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EFFECTS OF LIGHT SOURCES AND DRYING METHODS ON PLANT GROWTH AND STEVIOL GLYCOSIDES CONTENT OF STEVIA (Stevia rebaudiana Bertoni)

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By

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EFFECTS OF LIGHT SOURCES AND DRYING METHODS ON PLANT GROWTH AND STEVIOL GLYCOSIDES CONTENT OF STEVIA (*Stevia rebaudiana* Bertoni)

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June 2017

Chairman : **Phebe Ding, PhD**
Faculty : **Agriculture**

Stevia (*Stevia rebaudiana* Bertoni) has received great attention with the rise in demand for low-sugar food and beverage additives, and natural alternative to sugarcane sugar and artificial sweeteners. The leaves produce intensively sweet steviol glycosides (mainly stevioside and rebaudioside A). Stevia is an obligate short-day plant with a critical daylength of 13 h. Daylength in Malaysia which is less than 13 h causes stevia to flower early, resulting in a low leaf yield and a low percentage of sweetener content. In the first experiment, the effects of night interruption technique using incandescent, fluorescent cool day light (C.D.L.), fluorescent warm white (W.W.), light-emitting diode (LED) C.D.L. and LED W.W. for 60 min daily to lengthen vegetative phase, on plant biomass and steviol glycoside content of stevia were investigated. The study was carried out using a nested design with a 6 x 4 factorial arrangements of six light sources and four growing weeks after night interruption initiated (week 2, 4, 6 and 8) with four replications. Results showed that night interruption technique extended vegetative phase from 20 days (control) to 120 days; thus allowing accumulation of plant biomass and steviol glycosides content. Leaf biomass and steviol glycosides of all light sources treated plants increased significantly as compared to control plants especially in week 6 and 8. Fluorescent W.W. can be considered as the best light source for night interruption technique of stevia as total steviol glycosides content per plant increased by 190-270% as compared to other light sources. The second experiment was conducted to determine the effects of several drying methods on steviol glycosides content of stevia leaves. Six drying method treatments were arranged in a completely randomized design (CRD) with four replications. Stevioside content was not significantly affected by the drying of sun, air, oven and freeze at different temperature to obtain pre-determined moisture content (MC). However, rebaudioside A content was significantly reduced by 3.38% under oven drying at 70 ^{o}C to obtain 10% MC. The reduction indicated that thermal degradation of rebaudioside A occured at higher temperature. In conclusion, sun drying is the easiest and cheapest technique to dry stevia leaves without causing significant reduction of stevioside and rebaudioside A.

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

KESAN SUMBER CAHAYA DAN KAEDAH PENGERINGAN KEPADA PERTUMBUHAN POKOK DAN KANDUNGAN GLIKOSIDA STEVIOL STEVIA (*Stevia rebaudiana* Bertoni)

