UNIVERSITI PUTRA MALAYSIA

INFLUENCE OF MEDIA, NITROGEN, INDIGENOUS MICROORGANISM AND WATER STRESS ON BIOACTIVE COMPOUNDS AND BIOMASS YIELD OF Andrographis palliculata (Burm.f.) WALL. EX NEES

SHARA SALIH ALI

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By

SHARA SALIH ALI

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

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December 2014

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The current interest in reducing the herbal product importation by cultivating medicinal plants as Hempedu bumi, may have great impact on increasing the production of herbs in the local herbal industry. Growing plants under soilless media enable year round production of Hempedu bumi biomass. Although, some of soilless media (SM) components are cost effective and finding an alternative which is cheap and available locally in combination with indigenous microorganism might reduce applied rate of N chemical fertilizer. Besides the fact that organic and microbial amend d technique can enhance the growth of healthy herb, but also there is a need to increase the secondary metabolites in the pharmaceutical herb industry and this could be achieved by undergoing the plant through water stressed condition.

A preliminary study was carried out to determine the physical, chemical and microbiological properties of the soilless media that has used in the study which where; (coconut coir dust (CCD), empty fruit bunch compost (EFBC) and peat. Results of these study showed that the properties of some SM such as (EFBC) were suitable for the plant growth and development of *Andrographis paniculata*. In the first glass house experiment, five different combinations of SM were used as growing media which includes; C1 as control = CCD only (1: -), C2 = EFBC + CCD (7:3), C3 = EFBC + CCD (3:7), C4 = CCD + Peat (7:3), C5 = CCD + Peat (3:7) in combination with four nitrogen rates (NR) (0, 30, 60, and 90 kg ha^{-1}) along with and without indigenous microorganism (IMO) application. The growth performance, physiology, biomass production of root and shoot, macronutrient content of leaf tissue and microbial populations in rhizosphere were measured at 30 and 60 day after planting (DAP). At 30 and 60 DAP with application of IMO the soilless media that gave highest growth performance parameter was under C3 media with fertilization of 60 kg N ha^{-1} compared to control media C1 and other media C2, C4 and C5, plant

height (25, 40.8 cm, respectively), number of leaves (121, 132 leaves plant^{-1}) and total leaf area (122.8, 156.7 cm^2), dry matter biomass (dry weight of shoot (3.1, 5.1 g), root (0.5, 1.1 g) and total dry biomass (3.6, 6.2 g)) physiological response (photosynthetic rate (9, 10 µmol m^{-2} s^{-2}), stomatal conductance (410.4, 469.4 mmol m^{-2} s^{-1}) and transpiration rate (3.9, 4.2 mmol H_2O m^{-2} s^{-1})) and a decline in all parameter noticed at 90 kg N ha^{-1}. With 90 kg N ha^{-1} the parameters declined under C3 media in which plant height (19, 37.5 cm, respectively), number of leaves (74.8, 105.8 leaves plant^{-1}) and total leaf area (88, 93.2 cm^2), dry matter biomass (dry weight of shoot (1.7, 3.6 g), root (0.3, 0.9 g) and total dry biomass (2, 4.5 g)) physiological response (photosynthetic rate (8.5, 9 µmol m^{-2} s^{-2}), stomatal conductance (234.8, 363 mmol m^{-2} s^{-1}) and transpiration rate (2.3, 2.6 mmol H_2O m^{-2} s^{-1})).

