EARLY GROWTH, ESSENTIAL OIL AND ZERUMBONE CONCENTRATION OF *Zingiber zerumbet* (L.) SMITH AS AFFECTED BY DIFFERENT TYPES OF ORGANIC FERTILIZERS AND HARVESTING TIMES

BY

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A thesis submitted in fulfilment of the requirement for the degree of Master of Science (Biosciences)

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JUNE 2021

ABSTRACT

Zingiber zerumbet is a perennial tuberous herbal plant that has been traditionally used for medical and culinary flavor for centuries. Recently, the zerumbone compound in *Zingiber* oil has been intensively studied in the pharmacological field. The Malaysian government has encouraged local entrepreneurs to explore and commercialise herbal-based products derived from native herbal plants, including *Zingiber zerumbet*. Large-scale cultivation is required to provide a continuous supply of herbal plant derivatives for industrial purposes. However, little is known about *Zingiber zerumbet* cultivation. As herbal plants are utilized for health and nutrition, they must be cultivated employing good agricultural practices to assure purity, quality, and maximum quantity of bioactive compounds. Accurate harvesting time is also critical, as it impacts the amount of essential oil and its constituents. As a result, the purpose of this research was to investigate the effects of various types of organic fertilizers and harvesting times on the early stage of growth, essential oil, and zerumbone concentrations of *Zingiber zerumbet* (L.) Smith. Bat guano, goat manure, and quail litter were the organic fertilizers researched. The nine treatments were arranged in a split-plot design with five replications. The morphological growth parameters were regularly measured every two weeks. The plants were harvested for destructive data at 90, 120 and 150 DAT. The essential oil was extracted from *Zingiber zerumbet* rhizomes by means of Supercritical-Fluid-Extraction (SFE) and analysed by Gas Chromatography-Mass Spectrometry (GC-MS) and Gas Chromatography coupled with Flame Ionization Detection (GC-FID). Data were analysed by using SAS version 9.4 package. The mean values of the growth parameters and yield components of *Zingiber zerumbet* (L.) Smith were compared between treatments. The Duncan new multiple range test (α =0.05) was used for mean comparison. Based on this study, there were significant effect of different types of organic fertilizers and harvesting times on early growth parameter, essential oil and zerumbone concentration of *Zingiber zerumbet* (L.) Smith. Quail litter (F3) was significantly produced the plants with the maximum height, number of leaves and tillers and produced the highest fresh and dry weight of root and rhizome. Besides, T3 (150 DAT) gave the highest mean value for all parameters measured. Quail litter produced the highest percentage of essential oil, while plants treated with quail litter and goat manure had the highest percentage of zerumbone. Therefore, a combination of quail litter and 150 DAT was recommended in the cultivation of *Zingiber zerumbet* plants.

