UNIVERSITI PUTRA MALAYSIA

PROVIDING OPTIMUM NITROGEN, PHOSPHORUS AND POTASSIUM LEVELS TO OIL PALM AS A STRATEGY TO COMBAT GANODERMA DISEASE INFECTION

HASMAH BT. MOHIDIN

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By

HASMAH BT. MOHIDIN

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

July 2016

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DEDICATION

I dedicate this humble effort to
my beloved late parents,
Hjh Hamdiah Bol and Mohidin Bin Junit
My supportive and understanding husband, Sulaiman Bin Man,
my sisters and brothers, and in-laws
to whom I could never repay back their sacrifices.

My beloved children;
Mohamad Hazrin
Afiqah
Mohamad Haziq
Mohamad Hafiz
Mohamad Haiqal,
for their patience, with love and thank

Abstract of thesis submitted to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for Doctor of Philosophy

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July 2016

Chairman: Professor Mohamed Hanafi Musa, PhD
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Basal stem rot (BSR) of oil palm caused by *Ganoderma boninense*, has caused a serious economic impact to the oil palm industry. The BSR affects oil palms at all ages, are spreading to greater area yearly with increasing occurrence in peat soils. Nitrogen (N), phosphorus (P), and potassium (K) nutrition play a critical role in oil palm growth and are known to give beneficial effects on BSR disease control. Plants with optimum nutritional status have the highest resistance to pests and diseases. Therefore, it is important to determine these N, P and K optimum nutrient values to promote overall palm health and provide better protection against *Ganoderma* disease. This study investigated the optimum concentrations of N, P_2O_5 and K_2O in solution culture (soilless condition) followed by peat soil medium at nursery conditions for *dura* (D) × *pisifera* (P) oil palm progeny. These optimum values were adopted and formulated in various levels of N, P, and K nutrition as treatments for three months prior to challenge with *G. boninense* to assess their effect in relation to *Ganoderma* incidence and severity on oil palm seedlings grown on peat soil. Subsequently, their effect on nutritional and biochemical traits were then evaluated. Under Hoagland's solution culture, the optimum levels of N, P_2O_5 and K_2O were found at 100, 90 and 300 mg/L, respectively, at pH 5.5 ± 0.1 of 6.5 months, gave significant growth traits, nutrient uptake in plant tissues and biomass accumulation. On peat soil condition, the best nutrients concentrations N, P_2O_5 and K_2O of 21.72, 21.72 and 40.04 g/plant, respectively, for growth improvement at pH 4.3 ± 0.2, of 16.5 months. N, P_2O_5 and K_2O indicated significant difference ($p<0.5$) in term of dry biomass accumulation, nutrient concentration and partitioning in tissues at N2, P2, K3 levels. Pathological data revealed the single nutrient deficiency (low N, low K and low P) at 12.16, 12.16 and 22.26 g/plant gave negative effects and were highly infected with *Ganoderma*. Treatment low N was the most severely infected at the roots, stem boles and leaves with the lowest dry biomass accumulation. Generally, a positive correlation was observed at *G. boninense* inoculated palm roots for enzymes activity; namely, β-1,3-glucanase PAL, POX, and chitinase, which revealed higher activities ($p< 0.5$) suggesting their roles in induced defense related mechanism. All the enzyme activities ranked in ascending order were as followed : T5 > T2 > T10 > T8 > T6. In conclusion,

optimum N and P nutrient with high K at application of N, P_2O_5 and K_2O, 17.37, 17.37 and 41.34 g/plant, respectively, exhibited a better growth and promoted better protection against BSR for almost all the pathological parameters assessed. However, N, P and K treatments had provide fair protection by delaying *G. boninense* infection up to 6 month after inoculation but after and beyond this period, they seem to lose their protecting effect. The results could be useful for developing new strategies with proper nutrition, which may effectively reduce *Ganoderma* incidence and severity grown in peat soil.