While without IMO application, the above mentioned parameters increased with increasing rate of N fertilizer to 90 kg N ha^{-1} at both planting periods. The combined application of IMO and N fertilizer created positive impact on most of the plant character and total biomass yield of Hempedu bumi grown under C3 media compared to C1 (control). Despite of the fact that EFBC is slow in releasing nutrient but additional of IMO could positively enhance nutrient release from the compost and resulting in highest biomass production of the plant. In second experiment, two soilless media (C1 and C3) from previous studies were chosen base on their effects on the performance of plant growth, physiological parameter and biomass production to further determine their effect on bioactive compound of *Andrographis paniculata* under WS condition. Media C1 as control and C3 supplied with or without fertilizer. Plant with fertilizer was supplied with 60 kg N ha^{-1}, 15 kg P ha^{-1}, 20 kg K ha^{-1} and no fertilizer at all was given to the plant without fertilizer. The plants was subjected into three WS levels which include; well watered (WW), moderate water stressed (MS) and severe stressed (SS). The plant received equal amounts of water twice a day for plant establishment and WS treatments were introduced 60 DAP for a period of 21 days. During WS treatments, the leaf relative water content (LRWC) and stomatal conductance (g_s) were recorded at 5 days interval and the leaf increments were measured at 3 days interval. Data for biomass production and bioactive compound was determined after 21 days of WS treatment. Results showed that the highest plant biomass production (dry weight of shoot (11.5 and root 1.4 g) was observed from plants grown in C3 media under WW with application of fertilizer. Averaged overall WS treatment with fertilizer application the mean number of leaves and total leaf area (TLA) of plant grown under media C3 exhibited 67% and 31% higher than that of plants grown in C1. While without fertilizer application the number of leaves and total leaf area (TLA) of plant grown in C3 media was 69% and 33% higher than that of plants grown under control media C1. The biochemical compound of the plant was significantly affected by WS condition in which the highest content of andrographolide (0.06 µg mg^{-1} dry leaf), proline (3.4 µmole g^{-1} FW), total phenolic (282.4 (µg mg^{-1} extract) and flavonoid (381.8 µg mg^{-1} extract) content were observed in SS plants grown under media C3 .When averaged across different WS condition, the plant water status and chlorophyll content of plants grown under media C3 were adversely affected by stress condition when the result showed 16% and 57% higher compared to control either with or without fertilizer application, respectively

In conclusion, results of this study suggest that the best media proportion was C3 media which consisted of 7: 3 of CCD: EFBC which gave best growth performance as well as biomass production of *Andrographis paniculata*. Application of IMO as an amendment to soilless media resulted in reducing the used amount of chemical N fertilizer in which the best biomass production was determined when the plant applied with 60 kg N ha^{-1} and a decline in the biomass was observed with increasing N rate to 90 kg N ha^{-1}. The biochemical content and the secondary metabolite of the plant increased with subjecting the plant to water stress condition. Overall, improvement of SM amended with IMO and maximizing active compound of the herb can be implemented to increase herbal industry in Malaysia.

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

PENGARUH MEDIA, NITROGEN, MIKROORGANISMA ASLI DAN TEKANAN AIR KE ATAS SEBATIAN BIOAKTIF DAN HASIL BIOMASS DALAM *Andrographis paniculata* (Burm.f.) WALL. EX NEES

Oleh

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Disember 2014

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Tarikan utama dalam usaha mengurangkan import produk herba ialah melalui usaha penanaman tanaman herba seperti Hempedu bumi, usaha ini akan memberi kesan kepada peningkatan produktiviti herba dalam pasaran tempatan. Proses penanaman dengan menggunakan kultur media tanpa tanah mampu menyediakan penghasilan Hempedu bumi sepanjang tahun. Walaupun sesetengah komponen media tanpa tanah memerlukan kos yang tinggi tetapi terdapat alternatif dalam mengurangkan kos iaitu dengan melibatkan penggunaan produk tempatan dengan kombinasi bersama mikroorganisma bermanfaat yang boleh mengurangkan kadar penggunakan baja kimia Selain daripada penerapan teknik organik dan penggunaan mikrob untuk meningkatkan pertumbuhan herba, terdapat juga teknik lain yang boleh digunakan untuk meningkatkan penghasilan metabolik sekunder dalam industri farmaseutikal herba iaitu melalui pendedahan tumbuhan kepada keadaan ketegangan air. Kajian preliminari telah dijalankan untuk mengenalpasti sifat-sifat fizikal, kimia dan mikrobiologi dalam media tanpa tanah dengan melibatkan sabut kelapa (CCD), kompos tandan buah kosong (EFBC) dan tanah gambut. Hasil kajian menunjukkan beberapa sifat SM seperti EFBC sesuai untuk pertumbuhan dan perkembangan *Andrographis paniculata*. Dalam eksperimen rumah kaca yang pertama, lima kombinasi SM telah digunakan sebagai media pertumbuhan iaitu; C1 sebagai kawalan = CCD sahaja (1:-), C2 = EFBC + CCD (7:3), C3 = EFBC + CCD (3:7), C4= CCD + Gambut (7:3), C5 = CCD + Gambut (3:7) dalam kombinasi kadar N yang berbeza (0,30, 60,90 kg ha^{-1}) bersama dengan penggunaan IMO dan tanpa IMO. Kadar pertumbuhan, fisiologi, penghasilan biomass pucuk dan akar, kandungan micronutrient dan populasi rhizosfera diukur 30 dan 60 hari selepas menanam (DAP).

