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PHYSIOLOGICAL RESPONSE AND PHYTOCHEMICAL CONTENT OF Andrographis paniculata (Burm.) AS AFFECTED BY LIGHT AND NITROGEN

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By

KHAIRUL AZREE ROSLI

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

November 2013

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DEDICATION

To my parents, Kamariah Mahmood and Rosli Sufian. I love to learn. That wonderful gift is from them and I carry it with me always.

To knowledge seekers of all ages who want to be the best learners they can possibly be – and to parents who wish this gift for their children.

“Many of life’s failures are those people who did not realize how close they were to success when they gave up.”

Thomas Edison

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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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By

KHAIRUL AZREE ROSLI

November 2013

Chair: **Siti Aishah Hassan, PhD**
Faculty: **Agriculture**

Andrographis paniculata (Burm.) Nees or hempedu bumi is a traditional medicinal and aromatic plant with valuable phytochemical and pharmacological potential. Growth, physiological and phytochemical responses to light and nitrogen are useful measurements to determine favorable growing conditions for hempedu bumi. Despite numerous findings on other medicinal and aromatic plants, there are no literatures on how light and nitrogen affect growth, physiology and phytochemical content of hempedu bumi in the humid tropics of Malaysia. The objectives of this study were to determine the effects of shade and nitrogen on growth, physiology and phytochemical content of hempedu bumi. Plants were grown under two shade levels, 0% and 40%, and fertilized with five nitrogen rates, 90, 135, 180, 225 and 270 kg ha^{-1} in a nested design. Dependent variables were growth components, gas exchange, chlorophyll fluorescence, relative chlorophyll content, chlorophyll pigments and, leaf nutrient content and uptake, and, andrographolide, neoandrographolide and 14-deoxy-11, 12-didehydroandrographolide concentrations and yields. Shaded plants grew taller with greater total leaf area, specific leaf area, leaf area ratio, net assimilation rate and leaf andrographolide yield than sun-grown plants. Sun-grown plants had higher relative chlorophyll content, total chlorophyll, chlorophyll a, chlorophyll b, ratio of chlorophyll a to chlorophyll b and net photosynthesis rate, leaf concentrations of phosphorus, potassium, calcium and magnesium, and neoandrographolide than shaded plants. Fertilizing plants with increasing rate of nitrogen has increased their height, leaf area index, total leaf area, shoot and root dry mass, leaf mass ratio and root shoot ratio, intercellular carbon dioxide concentration and leaf temperature. There was a quadratic relationship between nitrogen rate and total dry mass of plants. Increasing rate of nitrogen has also increased leaf nitrogen

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and potassium content, and uptake of phosphorus, calcium and magnesium, and neoandrographolide yield. Shade interacted with nitrogen affecting plant height, leaf area index, total leaf area, leaf dry mass, leaf area ratio, and neoandrographolide and 14-deoxy-11, 12-didehydroandrographolide concentrations. Neoandrographolide and 14-deoxy-11, 12-didehydroandrographolide concentrations per extract were higher in sun-grown plants than in shaded plants. Shade and nitrogen did not affect relative growth rate, chlorophyll fluorescence and yield of 14-deoxy-11, 12-didehydroandrographolide. The goal in commercial hempedu bumi cultivation is to produce plants containing high dry mass and phytochemical content. Hempedu bumi could adapt to varying levels of shade and nitrogen by altering its morphology, physiology and biochemistry. Shading at 40% and fertilizing with nitrogen at 225 kg ha^{-1} can increase the dry mass and phytochemical content of hempedu bumi.

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

TINDAKBALAS FISIOLOGI DAN KANDUNGAN FITOKIMIA *Andrographis paniculata* (Burm.) TERHADAP CAHAYA DAN NITROGEN

Oleh

KHAIRUL AZREE ROSLI

November 2013

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Andrographis paniculata (Burm.) Nees atau hempedu bumi ialah tumbuhan ubatan dan beraroma tradisional yang mempunyai potensi fitokimia dan farmokologi bernilai. Tindakbalas pertumbuhan, fisiologi dan fitokimia terhadap cahaya dan nitrogen ialah ukuran berguna dalam menentukan habitat yang sesuai untuk penanaman hempedu bumi. Berbanding tumbuhan ubatan dan beraroma lain, tiada maklumat tentang kesan cahaya dan nitrogen terhadap pertumbuhan, fisiologi dan kandungan fitokimia hempedu bumi di rantau tropika lembap Malaysia. Objektik kajian ialah untuk menentukan kesan cahaya dan nitrogen terhadap pertumbuhan, fisiologi dan kandungan fitokimia di dalam hempedu bumi. Tumbuhan ditanam di bawah dua paras teduhan, 0% dan 40%, dan dibaja dengan lima kadar nitrogen, 90, 135, 180, 225 and 270 kg ha^{-1} dalam rekabentuk tersarang. Pembolehubah bersandar ialah komponen pertumbuhan, pertukaran gas daun, fluoresen klorofil, kandungan klorofil relatif, pigmen klorofil, pengambilan dan kepekatan nutrien di dalam daun, dan kepekatan dan hasil andrographolide, neoandrographolide dan 14-deoxy-11, 12-didehydroandrographolide. Tumbuhan teduhan telah tumbuh lebih tinggi dengan jumlah luas daun, luas daun spesifik, nisbah luas daun, kadar asimilasi bersih dan hasil andrographolide lebih tinggi berbanding tumbuhan matahari. Tumbuhan matahari mengandungi klorofil relatif, jumlah klorofil, klorofil a, klorofil b, nisbah klorofil a b, kadar fotosintesis, kepekatan fosforus, kalium, kalsium and magnesium daun, dan hasil neoandrographolide lebih tinggi berbanding tumbuhan teduhan. Kadar pembajaan nitrogen yang meningkat meningkatkan ketinggian, jumlah luas daun, jisim kering pucuk dan akar, nisbah jisim daun, nisbah akar daun, kepekatan karbon dioksida antara sel dan suhu daun. Terdapat hubungan kuadratik antara kadar nitrogen dan jumlah jisim tumbuhan. Kadar nitrogen meningkat juga telah meningkatkan kandungan nitrogen dan kalium daun, pengambilan fosforus, kalsium

