**UNIVERSITI PUTRA MALAYSIA**

# *GROWTH, YIELD AND PHYTOCHEMICAL CONTENTS OF SABAH SNAKE GRASS [Clinacanthus nutans (Burm.f.) Lindau] IN RELATION TO PLANT AGE, HARVESTING INTERVALS AND POTASSIUM APPLICATIONS*

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# GROWTH, YIELD AND PHYTOCHEMICAL CONTENTS OF SABAH SNAKE GRASS [*Clinacanthus nutans* (Burm.f.) Lindau] IN RELATION TO PLANT AGE, HARVESTING INTERVALS AND POTASSIUM APPLICATIONS

By

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

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**GROWTH, YIELD AND PHYTOCHEMICAL CONTENTS OF SABAH SNAKE GRASS [*Clinacanthus nutans* (Burm.f.) Lindau] IN RELATION TO PLANT AGE, HARVESTING INTERVALS AND POTASSIUM APPLICATIONS**

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**November 2017**

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*Clinacanthus nutans* or Sabah snake grass is a well reputed medicinal herb among locals which therapeutically used to cure various ailments and diseases for its phytochemicals and antioxidants. Various factors including cultural practices may contribute to the changes in growth and phytochemical contents, hence determine the quantity, quality and efficacy of an herb. Current studies focusing on the harvesting (age and intervals) and fertilizer (potassium) were undertaken evaluating growth, yield and phytochemical content of *C. nutans*. Plants were cultivated through stem cuttings and were harvested at three different age (week 8, week 12 and week 16 after transplanting) and three harvesting intervals (every 8, 12 and 16 weeks, for respective harvesting age) for three consecutive harvests (harvest 1, 2 and 3). After the completion of the first experiment, plants were then treated with five different rates of potassium fertilizer (0, 50, 100, 150, 200 and 250 kg $K_2O$/ha) using muriate of potash (MOP). The experiments were conducted based on Randomized complete block design (RCBD) with appropriate replications.

The results show a significant interaction between plant age and harvesting intervals observed in plant height, leaf area, fresh and dry weight of leaves, phenolic content and ferric reducing antioxidant potential activity (FRAP). The highest leaf fresh yield was recorded at the 16-week-old plants (144.13 g) during third harvesting intervals (154.32 g). The leaf to stem ratio, however decreased with plant age while increased with harvesting interval indicating an increment in stem proportion as the plant grow older. The phytochemical contents were also found to be increased with plant age giving the highest total flavonoid content (TFC) and total phenolic content (TPC) accumulated at 16-week-old plant of 7.32 mg GAE/g DW and 10.84 mg quercetin/g DW respectively. The TFC and TPC decreased following the repetitive harvesting

with the lowest recorded at first harvesting interval (TFC: 7.13 mg GAE/g DW, TPC: 10.30 mg quercetin/g DW). The antioxidant activities also significantly increased with plant age while decreased in a number of harvesting intervals. Of all identified compounds, shaftoside was found to be the most abundant in *C. nutans* at any levels of treatments. The order of C-glycosyl flavone compounds detected under different plant age and harvesting intervals based on concentration from high to low were as follows: shaftoside > iso-orientin > orientin > vitexin > iso-vitexin. In second experiment, all plant growth attributes and yield except for the number of branches were increased with increment of potassium rates. Referring to the trend analysis, the growth and development of *C. nutans* improved gradually with potassium in a positive and quadratic trend from 0 kg $K_2O$/ha to 200 kg $K_2O$/ha before decline slowly at 250 $K_2O$/ha in respective shoot fresh and dry weight, leaf gas exchange, phytochemical contents and antioxidant activities. The optimum rate and the maximum value, however, varied with the parameters.

All detected C-glycosyl flavone compounds were highest at 200 kg $K_2O$/ha given shaftoside as the major compound identified. These results obtained in both experiments indicates that the yield and phytochemical quality of *C. nutans* can be enhanced by regulating the cultural practices. Harvesting at week 12 with one-time interval and applying would be appropriate harvesting method as growth, yield and phytochemicals content of *C. nutans* were enhanced.An optimum potassium level of 154.75 kg $K_2O$/ha could be the appropriate K application for the cultivation of *C. nutans* as yield were enhanced and 143 kg $K_2O$/ha of muriate of potash would be the appropriate optimum K rate that would improve the production of phytochemical content in *C. nutans*.

