



UNIVERSITI PUTRA MALAYSIA

**EFFECTS OF EFB BIOCHAR ON GROWTH AND PHYTOCHEMICAL
CONTENT OF *Labisia pumila* Benth**

SITI NORAYU BINTI OMAR BAKI

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By

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**Thesis Submitted to the School of Graduated Studies, Universiti Putra
Malaysia, in Fulfillment of the Requirement for the Degree of
Master of Science**

December 2017

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

EFFECTS OF EFB BIOCHAR ON GROWTH AND PHYTOCHEMICAL CONTENT OF *Labisia pumila* Benth.

By

SITI NORAYU BTE OMAR BAKI

December 2017

Chairman : Associate Prof. Hawa ZE Jaafar, PhD
Faculty : Agriculture

Kacip Fatimah (*Labisia pumila*) is reputed to have great medical value for Malay women in Malaysia. As the national herbal industry, the demand is increasing rapidly, however, the supply of raw materials from the natural source is getting scarce, unreliable and unsustainable. Cultivation of *L. pumila* has many constraints especially of poor root development and sensitivity to the microenvironment. Biochar has been recognized to improve the rate of plant growth via media tilth and nutrient availability, thus may improve plant productivity. However, very few cases of biochar have been reported on medicinal plants. Since biochar has been recognized to improve plant growth and possibly quality of the herb, it will be useful to investigate the effect of EFB biochar mix with growing media of *L. pumila* in enhancing both the growth and quality of *L. pumila*, especially in terms of the phytochemical content. Therefore, two studies were conducted with the objectives, (i) to study the effect of different rates of EFB biochar on the growth and development of three varieties of *L. pumila* Benth. and (ii) to identify and classify the phytochemical compounds present in *L. pumila* varieties affected by the EFB biochar amendment. Prior to study, soilless media mixed with EFB biochar was characterized based on chemical properties. Result showed the pH, CEC, moisture content, water holding capacity of the soilless media of *L. pumila* were increased when added with EFB biochar with the following ranges: pH (5.6 to 6.1), CEC (64.2 to 72.2 cmol (+) kg⁻¹, moisture content (25.3% to 29.2%) and water holding capacity (40.5% to 43.8%). The bigger BET surface area and pore volume were found as 45.76 m²g⁻¹ and 0.008 ccg⁻¹, respectively. In first study, four rates of biochar (0%, 3%, 6% and 9% (w/w)) were applied to the three varieties of *L. pumila* (var *pumila*, var *alata* and var *lanceolata*) and data on *L. pumila* growth was collected in four harvesting times (30, 60, 90 and 120 days of treatment). Generally, plant applied with EFB biochar exhibited significant difference in leaf area, chlorophyll content (TCC), root surface area, total dry biomass (TDB), relative growth rate (RGR) and leaf macro-nutrient content. All these growth parameters except total leaf area showed that *L. pumila* plants treated with 3% and 6% shown no significant different. Meanwhile, total leaf area of *L. pumila* treated

with 6% of EFB biochar gained higher value compared to other treatments. The result indicates that growth parameters for *L. pumila* reduced significantly with the highest rate 9% of EFB biochar. *Labisia pumila* var *pumila* and var *lanceolata* showed higher improvement compared to var *alata*. The growth parameters showed higher values at 120 days after treatment. The result showed that the application of EFB biochar at rate 3% and 6% gave significantly higher growth of *L. pumila* compared to 9% of EFB biochar. In second study, one rate of EFB biochar 6% w/w was chosen and 0% (without biochar application) w/w to investigate the enhancement of secondary metabolites production of *L. pumila*. In study 2, an experiment of 2 rates of EFB biochar vs 3 varieties of *L. pumila* vs 3-time harvest has been conducted. The two rates of biochar (0% and 6% w/w) were applied to the three varieties of *L. pumila* (var *pumila*, var *alata* and var *lanceolata*) and the data has been taken in three harvesting times (40, 80 and 120 days of treatment). From the result showed that total phenolics (TP) content in 120 days treatment did not change in all the combined treatment except for var *lanceolata*. Meanwhile, total flavonoid (TF) content of *L. pumila* seedlings treated with 6% of EFB biochar had lower value compared to untreated plants. The highest production of TF content was obtained in var *alata* at 80 days after treatment when no EFB biochar applied. The secondary metabolites harvest index of *L. pumila* in non-treated plants and plants treated with 6% of EFB biochar were higher at 40 days of treatment. By using thin layer chromatography (TLC) method to the three varieties of *L. pumila* showed the presence of gallic acid, ferulic acid and caffeic acid in phenolic group, meanwhile in the flavonoid group presence of quercetin and kaempferol. The result from this study indicated the potential of EFB biochar in improving the *L. pumila* growth with the optimum application rate of 6% EFB biochar. From the research findings, it can be concluded that the application of EFB biochar as soilless media amendment can enhanced the growth of *L. pumila* plants, however there we no significant in the production of secondary metabolite on treated plants, specifically, total phenolics and total flavonoid content.