خلاصة البحث

نبات *Zingiber zerumbet* هي نبات عشبية درنية معمرة تستخدم تقليديًا للنكهة الطبية والطهي لعدة قرون. وتمت دراسة مركب zerumbone في زيت Zingiber بشكل مكثف في المجال الدوائي مؤخرا. شجعت الحكومة الماليزية رواد الأعمال المحليين على استكشاف وتسويق المنتجات العشبية المشتقة من النباتات العشبية المحلية، وبما في ذلك *Zingiber zerumbet*. الزراعة على نطاق واسع مطلوبة لتوفير إمدادات مستمرة من مشتقات النباتات العشبية للأغراض الصناعية. ومع ذلك، لا يُعرف الكثير عن زراعة *Zingiber zerumbet*. ويجب زراعة النباتات العشبية باستخدام الممارسات الزراعية الجيدة لضمان النقاء والجودة والكمية القصوى من المركبات النشطة بيولوجيًا، حيث يتم استخدامها للصحة والتغذية. ويعد وقت الحصاد الدقيق أمرًا بالغ الأهمية أيضًا لأنه يؤثر على كمية الزيوت العطرية ومكوناتها. إذن، الهدف من هذا البحث هو دراسة تأثير أنواع مختلفة من الأسمدة العضوية وأوقات الحصاد على المرحلة المبكرة من النمو والزيوت العطرية وتركيزات الزيرومبون من *Zingiber zerumbet (L.) Smith*. وكانت ذرق الخفافيش وروث الماعز وفضلات السمان هن الأسمدة العضوية التي تم البحث عنها. ورتبت العلاجات تسعة في تصميم انقسام مؤامرة مع خمسة مكررات، وتم قياس معايير النمو المورفولوجية بانتظام مرة كل أسبوعين. وقد تم حصاد النباتات لبيانات المدمرة في 90 و 120 و 150 DAT ثم تم استخراج الزيت العطري من جذور *Zingiber zerumbet* عن طريق استخراج السوائل فوق الحرجة (SFE) وتحليله بواسطة كروماتوجرافيا الغاز – مطياف الكتلة (GC–MS) وكروماتوجرافيا الغاز إلى جانب اكتشاف تأين اللهب (GC–FID)، وتم تحليل البيانات باستخدام حزمة الإصدار 9.4 من SAS. تمت مقارنة القيم المتوسطة لمعاملات النمو ومكونات المحصول لمركب *Zingiber zerumbet (L.) Smith* بين المعاملات ويستخدم اختبار Duncan الجديد متعدد المدى ($\alpha = 0.05$) للمقارنة المتوسطة. بناءً على هذه الدراسة، كان هناك تأثير معنوي لأنواع مختلفة من الأسمدة العضوية وأوقات الحصاد على معامل النمو المبكر والزيت العطري وتركيز الزيرومبون من *Zingiber zerumbet (L.) Smith*. وأنتجت السمان القمامة (F3) كثيرا من النباتات مع ارتفاع الحد الأقصى، عدد الأوراق والفلاحين وتنتج أعلى الطازجة والوزن الجاف للجذور وجذمور. إلى جانب ذلك، أعطى T3 150) (DAT أعلى قيمة متوسطة لجميع المعلمات المقاسة. وأنتجت فضلات السمان أعلى نسبة من الزيوت العطرية بينما سجلت النباتات المعالجة بفضلات السمان وروث الماعز أعلى نسبة من الزيرومبون. في الختام ، تمت التوصية بمزيج من فضلات السمان و 150 DAT في زراعة نباتات زنجبير زيرومبيت.

APPROVAL PAGE

I certify that I have supervised and read this study and that in my opinion, it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a thesis for the degree of Master of Science (Biosciences).

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ACKNOWLEDGEMENTS

Alhamdulillah, all praises be to Allah S. W. T., this thesis has been completed entirely with ease. First and foremost, I would like to convey my utmost infinite thanks to both of my supervisor and co-supervisor – Dr. Rozilawati Shahari and Assoc. Prof. Dr. Deny Susanti Darnis. A highest gratitude for all the commitments, time and knowledge shared and invested. Despite their commitments, they took time to listen and attend to me whenever requested. I feel indebted for all your kindness and help. May Allah S.W.T. put you both in His protections always.

Following this, I would like to express my sincere gratitude to the staffs of Plant Science Department, especially Br. Muzammil and Br. Halim, who have helped me so much, peculiarly in natural product extraction and chemical analysis. Warmest appreciations are given to Dr. Nur Shuhada Tajuddin and Dr. Mohd Hairul Azri Punori for all the assistive tips and comments in regard to thesis writing. A special thanks to Dr. Mohd Shukor Nordin and Dr. Norhanizan Usaizan for the constructive comments during PTEM. An expression of appreciation is given to Br. Taufiq, Br. Azim and Sr. Ila that have assisted so much in term of administrative works of semester registrations and thesis formatting. May Allah S.W.T. make ease all your endeavors.

