UNIVERSITI PUTRA MALAYSIA

GROWTH AND QUALITY ENHANCEMENT OF YOUNG Labisia pumila BENTH. AND HOOK. F. VARIETIES UNDER ORGANIC AND INORGANIC FERTILISATION

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By

NURRUL AKMAR BINTI ROSNI

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

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DEDICATION

I dedicate this thesis to ALLAH for the guidance, strength and power of mind to complete this thesis.

I also would like to dedicate to my parents, Rosni bin Tumin and Ramlah bt Bidin, whose unconditional love, support, and encouragement have enriched my soul and inspired me to pursue and complete this research.

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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Chairman : Assoc. Prof. Hawa binti Jaafar, PhD
Faculty : Agriculture

The application of organic and inorganic fertiliser to the plant is considered as a good agricultural practice because it improves the plant growth and its quality. A study was conducted with the objectives: (i) to determine growth and quality of different varieties of *Labisia pumila* with different sources of fertilisers at 90 kg N/ha; (ii) to determine the best rate of organic and inorganic fertiliser in enhancing growth and secondary metabolites production of *L. pumila* and (iii) to determine and characterize phytochemical quality of *L. pumila* leaves extract as the result of fertilizer application. There were two experiments conducted to fulfil the objectives. The treatments were designed in a randomized complete block design (RCBD).

In the first experiment, two varieties of *L. pumila* (*alata* and *pumila*) and four different sources of fertiliser at 90 kg N/ha (chicken manure, Gobi, NPK green and control) were used to determine the optimum growth and quality of *L. pumila*. There were higher interactions among all factors recorded on total biomass and photosynthesis activity on organic fertilisers ($p \leq 0.05$). Chicken manure and Gobi were statistically different on leaf gas exchange ($p \leq 0.01$) and secondary metabolites ($p \leq 0.05$) of *L. pumila.* The experimental results of this study have shown that the Gobi and chicken manure produced a higher effect leaf gas exchange and secondary metabolites of on *L. pumila* varieties compared to NPK green. High amount of secondary metabolites (total phenolic and flavonoid) and leaf gas exchange recorded give preference to the use of organic than inorganic fertiliser.

In the last experiment, four N rates (0, 30, 60 and 90 kg N/ha) and two types of fertiliser (Gobi and NPK green) were used to determine growth and quality of *L. pumila* var *alata* at different harvesting stages. The effect of the main interaction among all factors was particularly on nutrient content ($p \leq 0.01$) and secondary metabolites ($p \leq 0.01$). The NPK

green at 90 kg N/ha at 15 WAT had higher N content (1.24%) and at 60 kg N/ha at 10 WAT recorded higher nitrate content (3.715 mg/l). Gobi at 90 kg N/ha showed the higher secondary metabolites at 10 WAT. Thus, the phytochemical content of Gobi from the above treatment was determined quantitatively using TLC analysis with quercetin (88532 a.u), kaempferol (61226 a.u), gallic acid (84559.5 a.u), cinnamic acid (43828.5 a.u) and ferulic acid (63336 a.u) found abundant in plant extracts by using qTLC appweb. Gobi at 90 kg N/ha is a good resource on leaf gas exchange, secondary metabolites and the compositions in TLC analysis of *L. pumila* as compared to NPK green. Harvesting *L. pumila* at age from 26 to 31 weeks showed significant production of secondary metabolites probably due to plants were saturated with nitrogen.

Both experiments have proven that organic fertilisers can produce higher quality of *L. pumila* compared to NPK green when harvested at 15 weeks after transplanting. The availability of N due to the slower release of N from organic fertiliser during the growing season and through additional soil N availability may affect the photosynthesis of plant by altering the synthesis of secondary compounds by applying suitable complete fertiliser sources. The carbon surplus, which was not utilized for growth due to the slow release of nutrients from the organic fertiliser, was allocated to the production of secondary metabolites, in particular phenolics. This followed the principle of growth-differentiation balance (GDB) hypothesis that states an imbalance in carbon and nutrient will allow plant to excess resource for secondary metabolites production.

Keywords: *Labisia pumila*, slow-release fertiliser, organic fertiliser, Gobi, secondary metabolites

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

PEMBAJAAN SECARA ORGANIK DAN SINTETIK MEMPENGARUHI PERTUMBUHAN DAN KUALITI VARIETI ANAK POKOK *LABISIA PUMILA* BENTH. & HOOK. F.