Abstract tesis yang dikemukakan kepada Senat Universiti Putra Malaysia ini memenuhi keperluan untuk ijazah Doktor Falsafah

PENYEDIAAN TAHAP NITROGEN, FOSFORUS DAN POTASIUM OPTIMA SEBAGAI STRATEGI UNTUK MELAWAN JANGKITAN PENYAKIT *GANODERMA*

Oleh

HASMAH BT. MOHIDIN

Julai 2016

Pengerusi: Profesor Mohamed Hanafi Musa, PhD
Institut: Pertanian Tropika

Penyakit reput pangkal (BSR) kelapa sawit disebabkan oleh *Ganoderma boninense*, memberi kesan ekonomi yang serius kepada industri kelapa sawit. BSR menjejaskan sawit pada semua peringkat umur dan merebak ke kawasan yang lebih luas setiap tahun dengan peningkatan kejadian di tanah gambut. Nutrisi nitrogen (N), fosforus (P) dan kalium (K) memainkan peranan penting dalam perkembangan kelapa sawit dan memberi kesan baik bagi kawalan penyakit BSR. Tumbuhan yang status nutrisinya seimbang mampu memaksimakan ketahanan terhadap penyakit. Oleh itu,adalah penting untuk menentukan nilai optima nutrien N, P dan K untuk meningkat kesihatan sawit secara amnya dan memberi perlindungan awal terhadap *Ganoderma*. Kajian ini menyiasat tahap optima N, P_2O_5 dan K_2O dalam larutan nutrien (tanpa tanah) diikuti dengan media tanah gambut di nurseri bagi anak kelapa sawit. Nilai-nilai yang optima diformulasi pada pelbagai tahap nutrien N, P dan K sebagai perlindungan sebelum diuji keberkesanan terhadap *G. boninense* dari aspek insiden *Ganoderma* dan keseriusannya ke atas progeni *dura* (D) × *pisifera* (P) yang ditanam di kawasan tanah gambut. Nilai-nilai optima ini telah dirumus dalam pelbagai kombinasi N, P dan K sebagai perlindungan sebelum dicabar dengan *G. boninense* dan dinilai berdasarkan sifat pertumbuhan, fisiologi, nutrisi, patologi dan biokimia untuk insiden keseriusan *Ganoderma* yang ditanam pada tanah gambut. Pada larutan nutrient Hoagland, tahap optima N, P_2O_5 dan K_2O terdapat pada 100, 90 dan 300 mg/L (berkadaran dengan 150, 135 and 450 kg/ha), masing-masing pada pH 5.5 ± 0.1, 6.5 bulan, memberikan signifikan ($p<0.5$). pada ciri-ciri pertumbuhan, penyerapan nutrien dalam tisu-tisu tumbuhan dan pengumpulan biomas. Pada media tanah gambut, tahap nutrien yang terbaik untuk peningkatan pertumbuhan ($p<0.5$) N, P_2O_5 dan K_2O adalah pada 21.72, 21.72 dan 40.04 g/tumbuhan, masing-masing pada pH 4.3 ± 0.2, 16.5 bulan. Data patologi mendedahkan satu nutrient defisien (rendah NPK) memberikan kesan negatif dan dijangkiti *Ganoderma* dengan teruk. Nutrien N rendah memberi kesan yang paling serius bagi jangkitan akar, pangkal batang dan daun dengan biomas kering terendah. Hubungan positif diperhatikan pada akar kelapa sawit akibat jangkitan *G. boninense* bagi aktiviti enzim; iaitu β-1,3-glucanase PAL, POX, dan chitinase, menunjukkan aktiviti-aktiviti yang lebih tinggi, ($p< 0.5$) mencadangkan peranan mereka berkaitan

mekanisma pertahanan teraruh. Semua aktiviti enzim mengikut kedudukan susunan seperti berikut: T5 > T2 > T10 > T8 > T6. Sebagai kesimpulan, nutrien N dan P yang optima dengan K tinggi pada penggunaan nutrien N, P_2O_5 dan K_2O, pada kadar 17.37, 17.37 dan 41.34 g/tumbuhan, masing-masing mempamerkan pertumbuhan dan perlindungan yang lebih baik untuk penyakit BSR bagi hampir kesemua parameter patologi yang dinilai. Walau bagaimanapun, rawatan N, P dan K telah memberikan perlindungan yang seimbang dengan menunda jangkitan *G. boninense* jangkitan sehingga 6 bulan selepas dijangkiti tetapi selepas tempoh ini, ia seolah-olah kehilangan kesan perlindungan. Keputusan ini berguna untuk membangun strategi nutrisi yang baik dan berkesan untuk mengurang insiden dan keseriusan *Ganoderma* yang ditanam pada tanah gambut.