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dan magnesium, dan hasil neoandrographolide. Paras teduhan beinteraksi dengan nitrogen dan mempengaruhi ketinggian pokok, indeks luas daun, jumlah luas daun, jisim kering daun, nisbah luas daun, dan kandungan neoandrographolide dan 14-deoxy-11, 12-didehydroandrographolide. Teduhan dan nitrogen tidak mempengaruhi kadar pertumbuhan relatif, fluoresen klorofil dan hasil 14-deoxy-11, 12-didehydroandrographolide. Matlamat penanaman hempedu bumi secara komersil ialah untuk menghasilkan pokok yang mengandungi kandungan bahan kering dan fitokimia yang tinggi. Hempedu bumi boleh beradaptasi terhadap paras teduhan dan nitrogen yang berbeza dengan mengubahsuai morfologi, fisiologi dan biokimianya. Teduhan 40% dan pembajaan dengan 225 kg ha^{-1} nitrogen boleh meningkatkan jisim kering dan kandungan fitokimia di dalam hempedu bumi.

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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;
- supervision responsibilities as stated in the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) are adhered to.

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LIST OF ABBREVIATIONS

A	Net photosynthetic rate
AG	Andrographolide
BRIS	Beach ridges interspersed with swales
C/N	Carbon-Nitrogen
Ca	Calcium
Chl a	Chlorophyll a
Chl b	Chlorophyll b
Ci	Intercellular CO_2 concentration
CO_2	Carbon dioxide
DAT	Days after transplanting
DDAG	14-deoxy-11, 12-didehydroandrographolide
E	Transpiration rate
Fo	Baseline fluorescence
Fv	Variable fluorescence from dark-adapted leaf
Fm	Maximal fluorescence from dark-adapted leaf
Fv/Fm	Maximum quantum efficiency of photosystem II photochemistry
Fv/Fo	Water-splitting complex activity of photosystem II donor side
g_s	Stomatal conductance
h	Hour
H	Hydrogen
HIV	Human immunovirus
HPLC	High performance liquid chromatography
K	Potassium
L_A	Total leaf area per plant
LAI	Leaf area index
LAR	Leaf area ratio
LMR	Leaf mass ratio
MARDI	Malaysian Agricultural Research and Development Institute
Mg	Magnesium
MOP	Muriate of potash
N	Nitrogen
NAG	Neoandrographolide
NAR	Net assimilation rate
O	Oxygen
P	Phosphorus
PROC GLM	General linear model
Q.S.	Quantity sufficient
RGR	Relative growth rate
RP-HPLC	Reverse phase high performance liquid chromatography
RSR	Root shoot ratio
SLA	Specific leaf area
SPAD	Soil plant analysis development
T_{Leaf}	Leaf temperature
TSP	Triple superphosphate
UK	United Kingdom

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UPM	Universiti Putra Malaysia
USA	United States of America
UV-vis	Ultraviolet visible
v/v	Volume by volume
VPD	Vapor pressure deficit
WHO	World Health Organization

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CHAPTER 1

INTRODUCTION

Andrographis paniculata (Burm. f.) Nees or hempedu bumi is a popular medicinal and aromatic plant in the Malaysian traditional system of medicine. Hempedu bumi contains valuable phytochemicals that contribute to its healing properties. Researchers conduct many studies to evaluate the phytochemical and pharmacological potential of hempedu bumi (Ramlan et al. 2005; Khatun et al. 2011; Subramaniam et al. 2012). Although hempedu bumi is renown for its medicinal properties, researchers are also exploring the possibility of applying this plant in various fields. For example, fields such as agriculture, forestry, animal production, veterinary medicine or manufacturing (Valdiani et al. 2012). Hempedu bumi has received much attention in recent years because of its medicinal properties and other uses that offer tremendous economic benefits. Currently, there are attempts to cultivate hempedu bumi on a commercial scale to meet the exploding global market (Rahman 2012). Researchers are studying the agronomic requirements to enhance the growth and quality of hempedu bumi (Sanjutha et al. 2008; Ramesh et al. 2011; Mishra and Jain 2013).