Pada 30 dan 60 DAP dengan penggunaan IMO, media yang menunjukkan kadar pertumbuhan yang tertinggi ialah media C3 dengan pembajaan sebanyak 60 kg ha^{-1} berbanding dengan media kawalan C1, C2, C4 dan C5, peningkatan yang

ditunjukkan ialah ketinggian pertumbuhan (24, 40.8 cm), jumlah daun (121, 132 daun per pokok) dan jumlah luas daun (122.8 , 156.7 cm^2), berat kering biomass (berat kering pucuk (3.1, 5.1 g), akar (0.5, 1.1 g) dan jumlah bahan kering (3.6, 6.2 g)), tindak balas fisiologi ((kadar fotosintesis (9, 10 µmol m^{-2} s^{-2}), konduksi stomata (410.4, 469.4 mmol m^{-2} s^{-1}) dan kadar transpirasi (3.9, 4.2 mmol H_2O m^{-2} s^{-1})) dan semua parameter mengalami penurunan pada kadar baja 90 kg N ha^{-1}. Dengan penggunaan baja 90 kg N ha^{-1} dalam media C3 menunjukkan penurunan bagi ketinggian pokok (19, 37.5 cm), bilangan daun (74.8, 105.8 daun $pokok^{-1}$) dan luas daun (88, 93.2 cm^2), biomass bahan kering (berat kering pucuk (1.7, 3.6 g), akar (0.3, 0.9 g) dan jumlah berat biomass (2, 4.5 g)), manakala bagi respon fisiologi seperti (kadar fotosintesis (8.5, 9 µmol m^{-2} s^{-2}), konduksi stomata (234.8, 363 mmol m^{-2} s^{-1}) dan kadar transpirasi (2.3, 2.6 mmol H_2O m^{-2} s^{-1})). Walaupun tanpa kehadiran IMO, semua parameter yang dinyatakan diatas meningkat dengan penggunaan baja N sebanyak 90 kg N ha^{-1} sepanjang dua musim penanaman. Gabungan aplikasi IMO dan baja N memberi kesan positif kepada kebanyakan pertumbuhan pokok dan jumlah penghasilan biomass Hempedu bumi yang ditanam dalam media C3 berbanding dengan media C1 (kawalan). Walaupun terdapat fakta yang menyatakan bahawa EFBC lambat melepaskan nutrien kepada tumbuhan tetapi dengan penambahan IMO ia menunjukkan kesan positif dalam meningkatkan pembebasan nutrient dari baja dan penghasilan biomass yang tinggi bagi setiap tanaman.

