



**NEAR INFRARED CALIBRATIONS FOR RAPID SCREENING OF FOLIAR  
NITROGEN AND PHOSPHORUS IN *Eucalyptus pellita* F. Muell**

**By**

**AGUSTAN BIN ALWI**

**Thesis Submitted to the School of Graduate Studies, Universiti Putra  
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Science**

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in  
fulfilment of the requirement for the degree of Master of Science

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**October 2022**

**Chair : Hazandy Bin Abdul Hamid, PhD**  
**Institute : Tropical Forestry and Forest Products**

*Eucalyptus pellita* F. Muell. has become an important tree species in the forest plantations of South-East Asia, and in Malaysian Borneo in particular, to replace thousands of hectares of *Acacia mangium* Willd., which has suffered significant loss caused by *Ceratocystis manginecans* infection in Sabah, Malaysia.

Nitrogen (N) and phosphorus (P) fertilization at the time of planting are essential to optimize *E. pellita* establishment and growth in forest plantations. Traditionally, plant nutrient content is analyzed via wet chemistry methods using ground, dried foliar samples with the associated cost of analysis of many leaf samples. Generally, this chemical technique requires large amount of chemicals and reagents, which may be unfriendly to environment and hazardous to health.

This study aims to quantitatively estimate N and P elements in *E. pellita* foliage samples by means of near- infrared (NIR) reflectance spectroscopy. This method is non- destructive, rapid and cost-effective. In order to be used effectively, the NIR spectra must be calibrated with foliar samples that have also been analyzed using reference methods.

In this present study, the nutritional status of the foliage was investigated with the aim to develop near- infrared spectroscopic calibrations that can be used to monitor and quantify nutrient status, particularly total foliar nitrogen (N) and phosphorus (P) in the field. The spectrum data was captured using VIAVI 1700 hand-held spectrometer. Spectra acquired on fresh foliage in situ on the tree could be used to predict N and P with accuracy suitable for operational decision- making

with regards to fertilizer application. Accuracy of spectra acquired on dried ground foliage could be used to predict N and P within a relative error of 10% ( $R^2_c$ ,  $r^2_{CV}$ , RMSEP, RPD of = 0.77, 0.71, 0.02 g. 100 g<sup>-1</sup>, 1.9 for foliar P and = 0.90, 0.88, 0.21 g. 100 g<sup>-1</sup>, 3.0 for foliar N on dried ground foliage).

Therefore, the ultimate application of this near-infrared spectroscopic calibration is in situ nutrient monitoring, particularly in longitudinal studies in fertilizer trial plots and forest operations, as the non-destructive nature of NIR spectroscopy would enable regular monitoring of individual leaves over time without the need to destructively sample them. This would aid the temporal and spatial analysis of field data.



Abstrak tesis ini disampaikan kepada Senat Universiti Putra Malaysia bagi memenuhi syarat untuk ijazah master sains

## **KALIBRASI INFRA MERAH DEKAT UNTUK SARINGAN PANTAS FOLIAR NITROGEN DAN FOSFORUS DALAM *Eucalyptus pellita* F. Muell**

Oleh

**AGUSTAN BIN ALWI**

**Oktober 2022**

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*Eucalyptus pellita* F. Muell. telah menjadi spesies pokok yang penting di ladang hutan Asia Tenggara dan di Borneo Malaysia khususnya, untuk menggantikan beribu-ribu hektar *Acacia mangium* Willd. yang telah mengalami kerugian yang besar akibat serangan penyakit *Ceratocystis manginecans* di Sabah, Malaysia.

Pembajaan nitrogen (N) dan fosforus (P) pada masa penanaman adalah penting untuk mengoptimalkan kadar pertumbuhan *E. pellita* di ladang hutan. Secara tradisinya, kandungan nutrien tumbuhan dianalisis melalui kaedah bahan kimia menggunakan sampel daun kering yang dikisar dengan kos analisis yang memerlukan banyak sampel daun. Secara amnya, Teknik kimia ini memerlukan sejumlah besar bahan kimia dan reagen yang mungkin tidak mesra alam sekitar dan berbahaya kepada kesihatan.

Kajian ini bertujuan untuk menganggar secara kuantitatif bagi unsur N dan P dalam sampel daun *E. pellita* menggunakan spektroskopi pemantulan inframerah dekat (NIR). Kaedah ini tidak merosakkan, cepat dan kos efektif. Bagi menilai keberkesanan kaedah ini, spektrum NIR mesti ditentukan dengan sampel daun yang telah dianalisis menggunakan kaedah rujukan.

