estimated biodiesel yield of different feedstock species (Azam et al. 2005)

<table>
<thead>
<tr>
<th></th>
<th><em>Azadirachta indica</em></th>
<th><em>Calophyllum inophyllum</em></th>
<th><em>Jatropha curcas</em></th>
<th><em>Pongamia pinnata</em></th>
<th><em>Ziziphus mauritiana</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Average oil yield tree⁻¹ (kg)</td>
<td>6.6</td>
<td>11.7</td>
<td>1</td>
<td>4.95</td>
<td>4.95</td>
</tr>
<tr>
<td>Spacing of plantations (m²)</td>
<td>5 × 5</td>
<td>5 × 5</td>
<td>2 × 2</td>
<td>3 × 3</td>
<td>6 × 6</td>
</tr>
<tr>
<td>Number of Plants ha⁻¹</td>
<td>400</td>
<td>400</td>
<td>2500</td>
<td>1111</td>
<td>277</td>
</tr>
<tr>
<td>Average oil yield ha⁻¹ (kg)</td>
<td>2670</td>
<td>4680</td>
<td>2500</td>
<td>5499</td>
<td>1371</td>
</tr>
<tr>
<td>Biodiesel ha⁻¹ (kg)</td>
<td>2136</td>
<td>3744</td>
<td>2000</td>
<td>4399</td>
<td>1096</td>
</tr>
</tbody>
</table>
When compared to most common biodiesel feedstock species, *Calophyllum inophyllum* has the highest kernel oil content per tree (Table 11.1). Calophyllum oil has an ideal fatty acid composition to produce biodiesel (Azam et al. 2005; Hathurusingha and Ashwath 2007). Relatively high iodine value (composition of unsaturated fatty acids) in calophyllum oil improves cold flow properties of resultant biodiesel. This helps in maintaining the fuel in liquid form during cold temperatures. This characteristic is ideal for Australian conditions.

*Jatropha curcas*, commonly known as “Poison tree” is generally considered as an invasive species in Australia (Weeds Australia 2010). Establishment of *Jatropha* plantations may have adverse impacts on native Australian species. In terms of the number of multiple uses (resins, tannins, anti-cancer and anti-HIV compounds, timber, ornamental, apiculture and restoration) calophyllum is superior to the majority of the common biodiesel feedstock species.

According to Azam et al. (2005) 400 trees of *C. inophyllum* can yield 3744 kg of biodiesel in a given year. By using the new conversion protocol (Australian Patent: No.2010902733) 88% conversion rate can be achieved and then the yield can be further improved to 4118 kg ha⁻¹ year⁻¹. Considering all these positive aspects it is reasonable to suggest that *Calophyllum* is one of the best sources to produce biodiesel in Australia.

### 11.3 Present Status of “Beauty Leaf” Populations in Australia

Beauty Leaf Tree (*Calophyllum inophyllum*) is considered to be a coastal species of Northern Australia (Friday and Okano 2006). However, one record (observation) from inland Southeast Queensland appeared in Australia’s Virtual Herbarium. Scattered populations can be found along the coastal line from Yeppoon to Cape York. According to this study, size of the calophyllum populations in northern Australia ranged from 4 to 40 trees. There were four trees in Yeppoon; three relatively younger trees (around ten years) in Rosslyn bay and one 15 year old tree in Bell Park. In Townsville, four very old trees can be found in the botanic gardens, but majority (≥35) of the trees were found in the strand beach recreational area. Those in the Strand beach can be categorized into two age groups.
One group was roughly aged >25 years and the other group was around 8-12 years and they were mostly recreational plantings. The best age class distribution was found among the stands in Cardwell.

Cardwell had the largest Calophyllum trees in comparison to other selected provenances in Australia. The majority of the larger trees are naturally growing in that area. There were approximately 25 trees in Cardwell. Calophyllum populations in Cardwell were oriented fairly close to one another (2-4 km).

Calophyllum populations in Darwin were far more scattered compared to other selected provenances. They were growing on relatively less deep soils (Personal Communication 2008). Stands in Darwin also had good age class distribution. However, they had relatively low average tree height which may due to low soil depth.

Bowen had the largest calophyllum population (>40). The origin of those trees is unknown. All were planted 20 year ago and had uniform spacing. They had large crowns due to wide spacing and more fruit clusters than other selected northern Australian provenances. There were four solitary trees in the Mackay region, among them was a large very old (>50 years) tree with a very broad crown. A large number of seedlings were observed under that tree. This indicated that seed dispersal in Calophyllum inophyllum in Mackay is seriously limited. This may be due to the absence of animal species that usually disperse calophyllum seeds around that area. In Sri Lanka calophyllum seeds are said to be dispersed by fruit bats (Personal Communication 2009).