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I certify that a Thesis Examination Committee has met on Date Month Year to conduct the final examination of Hasmah Binti Mohidin on her thesis entitled "Providing Optimum Nitrogen, Phophorus and Potassium Levels To Oil Palm As A Strategy To Combat *Ganoderma* Disease Infection" \in accordance with the Universities and University College Act 1971 and the Constitution on the Universiti Putra Malaysia [P.U. (A) 106] 15 March 1998. The committee recommends that the student be awarded the Doctor of Philosophy.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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Name of Member of Supervisory Committee: Professor Dr. Mohd Rafii Yusop

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LIST OF ABBREVIATIONS

%	Percent
µg	Microgram
µl	Microliter
AA	Auto Analyzer
Ab	Absorbance
ANOVA	Analysis of Variance
AUDPC	Area under disease progress curve
BSR	Basal Stem Rot
CEC	Cation Exchange Capacity
cm	centimeter
CRD	Completely randomized design
CWDE	Cell wall degrading enzyme
DAB	3,3'-Diaminobenzidine
DAT	Day after treatment
D x P	Dura x Pisifera
DI	Disease incidence
DR	Disease reduction
DSI	Disease severity index
DSIF	Disease severity foliar symptom
DSIB	Disease severity index of bole stem
DSIR	Disease severity index of root
EDTA	Ethylenediaminetetraacetic acid
ER	Epidemic rate
FELDA	Federal Land Development Authority
G. boninense	*Ganoderma boninense*
GSM	*Ganoderma* Selective Medium
h	Hour
H_2O_2	Hydrogen peroxide
ha	Hectare
HCl	Hydrochloric acid
KOH	Potassium hydroxide
L	Litre
M	Molar
MAI	Month after inoculation
MEA	Malt extract agar
min	Minute
mL	Milliliter

mM	Millimolar
MPOB	Malaysia Palm Oil Board
N	Nitrogen
NAG	N-acetylglucosamine
NaOH	Sodium Hydroxide
nm	Nanometre
OD	Optical Density
PAL	Phenylalanine Ammonia - lyase
PDA	Potato Dextrose Agar
PER 71	*Ganoderma boninense*
POX	Peroxidase
PR Proteins	Pathogenesis-related proteins
PVP	Polyvinyl pyrrolidone
RCBD	Randomized complete block design
RWB	Rubber wood block
SAR	Systemic acquired resistance
SFS	Severity Foliar Symptom
s.d	Standard deviation
S.E	Standard error
SPAD	SPAD Chlorophyll value
t	Tonne
UPM	Universiti Putra Malaysia
WAI	Week after inoculation
w/v	Weight per volume
w/w	Weight per weight

CHAPTER 1

INTRODUCTION

1.1 Background Information

Oil palm (*Elaeis guineensis* Jacq.) is a perennial crop that originated from native West Africa and was introduced to Brazil and other tropical countries in the fifteen century by the Portuguese (Corley, 1976). It is planted as a commodity to be used for the purpose of international food, household and industry. Oil palm was introduced to Malaysia, formerly known as Malaya by the British in the year 1870 1980s as an ornamental plant. In 1917 marked as the beginning of the first commercial oil palm establishment in the Malaysian history. Palm oil is fast gaining recognition as it contributes 30 per cent to the world's edible oils and fats production and evidence has shown that palm oil is the best using minimal land to produce any given tonnage of vegetable oil (MPOC, 2015). As a comparison, soybean uses ten times more hectares than oil palm to produce the same tonnage of vegetable oil. Palm oil is highly productive and commercially profitable at large scales, and palm oil demand is rising. Though Malaysia is the second world largest producer of oil palm, it faces a difficult task to maintain the position. Among the limitations are lesser suitable soil for oil palm growing, sustainability and food health issues. However, above all, the basic challenges are to monitor the palm's health and maintain the economic production of the palm which is affected either by poor management practices or pest and disease problems.