Dalam eksperimen kedua , dua media telah dipilih (C1 dan C3) daripada eksperimen sebelum ini berdasarkan peningkatan tindak balas dalam pertumbuhan pokok, fisiologi dan penghasilan biomass yang bertujuan untuk menentukan kesan media terhadap kandungan komposisi bioaktif dalam *Andrographis paniculata* dalm keadaan WS. Media C1 sebagai kawalan and C3 digunakan dengan aplikasi baja dan tanpa pembajaan. Tumbuhan yang diberikan baja ialah dengan kadar sebanyak 60 kg N ha^{-1}, 15 kg P ha^{-1}, 20 kg K ha^{-1} dan tiada baja diberikan kepada pokok dalam kategori tanpa pembajaan. Tumbuhan diletakkan dalam 3 keadaan WS iaitu; disiram (WW), tegangan air sederhana (MS) dan tegangan air yang serius (SS). Sepanjang tempoh pengenalan rawatan, tanaman menerima jumlah air yang sama banyak iaitu dua kali sehari dan rawatan WS diberikan selepas 60 hari dalam tempoh 21 hari. Sepanjang rawatan WS, kandungan relatif air dalam daun dan konduksi stomata (g_s) dicatatkan selang 5 hari manakala pertambahan saiz daun diukur selang 3 hari. Data untuk penghasilan biomas dan komposisi bioaktif ditentukan selepas 21 hari rawatan. Keputusan menunjukkan penghasilan biomass yang tertinggi (berat kering pucuk (11.5 dan akar 1.4 g) oleh tumbuhan yang ditanam dalam media C3 dibawah WS dengan kombinasi aplikasi pembajaan. Purata keseluruhan rawatan WS dengan aplikasi baja menunjukkan min bilangan daun dan jumlah luas daun (TLA) bagi tumbuhan yang ditanam dalam media C3 menunjukkan peningkatan 67% dan 31% lebih tinggi daripada tumbuhan yang ditanam dalam media C1. Manakala rawatan tanpa pembajaan menunjukkan bilangan daun dan jumlah luas daun bagi tumbuhan yang ditanam dalam media C3 menunjukkan peningkatan 69% dan 33% lebih tinggi daripada tumbuhan yang ditanam dalam media C1. Sebatian kimia dalam tumbuhan turut menunjukkan kesan yang ketara dalam keadaan dimana jumlah tertinggi andrographolide (0.06 µg mg^{-1} daun kering), proline (3.4 µmole g^{-1} FW), jumlah phenolic (282.4 (µg mg^{-1} extrak) dan kandungan flavonoid (381.8 µg mg^{-1} extrak) direkodkan bagi tumbuhan yang ditanam dalam media C3 . Dalam purata keadaan WS yang berbeza, status kandungan air pokok dan klorofil daun bagi pokok yang ditanam dalam media C3 menunjukkan kesan tekanan yang berbeza seperti yang dicatatkan iaitu 16% dan 57% lebih tinggi daripada media kawalan sama ada dengan atau tanpa pembajaan.

Kesimpulannya , hasil kajian ini mencadangkan bahawa media C3 yang terdiri daripada 7: 3 daripada CCD: EFBC telah memberikan peningkatan pertumbuhan yang terbaik dan penghasilan biomass tertinggi bagi *Andrographis paniculata*. Penggunaan sebagai perapi media tanpa tanah menunjukkan pengurangan dalam kadar penggunaan baja N dimana penghasilan jumlah biomas yang tertinggi dicatatkan oleh tumbuhan yang menggunakan 60 kg N ha^{-1} dan penghasilan biomas meunjukkan penurunan apabila kadar baja N dinaikkan kepada 90 kg N ha^{-1}. Kandungan sebatian kimia dan metabolit sekunder bagi tumbuhan ini meningkatan dengan kenaikan kadar tegangan air yang dikenakan.secara keseluruhannya, penggunaan SM dengan kombinasi IMO boleh ditingkatkan penggunaannya unuk penghasilan komposisi bahan aktif yang maksimum bagi meningkatkan industri herba di Malaysia.

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I certify that a Thesis Examination Committee has met on 16 December 2014 to conduct the final examination of Shara Salih Ali on her thesis entitled "Influence of Media, Nitrogen, Indigenous Microorganism and Water Stress on Bioactive Compounds and Biomass Yield of *Andrographis paniculata* (Burm.f) Wall. ex Nees" 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 Science.