A large number of fallen fruits were found under most of the calophyllum trees in Australia, mostly devoid of exocarp. Tooth marks on those fruits indicated that the exocarps may have been eaten by some small animal. Germination tests revealed that those exocarp-eaten fruits are more likely to be viable than exocarp-intact fruits. Unlike in Australia, home gardens and farms in Sri Lanka had calophyllum seedlings which germinated from fruit bats drops (Personal Communication 2009). At the absence of effective seed dispersal mechanisms, calophyllum stands in Australia are naturally confined to the coastal zone.
11.4 Growth, Fruit and Oil Yield Variations and Their Implications

Significant country to country and provenance variations were observed in growth, fruit yield and estimated oil yield of calophyllum trees from Australia and Sri Lanka. Interpretation of these variations was limited to possible trend forecasting due to unknown life histories of some of the selected trees. Except for two provenances, most of the selected stands appeared to have good age class distribution. This strengthened the meaningfulness of those comparisons. Calophyllum trees in Sri Lanka were comparatively taller than those in Australian provenances.

Irrespective of the locality, calophyllum trees were found to maintain their identical form i.e. branch angles and crown diameters were independent of environmental variables. Nutrient rich soil conditions appeared to promote radial growth (DBHOB, crown diameter) and branching in calophyllum trees. Fruit yield ha\(^{-1}\) year\(^{-1}\) was only found to correlate with crown diameter and total soil carbon content. A strong relationship was found between crown diameter and DBHOB. Hence it can be assumed that fertilization may induce crown growth and consequently the fruit yield ha\(^{-1}\) year\(^{-1}\). Calophyllum trees in Sri Lanka had less spacing with adjoining tree species were taller and had smaller crowns compared to those in Australia. Fruit yields of Sri Lankan stands may therefore be improved by giving wide spacing.

Estimated values of oil yield ha\(^{-1}\) year\(^{-1}\) are slightly lower than the actual value due to reasons that were explained in section 4.2.2. This implies that calophyllum provenances in Australia have the capacity to produce oil yields that are closer to that reported by Azam et al. (2005). There appeared to be a strong relationship between the estimated oil yield and the long term drought period. Soil phosphorous content also seemed to have a positive influence on the estimated oil yield.

For an ideal feedstock plantation, it is best to have wide spacing and shoot apex removed to induce relatively less tall trees with larger crowns (Wilson 2000). Fertilizer rich in nitrogen and phosphorous may be administered to increase the fruit and oil yields. Establishing plantations in drier areas is also beneficial in achieving oil rich seeds.
11.5 Provenance and Seasonal Variations in Kernel Oil Content and Fatty Acid Composition

Calophyllum stands in Sri Lanka had significant provenance and seasonal variations in their kernel oil content (section 7.1.3). Seeds collected from Anuradhapura had significantly higher kernel oil content than the rest of the provenances. Calophyllum trees in Anuradhapura usually experience periodic drought. Mean annual rainfall and soil nitrogen levels showed significant negative correlations with kernel oil content. Kernel oil extracts from all selected provenances recorded a high percentage of unsaturated fatty acids. Unsaturated fatty acids are important to maintain the fuel in liquid form (Azam et al. 2005; Knothe 2005). Saturated fatty acid composition in kernel oil extracts from Sri Lanka was higher compared to those from Australia. Saturated fatty acids have high cetane numbers i.e. gives better ignition properties (Knothe et al. 2003). However, high amounts of saturated fatty acids lead to inferior cold flow properties such as high cloud points, pour points and cold filter plugging points (CFPP) (Knothe 2005).

Long chain saturated fatty acids can give high cold filter plugging points (CFPP). CFPP is the temperature at which fuel filters get clogged by precipitating long chain saturated fatty acids (Ramos et al. 2009). Kernel oil extracts from Kurunegala had the most ideal fatty acid profile and were predicted to have superior biodiesel properties compared to other provenances. Kernel oil extracts from Sri Lankan provenances were found to have superior fatty acid profiles for biodiesel production, compared to those of Australian provenances. However, the predicted biodiesel properties of kernel oil extracts from both Australian and Sri Lankan provenances were found to conform to Industrial standards (ASTM 6751, EN 14214).

