UNIVERSITI PUTRA MALAYSIA

RESPONSE SURFACE METHODOLOGY APPROACH FOR OPTIMIZATION OF GROWTH AND NUTRIENT COMPOSITION OF KENAF (Hibiscus cannabinus L.) CULTIVAR BY LIQUID BIO-FERTILIZER APPLICATION

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FP 2021 66

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By

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Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in fulfilment of the Requirements for the Degree of Master of Science

June 2020

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

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June 2020

Chairman : Tee Tuan Poy, PhD
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Feedstuff is an important input to sustain a livestock production. In Malaysia, animal feed for ruminant livestock, especially forages, are often inadequate in term of nutrients value. Kenaf (*Hibiscus cannabinus* L.) has been viewed as a potential alternative crop in the Malaysian agriculture in recent years for animal feeds. This study was conducted to evaluate the effect of plant maturity, planted density and soil fertility on morphological growth and nutrient composition of kenaf and to determine their optimum values by using RSM (Response Surface Methodology). In order to determine the optimum plant maturity denoted as (X_1), plant density, (X_2), and bio-fertilizer application rate, (X_3), a field experiment was carried out based on Central Composite Design (CCD) that fit a second order polynomial by a least squares technique and developed an equation that was used to describe how these variables effect the responses. The interrelationship among the variables were also determined. Three levels for each factor, X_1 (28 days, 42 days and 56 days), X_2 (100,000 plants/ha, 300,000 plants/ha and 500,000 plants/ha) and X_3 (30 kg N/ha, 60 kg N/ha and 90 kg N/ha) were selected to investigate the influence and interaction between these factors. The results showed that plant maturity had a significant ($P<0.01$) effect on kenaf plant height, yield, ash, OM, CP and ADL. For plant density, there was a significant ($P<0.05$) effect on kenaf plant height with no significant effect on other dependent variables. Whereas, fertilizer rate had significant ($P<0.05$) effect on kenaf plant height, yield and CP with no significant effect on other dependent variables. When optimization model and equations were developed by RSM based on obtained results (plant maturity = 28 days, plant density = 430,000 plants/ha and bio- fertilizer rate = 90 kg N/ha), another field experiment was conducted to validate the model. The validation experiment is vital to demonstrate evidences that a procedure, model and equations carried out in RSM are true within the system. The optimum plant density, plant maturity and bio-fertilizer rate were applied to the growing kenaf during validation experiment to get its optimum morphological growth and nutrient composition values,

which is suitable for ruminant livestock feeds. The effect of 28 days of plant maturity, 430,000 plants/ha of plant density and 90 kg N/ha of bio-fertilizer rate in validation experiment does positively affect the morphological growth (height and number of leaves; except for kenaf yield) and nutrient composition of kenaf (DM and CP; except ash, OM, ADF, NDF and ADL) in actual values when comparing them to predicted values. The results indicate that both bio-fertilizer rate and plant density positively affect kenaf plant growth in term of height, but the plants were more influenced by liquid bio-fertilizer rate even though the plant is still considered young to give any major morphological effect. These values are suitable and more likely to be used for ruminant livestock feed.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

PENDEKATAN METODOLOGI TINDAK BALAS PERMUKAAN UNTUK MENGOPTIMUMKAN PERTUMBUHAN DAN KOMPOSISI NUTRIEN KULTIVAR KENAF (*Hibiscus cannabinus* L.) DENGAN MENGGUNAKAN BAJA BIO CECAIR