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

**PERTUMBUHAN, HASIL TANAMAN DAN KANDUNGAN FITOKIMIA BELALAI GAJAH [*Clinacanthus nutans* (Burm.f.) Lindau] BERHUBUNG DENGAN UMUR POKOK, SELANGAN PENUAIAN DAN PEMBAJAAN POTASSIUM**

Oleh

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*Clinacanthus nutans* atau Belalai gajah adalah tanaman herba yang dikenali di antara penduduk tempatan dalam mengubati pelbagai penyakit oleh kerana adanya fitokimia dan antioksida. Pelbagai faktor termasuk amalan agronomi mungkin menyumbang kepada perubahan pertumbuhan dan kandungan fitokimia yang menentukan kuantiti, kualiti dan keberkesanan sesuatu tanaman herba. Kajian terkini yang menumpukan kepada penuaian (umur dan selang) dan pembajaan (potassium) telah dijalankan bagi menilai pertumbuhan, hasil tanaman dan kandungan fitokimia dalam Belalai gajah. Pokok telah ditanam menggunakan keratan batang dan telah dituai pada tiga umur yang berbeza (minggu 8, minggu 12 dan minggu 16 setelah transplant) dan tiga selang penuaian (setiap 8, 12 dan 16 minggu untuk umur masing-masing) untuk tiga kali penuaian berturut-turut (tuai 1, 2 dan 3). Setelah eksperimen pertama telah lengkap, pokok kemudiannya dibaja dengan lima kadar baja potassium yang berbeza (0, 50, 100, 150, 200 and 250 kg $K_2O$/ha) menggunakan muriate of potash (MOP). Eksperimen ini dijalankan dengan menggunakan reka bentuk blok lengkap rawak dengan replikasi yang sesuai.

Hasil kajian mendapati terdapat interaksi yang ketara di antara umur pokok dan selang penuaian pada tinggi pokok, luas daun, berat daun segar dan kering, kandungan fenolik dan aktiviti FRAP. Hasil berat daun yang segar telah direkodkan pada umur minggu ke-16 (144.13 g) pada selang penuaian yang ketiga (154.32 g). Walau bagaimanapun, nisbah daun kepada batang menurun mengikut umur pokok manakala ianya meningkat mengikut selang penuaian yang menunjukkan bahawa batang bertambah banyak apabila pokok semakin tua. Kandungan fitokimia juga didapati meningkat mengikut umur pokok dengan jumlah kandungan flavonoid (TFC) dan

jumlah kandungan fenolik (TPC) tertinggi terkumpul pada umur pokok ke-16 minggu dengan masing-masing 7.32 mg GAE/g DW dan 10.84 mg quercetin/g DW. TFC dan TPC menurun mengikut pengulangan penuaian dengan kadar terendah direkodkan pada selang penuaian pertama (TFC: 7.13 mg GAE/DW, TPC: 10.30 mg quercetin/DW). Aktiviti antioksida juga meningkat mengikut umur pokok dan menurun apabila kadar penuaian ditingkatkan. Diantara semua kandungan bioaktif, shaftoside banyak didapati dalam *C. nutans* pada mana-mana rawatan. Turutan C-glycosyl flavone pada umur pokok dan selang penuaian berdasarkan pada konsentrasi yang tinggi kepada rendah adalah yang tertera: shaftoside > iso-orientin > orientin > vitexin > iso-vitexin. Dalam kajian yang kedua, semua pertumbuhan dan hasil pokok kecuali bilangan cabang telah meningkat mengikut kadar pembajaan potassium. Berdasarkan kepada trend analisa, pertumbuhan dan perkembangan *C. nutans* meningkat mengikut kadar pembajaan potassium secara positif dan kuadratik bermula daripada 0 kg $K_2O$/ha kepada 200 kg $K_2O$/ha sebelum menurun secara perlahan pada 250 kg $K_2O$/ha yang dapat dilihat masing-masing pada berat basah dan kering tanaman, penukaran gas daun, kandungan fitokimia dan aktiviti antioksida. Akan tetapi, kadar pembajaan yang optima and maksimum adalah berbeza mengikut parameter.