Due to the time and legal constraints (Plant Protection Act 1994 of Sri Lanka), the study of seasonal variations was limited to the Australian provenances. Sri Lanka being a tropical country generally does not have distinct weather seasons as experienced in temperate countries. Australian provenances demonstrated significant seasonal variations in their fatty acid profiles (Section 7.2).
Kernel oil extracted from fruits harvested in autumn (April-May) appeared to have better fatty acid profiles for biodiesel production than those extracted in winter (June-July). A positive trend was observed between the temperature during fruit development and the kernel oil content. A strong negative relationship was found between mean annual rainfall (long term) and kernel oil content. However, the relationship between rainfall during fruit development (short term) and kernel oil content was found to be positive.

This study revealed that locations having warmer climatic conditions are more suitable for establishing calophyllum plantations in order to achieve high oil yields. Although nitrogen deficiency and periodic drought appeared to favour oil accumulation in calophyllum seeds, rainfall during anthesis and pre-anthesis also plays a major role in carbohydrate synthesis in seeds which directly impacts fat synthesis (Acetyl Co A). In Australia high oil yield in warm seasons could make up for low oil yield in cool seasons.

11.6 Technical Barriers to Mechanical Extraction

The oil extraction process has to be continuous for industrial scale biodiesel production. Continuous mechanical extraction of calophyllum oil is difficult to accomplish by a conventional screw press. Complications in cold-press extraction are mainly caused by the moisture content (MC) and oleoresin composition (Dweck and Meadows 2002; Seneviratne and Kotuwagedara 2009) of the kernels. In the experimental trials, approximately 65% oil recovery efficiency was achieved by preheating barrels of the screw press to 65 ºC, maintaining a medium compression ratio and by controlled feeding.

Early cream extrusions can be avoided by using a replaceable and washable mesh cover in the first cycle of each extraction trial. Once the optimum operating temperature and barrel pressure is reached, the operations are more likely to be satisfactory. At the best operational conditions, the cake/meal becomes extremely dry and papery (MC <2%). Oil content, recovery and extractability of calophyllum kernels can be slightly improved by drying bisected kernels for an optimum period (28 days).
Drying denatures protein structure in cells due to the simultaneous influence of heat and loss of water. As a result, fat drops lose their phosphoprotein envelopes and consolidate into larger globules (Fornal et al. 1994). Over-drying at temperatures >45 ºC is less desirable as it allows volatile oil to escape. Drying kernels at <45 ºC improves the oil extractability in calophyllum kernels. Steam and heat pre-treatment were also found to enhance the oil recovery and extractability. Steam breaks cell walls (Ward 1979).

Heat induced enzymatic hydrolysis simultaneously degrades cell walls and breaks down lipoproteins and lipopolysaccharides (Shanker et al. 1997). Most of the latest commercial scale oil seed crushing systems generally employ controlled drying, de-hulling and steam injection. In small-medium systems (i.e. farm based) batch to batch pre-treatments and extraction mechanisms (hydraulic pressing) can be employed to achieve high oil yields.

11.7 Complications in Conversion of Calophyllum Oil into Biodiesel

Conversion of highly viscous (62 cSt) and acidic (22 mg KOH/kg) Beauty Leaf oil into biodiesel is difficult to achieve by conventional protocols (Sahoo et al. 2007; Venkanna and Reddy 2009). Oleoresins and other organic inhibitors (e.g. phenolic compounds) found in Beauty Leaf oil (Adeyeye 1991; Seneviratne and Kotuwegedara 2009) make the conversion even more complicated.

Findings of the present study confirmed that the existing conversion protocols including the modified protocols proposed by Sahoo et al. (2007) and Venkanna and Reddy (2009) are not effective in completely converting Beauty Leaf oil into biodiesel. The modified protocol developed in this project recorded high conversion efficiency (89%), better cold flow properties and, the product was free of particles and free fatty acids (Section 8.2).

All modified protocols involve extra steps and extra reagents. They involve removing or suppressing organic inhibitors, reducing free fatty acids and dissolving and purifying fatty acid methyl esters (biodiesel). If the conversion becomes time consuming, the commercial viability of the process becomes less viable.
The new protocol consumes less time compared to the methods developed by Sahoo et al. (2007) and Venkanna and Reddy (2009) and yielded superior product. The new method also consumes low amounts of reagents.

Methanol is one of the major raw materials for biodiesel production. Industrial grade methanol can be purchased for 50 cents per litre. Under the present situation, the costs for reagents, extraction and processing costs make the production of biodiesel less viable. However in large scale production, B5 and B10 blends of Beauty Leaf biodiesel can still compete with commercial biodiesel made from tallow and waste vegetable oil (B5 and B10). Costs might also be reduced by adding a methanol plant to the production plant, where methanol is generated from natural gas (methane).