Dalam kajian ini, status nutrisi daun telah disiasat dengan tujuan untuk membangunkan penentuan spektroskopi inframerah yang boleh digunakan untuk memantau dan mengukur status nutrien, terutamanya bagi jumlah nitrogen (N) dan fosforus (P) daun di ladang. Pengambilan data spektrum adalah menggunakan spektrometer VIAVI 1700. Spektrum yang diperolehi daripada daun segar "in situ" pada pokok boleh digunakan untuk meramalkan nilai N dan P dengan ketepatan yang sesuai untuk membuat keputusan operasi yang berkaitan

penggunaan baja. Jika ketepatan yang lebih tinggi diperlukan, spektrum yang diperoleh pada dedaun kering yang digiling boleh digunakan untuk meramalkan N dan P dimana ralat relatifnya adalah 10% ( $R^2_c$ ,  $r^2_{cv}$ , RMSEP, RPD = 0.77, 0.71, 0.02 g. 100 g<sup>-1</sup>, 1.9 untuk daun P dan = 0.90, 0.88, 0.21 g. 100 g<sup>-1</sup>, 3.0 untuk dedaun N pada dedaun kering yang digiling).

Oleh itu, aplikasi kajian ini bagi pemantauan nutrien in situ, terutamanya untuk membantu kajian menyeluruh dalam percubaan baja dan operasi hutan, dimana kaedah spektroskopi NIR yang tidak merosakkan akan membolehkan pemantauan tetap daun secara individu dari masa ke masa tanpa merosakkan sampel. Ini juga akan membantu dalam analisis temporal dan spatial data di ladang.



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I am extremely indebted to my parents and my wife for fully supporting my Master's program. Without their support, I would not be able to enhance my knowledge, particularly in forest plantation management.

Thank you very much and hope everyone doing great.

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## TABLE OF CONTENTS

	<b>ABSTRACT</b>	<b>Page</b>
	<b>ABSTRAK</b>	i
	<b>ACKNOWLEDGEMENTS</b>	iii
	<b>APPROVAL</b>	v
	<b>DECLARATION</b>	vi
	<b>LIST OF TABLES</b>	viii
	<b>LIST OF FIGURES</b>	xii
	<b>LIST OF ABBREVIATIONS</b>	xiii
		xv
<b>CHAPTER</b>		
<b>1</b>	<b>INTRODUCTION</b>	<b>1</b>
	1.1 Background	1
	1.2 Statement of Problems and Justifications	6
	1.3 Objectives	10
	1.4 Organization of Chapters	10
<b>2</b>	<b>LITERATURE REVIEW</b>	<b>7</b>
	2.1 Nutrient Requirements in Plantation Forests	7
	2.2 <i>Eucalyptus pellita</i>	8
	2.2.1 The wood quality of <i>E. pellita</i>	9
	2.2.2 <i>Eucalyptus pellita</i> performance in Sabah	10
	2.3 Foliar Sampling Approach for Plant Nutrient Monitoring	10
	2.4 Near-Infrared Spectroscopy	11
	2.4.1 History of NIR spectroscopy	12
	2.4.2 Principle of NIR spectroscopy	13
	2.4.3 Spectrometer and spectral acquisition	15
	2.4.4 Spectral data analysis	16
	2.4.4.1 Transformation of spectral data	16
	2.4.4.2 Principal component analysis	17
	2.4.4.3 Partial least square regression	17
	2.5 Calibration and Validation Statistics	18
	2.6 NIR Spectroscopy in Foliar Nutrients	20
	2.7 NIR Spectroscopy in Wood	21
	2.7.1 Chemical composition of woods	22
	2.7.2 Wood density	22
	2.7.3 Wood pulp and paper	23
	2.7.4 Wood species and classification	23
<b>3</b>	<b>METHODOLOGY</b>	<b>24</b>
	3.1 Research Site	24
	3.1.1 Soil Type	25
	3.1.2 Plot Information	25
	3.1.3 Site Preparation	27
	3.2 Planting Material	28
	3.3 Treatment Design	29
	3.3.1 Field Planting	30