Tambahan pula, semua kandungan C-glycosyl flavon tertinggi direkodkan pada 200 kg $K_2O$/ha dengan shaftoside sebagai konstitusi yang utama. Semua hasil kajian pada kedua-dua eksperimen menunjukkan bahawa hasil tanaman dan fetokimia *C. nutans* boleh ditingkatkan dengan memperbaiki amalan agronomi. Penuaian pada umur minggu ke-12 dengan sekali selang penuaian adalah teknik penuaian yang sesuai kerana pertumbuhan, hasil tanaman serta kandungan fitokimia meningkat pada tanaman *C. nutans*. Pembajaan potassium yang optima pada 154.75 kg $K_2O$/ha adalah pembajaan yang sesuai bagi penanaman *C. nutans* kerana hasil tanaman meningkat dan 143 kg $K_2O$/ha adalah kadar pembajaan potassium yang optima dalam meningkatkan kandungan fitokimia dalam *C. nutans*.

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I certify that a Thesis Examination Committee has met on 20 November 2017 to conduct the final examination of Nur Mardhiati Afifa Abd Samat on her thesis entitled "Growth, Yield and Phytochemical Contents of Sabah Snake Grass [*Clinacanthus nutans* (Burm.f.) Lindau] in Relation to Plant Age, Harvesting Intervals and Potassium Applications" 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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This thesis was submitted to the Senate of the 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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| | |
|---|---|
| cm | Centimetre |
| ROS | Reactive oxygen species |
| AAPH | 2,2'-azobis (2-amidinopropane) dihydrochloride |
| DPPH | α, α-diphenyl-β-picrylhydrazyl |
| ABTS | 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) |
| TLR | Toll-like receptor |
| % | Percentage |
| VZV | Varicella-zoster virus |
| HSV | Herpes simplex virus |
| ALS | Automatic liquid sampler |
| UHPLC | Ultra-high pressure liquid chromatography |
| HPLC | High pressure liquid chromatography |
| GC | Gas chromatography |
| GA | Gibberellic acid |
| kg | Kilogram |
| ha | Hectare |
| $K^+$ | Potassium ion |
| K, $K_2O$ | Potassium |
| mmol | Milimoles |
| L | Liter |
| $CO_2$ | Carbon dioxide |
| PAL | Phenylalanine ammonia-lyase |
| µg | microgram |

| | |
|---|---|
| G | gram |
| CEC | Cation exchange capacity |
| N | Nitrogen |
| P, $P_2O_5$ | Phosphorus |
| Ca | Calcium |
| Mg | Magnesium |
| Cu | Copper |
| Fe | Iron |
| Mn | Manganese |
| Zn | Zinc |
| TSP | Triple superphosphate |
| MOP | Muriate of potash |
| mm | Millimeter |
| $^oC$ | Celcius |
| $Na_2CO_3$ | Sodium carbonate |
| UV | Ultraviolet visible |
| GAE | Gallic acid equivalent |
| Nm | Nanometer |
| $NaNO_3$ | Sodium nitrite |
| $AlCl_3$ | Aluminium chloride |
| NaOH | Sodium hydroxide |
| mg | Milligram |
| mM | Millimolar |
| TPTZ | 2,4,6- tri [ 2-pyridyl]-s- triazine |
| $FeCl_3$ | Iron chloride |

| | |
|---|---|
| µM | Micromole |
| µL | Microlitre |
| $Cm^2$ | Square centimeter |
| DW | Dry weight |
| FRAP | Ferric reducing antioxidant power |
| mmol $m^{-2}$ $s^{-1}$ | Millimole meter square per second |
| µmol $m^{-2}$ $s^{-1}$ | Micromole meter square per second |

# CHAPTER 1

# INTRODUCTION

As herbal consist of a valuable source of bioactive compounds, it has been contributed significantly to human welfares which widely utilized across the world of nutraceuticals either traditionally, complementary and alternatively. More than 80% of the world population especially in developing countries has been appraised to rely predominantly on herbal remedies to fulfil healthcare necessity (WHO, 2002) and such medicines are acquired mostly from plant extracts. These plant extracts are known to be rich with phytochemicals which appear to be the antioxidant nature of aromatic phenolic acids and flavonoids structures. Flavonoids, as the largest family having low molecular structure of phenolic secondary metabolites are well known to be associated with great length of pharmacological activities which widely spread throughout the natural plants (Saxena et al., 2013). These compounds are receiving considerable attention due to documented protective role against many diseases which may attributed to the biological effects of antioxidant activity against reactive oxygen species (Schijlen et al., 2004).