Oleh

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Makanan ternakan merupakan input penting untuk mengekalkan pengeluaran ternakan. Di Malaysia, makanan haiwan ternakan ruminan, terutamanya foraj, sering kali tidak mencukupi dari segi nilai kandungan nutrien. Pada kebelakangan ini, kenaf (*Hibiscus cannabinus* L.) dilihat sebagai tanaman alternatif yang berpotensi di dalam bidang pertanian Malaysia sebagai makanan haiwan. Kajian ini dijalankan untuk menilai kesan kematangan tumbuhan, ketumpatan tanaman dan kesuburan tanah untuk pertumbuhan dan komposisi nutrien kenaf organik selain menentukan nilai optimum dengan menggunakan RSM (response surface methodology). Untuk menentukan kadar optimum kematangan tanaman kenaf dirujukkan sebagai (X_1), ketumpatan tumbuhan, (X_2), dan kadar aplikasi baja bio, (X_3), satu eksperimen lapangan perlu dijalankan berdasarkan Central Composite Design (CCD) yang memuatkan urutan kedua polinomial dengan teknik least squares dan menghasilkan rumus yang digunakan untuk menggambarkan bagaimana pembolehubah ini mempengaruhi tindak balasnya. Hubungan antara pembolehubah juga turut ditentukan. Tiga peringkat untuk setiap faktor, X_1 (28 hari, 42 hari dan 56 hari), X_2 (100,000 tanaman/ha, 300,000 tanaman/ha dan 500,000 tanaman/ha) dan X_3 (30 kg N/ha, 60 kg N/ha dan 90 kg N/ha) dipilih untuk mengkaji pengaruh dan interaksi antara faktor-faktor ini. Hasil daripada kajian ini menunjukkan bahawa kematangan tanaman mempunyai pengaruh yang signifikan ($P < 0.01$) terhadap ketinggian tanaman, hasil, abu, OM, CP dan ADL. Kepadatan tanaman juga mempengaruhi ketinggian tanaman kenaf secara signifikan ($P < 0.05$) tanpa mempengaruhi pembolehubah yang lain secara signifikan. Manakala, kadar baja pula mempengaruhi secara signifikan ($P < 0.05$) terhadap ketinggian, hasil tanaman dan CP kenaf tanpa mempengaruhi pemboleh ubah yang lain. Selepas model dan rumus yang optimum dibangunkan oleh RSM berdasarkan hasil yang diperolehi, satu lagi eksperimen lapangan dijalankan bagi mengesahkan model tersebut. Pengesahan eksperimen ini sangat penting bagi membuktikan bahawa prosedur, model dan rumusan yang dibangunkan menggunakan RSM adalah benar mengikut sistem. Kematangan

tumbuhan = 28 hari, kepadatan tumbuhan = 430,000 dan kadar baja bio = 90 kg N /ha menghasilkan hasil yang optimum. Model dan rumusan ini telah digunakan untuk penanaman kenaf semasa pengesahan kajian bagi mendapatkan morfologi pertumbuhan yang optimum dan nilai komposisi nutrien yang sesuai untuk makanan ternakan ruminan. Kesan kematangan tumbuhan selama 28 hari, tanaman pada kepadatan 430,000 pokok/ha dan baja bio dengan kadar 90 kg N/ha dalam eksperimen ini telah mengesahkan secara positif bahawa ianya mempengaruhi pertumbuhan morfologi (ketinggian dan jumlah daun kecuali hasil kenaf) dan komposisi nutrien kenaf (DM dan CP kecuali abu, OM, ADF, NDF dan ADL) dalam nilai yang sebenar dalam tanaman lapangan berbanding nilai yang diramalkan. Hasil daripada kajian ini menunjukkan bahawa kedua-dua kadar baja bio dan kepadatan tanaman mempengaruhi pertumbuhan tanaman kenaf secara positif dari segi ketinggian pokok. Walaubagaimanapun tanaman tersebut lebih dipengaruhi oleh kadar baja bio walaupun tanaman tersebut masih dianggap muda untuk memberikan kesan yang besar terhadap morfologi pokok. Dapatan hasil nilai-nilai ini sesuai dan lebih diutamakan untuk dijadikan sebagai makanan ternakan ruminan.