3.3.2	Sample Preparation	32
3.3.3	NIR Spectroscopy	34
3.3.4	Foliar Chemical Analysis	36
3.3.5	Spectral Data Analysis	36
<b>4</b>	<b>RESULTS AND DISCUSSION</b>	<b>38</b>
4.1	Soil Data Results	38
4.2	Foliage Nitrogen and Phosphorus Levels	39
4.3	Fresh and Dry Foliar Spectral Performance	40
4.4	Prediction using Spectra from Fresh Foliage	40
4.5	Prediction using Spectra from Dry Foliage	41
<b>5</b>	<b>CONCLUSION AND RECOMMENDATION</b>	<b>46</b>
	<b>REFERENCES</b>	<b>47</b>
	<b>APPENDICES</b>	<b>59</b>
	<b>BIODATA OF STUDENT</b>	<b>91</b>
	<b>PUBLICATION</b>	<b>92</b>

## LIST OF TABLES

Table	Page
2.1 Scientific classification of <i>Eucalyptus pellita</i>	8
2.2 Classification of model performance according to William (2010)	19
2.3 Summary of wavelength ranges and RPD of various plant materials based on foliar samples used in Visible-NIR and NIR calibrations to estimate nutritional status.	20
3.1 Summary of research site information	26
3.2 Fertilizer treatments for the seedlings at the nursery stage	29
3.3 Fertilizer treatments for the field trial plot at establishment	32
4.1 Soil chemical analysis at two different depths from 0 to 40 cm at 0-10 cm (top soils) and 20-40 cm (subsoils)	38
4.2 PLS regression statistics for calibrations developed from <i>E. pellita</i> fresh foliage using nursery (ten weeks), three-month, and six-month samples, and the prediction of the nine-month samples as an independent test set	41
4.3 PLS regression statistics for calibrations developed from <i>E. pellita</i> dried ground foliage using nursery (ten weeks), three-month, and six-month samples, and the prediction of the nine-month samples as an independent test set.	42
4.4 Final cross-validated calibrations using nursery (ten weeks), three-month, six-month, nine-month, and 12-month samples	44

## LIST OF FIGURES

Figure		Page
1.1	Overview of spectrometer, (a) is The MicroNIR™ spectrometer (Viavi Solutions Inc., Milpitas, CA, USA), (b) an example of spectrum captured from sample and (c) overview of MicroNIR sections.	5
2.1	<i>Eucalyptus pellita</i> (a) seeds, (b) seedlings at ten weeks in nursery, and (c) physical appearance at six months of age in the field.	8
2.2	The electromagnetic spectrum showing the spectral range and location of NIR relative to other types of electromagnetic radiation.	11
2.3	Electromagnetic radiation dispersion, Herschel's experiment, demonstrated the existence of near-infrared energy. The dotted line indicates the human eye's relative reaction to wavelengths of radiation, while the solid line depicts the heating impact.	12
2.4	Example of a spectrum of samples with the main areas of absorption identified.	14
2.5	An incremental number of publications on NIR spectroscopy in forestry from 1992 to 2018.	22
3.1	Research site showing (a) Sabah map and local administrative districts, (b) location of the site study and (c) plot layout.	24
3.2	Soil physical properties (a) and procedure of soil sampling in the field before planting. Each pit consists of two samples (b) where A: 0 – 10 cm and B: 20 – 40 cm depths. The samples were placed in a zip-locked plastic bags (c) and then temporarily stored in the toolbox (d).	25
3.3	Site preparation using chemical weeding (a), plotting and lining (b), holing and planting (c), fertiliser placement during planting (d) and mulching (e).	28
3.4	The physical appearance of seedlings according to different rates application at ten weeks at the nursery	30