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

KESAN BIOCAR EFB TERHADAP PERTUMBUHAN DAN KANDUNGAN FITOKIMIA *Labisia pumila* Benth.

Oleh

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Labisia pumila (Kacip Fatimah) mempunyai nilai perubatan yang sangat bagus dikalangan perempuan Melayu di Malaysia. Industri tanaman herba mempunyai permintaan yang tinggi tetapi pengeluaran bahan mentah dari sumber hutan amat sedikit dan tidak mencukupi. Penanaman Kacip Fatimah mempunyai pelbagai halangan seperti pertumbuhan akar yang perlahan dan amat sensitif terhadap keadaan persekitaran mikro. Biocar telah dikenalpasti boleh meningkatkan pertumbuhan tanaman dengan menyuburkan media penanaman, keberadaan zat makanan dalam media penanaman seterusnya meningkatkan pengeluaran tanaman. Walaubagaimanapun, hanya sedikit kajian yang dijalankan mengenai kesan biocar pada tanaman ubatan. Disebabkan biocar telah dikenali dapat meningkatkan pertumbuhan tanaman dan kemungkinan juga kualiti herba, ia akan memberi manfaat untuk mengkaji kesan biocar EFB yang dicampurkan dengan media tanaman Kacip Fatimah dalam meningkatkan pertumbuhan dan kualiti Kacip Fatimah terutama kandungan fitokimia. Disebabkan itu, satu penyelidikan telah dijalankan dengan dua objektif; (i) untuk mengkaji kesan pelbagai kadar biocar EFB pada pertumbuhan tiga variasi Kacip Fatimah (ii) untuk mengenalpasti kandungan fitokimia pada tiga variasi Kacip Fatimah kesan daripada penambahan biocar EFB. Sebelum kajian dijalankan, media tanpa tanah dicampurkan bersama biocar EFB dan dianalisis dari segi sifat kemikal. Keputusan menunjukkan pH, kapasiti pertukaran kation, kandungan kelembapan, kapasiti memegang air bagi media penanaman Kacip Fatimah meningkat setelah ditambah dengan biocar EFB dengan nilai pH (5.6 to 6.1), kapasiti pertukaran kation (64.2 to 72.2 cmol (+) kg⁻¹, kandungan kelembapan (25.3% to 29.2%), kapasiti memegang air (40.5% to 43.8%). Luas permukaan BET dan isipadu pori bagi biocar adalah 45.76 m²g⁻¹ dan 0.008 ccg⁻¹. Dalam eksperimen yang pertama, 4 kadar biocar EFB (0%, 3%, 6% dan 9% (g/g)) diletakkan pada 3 variasi Kacip Fatimah (var *pumila*, var *alata* dan var *lanceolata*) dan data pertumbuhan Kacip Fatimah diambil pada 4 masa menuai (30, 60, 90 dan 120 hari rawatan). Secara umumnya, pokok Kacip Fatimah yang ditambah dengan biocar EFB menunjukkan perubahan pada luas permukaan daun, kandungan klorofil, luas permukaan akar, jumlah berat kering, kadar pertumbuhan

