COMPARISON OF CHICKEN MANURE RATE, PLANTING DENSITY AND PLANT SUPPORT SYSTEM ON GROWTH PERFORMANCE AND PHYTOCHEMICAL COMPOUNDS OF *Gynura procumbens* (Lour.) Merr.

By

WAN HUDA DINIE BINTI WAN MAJID

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

August 2021

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Specially dedicated to my beloved mother, Sarifah binti Husain, my late father, Wan Majid bin Wan Harun and my lovely husband, Mohammad Hanafi Alias.
Mama, I love you..
Abah, I miss you..
Abang, I love you to the moon and back..

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

COMPARISON OF CHICKEN MANURE RATE, PLANTING DENSITY AND PLANT SUPPORT SYSTEM ON GROWTH PERFORMANCE AND PHYTOCHEMICAL COMPOUNDS OF Gynura procumbens (Lour.) Merr.

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WAN HUDA DINIE BINTI WAN MAJID

August 2021

Chair : Martini Mohammad Yusoff, PhD
Faculty : Agriculture

Gynura procumbens is a herbal medicinal plant that has a range of benefits for medical purposes. Traditionally, it is used for the treatment of several diseases inflicting humans and contains high phenolic compounds such as myricetin and kaempferol derivatives. Agronomic practices have been known to affect greatly on the composition of the phytochemical compounds in plants. Hence, this study was conducted to evaluate the effects of different rates of chicken manure, leaf maturity, planting density and requirement of plant support system on growth, physiological characteristics, total dry matter yield and phytochemical compounds of *G. procumbens*. In Experiment 1, *G. procumbens* plants was grown with five different rates of chicken manure; 0, 100, 200, 300 and 400 kg ha^{-1}. Harvested leaves were divided into matured and young leaves according to the leaf maturity for phytochemical analyses. Plants receiving 300 and 400 kg ha^{-1} of chicken manure recorded the highest growth, crop growth rate, leaf area index and total dry matter yield. Total phenolic and total flavonoids content also DPPH activity were significantly affected by the interaction of different rates of chicken manure and leaf maturity. Total phenolic content in mature leaves significantly decreased with increasing rate of chicken manure. Young leaves recorded increased total phenolic content up to the optimum level and decreased with the application of more than 200 kg ha^{-1} of chicken manure. Total flavonoids content decreased in mature leaves while young leaves recorded the highest total flavonoids content with 100 and 200 kg ha^{-1}. DPPH activity in young leaves from plants treated with 100, 200 and 300 kg ha^{-1} of chicken manure were high. Kaempferol-3-rutinoside and kaempferol-3-glucoside contents were also significantly affected by the interaction between different application rate of chicken manure and leaf maturity. By increasing the rate of chicken manure, young leaves tended to show a declined trend in both compounds. Considering the effects of chicken manure rate and leaf maturity on growth performance, physiological characteristics, total dry matter production and phytochemical content, 300 kg ha^{-1} chicken manure rate is selected

as the suitable fertilizer rate for cultivation of *G. procumbens.* Experiment 2 was conducted to determine the suitable planting density of *G. procumbens* when grown with or without plant support. Plants was grown with two planting densities: 15 plants m^{-2} and 25 plants m^{-2} and with and without plant support. Hortonova trellis netting is the type of plant support and has been applied in the second experiment. Plant growth, physiological characteristics, total dry matter yield and phytochemical content were determined based on the selected fertilizer rate (300 kg ha^{-1}) from the first experiment. Planting density of 15 plants m^{-2} resulted in higher plant growth and total dry matter production per plant. However, leaf area index, crop growth rate and phytochemical content produced were higher on 25 plants m^{-2} planting density in comparison with 15 plants m^{-2}. Plants grown with and without plant support system on the other hand revealed no significant effect for each of the measured variables. Based on the results from both experiments, optimum growth performance, physiological characteristics, total dry matter production and phytochemical content of *G. procumbens* could be obtained with an application of 300 kg ha^{-1} of chicken manure with 25 plants m^{-2} planting density.

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

PERBEZAAN KADAR BAJA TAHI AYAM, KEPADATAN TANAMAN DAN SISTEM SOKONGAN TUMBUHAN TERHADAP PRESTASI PERTUMBUHAN DAN KANDUNGAN FITOKIMIA *Gynura procumbens* (Lour.) Merr.