	stage.	
3.5	Plot design using randomized complete block design (RCBD).	31
3.6	Fresh leaf scanned using spectrometer MicroNIR 1700 viavi (a) and leaf was selected (b) in the nursery before oven dried and ground.	33
3.7	In-situ fresh leaf scanned using spectrometer microNIR 1700 viavi (a) at three months of age, nine months old leaf sampling (b1) – the upper third of the canopy then the third or fourth node leaf (b2) and (b3) in the field were taken in the field.	34
3.8	The leaf region (fresh sample) was scanned using a spectrometer Micro-NIR 1700.	35
3.9	The dried ground sample was scanned for spectral data recording.	35
3.10	Schematic diagram of an overview of the research flow in developing of calibration and validation model.	37
4.1	Box–Whisker plots showing the distribution of nitrogen and phosphorus concentrations collected from <i>E. pellita</i> leaves at each stage of sampling in the nursery and at three, six, nine, and 12-months post planting August 2019). Note that May 2020 and August 2020 values are NIR (predicted values using calibrations built with six- and six-month (May 2020), and three-, and nine-month (August 2020) data.	39
4.2	Mean spectra with standard deviation for NIR spectra acquired from <i>E. pellita</i> fresh foliage in blue-line and dried ground foliage in red-line.	40
4.3	NIR-predicted vs measured calibration plots of dry <i>E. pellita</i> foliar nitrogen (a-c) and phosphorous (d-f) for: (a, d) nursery-stage and three- month-old data, (b, e) nursery-stage, three-month-old and six-month-old data, and (c, f) predicted foliar nitrogen in nine-month-old samples (□) superimposed on the calibration. N: raw spectra, P: spectra with 1st derivative + SNV transformation.	43

## LIST OF ABBREVIATIONS

AFC	Borneo Forestry Cooperative
Bhd	Berhad
cm	Centimeter
cm <sup>-1</sup>	Reciprocal Centimeter
CRD	Completely Randomized Design
DBH	Diameter at Breast Height
EMSC	Extended Multiplicative Scatter Correction
FAO	Food and Agriculture Organization
g.100g <sup>-1</sup>	Gram per Hundred Grams
ha	Hectare
i.e.	That is to say (Latin: <i>id est</i> )
In	Indium
GaAs	Gallium Arsenide
IR	Infrared
Kg m <sup>-3</sup>	Kilogram per Cubic Meter
LV LVF	Latent Variable Linear Variable Filter
m	Meter
m <sup>3</sup>	Cubic Meter
m <sup>3</sup> ha <sup>-1</sup> year <sup>-1</sup>	Multiplicative Scatter Correction
mm	Milimeter
MAIV	Mean Annual Increment of Volume
MIR	Mid Infrared
MOE	Modulus of Elasticity
MSC	Multiplicative Scatter Correction
MTH	Mixed Tropical Hardwood
N	Nitrogen
NIR	Near Infrared

Nm	Nanometer
P	Phosphorus
PCA	Principal Component Analysis
pH	Potential of Hydrogen
PLS	Partial Least Squares
RCBD	Randomised Complete Block Design
RMSE	Root Mean Square Error
RMSEC	Root Mean Square Error of Calibration
RMSECV	Root Mean Square Error of Cross Validation
RMSEP	Root Mean Square Error of Prediction
RPD	Ratio of Prediction of Determination
SAFODA	Sabah Forest Development Authority
SD	Standard Deviation
SEC	Standard Error of Calibration
SEP	Standard Error of Prediction
Sdn	Sendirian
SG	Savitzky-Golay
SNV	Standard Normal Variate
Spha	Stems per Hectare
SSB	Sabah Softwoods Berhad
Tn	Treatment Number
YFEL	Young Fully Expanded Leaf
"	Inches
%	Percentage
>	More than
<	Less than

## CHAPTER 1

### INTRODUCTION

#### 1.1 Background

Sabah forest industry has been reliant on raw wood acquired locally from natural forest stands for decades, also known as a mixed tropical hardwood (MTH). However, as natural forest harvest declines around the world, sustainably-managed forest plantations are gradually replacing this scenario as the primary source of wood supplies (FAO, 2020), prompting the increased expansion of forest plantations around the world.

The mission and vision of forest plantations varied markedly among regions. Nonetheless, the general consensus on establishing forest plantations is to provide raw materials for industrial timber processing operations, such as solid-wood products, panel products and furnishings, and pulpwood for paper. Forest plantations, also known as planted forests, were established to fulfill the global demand for timber products as a replacement for natural timber products. In 2000, forest plantations only made up 5% of global forest cover, but they produced 35% of global round-wood production (FAO, 2010; 2016). Globally, forest plantation area has increased by 1.85% per year on average since 1990, and is currently approaching 294 million hectares.

The greatest annual average increase occurred in North and Central America, South America, and Asia, with around 2.5%, 2.4% and 2.3%, respectively (FAO, 2020). Both *Pinus* species and *Eucalyptus* species have been deployed, to a point where *Eucalypt* trees occupy around 20 million hectares in more than 90 countries, with the largest plantation areas being in Brazil (more than seven million ha), India (more than six million ha), and China (more than five million ha) (Elli et al., 2019; Arnold et al., Japarudin et al., 2020).