relatif dan kandungan zat makro. Semua parameter pertumbuhan Kacip Fatimah ini kecuali luas permukaan daun menunjukkan tiada perbezaan pada pertumbuhan pokok yang dirawat dengan biocar EFB pada kadar 3% dan 6%. Manakala, luas permukaan daun Kacip Fatimah yang dirawat dengan biocar EFB pada kadar 6% menunjukkan nilai lebih tinggi berbanding kadar rawatan yang lain. Hasil daripada kajian ini menunjukkan pertumbuhan Kacip Fatimah menurun apabila dirawat dengan biocar EFB pada kadar paling tinggi iaitu 9%. Kacip Fatimah variasi *pumila* dan variasi *lanceolata* menunjukkan perubahan yang lebih tinggi dari variasi *alata*. Parameter pertumbuhan paling tinggi ialah pada hari ke-120 selepas rawatan. Hasil kajian menunjukkan penambahan biochar EFB pada kadar 3% dan 6% memberi pertumbuhan Kacip Fatimah yang lebih tinggi berbanding 9% biocar EFB. Dalam eksperimen kedua, satu kadar biocar EFB 6% (g/g) telah dipilih dan satu kadar kawalan 0% (g/g) untuk mengkaji penambahbaikan pada penghasilan metabolit sekunder pada pokok Kacip Fatimah. Eksperimen ini terdiri daripada 2 kadar biocar EFB vs 3 variasi Kacip Fatimah vs 3 masa menuai telah dijalankan. Dua kadar biocar EFB (0% dan 6 % (g/g)) diletakkan pada 3 variasi Kacip Fatimah (var *pumila*, var *alata* dan var *lanceolata*) dan data diambil pada 3 masa menuai (40, 80 dan 120 hari rawatan). Keputusan menunjukkan kandungan fenolik tidak berubah pada semua kombinasi rawatan kecuali pada pokok variasi *lanceolata*. Manakala, kandungan flavonoid dalam pokok Kacip Fatimah yang dirawat dengan biocar EFB dengan kadar 6% mempunyai nilai yang lebih rendah daripada pokok Kacip Fatimah yang tidak dirawat dengan biocar EFB. Penghasilan kandungan flavonoid paling tinggi didapati dalam pokok Kacip Fatimah variasi *alata* yang tidak dirawat pada hari ke-80. Index penuaian metabolit sekunder paling tinggi didapati pada hari ke-40 pada kedua-dua pokok Kacip Fatimah yang tidak dirawat dan pokok yang dirawat dengan biocar EFB 6%. Dengan menggunakan kaedah lapisan kromatografi nipis ke atas tiga variasi Kacip Fatimah juga menunjukkan kewujudan beberapa kandungan fitokimia seperti acid galik, acid ferulik dan acid kafik iaitu dalam kumpulan fenolik. Manakala dalam kumpulan flavonoid terdapat kandungan kuersetin dan kaemferol. Hasil daripada kajian menunjukkan potensi biocar EFB dalam meningkatkan pertumbuhan tanaman Kacip Fatimah dengan kadar optima 6%. Hasil daripada penemuan kajian ini, dapat disimpulkan bahawa penggunaan biocar EFB sebagai pembaikpulih media tanpa tanah boleh meningkatkan pertumbuhan tanaman Kacip Fatimah, walaubagaimanapun, tiada peningkatan pada penghasilan metabolit sekunder pada pokok yang dirawat dengan biocar, secara spesifiknya pada kandungan fenolik dan flavonoid.