Oleh

MOHD AZIZ BIN RASHID

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Pengerusi : **Phebe Ding, PhD**
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Stevia (*Stevia rebaudiana* Bertoni) telah menerima banyak perhatian dengan pertambahan permintaan untuk makanan rendah gula dan bahan tambahan minuman, dan alternatif semulajadi kepada gula tebu dan pemanis tiruan. Daunnya menghasilkan glikosida steviol yang sangat manis (terutamanya steviosida dan rebaudiosida A). Stevia ialah tumbuhan hari pendek obligat dengan tempoh siang kritikal sebanyak 13 jam. Tempoh siang di Malaysia yang kurang dari 13 jam menyebabkan pokok stevia berbunga cepat, merendahkan hasil daun dan kandungan peratus pemanis yang rendah. Dalam eksperimen pertama, kesan teknik pecahan malam menggunakan lampu pijar, pendarfluor cool day light (C.D.L.), pendarfluor warm white (W.W.), diod pancaran cahaya (LED) C.D.L. and LED W.W. selama 60 minit pada setiap hari untuk memanjangkan fasa vegetatif, ke atas biomasa tumbuhan dan kandungan glikosida steviol telah dikaji. Kajian telah dijalankan menggunakan rekabentuk tersarang dengan 6 x 4 susunan faktorial iaitu enam sumber cahaya dan empat minggu pertumbuhan selepas pecahan malam bermula (minggu 2, 4, 6, dan 8) dengan empat replikasi. Keputusan menunjukkan teknik pecahan malam memanjangkan fasa vegetatif dari 20 hari (kawalan) kepada 120 hari; seterusnya membenarkan pengumpulan biomasa tumbuhan dan kandungan glikosida steviol. Biomasa daun dan glikosida steviol bagi pokok-pokok yang didedahkan dengan semua sumber cahaya menunjukkan peningkatan signifikan berbanding kawalan terutamanya pada minggu pertumbuhan ke 6 dan 8. Pendarfluor W.W. adalah sumber cahaya terbaik untuk teknik pecahan malam bagi stevia dengan peningkatan kandungan glikosida steviol sepokok sebanyak 190-270% berbanding sumber cahaya lain. Eksperimen kedua dijalankan untuk menentukan kesan beberapa kaedah pengeringan terhadap kandungan glikosida steviol daun stevia. Enam kaedah pengeringan disusun dalam rekabentuk penuh rawak (CRD) dengan empat replikasi. Kandungan steviosida tidak terjejas secara signifikan oleh pengeringan matahari, oven dan pembekuan pada suhu berbeza untuk mencapai kandungan kelembapan (MC) yang ditentukan lebih awal. Walau bagaimanapun, kandungan rebaudiosida A telah mengurang secara signifikan sebanyak 3.38% secara pengeringan oven pada 70 ^{o}C untuk mencapai 10% MC. Pengurangan ini menunjukkan degradasi

terma berlaku pada suhu tinggi. Kesimpulannya, pengeringan matahari adalah teknik paling mudah dan murah untuk mengeringkan daun stevia tanpa menyebabkan pengurangan signifikan kepada steviosida dan rebaudiosida A.

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I certify that a Thesis Examination Committee has met on 14 June 2017 to conduct the final examination of Mohd Aziz bin Rashid on his thesis entitled "Effects of Light Sources and Drying Methods on Plant Growth and Steviol Glycosides Content of Stevia (*Stevia rebaudiana* Bertoni)" 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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LIST OF ABBREVIATIONS

AGR	Absolute growth rate
ANOVA	Analysis of Variance
AOP	Antioxidant properties
C.D.L.	Cool day light
CRD	Completely Randomized Design
CV	Coefficient of variation
DMRT	Duncan's Multiple Range Test
ELSD	Evaporative Light Scattering Detector
FDA	Food and Drug Administration
Fm	Maximum fluorescence
Fo	Initial fluorescence
Fv	Variable fluorescence
GA	Gibberellins
GRAS	Generally Recognized as Safe
HPLC	High Performance Liquid Chromatography
IBA	Indole-3-butyric acid
LAI	Leaf Area Index
LAR	Leaf area ratio
LED	Light-emitting diode
LD	Long-day
LSD	Least Significant Differences
MARDI	Malaysian Agricultural Research and Development Institute
MC	Moisture content
NPK	Nitrogen, phosphorus and potassium

NS	Non-significant
P	Probability
PAR	Photosynthetically Active Radiation
Pfr	Phytochrome far red
ppm	parts per million
Pr	Phytochrome red
rpm	revolutions per minute
RT	Retention time
r	Pearson's correlation coefficients
SAS	Statistical Analysis System
S.E.	Standard error
SLA	Specific leaf area
SD	Short-day
TPC	Total phenolic content
U.S.A.	United States of America
UV	Ultra-violet
WHO	World Health Organization
W.W.	Warm white
µl	Microliter

CHAPTER 1

INTRODUCTION

1.1 Background

Stevia has received great attention with the rise in demand for low-carbohydrate, low-sugar food and beverage additives. Leaves of this plant produce a zero-calorie, non-nutritive, high potency sweetener with a sweetening value which is 150-450 times (by weight) higher than sucrose (Kinghorn, 1987). The sweeteners, containing steviol glycosides can be used as a natural alternative sweetener to other synthetic sweeteners such as aspartame, saccharine or acesulfame-K that constantly being associated with health concerns (Puri et al., 2011). Steviol glycosides are considered safe for consumption with no major contradictions, warnings and side effects reported (Ferri et al., 2006; Geuns, 2000).