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×	Multiply/interaction between
+	Plus
-	Minus
>	Greater than
<	Less than
≥	Greater than or equal to
≤	Less than or equal to
±	Plus minus
/	Divide
=	Equal to
°	Degree
°C	Degree celcious
%	Per cent
λ	Lambda
µg	Microgram(s)
µL	Microlitre(s)
µM	Micromolar(s)
µm	Micrometre(s)
µmol	Micromole(s)
ABA	Abscisic acid
ADP	Adenosine diphosphate
$AlCl_3$	Aluminum Trichloride
AM	Arbuscular mycorrhizal
ANOVA	Analysis of variance
AU	Absorbance units

C Carbon

Ca Calcium

CaCl2 Calcium chloride

CCD Coconut coir dust

CEC Cation exchange capacity

Cfu Colony forming unit

CO_2 Carbon dioxide / respiration

conc. Concentration

CIRP Christmas Island rock phosphate

d Day(s)

DAP Days after planting

dH_2O Distilled water

DPPH 2, 2-diphenyl-1-picryl-hydrazyl

Dr. Doctor

EC Electrical conductivity

EFBC Empty fruit bunch compost

EM Effective microorganism

FFTC Food and fertilizer technology center

g Gram(s)

GA Gibberellic acid

GA Gallic Acid

h Hour(s)

H Hydrogen

ha Hectare(s)

HCl Hydrochloric acid

H_2O Water

H_2O_2	Hydrogen peroxide
H_2SO_4	Sulfuric acid
IMO	Indigenous microorganism
INM	Integrated nutrient management
K	Potasium
kg	Kilogram(s)
KOH	Potassium hydroxide
KNF	Korean Natural Farming
L	Liter(s)
LI	Leaf increment
Log	Logarithm
m	Metre(s)
M	Molar
mAU	Milliabsorbance unit
MeOH	Methanol
mg	Milligram(s)
Mg	Magnesium
min	Minute(s)
mL	Millilitre(s)
mm	Millimetre(s)
mM	Millimolar(s)
mmol	Millimole(s)
MPOB	Malaysian palm oil board
N	Nitrogen
NaCl	Sodium chloride
NaF	Sodium fluoride

$NaHSO_3$	Sodium hydrogen sulphite
NaOH	Sodium hydroxide
NaOCl	Sodium hypochlorite
NO_3	Nitrate
NR	Nitrogen rate
NS	Not significant
Pa	Pascals
O_2	Oxygen
OPT	Oil palm trunk
P	Probability
P	Peat
P	Phosphorus
pH	Symbol denoting hydrogen ion in a solution
PGPR	Plant growth promoting rhizobacteria
POME	Palm oil mill effluent
Prof.	Professor
PSB	Phosphorus solubilizing bacteria
RD	Root diameter
RDW	Root dry weight
RH	Relative humidity
RL	Root length
RLWC	Relative leaf water content
RM	Malaysian ringgit
RSA	Root surface area
RV	Root volume
R: S	Root to shoot ratio

RU	Rutin
s	Second(s)
SAS	Statistical Analysis System
SDW	Shoot dry weight
S.E.	Standard error
sp.	Species
SM	Soilless media
Std	Standard
UFLC	Ultra-fast liquid chromatography
UK	United Kingdom
USA	United States of America
UV	Ultra-violet
Vol.	Volume(s)
v/v	Volume by volume
viz.	Videlicet (namely)
WS	Water stress
w/w	Weight by weight

CHAPTER 1

INTRODUCTION

In Malaysia, *Andrographis paniculata* is known as 'Hempedu bumi', which means 'bile of the earth'. Commonly it is known as 'king of bitter' since it is one of the bitterest herbs that is regularly used in traditional medicine. Before antibiotics were invented Indian people used this plant for natural ailment remedy (Kumar, Dora, Singh, & Tripathi, 2012). The pharmaceutical value of this plant is in the aerial part and the root which have a very bitter taste due to the existence of active ingredient viz. andrographolide, 14-deoxy-11, 12 didehydroandrographolide and neoandrographolide. However, maximum amount of these compounds contained in mature leaves of the plant (Suriyo et al., 2014). Whole plant parts traditionally used for the treatment of hepatitis, cough, fever, mouth ulcers, sores, venomous snake bites, common cold, and urinary tract infections. It is also discovered that the plant has anticancer and immune stimulatory (Bhattacharya, Puri, Jamwal, & Sharma, 2012). Besides applications of aerial part of the plant for liver disease, dysentery and common colds, it also applied in the therapy of hypertension and diabetes mellitus (Agarwal, Sulaiman, & Mohamed, 2005; Wiart, 2002; Zhang & Tan, 1996). Study have found that the methanolic extract of *Andrographis paniculata* have high potential as an antiviral activity on dengue fever (Tang, Ling, Koh, Chye, & Voon, 2012).