Industrial tree plantation (ITP) in Malaysia began in the 1920's for research purposes (Selvaraj and Muhammad, 1980), with the then anticipated establishment of an integrated pulp and paper mill in Peninsular Malaysia in 1967, at which time the first large-scale commercial forest plantation was developed. Given the depleting availability of timber resources, the government turned its focus on expanding the forest plantations through Malaysia's afforestation program in the 1980's (Ratnasingam et al., 2020).

In the 1970's, the government of Sabah played a key role in establishing firms, mainly Sabah Softwoods Bhd. (SSB), the Sabah Forestry Development Authority (SAFODA), and Sabah Forest Industries Sdn. Bhd. (SFI) to spearhead the forest



plantation program. As a result, four fast-growing hardwood species were chosen, such as *Acacia mangium* Willd., *Gmelina arborea* Roxb., *Falcataria moluccana* (Miq.) Barneby & J.W. Grimes (formerly known as *Albizia falcataria* (L.) Fosberg), and *Eucalyptus deglupta* Blume. Both *G. arborea* and *E. deglupta* were phased out in favour of *A. mangium* and *F. moluccana* (Sabah Forestry Department, undated).

Over the years, the timber industries in Sabah became more aware of the need for raw materials; hence, the expansion of the tree plantation areas with approximately 55,560 ha of *A. mangium* had been established by 2002 (Krishnapillay, 2002), which then further increased to 75,120 ha by 2004 (Hashim et al., 2015). In the excitement of increasing production, the forest plantation industry, however, was shocked by the infestation of *Ceratocystis manginecens* fungi (vascular wilt disease) on *A. mangium* plantations (Tarigan et al., 2010; 2011; Brawner et al., 2015). This has led to the focus on increased research and understanding of *Eucalyptus* as an alternative species. While there are more than 700 varieties of *Eucalyptus*, only a dozen or so species are deployed in commercial tree plantations, particularly in tropical regions to continuously provide wood product. Interestingly, this genus has a wide range of adaptation based on species variety, for example, *Eucalyptus nitens* and *E. globulus* are more suitable in temperate regions, while *E. pellita*, *E. urophylla* and *E. camaldulensis* are adapted to tropical and sub-tropical regions. Nowadays, the utilization of *Eucalyptus* has been proven in Asia, where *Eucalyptus* plantations have increased in a number of countries, particularly India, China, Vietnam and Thailand, where they support major fundamentals to the industries and contribute to mitigation of rural poverty (FAO, 2020).

The development of *Eucalyptus* plantations, particularly *E. pellita*, is influenced by the quality of the planting materials and silviculture practices, such as nutrition management. Generally, nutrition management is essential when planting tree seedlings in commercial *Eucalyptus* plantations. By highlighting the growth limitation factor, nutrients such as Nitrogen (N) and Phosphorus (P) are crucial depending on the site condition (Garciano et al., 2006; Inail et al.; Crous et al., 2015; 2019)., and it is a viable silvicultural method for increasing stand nutrient availability (Schonau et al., 1989, Fox et al., 2007, Viera et al., 2016).

*Eucalyptus pellita* F. Muell., also known as red mahogany, occurs naturally in the tropical regions of north-eastern Queensland, Australia and the island of New Guinea. It grows in gentle to moderate sloping terrain mixed with other eucalypt species in tall open forest and at the fringes to tropical rainforest. It prefers sand to loam soil types with good drainage at altitudes from sea level to 800 m asl with moderate to high rainfall of 900 to 4000 mm annually (Brooker and Kleinig, 2012). It is one of the few eucalyptus species that is adapted to the wet tropical climate and is thus less susceptible to fungal leaf pathogens (Harwood et al., 1997). This adaptation together with other positive traits make it suitable for plantation establishment in wet tropical regions outside of its natural range.

At planting stage, both N and P are generally applied, along with other macro- and micro- nutrients, in Eucalyptus plantations depending on the site and soil condition. To observe and quantify the response to these applications on the growth of trees over time, the collection of foliar samples is required to generate some information on the level of availability of the nutrients. In addition, before site establishment in the planted forest, weed control is crucial to reduce the competition in terms of nutrition and space to optimize tree growth (Alwi et al., 2020). However, there are several problems associated with monitoring foliar nutrition. The traditional analysis using wet chemical methods takes a long time, is destructive, has a high cost of analysis, and the consumption of chemical reagents is not environmentally friendly (Samborski et al., 2009; Minesatti et al., 2010).