I would also like to express my thankfulness to my colleagues for the shared energy, insights and encouragement – Br. Muhammad Syufihuddin Shamsuddin, Sr. Nik Nadira Nazua, Sr. Fatin Munirah and Br. Shahmi Salleh. Not also to forget to my administrator and colleagues at Kolej Matrikulasi Pahang for the supports and advice. Next, I am very much indebted to the rest of my postgraduate peers for all your prayers, and also for all the assistance and care that they have given during my thick and thin moments throughout my study and research period. At most that I can do to repay all your kindness is with my prayers so that you may be successful in this life and hereafter.

Lastly, but not the least, my deepest appreciations are conveyed to the special figures that I most beloved; they are my husband and kids – Mohd Redzuan Ismail, Riyadh Iskandar and Dhia Nur Imani for the constant encouragements, patience, motivation, inspiration and endless prayers for my success and well-being. Not to forget, thanks to my *Abah* and *Mak*, father and mother in law, and siblings that I cared so much for the undivided prayers and support. Greatest thanks to all individuals who directly or indirectly contributed to the successful completion of my graduate work. May Allah S.W.T. blesses all of you, and showers you with His Rahmah.

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LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
DNMRT	Duncan New Multiple Range Test
GC-FID	Gas Chromatography Coupled with Flame Ionization Detection
GC-MS	Gas Chromatography-Mass Spectrometry
ICPMS	Inductively Coupled Plasma Mass Spectrometry
SAS	Statistical Software Analysis
SFE	Supercritical Fluid Extraction
TOC	Total Organic Carbon
WHC	Water Holding Capacity

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND OF THE STUDY

Medicinal plants have been extensively used for different purposes especially for the treatment of different ailments in many regions of the world and was pioneered over hundreds of centuries ago. In China, the traditional Chinese physician or herbalist known as *sinseh* will issue the prescription of ginseng-based medicine to treat the diseases of their patients while Indonesian community is popular for their *jamu* (Zakaria, 2015). The herbs based medicinal system called Ayurveda and the Hippocratic (Greek) elemental healing system are widely used in India and Western cultures respectively (Ahmad & Othman, 2014). In Malaysia, herbs are very prominent in Malay and Indian community and have long been widely consumed as a raw material and often used in daily cuisines (Yob et al., 2011). Herbs can be taken orally either as a whole or in the form of tea or syrup, powdered or dried extract or can be processed to form essential oil and ointments. In general, herbs are plants that are used in many ways such as for self-medication, food, flavoring or perfume purposes. The raw herbal remedies are also form the basis of the modern world of pharmaceuticals (Ahmad & Othman, 2014) and to this day, some drugs such as warfarin, indinavir, cyclosporine, amitriptyline, tacrolimus and irinotecan have been identified to have been added with herbs isolated compounds (Zhou Shu-Feng et al., 2007).

With regards to many traditional applications, herbal benefits, and greater awareness of healthcare, it influences the current global herbal medicine market scenario. Worldwide herbal industry is one of the fast-growing industry due to the

consumers' demand for natural health care products (Ahmad et al., 2015). The Malaysian government has acknowledged the herbal industry as one of the most promising industries in the future. Policies related to the herbal industry have been carefully discussed and planned in Industrial Master Plan, Biodiversity Policy, National Agricultural Policy, National Agro-Food Policy (NAFP), Science and Technology Policy as well as in National Key Economic Area (NKEA) (Zakaria, 2015).

Under the NKEA, Malaysia is positioned as high-potential country to produce, develop and manufacture the high-quality herbal products due to its diversity of resources and forest heritage and also regarded as 'jungle of pharmacy' (Ahmad et al., 2015). Malaysia is rich in tropical rain forest consists of various flora with over 3000 species of medicinal herbal plants (Ahmad & Othman, 2014). Fifteen thousand species of flowering plants was reported in Malaysia and more than 2000 species have healing qualities and highly potential to be commercialized (Ministry of Natural Resources and Environment, 2006).

According to Ministry of Agriculture and Agro-based Industry (2016), making the herbal industry as one of the National Key Economic Areas (NKEAs) was in line with the government's aspiration to increase the production of herbal products in Malaysia. The Entry Point Project 1 (EPP 1) under NKEA focuses in ensuring sufficient supply of raw herbal materials to fulfill the market demands. Large-scale of herbs cultivation is a smart way to sustain the supply of raw materials and ensure the survival of the herbal industry. Table 1.1 shows the number of herbal cultivators according to states in Peninsular Malaysia (FRIM, 2015).