For the last four decades, there has been a rapid expansion of the hectares planted with oil palm in Malaysia. Hence, in the recent years, due to shortage of suitable land and limited alluvial soil, there is an increasing trend of oil palm cultivation utilization on tropical peat soils. Tropical peat soil, though a marginal soil, has been viewed as potential medium for oil palm development due to it homogenous soil characteristics, its constant availability of water and its flatness. However, in spite of these positive soil traits, peat soil does have their limitations, too. Tropical peat soil which is clustered under oligotrophic group, is naturally poor in nutrient contents (Andriesse, 1988; Okazaki and Yonebayashi, 1992). There are certain degree of skeptism expressed by certain quarters based on their findings on peat soil fertility status, physical barriers and chemical constraints (Singh et al., 1987). Above all, like other crops, oil palm is susceptible to disease, mainly fungal *Ganoderma* which will pose serious losses in yield production.

Nevertheless, improvement in palm oil production in the world is governed not only by the implementation of new plantations, the regeneration of old plantings, and the availability of high yielding planting materials, but also by good pest and disease control measures to fill the yield gap existing between field trials and plantations (Jalani et al., 2003). Severe attacks by *G. boninense* causing basal stem rot (BSR) disease on oil palm crops resulted in significant yield losses. This disease was first reported by Thompson in the year 1931 when oil palm was first introduced in Malaysia. Recent BSR survey in 2009-2010 reported that BSR in Malaysia was 3.7%

corresponding to 59, 148 ha and about 45.0% of estates in Malaysia (Idris et al., 2011). The estimated yield losses due to BSR disease was RM1.5 billion in Malaysia and RM3.0 billion in Indonesia (MPOB, 2010; Arif et al., 2011). The situation is made even more critical with the active replanting of oil palm in areas with bad history of *Ganoderma* and increasing disease incidence on tropical peat soil.

1.2 Problem Statement

The BSR disease of oil palm caused by white rot basidiomycetous fungus *G. boninense*, is the most destructive disease and caused a serious economic impact on the Malaysian oil palm industry and other parts of the world as the disease tends to become cumulative over successive planting cycles (Susanto, 2009). High incidence of BSR results in economic losses, due to zero yield from dead palm, and significantly reduced weight and number of fruit bunches in infected but living palms (Chong, 2010). The BSR affects oil palms at all ages, from one year old seedlings in the field to advanced old trees and is spreading to greater area yearly (Singh, 1991) especially in tropical peat soils (Rao, 1990; Benjamin and Chee, 1995; Ariffin et al., 1996). The high occurrence of *Ganoderma* disease in peat lands is mostly caused by basidiospore as the dispersal agent, and conditions of the peat are suitable for *Ganoderma* to develop.

Despite the many approaches that have been adopted by many researchers the solution to the *Ganoderma* problem has not been developed. One of the important disciplines is in the area of cultural method through nutrient manipulation. There were some agronomic trials conducted to investigate nitrogen (N), phosphorus (P) and potassium (K) nutrition on oil palm growth in various soil types and *Ganoderma* BSR disease responses (Turner and Poon, 1968; Singh, 1990; Tayeb 1999; Tayeb et al., 2003; Idris et al., 2006). Generally, their results revealed variable and seemingly different outcomes as assessed by visual observation. Fertilizer trials carried out with palms infected by *Ganoderma* by Mohd Tayeb and Hamdan (1999) reported that on peat, recent marine alluvial soil (Bernam series) and inland lateritic soil (Malacca series), N fertilizer increased the incidence of BSR. Phosphorus fertilizer increased BSR in all trials except on the lateritic Malacca series soil. Even on this soil the opposite effect of increased P with reduced BSR incidence was only observed in plots where K fertilizer was applied. Increasing rate of application of K fertilizer significantly decreased BSR incidence of oil palm on peat and the recent marine alluvial soil. In the lateritic Malacca series soil, K fertilizer also reduced BSR incidence but only at higher N and P rates.