Stevia was first introduced into Malaysia in the mid-1970's but failed to turn into a commercial crop mainly due to lack of suitable varieties, crop management and production technology (Ghawas et al., 2009). All variety of stevia introduced from other producing countries are photoperiod-sensitive. Daylength in Malaysia (which is less than 13 h) causes stevia to flower early, resulting in a low leaf yield per harvest and a low percentage of sweetener content. Early flowering also causes frequent harvesting and this leads to higher labour requirement and increases in production cost (Tan et al., 2008). The plants were harvested approximately every month (with lower leaf yield) as compared to 3-5 times annually in countries with longer daylength.

The use of classic incandescent lamps (rich in far-red light) as night interruption delayed the flowering and induced stevioside content of stevia (Zaidan et al., 1980) but it was considered not economical due to long hours of exposure required and less energy-efficiency. Commercial bulbs such as fluorescent lamps and light-emitting diode (LED) are more energy efficient, however its effect on plant biomass and flowering of stevia is still lacking. In this study, several types of commercial bulbs were used to impose night interruption in stevia plants and its effects to increase leaf biomass, delay flowering and induce steviol glycosides contents were determined.

Several drying methods are used to dry stevia leaves but the most preferable and practiced method is by sun drying (Samsudin and Aziz, 2013; Gates, 1997). However, sun drying will probably reduce the steviol glycosides content in the leaves. Abou-Arab et al. (2010) found that sun drying for 48 h caused 0.81% reduction of stevioside in stevia leaves. According to Steve Marsden of Herbal Advantage Inc., very little steviol glycoside will be lost if sun-drying for 8 h or less (Richard, 1996). However, proper experiment and scientific data are required to confirm the significance of steviol glycosides reduction by drying methods. The study on the effects of drying methods on steviol glycosides content is still lacking. In the second study, the effects of several drying methods on steviol glycosides content were determined.

1.2 Objectives

The objectives of the study were:

1. To determine the effects of different light sources as night interruption on vegetative and reproductive growth, and steviol glycosides content of stevia.
2. To determine the effects of several drying methods on steviol glycosides content of stevia.

1.3 Significance of study

The demand for stevia is growing due to increase cases of *Diabetes mellitus,* cardiovascular diseases, hypertension, obesity and other health problems among Malaysians, as well as concern over the safety of chemical sweeteners. Sweeteners from stevia plant are natural, zero-calorie, has a low glycemic index, can reduce blood sugar level and safe for consumption. However, the photoperiod-sensitive in stevia causes the plants to flower early that leads to low leaf yield and low sweetener content, thus preventing the commercial and large-scale production under local conditions. Previously, the study using light sources were merely to investigate the photoperiodism characteristics in stevia and not meant for agronomic purposes and technique for large scale production.

The technique of night interruption using commercial light sources in this study can be used to manipulate photoperiodism of stevia. The success of this technique will prevent early flowering, increase plant growth, leaf yield and sweetener content of stevia. Short duration of light exposure as night interruption will be cost effective and more environment friendly as compared to other technique such as day lengthening by continuous long hours of light exposure. Therefore, the findings of this study will give many benefits for local farmers and producers as well as researchers. The farmers and stevia producers can adopt this technique to overcome the agronomic problem and plant physiological challenges; thus will increase the production of stevia in Malaysia. The results from second study, which is the effects of drying methods on sweetener content, will benefit stevia producers in maintaining good quality of stevia at post harvest stage.

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