ACKNOWLEDGEMENTS

I am thankful to Allah S.W.T for giving me the strength and continuous motivation to complete this thesis of master as a requirement. I would like to express my appreciation to my supervisor, Dr. Tee Tuan Poy, and co-supervisor, Prof. Ir. Dr. Chin Nyuk Ling and Dr. Shokri Bin Jusoh for all the advice and valuable guidance throughout the duration of the research.

I also want to express my sincere gratitude to all the staff of Animal Science Department, Faculty of Agriculture for their help and guidance. A grateful dedicates was specially made for my team under same supervisor for the help, cooperation and guidance during the analysis work.

I also love to express my sincere appreciate to my beloved family especially my parents. Finally, I wish to express my deepest appreciation and sincere gratitude to all the people involved directly or indirectly in completing my research.

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

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Declaration by Members of Supervisory Committee

This is to confirm that:

- the research conducted and the writing of this thesis was under our supervision;
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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF APPENDICIES

LIST OF ABBREVIATIONS

ADF	Acid detergent fibre
ADL	Acid detergent lignin
ANOVA	Analysis of variance
°C	Degree Celsius
CCD	Complete composite design
cm	Centimetre
CP	Crude protein
DM	Dry matter
Ha	Hectare
kg	Kilogram
m	Metre
m^2	Metre square
ml	Millilitre
N	Nitrogen
NDF	Nitrogen detergent fibre
NUE	Nitrogen uptake efficiency
OM	Organic matter
r^2	Coefficient of determination
RSM	Response surface methodology
Tg	Total global
%	Percentage

CHAPTER 1

INTRODUCTION

Feedstuff in Malaysia, especially fodder and forage often provides inadequate nutrient for animal production. This problem came from the depletion of the soil nutrients and overgrazing as a result of poor management. Overgrazing is very common in Malaysia, and it leads to fast degradation of the soil and natural pastures. A supply of good quality forage is necessary in order to meet the nutrient requirements of the animal. Napier grass, cassava and leucaena are some example of fodder crops that are mainly given to livestock. Among all, kenaf is another fodder crop that can be used to feed the livestock.

Kenaf (*Hibiscus cannabinus* L.) has been viewed as a potential alternative crop of significance in the Malaysian agriculture of the 21st century (Wong *et al*., 2008). Kenaf is a short seasonal plant and is commonly produced for its fibre. It is also a natural versatile plant which can make product such as paper, textile, insulator and bio composite (Khalil *et al*., 2010). The high nutritional values in early harvested kenaf are what made it suitable as animal feed. The whole kenaf plant has high protein, very good digestibility and may be pelletized (Alexopoulou *et al.,* 2013) for ease of application to livestock.

The nutrient composition of a feed is a detailed set of information on the nutrient components and provide values for energy and nutrients. A good quality feed is a key to a good performance for the animal to be efficient and productive. Achieving optimal production of kenaf fodder can be profitable and give good economic returns to the farmers. Many experimental researches have been done to study the factors affecting nutrient composition of kenaf. However, the experiments were run using conventional multifactor and were very costly and time consuming in getting the results due to a broad range of treatment combinations. The response surface methodology (RSM) has been established to determine the influences of individual factors and their interactions (Mansouri *et al*., 2014) which is more favourable and saves time in getting the results.

RSM could tell the optimum conditions in obtaining the desired responses, as well as the development of mathematical model in explaining the relationship between the experiment variables and its responses (Sindhu *et al*., 2014). In general, it applies an experimental design such as Central Composite Design (CCD) to fit a second order

polynomial by a least squares technique, and an equation is used to describe how the test variables affect the response and determine the interrelationship among the variables (Wu *et al*., 2007).