11.8 Industrial Compliance and Mechanical Reliability of COME

Transportation fuels have to pass strict industrial quality standards before handing them over to the consumers. Biodiesel has to essentially comply with ASTM D6751-01 and EN 14214 biodiesel quality specifications. Apart from marginal deviations from the standards for total acid number, total glycerol and carbon residue, calophyllum oil methyl ester (COME) conformed to the majority of the ASTM and EN standards (Section 8.2).

COME derived from three existing methods and the new method was tested for metal component degradation, using the copper strip corrosion test specified by ASTM. The product from the new method was found to cause the least corrosion in copper wires. Neat COME was found to swell elastomer components. However, the degree of swelling caused by B100 of COME was lower than common biodiesel types (e.g. Palm oil methyl ester). The elastomer swelling caused by the B20 blend of was found to be similar to that of mineral diesel. These observations suggest that B20 blend of COME is compatible with modern engine components. Engine performance of COME is one of the most important factors that have to be tested before end-use.

The most common method of testing engine performance involves a controlled engine dynamometer setup. In this study MAHA LPS 2000 chassis dynamometer was employed to test the engine performance of COME, since it resembles actual operational conditions.
The performance of COME B20 was also demonstrated in a three wheel drive in Uva Wellassa University, Badulla, Sri Lanka (Fig 11.1) and in a 4WD in CQU, Rockhampton, Australia (Fig 11.2). COME B20 performed equally well when compared with high speed diesel.
11.9 COME as a Fuel Additive

Combustion knock in compression ignition (CI) engines is generally caused by a sharp increase in pressure which forms a shock wave that generates heavy vibrations and a knocking sound. Excessive knock can overheat the piston and the cylinder head, damage bearing and possibly seize the piston (Gupta 2006).

Irregular or incomplete combustion is one of the major causes for diesel knock (Van Zanten 1985). Biodiesel has lower calorific value than mineral diesel due to the higher oxygen content (Ghadge and Raheman 2005; Sahoo et al. 2009). However, the presence of oxygen promotes complete combustion of biodiesel (Sahoo et al. 2009).

Cetane number (CN) reflects the ignition properties of a fuel (Knothe 2005). Higher cetane numbers indicate better ignition properties and low ignition delay. Hathurusingha et al. (2010b) found that fatty acid methyl esters derived from kernel oil of a native plant species *Calophyllum inophyllum* possess a high cetane number (CN 56). These preliminary experiments showed that the addition of high CN biodiesel can boost the CN of diesel and has the ability to reduce vibration and diesel knock much more efficiently than the additives used by CALTEX Australia.

Further tests are required to determine the optimum percentage of COME that cause the maximum vibration reduction effect. Apart from the issues mentioned above, reduction in vibration also indicates the smoothness of the combustion. Consequently, smooth combustion leads to reduced exhaust emissions. These observations indicate the superior engine performance of COME compared to commercially available biodiesel blends and mineral diesel.
Appendix

12. Making Use of the Transesterification By-products

12.1 Background

Transesterification by-products mainly consist of water, glycerine, KOH/NaOH, H₂SO₄/HCl, un-reacted oil and methanol. Methanol can be recovered by distillation and water is usually removed by heating and vacuum drying. Washed potassium hydroxide or potassium sulphate can be used as a fertilizer (Journey to Forever 1999). Unreacted oil and glycerine can be used to make good industrial soaps. The following has been done as a preliminary exercise rather than elaborate experiment.

12.2 Materials and Methods

Glycerine was separated from the ester layer and then purified before saponification. Then 3.85 g of NaOH was dissolved in 25 ml of water at 40 °C. Subsequently 100 ml glycerine was heated at 66 °C for 15 minutes and immediately after that NaOH solution was added to the glycerine solution while stirring. Stirring was continued for up to 20 minutes and then the soap was poured into a block and allowed to cool.

![A soap cube made from the transesterification by-products](image)
12.3  Results and Discussion

A fine textured solid soap cube was achieved (Fig 12.1). It was found to be effective in cleaning laboratory apparatus and glassware having oily stains. After three weeks it was found to accumulate water. This indicated that there were traces of NaOH remaining in the soap. The quality of the soap can be improved by optimizing NaOH required to completely saponify the sample.

12.4  Conclusion

By-products resulting from the transesterification of calophyllum oil can be used to make high quality industrial soap. Further testing and experimentation is required to determine the quality of the by-product soap.
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