Oleh

WAN HUDA DINIE BINTI WAN MAJID

Ogos 2021

Pengerusi : Martini Mohammad Yusoff, PhD
Fakulti : Pertanian

Gynura procumbens merupakan tumbuhan herba yang mempunyai perbagai manfaat bagi tujuan perubatan. Secara tradisional, tumbuhan ini digunakan untuk mengubati masalah kesihatan dan mengandungi kandungan fitokimia yang tinggi seperti myricetin dan kaempferol. Amalan pertanian telah terbukti mempengaruhi kadar penghasilan kualiti dan kuantiti kandungan metabolit di dalam tumbuhan. Oleh itu, kajian ini telah dijalankan bagi menilai kesan kadar baja tahi ayam yang berbeza, kematangan daun, kepadatan tanaman, dan keperluan sistem sokongan tumbuhan terhadap pertumbuhan tumbuhan, ciri-ciri fisiologi, jumlah hasil kering dan kandungan fitokimia di dalam *Gynura procumbens*. Di dalam kajian 1, tumbuhan telah ditanam dengan lima kadar baja tahi ayam yang berbeza; 0, 100, 200 300 dan 400 kg ha^{-1}. Daun yang dituai dibahagaikan kepada dua bahagian kematangan daun iaitu daun muda dan daun matang. Pokok-pokok yang menerima kadar baja 300 dan 400 kg ha^{-1} telah merekodkan kesan yang tinggi terhadap pertumbuhan pokok, kadar pertumbuhan pokok, indeks keluasan daun dan jumlah hasil kering. Jumlah kandungan fenolik dan flavonoid dan aktiviti DPPH di dalam daun terkesan dengan ketara terhadap interaksi antara kadar baja tahi ayam dan kematangan daun. Kandungan fenolik di dalam daun matang menunjukkan penurunan kandungan fenolik yang ketara apabila kadar baja meningkat. Manakala daun muda pula merekodkan peningkatan sehingga tahap optimum dan berkurangan selepas penggunaan baja melebihi 200 kg ha^{-1}. Jumlah kandungan fenolik pula menunjukkan tren penurunan pada daun matang manakala daun muda pula merekodkan jumlah kandungan fenolik yang tinggi pada kadar baja 100 dan 200 kg ha^{-1}. Aktiviti DPPH di dalam daun muda mencatatkan nilai yang tinggi pada pokok yang diberi kadar baja 100, 200 dan 300 kg ha^{-1}. Kandungan kaempferol-3-rutinoside dan kampferol-3-glucoside turut terkesan secara ketara oleh gabungan antara kadar baja organik dan kematangan daun. Dengan peningkatan kadar baja organik, daun muda cenderung untuk menunjukkan penurunan bagi kedua-dua kompoun. Dengan mengambilkira kesan kadar baja tahi ayam dan kematangan daun pertumbuhan tanaman, ciri-ciri fisiologi, jumlah hasil kering dan kandungan fitokimia, kadar baja 300 kg ha^{-1} dipilih sebagai kadar baja yang sesuai bagi

penanaman *Gynura procumbens*. Kajian kedua pula dijalankan bagi menentukan kepadatan tanaman yang sesuai bagi *Gynura procumbens* apabila ditanam dengan atau tanpa sistem sokongan tumbuhan. Pokok ditanam dengan dua kepadatan tanaman yang berbeza bersamaan 15 dan 25 pokok m^{-2} dengan dan tanpa sistem sokongan tumbuhan. Jaring teralis Hortonova merupakan jenis sistem sokongan tumbuhan yang telah digunakan dalam eksperimen kedua. Pertumbuhan pokok, ciri-ciri fisiologi, jumlah hasil kering dan kandungan fitokimia ditentukan berdasarkan kadar baja yang terpilih (300 kg ha^{-1}) daripada Kajian 1. Kepadatan tanaman sebanyak 15 pokok m^{-2} menghasilkan pertumbuhan pokok yang tinggi dan jumlah hasil kering per pokok. Keputusan menunjukkan kepadatan tanaman 15 pokok m^{-2} menghasilkan kadar pertumbuhan pokok serta pengeluaran hasil kering per pokok yang tinggi. Walaubagaimanapun, kepadatan tumbuhan 25 pokok m^{-2} menghasilkan indeks keluasan daun dan kandungan fitokimia yang tinggi berbanding kepadatan tanaman 15 pokok m^{-2}. Pokok-pokok yang ditanam dengan atau tanpa menggunakan sistem sokongan telah menunjukkan tidak ada kesan yang ketara bagi setiap parameter yang dicatatkan. Kesimpulan daripada kedua-dua kajian menunjukkan kadar pertumbuhan pokok, ciri-ciri fisiologi, jumlah hasil kering dan kandungan fitokimia *Gynura procumbens* yang optima boleh diperolehi dengan menggunakan kadar baja organik sebanyak 300 kg ha^{-1} dengan kepadatan tanaman 25 pokok $m^{-2.}$