As an alternative method to monitor and evaluate the nutrition elements in the trees, this study focused on the development of a Near- Infrared (NIR) spectroscopic method for the rapid, cost-effective determination of nutritional nitrogen and phosphorus in *E. pellita* foliage. Studies have been undertaken using this method to quantify and qualify nitrogen and phosphorus in agriculture, pharmaceutical, and soil, however, no evidence has been found for NIR application in *E. pellita* plant nutrition, particularly on specific nutrients such as N and P.

## **1.2 Statement of Problems and Justifications**

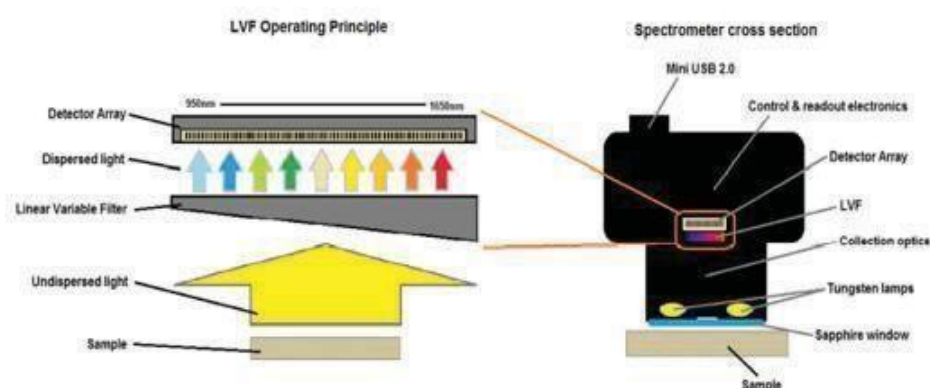
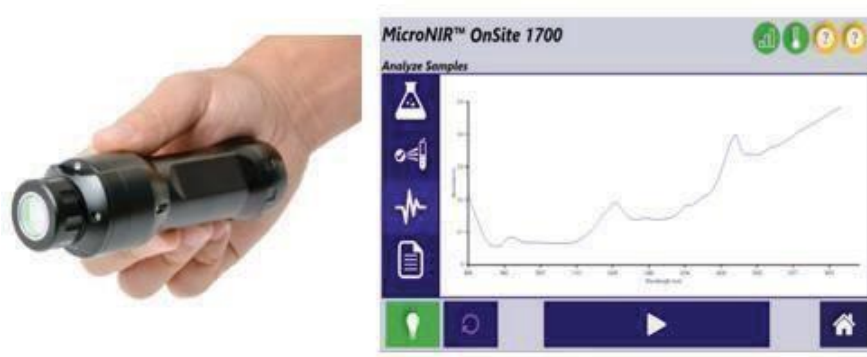
Nitrogen (N) and phosphorus (P) are the two macro-elements that influence the initial growth of plants among the seventeen essential nutrients required (Elser et al., 2007; Harpole et al., 2011), particularly in *E. pellita* due to their key roles in many cellular functions of plant metabolism (Inail et al., 2019). Generally, foliar chemical analysis is the main option in forestry to determine the nutritional plant status in conjunction with soil chemical analysis. Traditionally, foliar chemical analysis is used to obtain the nutrients information, however, it is challenging because of the destructive nature of the sampling, which makes it impossible to track individual leaves over time, making longitudinal investigations impossible. "Matched pairs" or bulked samples are employed instead of monitoring an individual leaf (or leaves) over time, which increases sampling errors related to sample representation and heterogeneity (Esbensen, 2019).

The capacity to monitor the foliar response over time to correlate nutrient status with tree growth will considerably help research into the nutritional response of plantation tree species such as *E. pellita*. However, traditional foliar chemical analysis requires a large number of samples for analysis, which is not only time-consuming and, expensive, but also has a negative impact on the environment due to the chemical reagents used during the chemical analysis in the laboratory (Kiwfo et al., 2021).