Traditional medicine uses medicinal and aromatic plants to prevent and treat diseases. It ranges from traditional and popular medicines of every country to using standardized and tested herbal extracts (Firenzuoli and Gori 2007). The World Health Organization has projected that more than 80% of the global population in developing countries use traditional medicine as their main form of therapy (Merican 2002; WHO 2002). The use of traditional medicine is also growing in developed countries such as the United States, United Kingdom and Japan (Craker and Gardner 2005; Gurib-Fakim 2006; Benzie and Wachtel-Galor 2011). In Malaysia, traditional medicine has been the main source of natural therapies since time immemorial (Siti et al. 2009; Adnan and Othman 2012; Yahya and Ali 2012). Use of traditional medicine had declined when synthetic medicines arrived (Daniell et al. 2001). However, Malaysians these days are becoming more aware of the benefits of traditional medicine especially the medicinal and aromatic plants (Aziz and Tey 2009; Hasan et al. 2009, 2011).

The international trade of medicinal and aromatic plants is becoming a leading force in the global economy. Malaysia exported RM46.4 billion worth of medicinal and aromatic plants in 1995, and the value escalated to RM83.1 billion by 1999. Singapore and Thailand were the major importers of medicinal and aromatic plants from Malaysia (FAO 2005). The Malaysian herbal industry has grown rapidly and has the potential to become a significant business in Malaysian agriculture. The

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Minister for Agriculture and Agro-Based Industry stated that the local herbal industry can contribute RM2.2 billion to Malaysia's Gross Domestic Product by 2020 (Bernama 2012). Furthermore, the Malaysian government aims to produce high-value herbal products from four local medicinal and aromatic plants under the Economic Transformation Program. One of them is hempedu bumi (Rahman 2012). Because of the enormous economic benefits, the demands for hempedu bumi are rapidly growing. In Peninsular Malaysia, traditional medicine companies use 7.8 ton of hempedu bumi a year as their main raw materials (Mohd and Mansor 2001). In India, the market for hempedu bumi between 2001 and 2002 was at 2005 ton with an annual growth rate of 3.1% (FAO 2005). However, between 2004 and 2005, the local and international market for hempedu bumi soared to 2197.3 ton (Rath 2005). India consumes almost 250 ton of aerial parts of hempedu bumi each year (Sharma et al. 2008). To meet the rising demand, there is a need to increase the supply of hempedu bumi without compromising its quality.

Superior genetic traits, high dry mass with high and consistent phytochemical content determine the quality of hempedu bumi (Canter et al. 2005; Mathe and Mathe 2008; Zhang et al. 2012; Traka et al. 2013). Good quality dried leaves of hempedu bumi could sell for USD 5 per kg while the pure active ingredients, such as andrographolide and its derivatives could price at up to USD 100, 000 per kg (Valdiani et al. 2012). Producing high quality hempedu bumi requires precise growing conditions (Mathe and Franz 1999). Although traditional medicine companies in Malaysia sources hempedu bumi locally, they harvest the plants mostly from the wild. There is a growing concern about over-harvesting hempedu bumi from the wild (Mohd and Mansor 2001). A more realistic long-term approach is to promote domestic cultivation of hempedu bumi. To cultivate hempedu bumi successfully, identifying their proper growth conditions is necessary.

Plant growth and development depend on abiotic and biotic factors. Abiotic factors include the physical environmental conditions and biotic factors include animals, insects, and diseases (Mittler 2006; Szakiel et al. 2011; Gouvea et al. 2012). Plants require specific environmental conditions to thrive and maximize their potential yields. Unfavorable environmental conditions can stress plants resulting in lower yields. In such cases, the environment can be artificially modified, such as in greenhouses, to meet the crop requirements (Decoteau 1998; Sharma 2004; Prasad et al. 2012). Researchers proposed various theories and models to explain how environmental factors affect growth and phytochemistry of plants. However, because of conflicting results, researchers are still debating whether there is a definite link between the two (Muzika 1993; Nitao et al. 2002; Lerdau and Coley 2002; Koricheva 2002; Stamp 2003; Massad et al. 2012).

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Light and nitrogen are important environmental factors that control plant growth and development. Modifying these environmental conditions would affect the growth, physiology and phytochemistry of hempedu bumi in several ways. Previous study reported that sun-grown hempedu bumi contained the highest concentration of flavonoid than shaded ones (Kumar et al. 2012). Another study found that hempedu bumi has increased total dry mass when fertilized with 200 kg N ha^{-1} and grown in a mixture of regosol soil, rice husk charcoal and organic fertilizer (Ambarwati 2008). Understanding how hempedu bumi responds to light and nitrogen may improve the management practices under different conditions. Although findings are available on the effects of light and nitrogen on hempedu bumi, the information is extremely lacking. Therefore, the objectives of this study were to determine the effects of varying levels of shade and nitrogen on:

(1) the growth response of hempedu bumi,

(2) physiological response of hempedu bumi,

(3) and phytochemical content of hempedu bumi.

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