Fertilizer is used to provide extra nutrients for the plant. Biologically, fertilizer helps in promoting the development of plant growth which directly improves crop production and make farming business profitable. However, optimum quantities of chemical fertilizers at appropriate time will have to be ensured to avoid the devastating of the soil fertility in the long term application (Iqbal *et al*., 2015). Due to the long term of chemical fertilizer utilization, it has caused soil degradation problems by depleting soil organic matter and inducing erosion, "compacting" subsequently makes it poor in soil fertility. It was obviously found on current farming issues (soil depleting) after the implementation of chemical fertilizer over 50 years. Hence, the concept of replenishing soil fertility by using organic fertilizer, compost, bio-fertilizer, or practicing mulching system has been recommended. Especially the organic farming system, which utilized the abundance of agriculture by-products and animal manure availability from intensive farming practices.

In quest of producing more food with over increasing population, the use of chemical fertilizers at extensive rate has significantly destroyed the soil fertility (Jan and Boswal, 2015). The impact of soil fertility depletion on organic matter, humus and others can be improved by using bio-fertilizer. In previous study reported by Mondal *et al*. (2015), application of bio-fertilizer and compost soil can significantly improves the soil fertility in terms of soil macronutrient status as well as soil health in terms of increased microbial population.

Organic farming system is getting increased attention and is a safe approach to handle these problems. The organic farming system is based on diverse biological development and replenishment of soil productivity (Vinitha and Swapna, 2015). The living microbes in the soil contribute to the soil fertility on a sustained basis by decomposing the organic matters and nutrients. Bio-fertilizer was found to be the best alternative to maintain soil health with comparative results. Application of bio-fertilizer is accompanied by reducing pollution in the surrounding and are safe for human, animal and environment (Shaheen *et al*., 2013). Bio-fertilizer in liquid or solid form can be produced by agricultural by- products through composting process and can be a good alternative to substitute mineral fertilizer in order to maintain soil fertility and texture.

However, since the data on growth and nutrient composition of kenaf with bio-fertilizer application is still minimal, the objective of this research is to use RSM as an approach to optimize the independent variables which includes plant maturity, plant density and

fertilizer rate, particularly the utilization of bio-fertilizer and optimizing the cultivation process parameters of kenaf in terms of growth performance (height, number of leaves and yield) and its nutrient composition (dry matter, ash, organic matter, crude protein and fibers) based on the CCD experiments. In this study, liquid bio-fertilizer from Lim *et al*., (2016) was used which resulted from composting food waste and cattle manure with the composition of 1.92% nitrogen (N), 38.08% carbon (C), and pH of 7.83.

1.1 Problem statement

There is still lack of research in RSM application to optimize the effect of bio-fertilizer on growth and nutritive values of kenaf as fodder for livestock. It is important to determine the optimum plant maturity, plant density and fertilizer rate, particularly the utilization of bio-fertilizer that affect the growth and nutrient composition of organic kenaf cultivar by using RSM.

1.2 Research hypothesis

The use of bio-fertilizer may improve growth and nutrient composition of kenaf. The optimization of RSM application may provide the optimal interactions in plant maturity, plant density and liquid bio-fertilizer rate that significantly affect the growth and nutrient composition of kenaf.

1.3 General Objective

The general objective of this experiment is to evaluate the approach of RSM optimization on kenaf for the effect and relationship between plant maturity, plant density and liquid bio-fertilizer application on growth and nutrient composition of kenaf.

1.4 Specific objectives

1) To determine the RSM optimization effect on growth performance of kenaf cultivar by using liquid bio-fertilizer application.

2) To determine the RSM optimization effect on nutrient composition of kenaf cultivar by using liquid bio-fertilizer application.

3) To determine the optimum plant maturity, plant density and liquid bio-fertilizer application that affect the growth and nutrient composition of kenaf cultivar as fodder for livestock by using RSM optimization.

1.5 Significance of study

Desired production and good forage quality can be achieved if the optimization model of organic kenaf has been developed. This study aims at improving the use of RSM to optimize kenaf production as fodder. This research will give new information about the relationship between plant maturity, plant density and bio-fertilizer rate, particularly the utilization of bio-fertilizer and its optimization on growth performance and nutrient composition of kenaf.

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