Until today, many intensive research and extensive studies are still carried out to exploit plants capabilities as alternative regiments against various ailments and diseases. To date, around 21,000 plant species have been identified to have medicinal potency and the number is still increasing (WHO, 2009). *Clinacanthus nutans* or commonly known as sabah snake grass has been recognized as one of the potent medicinal plants and highly reported to have a broad spectrum of pharmacological properties such as anti-inflammatory (Shyuprom, 2004), antioxidant (Wanakiat et al., 2007) and antiviral against (herpes simplex virus (HSV), varicalle-zoster virus, papillomavirus, and dengue virus) (Kunson et al., 2013). Native to tropical Asia countries, this herb is well reputed in Thai folklore medicine due to its ability to cure ailments such as skin inflammation, snake and insect bites. In Malaysia, *C. nutans* has attracted public interest recently for its high medicinal properties specifically as anticancer regimens due to the presence of natural antioxidant and anti-proliferative properties (Yong et al., 2013). Owing to the fact that this herb give significant economic benefits towards human health, the demand of this herb has been increasing in recent years.

Although these constituents offer many substantial effect in human well-being, the polyphenolic compounds attributed the biological activities may often modulated by various factors which includes cultivars, genotype, environmental condition such as light intensity, relative humidity and cultural practices. In all of these factors, cultural techniques considered to be the highly applicable long-term alternative practices which provide ample favourable circumstances to overcome difficulties inherited by medicinal plant including manipulating the genetic and phenotypic variation of polyphenolic compounds, contamination and toxicity of constituents as well as misidentification (Canter et al., 2005). Through a proper and controlled growing

condition, plant growth development may be enhanced and largen the biomass production with high and polyphenolic content consistency can be optimized (Mathe & Mathe, 2008). Considering these significant advantages, cultural techniques can be used as an alternative means to produce high quality herbage yield. These conditions cover plant age, harvesting interval, soil composition and fertilizer applications.

Fertilization or plant nutrient availability in particular, has been highlighted in countless number of studies to give a profound effect in plant performances as well as regulating the secondary metabolites and antioxidant activity within plants (Prange & Dell, 1997; Mudau, 2007; Strik, 2008; and Ibrahim et al., 2012). Potassium as one of the macronutrients plays a vital determinant for yield and quality in many crops (Cassman et al., 1990; Pettigrew, 2008 and Cakmak, 2010). In physiological aspect, potassium responsible in promoting the photosynthesis process and important mineral in phloem transportation and osmotic regulation (Ghazemsadeh et al., 2012). The development of photosynthesis process will improve the accumulation of primary metabolites (starch and soluble sugar) which then be devoted to secondary metabolites of aromatic phenolics and flavonoids. It has been found by Ibrahim et al. (2012) whose stated that enhancement of phytochemicals typically accompanied by an increment of potassium supplementation. Similar occurrence has been found related to plant maturity where upsurges in polyphenolic compounds and antioxidant activity is plant age and harvesting interval dependent (Ghazemsadeh et al., 2012 and Hue et al., 2012). The dynamic changes in secondary metabolites may be attributed to the levels of certain enzymes which are present in plants. As such, plant's progression and productivity have been seen to be vary with respect to plant maturity, harvesting intervals and fertilizer application.

Most of reported studies previously on *C. nutans* are more emphasized on phytochemistry, biological aspect and pharmaceutical quality but relatively few studies covering the agronomic attribution. Studies performed with different levels of nitrogen fertilization, plant density, and light intensity particularly on *C. nutans* are well demonstrated yet no documentation of plant development, yield and phytochemical responses in various plant maturity, harvesting intervals and fertilization, especially potassium, have been recorded (Khaulah, 2014 & Nasiri, 2016). It is desirable to gather relevant information through cultivation practices specifically in harvesting and fertilizer management as alternative ways to intensify the production while sustaining the medicinal potency of the plants. Therefore, current studies were undertaken to evaluate the effect of harvest age, harvest intervals and potassium on growth, yield and phytochemicals of *C. nutans*.

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