Near-infrared (NIR) spectroscopy has great potential for rapid and real-time, non-destructive determination of N and P in foliage, and can offer high throughput analysis at a lower-cost for the element of interest (Prananto et al., 2021). As recently evaluated by multiple academics globally, the growing number of publications on this technique and the worldwide adoption of this technology by several research organizations indicated the growing interest in NIR spectroscopy application in bio analysis. However, in order to accurately estimate the concentration of targeted elements in a sample using NIR spectroscopy, the spectral analysis must first be calibrated (Agelet and Hurburgh, 2010) and validated against the spectral absorption of targeted elements of the foliar samples, in which this study aimed for the elements of nitrogen (N) and phosphorus (P). One way to obtain this value data set is to establish fertilizer trials at nursery and field stages and review the results once they are fully assessed to validate the range across the stages.

Another issue in determining the reliability of NIR calibration is the condition of samples with high moisture content (water) levels or samples analyzed in the field, because the fundamental of NIR spectroscopy applications is predominantly based on the use of dry and ground samples (Malley et al., 2004). In the NIR region, water contains intense absorption bands, with the major absorptions between 1400 and 1900 nm (Roberts and Cozzolino, 2017). The presence of water in samples tends to lower the sample's reflective index, resulting in less scattering of light incidence (Minasny et al., 2011; Ogen et al., 2019). Thus, the sample's spectral pre-processing and physical state (either dry or fresh) are crucial factors that determine the accuracy of the analysis in relation to the target outcome.

The spectral data are measured using a spectrometer. In this study, a hand-held spectrometer was used, which is known widely as the Micro-NIR 1700. The Micro-NIR 1700 (Viavi Solutions Inc., Milpitas, CA, USA) spectrometer comprises the light source, collection optics, electronics, and detector in a device that is less than 2" in diameter (50 mm) and weighs 58 g, using Viavi linear variable filter (LVF) technology as the dispersion element. An LVF is a band-pass filter coating intentionally wedged in one direction. As a result of the varying film thickness, the wavelength transmitted through the filter varies linearly in the direction of the wedge. The LVF is coupled to a linear detector array (128-pixel uncooled InGaAs photodiode array). The light source is a pair of integrated vacuum tungsten lamps. A 16-bit analogue-to-digital converter (ADC) is used for the analogue conversion, as shown in Figure 1.1. This spectrometer can be used in diffuse reflection, transmission, or transflection modes powered by USB.



**Figure 1.1: Overview of spectrometer, (a) the MicroNIR™ spectrometer (Viavi Solutions Inc., Milpitas, CA, USA), (b) an example of spectrum captured from sample, and (c) overview of MicroNIR sections.**  
(Source: [www.viavisolution.com](http://www.viavisolution.com))

### 1.3 Objectives

This study aimed to develop NIR spectral calibrations for the determination of foliar nitrogen (N) and phosphorus (P) in *E. pellita*. The specific objectives of this study were:

- i) to predict foliar N and P in *E. pellita* based on robust NIR spectral calibrations,
- ii) to improve the nutrient level monitoring, particularly N and P, through foliar assessment, and
- iii) to develop low-cost and fast methods for foliar N and P monitoring.

The information from this study will help to improve the nutrient management of *E. pellita* tree plantations and may enhance productions. This is important in diagnosing the nutrient level in a forest plantation.

#### **1.4 Organization of Chapters**

This thesis contains the following chapters:

##### **Chapter 1: Introduction**

The introduction summarizes and outlines the current state of forest plantation, both globally and in Malaysia (with particular emphasis in Sabah), and the key research problems met by this study.

##### **Chapter 2: Literature review**

This chapter presents an overview of the Eucalyptus plantation, information on species studied, and an insight into the theory and fundamentals of Near-Infrared spectroscopy. The literature review examines the conventional laboratory techniques analysis based on foliar (plant tissue) used in this project, and mathematical (chemometrics) techniques applicable to predict the value of nitrogen and phosphorus using through fresh and dry-ground samples of *E. pellita* foliage.

##### **Chapter 3: Methodology**

This chapter elaborates on the strategies employed in this research to achieve the objectives, with a detailed description of the study site, spectrometer as the (tool), and methodologies of foliar sample selection and preparation, N and P chemical analysis, spectroscopic and analytical techniques used in the assessment of calibration model development.

##### **Chapter 4: Results and discussion**

This chapter presents the results obtained for N and P of fresh and dry-ground *E. pellita* foliage, their correlation with NIR spectra and the calibration development and validation.

##### **Chapter 5: Conclusions and recommendation**

This chapter summarizes the results of the study and indicates how these results have fulfilled the objective of this study. It also consolidates the outcome and benefits to the industries, particularly in the nutrition management of *E. pellita*.



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