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

Martini Mohammad Yusoff, PhD
Senior Lecturer
Faculty of Agriculture
Universiti Putra Malaysia
(Chairman)

Azizah Misran
Senior Lecturer
Faculty of Agriculture
Universiti Putra Malaysia
(Member)

_____
ZALILAH MOHD SHARIF, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 09 March 2023

Declaration by the Graduate Student

I hereby confirm that:

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Signature: _____
Name of Chairman of Supervisory Committee: Martini Mohammad Yusoff

Signature: _____
Name of Member of Supervisory Committee: Azizah Misran

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ETP	Economic Transformation Programme
NKEA	National Key Economic Area
m	Meter
cm	Centimeter
NH3	Ammonia
GSK-3 β	Glycogen synthase kinase-3β
CO^2	Carbon dioxide
N	Nitrogen
P	Phosphorus
K	Potassium
Mg	Magnesium
Ca	Calcium
kg	Kilogram
kg ha^{-1}	Kilogram per hectare
EFB	Empty fruit bunch
Cu	Copper
Pb	Lead
Zn	Zinc
Cd	Cadmium
CEC	Cation exchange capacity
^oC	Degree Celsius
PPFD	Photosynthetic photon flux density
LAI	Leaf area index
m^2	Meter square

MWC	Mid-wire cordon
UK	Umbrella kniffin
TNF-α	Tumor necrosis factor-alpha
ROS	Reactive oxygen species
DPPH	2,2-diphenyl-1-pierylhydrazyl
FRAP	Ferric reducing antioxidant power
mm	Millimeter
kg ha^{-1}	Kilogram per hectare
RCBD	Randomized complete block design
H_2SO_4	Sulphuric acid
H_2O_2	Hydrogen peroxide
ml	Milliliter
AAS	Atomic absorption spectrophotometer
TPC	Total phenolic content
TFC	Total flavonoids content
v v^{-1}	Volume per volume
w v^{-1}	Weight per volume
µl	Microliter
nm	Nanometer
$NaNO_2$	Sodium nitrite
$AlCl_3$	Aluminium chloride solution
M	Molar
NaOH	Sodium hydroxide
mM	Millimolar
TPTZ	2,4,6-tri[2-pyridyl]-s-triazine
$FeSO_4.7H_2O$	Ferrous sulphate heptahydrate

HPLC	High performance liquid chromatography
min	Minutes
ANOVA	Analysis of variance
LSD	Least significant difference
kg ha^{-1}	Kilogram per hectare
g $week^{-1}$	Gram per week
DW	Dry weight
GAE	Gallic acid equivalent
QUE	Quercetin equivalent
K-3-R	Kaempferol-3-rutinoside
K-3-G	Kaempferol-3-glucoside
mg	Milligram
g $plant^{-1}$	Gram per plant
PAR	Photosynthetically active radiation
RUE	Radiation use efficiency
CGR	Crop growth rate

CHAPTER 1

INTRODUCTION

Herbal industry contributes hugely to the economic development of the country. Under the Economic Transformation Programme (ETP) of National Key Economic Area (NKEA) Entry Point Project 1 (EPP1), Malaysia has initiated research and development strategies of high value herbal products. The ETP is under the governance of Ministry of Agriculture and Food Industries Malaysia (Ahmad et al., 2015). There are 15 herbs which have been selected to be included in the focused list of herbs to be researched where *Gynura procumbens* is listed as one of the important herbs in NKEA. The extract of *G. procumbens* contains several important phenolic compounds which have been shown to have potential to be developed as target drugs with beneficial properties for medical purposes (Afandi, 2014).