In Malaysia the production of traditional and herbal medicine product is expands as a result of increased dependent on the import of raw materials for the local herbal industry which is an economic scarcity. Charles et al. (1993) reported that in 2009 the trading of herbal based products in Malaysia reached RM777 billion which is expected to triple by 2020. Among the valuable herbal plants in high demands in Peninsular Malaysia is 'Hempedu bumi' which has enormous pharmaceutical utilization. Therefore, it would be justifiable to increase or enhance the production of safety products in the local herbal industry by using new cultivation technology of organic based soilless media. Soilless media is an artificial method of providing plants with support and a reservoir for nutrients and water. There are very limited types of organic soilless media that can be used as potting media for plant production such as peat, coconut coir dust (CCD) and inorganic substrate such as rockwool and perlite. Currently the environmental and ecological concerns against using peat as a growth substrate is increasing due to its contribution in increasing global warming gases (Kip et al., 2010) . Moreover, the rising costs for peat as a growing media in horticulture have led to search for high quality and cost effective alternative for peat (Saffigna, Powlson, Brookes, & Thomas, 1989). The price of peat is increasing because they have to be imported (Dash & Mohanty, 2001). However the price of CCD is reasonable in comparison with peat but still there is a need to find out other substrates that are more reasonable. Oil palm is the most essential product in Malaysia and empty fruit bunch (EFB) is a raw by-product of palm oil that can be utilized as a growing media to be used in agriculture industries. Recycling and managing these residue of oil palm is targeted to reduce the major disposal problem and recover the energy (Wiart, 2002). Empty fruit bunch compost (EFBC) is one of the by-products that generated from palm oil which is rich in organic matter and nutrient content (Caliman, Hardianto, & Ng, 2001). The application of EFBC as media enrichment increased the nutrient uptake and yield of oil palm in palm oil plantation (Zaharah & Lim, 2000). Hence, as an alternative, empty fruit bunch compost (EFBC) probably can be fully utilized as a growing media.

The characteristic of growing media is one of the important factors that affect the growth performance of the plant because plant requires sufficient support, nutrients and moisture from the media in which they are grown. Use of this organic substrate as potting media could reduce the decline in fertilizer availability, reduce the rate of excessive and continues usage of these fertilizers which contaminate the environment and disturbs the quality of soil and increase economic return to the farmer (Agarwal et al., 2005). However soilless media may have good water holding and aeration properties, but can be limited on their capability to hold nutrients. Fertility management of this media is extremely important so that, the nutrient levels should be optimized in the growing media prior to planting in order to gain maximum crop growth. Balance among the essential plant nutrients, especially potassium, calcium, and magnesium is important. The application of inorganic and organic source of nutrient to increase the biomass production of plant is called integrated nutrient management (INM), a technique which modifies the plant nutrient supply to an optimum level. The highest growth parameters, nutrient uptake, yield and andrographolide content of *Andrographis paniculata* were observed when cultivated under INM system (Abdullah & Sulaiman, 2013).

The nutrient release of EFBC is slow during high nutrients appeal at peak time and in order to release nutrients from EFBC, microbial assisted biodegradation should be increased. Application of microbial component such as indigenous microorganism (IMO) could increase the nutrient emission from the compost (Abdul Mutalib, 2009). The IMO is a beneficial microorganism with the ability of releasing nutrient from the media. Due to the availability of filamentous fungi, yeasts and bacteria, IMO have many important functions which are fermentation, decomposition and N fixation (Jensen, Guilaran, Jaranilla, & Garingalao, 2006). In addition, effect on the microbial biomass and growing media health when combination of organic and chemical fertilizer is applied has been reported earlier (Dutta, Pal, Chakeraborty, & Chakrabarti, 2003).