Oleh

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.

Penggunaan baja organik dan baja sintetik untuk tumbuhan dianggap sebagai amalan pertanian yang baik kerana ia meningkatkan pertumbuhan tumbuhan dan kualiti tumbuhan. Satu kajian telah dijalankan dengan objektif: (i) menentukan pertumbuhan dan kualiti varieti pokok *L. Pumila* dengan menggunakan sumber baja yang berbeza pada kadar 90 kg N/ha; (ii) untuk menentukan kadar baja organik dan baja sintetitk yang optimum untuk pertumbuhan *L. pumila* dan pengeluaran metabolit sekunder dan (iii) untuk menentukan kualiti dan klasifikasikan komponen sebatian kimia yang diekstrak daripada daun *L. pumila*. Terdapat dua eksperimen yang dijalankan untuk memenuhi objektif. Experimen disusun atur mengikut reka bentuk blok lengkap rawak (RCBD).

Eksperimen pertama dijalankan dengan menggunakan dua jenis varieti *L. pumila* (*alata* dan *pumila*) dan empat sumber baja nitrogen yang berbeza pada kadar 90 kg N/ha (baja tahi ayam, Gobi, NPK hijau dan kawalan) telah dijalankan untuk menentukan pertumbuhan optimum dan kualiti pokok *L. pumila*. Keadaan saling bertindak balas terjadi pada semua faktor dapat dicatatkan pada jumlah berat kering dan kadar fotosintesis pada pokok *L. pumila* ($p \leq 0.05$). Baja tahi ayam dan Gobi memberi impak yang lebih tinggi pada data pertukaran gas daun ($p \leq 0.01$) dan metabolit sekunder ($p \leq 0.05$). berbanding NPK hijau. Hasil kajian daripada experimen ini, menunjukkan bahawa Gobi dan baja tahi ayam menghasilkan kesan yang lebih signifikan terhadap varieti pokok *L. pumila* berbanding NPK hijau. Kesimpulannya, jumlah kandungan metabolit sekunder dan pertukaran gas daun yang lebih tinggi menunjukkan bahawa penggunaan baja organik adalah lebih baik daripada baja sintetik.

Pada eksperimen terakhir, kadar baja nitrogen digunakan sebanyak 4 peringkat (0, 30, 60 dan 90 kg N / ha) dan dua jenis baja nitrogen (Gobi dan NPK hijau) digunakan untuk membentuk mekanisme pertumbuhan dan kualiti pokok di bawah kadar N yang berbeza dan jenis baja N yang berbeza pada peringkat penuaian yang berbeza. Hubungkait

kesemua faktor-faktor telah dipengaruhi oleh kandungan nutrien dan metabolit sekunder. Baja NPK hijau pada kadar 90 kg N / ha yang dituai pada minggu 15 selepas tanam(1.24%) dan kandungan nitrat pada 60 kg N / ha yang dituai pada minggu 10 selepas tanam (3.715 mg / l) mencatatkan pengumpulan kandungan nutrien yang lebih tinggi Manakala Gobi pada kadar 90 kg N/ha menunjukkan metabolit sekunder yang lebih tinggi pada minggu ke 10 selepas ditanam. Seterusnya, analisis melalui TLC menunjukkan Gobi memberi impak kepada tingginya kandungan quercetin (88532 a.u), kaempefrol (61226 a.u), asid gallic (84559.5 a.u), asid cinnamic (43828.5 a.u) dan asid ferrulic (63336 a.u) dengan menggunakan appweb qTLC yang diekstrak di dalam daun berbanding NPK hijau. Penuaian *L. pumila* pada umur 26 hingga 31 minggu menunjukkan pengeluaran metabolit sekunder yang ketara disebabkan oleh ketepuan kandungan N di dalam tumbuhan.