The phytochemical studies in *G. procumbens* have shown that the plant species contains phenolic compounds such as phenols, chlorogenic acid, rutin, astragalin and kaempferol-3-o-rutinoside (Kaewseejan & Siriamornpun, 2015; Kaewseejan et al., 2015; Rosidah et al., 2009; Rosidah et al., 2008). According to the previous studies, most of research on *G. procumbens* had only focused on its content of bioactive compounds (Kaewseejan & Siriamornpun, 2015; Kaewseejan et al., 2015; Rahman & Asad, 2013; Rosidah et al., 2008). However, none of the research has been conducted on many aspects related to the suitable agronomic practices to ensure high production of bioactive compounds. It was reported that the cultivation aspect requires a specific standard of practices or conditions such as nutrient sources to produce high quantity of bioactive compounds. Not only the source of nutrient, determination of planting distance as well as the suitable support system for *G. procumbens* are also crucial to reach the target yield.

Choosing a suitable nitrogen in fertilizer rate is most crucial elements in plants. Suitable amount of nitrogen influence each stage of plant growth and markedly affects the amount of photosynthesis activity (Zhou et al., 2011). Plants were metabolized nitrogen in two ionic forms NH_4^+ and NO_3^- which affect in plant growth development as well as nutritional quality (Olfati et al., 2012; Zhou et al., 2011). Nitrogen also is one of great importance as it influences both the primary and secondary metabolic pathways, thus aiding in accumulation of plant secondary metabolites (Chen et al., 2011). Deficiency of nitrogen, was found to enhance the accumulation of secondary metabolites in the plant tissues (Munene et al., 2017) while in contrast, chlorophyll content in leaves and plant biomass accumulation were dramatically restricted (Salahas et al., 2011). However, these claims were contended by Esther (2016) and Ibrahim et al. (2013) who indicated that the accumulation of plant secondary metabolites in herbs was higher under optimum level of application of nitrogen. These studies showed the inconsistent results on phytochemical and antioxidant activities of herbal species. Suitable nitrogen fertilizer rates are crucial in promoting the most favorable plant growth and yield of health promoting compounds and thus, it is important to determine the suitable fertilizer rate particularly in planting herbs.

Besides determination of suitable fertilizer rates, other agronomic practices such as implementation of suitable planting density are also crucial to be determined. Production of crop produced in any farming system needs to be conducted with an appropriate planting density of the crop besides other factors such as plant support system to ensure production of high plant biomass and secondary metabolites. Liu et al. (2011) reported that planting pattern including plant spacing affected the physiological characteristics and canopy structure of plant. In addition, Mahmood et al. (2014) reported that maximum crop yield of plants could be achieved by having optimum leaf area index (LAI) which was affected by planting density. Furthermore, studies have been done on the impact of plant spacing on chemical constituents of *Dracocephalum moldavica* which indicated increasing essential oil production with decreasing plant spacing in first planting season. However, in second planting season, it shown that the percentage of essential oil production decreased with close plant spacing (Hussein et al., 2006).

To produce maximum biomass yield with high amount of phytochemical contents in *G. procumbens*, there is a need to identify the suitability of application of plant support with combination of suitable planting density. Recently, most of the farmers had planted *G. procumbens* without the application of plant support system even though this plant has scandent growth habit (Flora and Fauna, 2020). As reported by Jovicich et al. (2004), optimum marketable yield of plants per unit area can be a consequence of plant canopy structure created by a combination of planting density and plant support system. The plant canopy structure as affected by different plant support systems may have different efficiencies in light interception which may affect the quantity and quality of plant production (Jovicich et al., 2004). Yusoff (2006) conducted a study to determine the effects of plant support system and intercropping of dragon fruit and long bean and reported that no significant effect of plant support system on vegetative stage of dragon fruit.

To date, metabolic activities and phytochemical content of the *G. procumbens* as affected by fertilizer application, planting density and plant support system are less known. Thus, understanding their response to agronomic practices is desirable for the commercial cultivation of *G. procumbens* and downstream processing of its down-stream products of by growing this herbal plant with high phytochemical contents. Therefore, the present study was conducted with the following objectives:

1. To investigate the effect of different rates of chicken manure on growth performance, physiological characteristics and total dry matter production of *G. procumbens.*
2. To measure the phytochemical compounds of *G. procumbens* as affected by different rates of chicken manure and leaf maturity.
3. To determine the effect of different planting densities and application of plant support system on growth performances, total dry matter productions, and phytochemical compounds of *G. procumbens* grown with suitable rate of chicken manure.

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