Disease control through nutrient manipulation and balanced nutrition is cost-effective and environmentally friendly. There can exist different relationships between nutritional status of plants, growth and disease incidence. As a rule, a 'balanced' nutrient supply ensuring optimum plant growth is also optimal for plant resistance (Huber et al., 2012). If a high nutrient supply stimulates growth and decreases disease incidence, it can be considered as ideal because optimal growth is combined with high resistance. There are cases where nutrient application higher or lower than needed for optimum growth, can result in improved disease resistance. However, in the case of oil palm and BSR diseases, it has not been well studied as to whether the optimum levels

of N, P and K for oil palm growth are also sufficient to enable the oil palm to maximize BSR disease resistance. Hence, this study focused on determining the optimum N, P and K nutrition with the hope that these nutrients could provide early protection and strengthened oil palm natural defense mechanism before challenging them against *Ganoderma* BSR disease.

1.3 Research Objectives

The general objective was to examine the agronomic and nutritional status of oil palm seedlings grown in peat soil with reference to macronutrients nitrogen (N), phosphorus (P) and potassium (K) to see whether their manipulation can reduce BSR disease. The specific objectives of the study were:

1. To determine the optimum levels of N, P and K nutrition for the growth of oil palm seedlings in solution culture (soilless condition)
2. To determine the optimum concentrations of N, P and K nutrition for oil palm seedlings grown on peat soil based on growth traits, biomass accumulation and nutrient uptake in plant tissues.
3. To evaluate the optimum N, P and K nutrition against *Ganoderma* disease incidence and severity based on growth and pathological traits for oil palm seedlings grown under peat soil.
4. To detect and assess nutritional and biochemical traits in relation to *Ganoderma* disease incidence and severity.

The findings provided the knowledge to develop practical approach for early protection of *Ganoderma* infection and the good prospect in the use of N, P and K nutrition to reduce incidence of *Ganoderma* in oil palm in peat soil.

1.4 Outline of the Thesis

Despite many agronomic trials on various macronutrients response of oil palm growth and BSR disease, there are few published information on the effect of N, P and K nutrition on oil palm seedlings grown in peat soil. In Chapter 1, the problem and research objectives were stated. In Chapter 2, information on oil palm, its economic importance in Malaysian scenario, economic impact of BSR disease, causal agent, symptom, epidemiology and spread, current control and management of BSR, nutrient management and their roles on plant growth, nutritional and physiology, characteristics of palm planted in peat soil, the nutritional effects of N, P and K on disease control were reviewed. Chapter 3 presents the methodology and discusses the results of the determination of optimum levels of N, P and K in Hoagland solution culture (soilless condition). In Chapter 4, the methodology was described in view toward advising the incorporation of macronutrients in the oil palm fertilization program with regard to their importance in growth and their potential role in plant defense against pests and diseases. Different concentrations of N, P and K respectively, were tested to identify their optimum concentrations for optimum and improved growth of oil palm seedlings in peat soil medium based on their growth and nutritional parameters. In Chapter 5, the optimum concentrations of N, P and K nutrients identified in chapters 3 and 4 in forms of fertilizers are tested as three levels (low, optimum and high) of N, P, K nutrition

with respective element in mixed fertilizers on healthy and inoculated oil palms seedlings to study their effect on growth, nutritional and pathological parameters in relation to *Ganoderma* incidence and severity and to recommend the overall best treatment in term of growth and reduction of BSR. In Chapter 6, this chapter discusses the nutritional and biochemical analysis of oil palm roots subjected to *Ganoderma* infection and detection of the presence of PR-protein in the oil palm seedlings during the early development of *Ganoderma* infection. Chapter 7 presents the summary of all the results obtained in this study, conclusion, and recommendations for future research.

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