Kedua-dua eksperimen telah membuktikan bahawa baja organik dapat menghasilkan kualiti pokok *L. pumila* yang lebih baik berbanding dengan NPK hijau**.** Ketersediaan N disebabkan oleh pelepasan N secara perlahan daripada baja organik semasa pertumbuhan pokok iaitu melalui ketersediaan tambahan N pada tanah boleh menyebabkan kesan daripada proses fotosintesis untuk mengubah sintesis metabolit sekunder dengan menggunakan sumber baja lengkap yang sesuai. Lebihan karbon, yang tidak digunakan untuk pertumbuhan kerana pelepasan nutrien yang perlahan dari baja organik, diperuntukkan kepada pengeluaran metabolit sekunder, khususnya fenolik. Ini mengikuti prinsip hipotesis keseimbangan pertumbuhan (GDB) bahawa ketidakseimbangan karbon dan nutrien akan membolehkan tumbuhan menggunakan lebihan karbon dalam pengeluaran metabolit sekunder.

Kata kunci: *Labisia pumila*, baja pelepasan perlahan, baja organic, Gobi, metabolit sekunder

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I certify that a Thesis Examination Committee has met on 4 October 2019 to conduct the final examination of Nurrul Akmar binti Rosni on her thesis entitled growth and quality enhancement of *young Labisia pumila* varieties under organic and inorganic fertilization in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Crop Production and Physiology.

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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 Master of Science. The members of the Supervisory Committee were as follows:

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%	percent
µmol	micro mole
µmol/m^2/s	micro mole per meter square per second
µmol/mol	micro mole carbon dioxide per mole air
^oC	degree – celcius
A	photosynthesis rates
AAS	atomic adsorption spectroscopy
$AlCl_3$	aluminium chloride
$Al_2 (SO_4)_3$	aluminium sulfate
ANOVA	analysis of variance
a.u.	arbitrary unit
C	carbon
Ca	calcium
CC	chlorophyll content
cm	centimeter
CM	chicken manure
cm^2	centimeter square
CNBH	carbon nutrient balance hypothesis
E	evapotranspiration
EPP1	Entry Point Project number one
F	fertiliser
g	gram
GAE	gallic acid equivalent
GDB	growth differentiation balance
GDBH	growth differentiation balance hyphotesis
GNI	Gross National Income
g/plant	gram per plant
g_s	stomatal conductance
H	harvest
H_2O_2	hydrogen peroxide
H_2SO_4	acid sulphuric
HCl	hydrochloric acid

HITF	harvest index total flavonoid
HITP	harvest index total phenolic
HPLC	high performance layer chromatography
K	potassium
KF	Kacip Fatimah
kg	kilogram
kg/N/ha	kilogram nitrogen per hectares
LA	leaf area
m	meter
mg	magnesium
mL	millilitre
$mol/m^2/s$	mole per meter square per second
N	nitrogen
n	number of samples
nm	nanometer
n.s	not significant
NaOH	sodium hydroxide
$NaNO_3$	sodium nitrate
NH_4^+	ammonium
NO^-_3	nitrate
NO_3-N	nitrate
P	phosphorus
PH	plant height
PPFD	photosynthesis photon flux density
qTLC	quantitative thin layer chromatography
TB	total biomass
TF	total flavonoid
TLC	thin layer chromatography
TP	total phenolic
V	variety

CHAPTER 1

INTRODUCTION

Labisia pumila (Blume) Fern – Vill., is a herbal plant namely known as *Kacip Fatimah*, Rumput *Siti Fatimah, Selusoh Fatimah*, *Akar Kacip Fatimah*, *Pokok Pinggang* and *Bunga Belangkas Hutan* (Jamia *et al*. 2003) and also named as 'queen of herbs' in Malaysia. This plant is growing well in the natural forest under 70% to 90% shade and known as shade-loving plants (Ibrahim and Jaafar, 2011a). It is also known as a high natural aphrodisiac potential plant in Malaysia, especially for women. *Labisia pumila* is a part of a small genus of the slightly woody plant (Karimi *et al*., 2011; Ibrahim and Jaafar, 2012). Kacip Fatimah extract has been used traditionally by women to maintain a healthy female reproductive system, help tighten and lubricate the vagina (Zakaria and Mohd, 1994; Bodeker, 1999; Ibrahim *et al*., 2014) and to tone the abdominal muscles and to regain body strength (Wan Ezumi *et al*., 2007). It is also a source of specific bioactive molecules, which act as antioxidants that are important in promoting health, primarily for women use and protection from coronary disease and cancer (Karimi *et al*., 2011; Ibrahim *et al*., 2011b). These phytochemicals are known to be involved in many medicinal roles in the human body.