As mentioned earlier due to the availability of many active compounds in ‘Hempedu bumi’ the plant is important as a pharmaceutical herb. Increase in the secondary metabolite or active compounds of the plant reached the demand of safety plant production for human health as well as environment. The productivity of the secondary metabolite in the plant can be enhanced by subjecting the plant through abiotic stress condition. Although, drought reduce the growth performance of the plant (Flexas, Bota, Loreto, Cornic, & Sharkey, 2004) but with application of appropriate fertilizer and microbial consortium the adverse effect of water stress on growth of plant could be alleviated. In addition, drought is one of the most essential abiotic stress factors in order to enhance the biochemical components of the plants

(Zobayed, Afreen, & Kozai, 2007). It has been reported that water stress or drought stress increased the production of active compound in many medicinal plant such as in *Artemisia annua* (Charles et al., 1993), *Hypericum perforatum* (Zobayed et al., 2007). In addition, adjusting plants to drought stressed condition will increase water used efficiency and water availability for human supply. Researcher have reported that the agricultural irrigation used over 70% of the world supplies of clean water and most of the water is especially used in the protected environments (Ali, Ismail, Mohd Saud, & Manan, 2004). By incorporating with IMO, the amendment of EFBC in soilless media is hoping to provide a sustainable growing media which can be used to increase the growth performance of 'Hempedu bumi'.

The current interest in reducing the herbal product importation by cultivating medicinal plants as 'Hempedu bumi', may have great impact on increasing the production of herbs in the local herbal industry. Growing plants under soilless media enable year round production of Hempedu bumi biomass. Although, some of soilless media (SM) components are cost effective and finding an alternative which is cheap and available locally in combination with indigenous microorganism might reduce applied rate of N chemical fertilizer. Besides the fact that organic and microbial amend d technique can enhance the growth of healthy herb, but also there is a need to increase the secondary metabolites in the pharmaceutical herb industry and this could be achieved by undergoing the plant through water stressed condition. Traditionally choices of soilless media that are available in the market are limited mainly to peat (Ramahsamy, Bakar, Abdullah, & Ishak, 2012a) and coconut (Mazuela, Urrestarazu, & Bastias, 2012). Although, EFBC have been used in agriculture but very little work has been reported on using EFBC as an alternative soilless media which increase recycling residue of oil palm and leads to use locally available residue. It has been reported that the empty fruit bunch compost (EFBC) increased nutrient uptake and yield of palm oil plantation when used as cultivation media (Zaharah & Lim, 2000). Abdul Mutalib (2009) reported that EFBC has great potential to be used in organic cabbage production replacing inorganic fertilizer. The choices of combination of soilless media have great effect on the plant growth as in previous study reported by Wira, Razi, and Jamil (2011a) which claimed that combination of EFC and CCD soilless media in 3:7, v/v ratio gave highest shoot biomass of rock melon while, combination of CCD and peat in 7:3, v/v ratio gave lowest shoot biomass compared to 100% CCD as control media.

However, studies on the application of IMO with soilless media to decrease the applied rate of N fertilizer and their effect on the biomass of Hempedu bumi have not been done. To add to that, study on the effect of water stress on the secondary metabolite of Hempedu bumi has not been done yet. In view on the potential benefit of EFBC as growing media it was therefore hypothesized that the cultivation of 'Hempedu bumi' under different combination of soilless media (EFBC, CCD and peat) with application of indigenous microorganism (IMO) and different nitrogen rates enhance the growth performance, as well as the secondary metabolite of the plant might improves by subjecting the plant to water stress condition.

Therefore, the aims of this research work were:

1. To determine the physical, chemical and microbiological properties of soilless media before planting.

2. To determine the best soilless media, optimum rate of N fertilizer and effect of IMO application on the growth and biomass production of *Andrographis paniculata* grown under different combination of soilless.

3. To evaluate the effect of water stress on the biomass and bioactive compound of *Andrographis paniculata* grown under selected soilless media and optimum level of N fertilizer.

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