Table 1.1 Number of Cultivators According to States in Peninsular Malaysia (Source: FRIM, 2015)

State	Number of Cultivators (%)
Johor	69 (14.9)
Kedah	19 (4.1)
Kelantan	29 (6.3)
Melaka	27 (5.8)
Negeri Sembilan	28 (6.1)
Pahang	110 (23.8)
Perak	67 (14.5)
Perlis	4 (0.87)
Pulau Pinang	19 (4.1)
Selangor	83 (18.0)
Terengganu	7 (1.5)
Federal Territory	0 (0)
Total	**462 (100)**

According to FRIM (2015), 462 herbal cultivators were identified in Peninsular Malaysia in 2015 and they were mainly located in Pahang, Selangor, Johor and Perak. Pahang recorded the highest number of herbal cultivators with 110 cultivators (23.8%). The most widely cultivated herb species in Pahang was *Hibiscus sabdariffa* (*Roselle)* which also be the highest cultivated species in Peninsular Malaysia. Besides, *Piper betle* (*Sireh*), *Aquilaria malaccensis* (*Karas*) and *Orthosiphon stamineus* (*Misai kucing*) were among most cultivated species.

Currently, Zingiberaceae plant has received much attention from many researchers in the entire nations. Zingiberaceae also has high potential to be used in various scientific investigations and industrial commercialization. Many species from Zingiberaceae family such as *Zingiber cassumunar* (bonglai), *Zingiber officinale* (ginger) and *Zingiber spectabile* (ginger wort) are among of economic potential value plants. *Zingiber zerumbet* or *lempoyang*, a perennial, tuberous root herb has been ranked 14th (MOA, 2014) under 18 identified herbs species proposed to be commercialized by Malaysian government due to its high value market (Government Transformation Programme, 2015).

Over recent years, the worldwide interest on *Zingiber zerumbet* became aroused as it has various applications especially in ethnopharmacological to serve the basic reference and guidelines for developing the new drugs (Koga et al., 2016). Traditionally, *Zingiber zerumbet* frequently used by women during confinement to return the best stage of body health after giving birth (Nawawi et al., 2017). This plant also used for ornamental purposes due to its attractive appearance (Blázquez, 2014; Koga et al., 2016) and it is also categorized as aromatic plant due to the presence of essential oils; volatile aromatic compound which accumulate in specialized structures such as oil cells (Joy, 2016).

There are several *lempoyang*-based products have been developed and launched in the local or worldwide market. *Monoi de Tahiti* is a skin and hair care products from French Polynesia which gives effect of repairing, shining and antidandruff (Tahiti, 2011). In Malaysia, Scientific and Industrial Research Institute of Malaysia (SIRIM) has developed and manufactured “Xanzwhite”; a wide range of skincare and lightening products including facial cleansers, toners, SPF 15 lightening fluid and multifunction cream which helps to overcome the skin hyperpigmentation (SIRIM, 2012). Therefore,

the quality of *Zingiber zerumbet* and other herbal plants is crucial to enable the production of high impact of natural and herbal-based products for commercialization.

1.2 PROBLEM STATEMENTS

The campaign to reduce the number of herbal imports from other countries has been announced which aimed to educate the community about the local herbs quality and encouraged the local entrepreneurs to explore and expand the local herbal industry (BERNAMA, 2009). However, Malaysia herbal industry is slow in progress and falls behind other countries such as China and India (Ahmad & Othman, 2014; Khamis, 2015). Most *Zingiber* spp. plants are cultivated in the village garden and home yards since long ago in many places throughout Southeast Asia, the Pacific, and Oceania (Sharifi-Rad et al., 2017; Yob et al., 2011). However, a large-scale cultivation should be made to provide continuous herbal plant material for industrial use (Ahmad & Othman, 2014; Zakaria, 2015).