Labisia pumila is one of the commercial herbal plants in Malaysia. Lately, *L. pumila* has received considerable interest because of its antioxidants and protection traits from anti-carcinogenic activity (Pihie *et al*., 2011; Ibrahim *et al*., 2012). *Labisia pumila* was identified as most of the top five medicinal plants in Malaysia under the Entry Point Project number one (EPP1) of the National Key Economic Area for the Ministry of Agriculture and Agrobased Industry, to be developed into a high value product for international market with the proposed Gross National Income (GNI) totaling to RM2.2 billion by 2020 (PEMANDU, 2010). This herb is relatively very slow-growing, and it usually takes about 16 to 36 months under forest conditions for the plant to be ready for use in the local pharmaceutical industry. It shows that there is a need to increase the growth of this unique herb to meet the demand of our domestic industry. One of the possible ways to enhance the growth of *Labisia pumila* and possibly its bioactive metabolite accumulation is to manipulate the fertilisation management, especially nitrogen sources of both inorganic and organic fertilisers. Therefore, a project on two common *L. pumila* varieties (*alata* and *pumila*) was fertilised with organic and inorganic fertilisers in the soilless media in the glasshouse. It is possible to achieve high production of secondary metabolite within in a short period of cultivation under glasshouse compared to the field (Radusiene *et al*., 2012)

Nitrogen is the crucially mineral element in fertilisation programs because plants typically need N in a more significant amount than another mineral nutrient for growth and development. It applied in the form of organic or inorganic sources. Sufficient nitrogen supply is often the primary constraint for most plants and often used to increase crop yields. Even though nitrogen fertilisation was documented to improve plant growth

(Golcz *et al*., 2006; Sifola and Barbieri, 2006), its influence to enhance secondary metabolite is still inconclusive. Decades of research proved that secondary metabolites are an indicator of plant N status as nitrogen fertilisation can increase secondary metabolites production in herbal plants (Babalar *et al*., 2010; Ibrahim *et al*., 2014). However, results are inconclusive because results also showed at some rates of nitrogen application, secondary metabolites decrease (Ibrahim *et al*., 2011; Selmar and Kleinwachter, 2013). It also has been reported some fertiliser treatments have been documented to cause increased in the total phenolic compound (Asami *et al*., 2003; Zheng *et al*., 2006) and some results were observed total phenolic to be decreased by fertiliser treatment (Khalil *et al*., 2007; Grevesen *et al*., 2008). Hence, there is a need for further on N sources and fertiliser type examination.

The recommended N fertilisation for production of *L. pumila* was at 90 kg N/ha from urea fertiliser can be increased total phenolic and flavonoid production of this herb (Ibrahim et al., 2011). Therefore, a study was conducted to examine the effect of fertilisation program of two common varieties of *L. pumila* (*alata* and *pumila*) using different sources of organic and inorganic fertilisers. Hence, two various sources of organic were used to supply nitrogen namely from an animal (chicken manure) and plant-based (Gobi). Chicken manure is the regular practice fertiliser applied in cultivation and Gobi is a more stable organic fertiliser. All components in herbal plants fresh, dried or processed for diagnosis, treatment, prevention, helping to aid the health of humans or animals and other physiological functions of the plant used process (Sartip *et al*., 2015). Thus, the important role of herbal plants in different industries, it is vital to increase the production of total biomass produced (Sartip *et al*., 2015). The use of organic fertiliser is the improving and competent practise of herbal cultivation.

Meanwhile, inorganic fertiliser also used as the nitrogen source, namely from compound fertilisers (NPK green). Commonly, NPK green applied in the leafy herbal plant. The main objective of the study was to examine how nitrogen from different sources of organic and inorganic fertilisers can influence plant growth and secondary metabolites production of *Labisia pumila.* The main objective of this study was to observe the influence of nitrogen fertilisation from both organic and inorganic fertiliser sources on plant growth and secondary metabolites of *L. pumila*. The specific objectives in this study were: 1) to determine growth and quality of different varieties of *L. pumila* under different sources of organic and inorganic fertilisers; 2) to determine the best rate of organic and inorganic fertiliser on *L. pumila* growth and secondary metabolites production; and 3) to identify the compositions of secondary metabolites (total phenolics and total flavonoid) in *L. pumila* under the best fertiliser practice obtained.

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LIST OF PUBLICATION

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