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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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LIST OF ABBREVIATIONS

EFB	empty fruit bunch
FFB	fresh fruit bunch
AA	auto-analyzer
AAS	atomic absorption spectrophotometer
SEM	scanning electron microscope
RCBD	randomized complete block design
SOM	soil organic matter
FTIR	fourier transform infra-red spectroscopy
BET	brunnauer emmet and teller
C/N	carbon and nitrogen ratio
WHC	water holding capacity
ANOVA	analysis of variance
LSD	least significant different
GAE	gallic acid equivalent

CHAPTER 1

INTRODUCTION

Labisia pumila commonly known as Kacip Fatimah has been identified in the Economic Transformation Program (ETP) under the National Key Economic Areas (NKEA) as one of the top five important herbs to be developed into high value herbal products. There are three varieties namely *pumila*, *alata* and *lanceolata* (Jaafar *et al.*, 2008) commonly found in Peninsular Malaysia and each of them contains a different composition of flavonoids and phenolics. In flavonoids group *L. pumila* contains apigenin, kaempferol, rutin and myricetin. For phenol group *L. pumila* contains gallic acid, pyrogallol and caffeic acid. This phytochemical content gives different medicinal use. Traditionally, the decoction of this plant is consumed by Malay women to facilitate child birth delivery and to improve their post-partum health. Men also can consume this herb to maintain and increase stamina (Norhanisah *et al.*, 2013).

Mostly, *L. pumila* plant is harvested from its natural habitat in tropical forest. The plant propagates from its seed in natural habitat. The dependence on seeds for propagation is inappropriate since the seeds are extremely scarce. With increasing demand, the supply of the plant from natural habitat is getting limited. Owing to the high demand and relatively slow growth rate of the plant, several researchers have studied new propagation techniques to grow the plant in larger scale and in shorter time. Therefore, *L. pumila* has been propagated through the leaf, stem and petiole cuttings. As another alternative to grow *L. pumila* as similar to their habitat, Rozihawati *et al.* (2003) reported the significantly satisfying result in cultivating the herb either in sand, sawdust, mixture of sand and sawdust media, mixture of cocoa peat and burnt rice husk with and without the supply of hormone treatment by using the cut leaf, petiole or stem from the plant. These cuttings were placed in the soilless media of cocoa peat, burnt rice husk and chicken dung. The cultivation effort still cannot produce this herb in commercial scale for local demand because the growth rate of this herb is relatively slow due to poor rooting environment.

Suitable planting media should have high a pore size to provide adequate aeration and moisture. Biochar has been recognized as a growing media amendment due to its potential to improve media physico-chemical properties. Biochar is formed through pyrolysis process whereas biomass is heated at the low temperature with the absence or limited supply of oxygen and simultaneously produces syngas and bio-oil. Biochar has been found to improve media structure, enhance nutrient retention and availability, and enhance the crop productivity (Yamato *et al.*, 2006; Asai *et al.*, 2009; Abebe *et al.*, 2012). The highly porous structure with alkaline properties and high surface area are believed to be potentially influential on planting media properties such as media pH, porosity and water holding capacity (Glaser *et al.*, 2002; Chan *et al.*, 2007). Biochar also enable the improvement of media fertility with the presence of high surface charge density that retains cations

(Liang *et al.*, 2006) and reduce the leaching nutrients (Steiner *et al.*, 2007; Novak *et al.*, 2009). Biochar also could directly provide nutrient since biochar itself contains several nutrient compositions.

There was many research of the effect of biochar on plant growth, but with different feedstock and pyrolysis condition, the result may be varied. In Malaysia, biomasses from oil palm empty fruit bunches (EFB) were used as biochar feedstock. However, there is no data on EFB biochar application to improve the growth performance and simultaneously increase the phytochemical content of *L. pumila* plant. Therefore, greater evaluation on the potential of EFB biochar in enhancing the growth performance and phytochemical content of *L. pumila* plant is required. Thus, the overall objective of this study was to study the effects of EFB biochar on growth and quality enhancement of three varieties of *L. pumila* (var *pumila*, *alata* and *lanceolata*). In order to achieve this main aim, two specific objectives were carried out, (i) to study the effect of different rates of EFB biochar on the growth and development of three varieties of *L. pumila* Benth. and (ii) to identify and classify the phytochemical compounds present in *L. pumila* varieties affected by the EFB biochar amendment.

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LIST OF PUBLICATIONS

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