Based on the survey conducted by FRIM (2015), most of the large-scale herbal cultivators work on *karas*, *belalai gajah* and roselle. Besides, there is not much information on the location of herbs cultivation in Malaysia (FRIM, 2015). As a result, little information about *Zingiber zerumbet* is known and less chances of sharing the knowledge with societies regarding the best cultivation technique for this plant to improve the growth and yield of this plant under natural environment. Without such partnership, it may be hard to educate others or small farmers towards large-scale cultivation because herbs cultivation is the critical aspect to produce high quality herbal products.

Quality of the herbal product depends on the raw material. Even when the herbs species are cultivated, the quality cannot be ensured in cases of poor agricultural

practices. It is therefore, critical to have guideline on the best cultivation method of herbal plant to ensure the purity and maximize the quality and quantity of its bioactive compounds (Abd Aziz, 2003). In addition to that, the requirement to optimize and maintain the sufficient levels of organic matter in soils are prerequisite for sustainable and high production of plant (Ati et al., 2018). In general, the use of fertilizer strongly influences the plant growth and crop yield. It is expected that the use of chemical fertilizers will continue to rise to increase the plant growth and yield with the increasing in the number of cultivated areas (Savci, 2012). However, the excessive use of chemical fertilizers will lead to soil and water pollution and resulting in a serious environmental and health problems (Savci, 2012).

Greater awareness on environmental and food safety issues has led to the growth and practice of organic agriculture (Ng, 2016; Savci, 2012; Tiraieyari et al., 2017). Farmers have been encouraged to explore the possibilities of implementing organic farming to produce organic plants (Shamrao et al., 2013; Tiraieyari et al., 2017). Organic farms in Malaysia are recognized through the Scheme of Certification program, Organic Malaysia (myOrganic) which has been introduced by the Department of Agriculture Malaysia. There are about 142 farms who managed to receive the MyOrganic certificate since it was launched in 2003 until 2014. However, the total number of organic farm is still low and incapable to accommodate the high demand for organic food in the market (Ministry of Agriculture and Agro-based Industry, 2016).

Organic agriculture is used to boost the level of organic matter in soil by replacing the inorganic fertilizers with animal manure to provide the required nutrients for plant growth and development (Seghatoleslami, 2013). Various kinds of organic materials such as animal manures can be applied as fertilizer to soil to improve physical, chemical and biological properties of the soil (Jannoura et al., 2014). Different organic

manures derived from different animals such as goat, cow, horse, quail, bat and chicken have different influence to the growth and yield of plant. Thus, it is necessary to know the best type of organic manure which help the most in increasing the growth and yield of *Zingiber zerumbet* plant.

Another factor which is harvesting time might also influences and gives effect to the quantity and quality of herbal plant. A research on the effect of harvesting times to the growth variations and the concentration of biochemical compound which include the essential oil is very crucial as it is heavily relies on the accurate harvesting times to gain much yield production. Previous studies revealed that as harvesting time increases, it will also increase the growth and yield of the plant (Asghari et al., 2009; Bahadirli et al., 2018; Beltrán et al., 2004; Singh et al., 2014; Tiryakioglu & Turk, 2012). Another study reported that the most suitable time for harvesting Zingiberaceae plant such as turmeric is at twelve months (Adi & Mulyaningsih, 2019). It also revealed that the different percentage of essential oil components such as zerumbone from Zingiber zerumbet plant was due to agricultural practices and plant age when harvested (Sabri, 2009).

1.3 JUSTIFICATION OF STUDY

Conventional farming requires the application of fertilizers to enhance the quantity of plant yield to reach the market demands. As herbal products are used for health and nutrition purposes, cultivation must be done with good agricultural practices to ensure the safety beside of gaining the highest yield. To achieve the goal, the researcher thought that it is very important for this study to provide the basic knowledge to the local farmers on the best organic fertilizer to fertilize their *Zingiber zerumbet* plant to satisfy the preference and demand of consumers on the organic foods and products who