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Preface

Research in the Faculty of Agriculture and Food Sciences, UPM Bintulu Sarawak Campus, Malaysia involves broad field of expertise such as agriculture, forestry, fishery and food sciences. This book communicates a distillation of researches that have been carried out at Faculty of Agriculture and Food Sciences in the year 2015 and 2016. It may be of benefit to those in applied sciences including agriculturists, food technologists, undergraduates and postgraduates as it provides current research findings of the faculty. Additionally, the book could be useful for the state as it provides information on research findings which are related to the natural resources of Sarawak, Malaysia. We would like to thank all contributors for their commitment in providing informative data in their chapters.

Nur Ashikin Psyquay Abdullah
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Serdang, 2017

1

Food and Nutrition in a Changing Climate: A Malaysian Scenario

Nur Ashikin Psyquay Abdullah and Shahrul Razid Sarbini

According to the US Census Bureau, the world population will reached 7.5 billion by 2020 and will continue increasing to 9 billion by 2042. With life expectancy reaching 77 years old, providing food for the population will be a challenge. In order to feed more, agriculture sectors incorporate research and development in food production which include sustainable land use, efficient waste management, focusing on food security, emphasizing on food diversity and natural resources, halal produces and downstream food processing. Malaysia is a developing country which also see hike in human population and increase in life expectancy, thus food security has become an important issue and policy in this country. Increasing food productions are often related to economics, land use and environmental factors.

Malaysia is one of the leading oil palm producer. However, oil palm plantation in Malaysia faced many challenges such as competition from neighboring countries and in particularly with the environmental issues. Clearing and opening up natural forest for cultivation received unwelcoming reviews from many NGOs in related to climate change, destruction of wildlife habitat and loss of plant diversity. Intensive use of land is not only seen in oil palm cultivation but also in rice. Malaysia is still unable to self-sustain in term of rice production and requires import from other countries. Food scarcity faced new challenges when crops are cultivated for bioethanol production rather than food, creating issues in food security. Food security exists ‘when all people at all times have access to sufficient, safe, nutritious food to maintain a healthy and active

life' consisting of three pillars, i.e., food availability, food access and food use. With the growing concern on the uses of pesticides on our crops, consumers are getting more awareness of the importance on the safety of their food. Food safety involves a wide range of discipline taking account on how the food comes from the farm and reached the households or supermarket shelf. Along with this, emphasis should be also given to the importance of biodiversity and its conservation, source of new crops for food and some focus on underutilize plants.

Food security policy in Malaysia is largely about ensuring the availability, accessibility and utilization of rice to the society (Bala *et al.,* 2014). Three policy objectives were set since 1970s, namely: to ensure high price to paddy farmers to produce rice, to achieve a certain level of self-sufficiency in rice and to ensure a stable and high quality of rice to the consumers. Bala *et al.* (2014) suggest that gradual transition to bio-fertilizers, funding for research and development for high yielding hybrid rice and increasing the cropping intensity, hold big promise towards productivity enhancement. The consumption of rice increase with the rise of income per capita but with social changes, rice are replace with alternative source of carbohydrate mainly wheat.

Gearing up in facing the coming challenges, more studies are undertaken to aid with the lack of food and increase the quality of food. This is also to provide and implement strategies to improve food productions in tropical agriculture. Improvement of yield and quality begins from the soil as this is the source of nutrient and water that will ensure best growth performance of any crops. Soil improvement or amendment using biochar derived from rice husk enhanced soil respiration as the rate of biochar's application increased. Biochar also has the potential to increase retention of N and increase its availability in soil. Apart from edible food, improvement of planting media for non-food crops such as herbs, livestock feed and timber are also in focus. Gaharu is the resin produced from injured cells of *Aquilaria* spp. and serves as raw material for the production of many aromatic medicinal products, stimulants and tonics. It was observed that the suitable amount of fertilizer application is 100 g of NPK, organic compost and slow-release fertilizer.

Despite providing enough food for the nation, concerns are also put into ensuring quality in term of nutritional value, safety and halal. Sourcing for new plants as food crops and promoting underutilized crops is part of overseeing issues in the food security. In Sarawak, there are various plants collected from the forests for food which is also known as jungle products but are not cultivated with proper agronomic management. Indigenous leafy vegetables have potential to be commercialized as food crops as they contain great sources of nutritional value and pesticide free. As an example, despite the uses of *Neptunia oleracea* as food by the local people in Sarawak, Malaysia, the plant have not been given due attention in terms of its propagation modes, availability and nutritional content.

In essence, agriculture and food still highly relies on the availability of natural resources. Malaysia holds abundant natural resources for agriculture and food production. Through continuous research and innovation, the agriculture sector will be able to bring wealth to and many benefits to the welfare of its community.

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2

Effects of Biochar Amendment and Rainfall Events on Soil Respiration

Lee Yin Yin, Normah Awang Besar, Baba Musta, Azwan Awang, Lum Mok Sam, Osumanu Haruna Ahmed, Nik Muhamad Nik Ab. Majid, Markus Atong and Mohamadu Boyie Jalloh

INTRODUCTION

Soil has been referred to as the "Earth's Living Skin" (Dent *et al*., 2005). It serves as anchorage to plant roots, holds water, stores nutrients and also provides a home and active sites for microorganisms. Soils are complex and dynamic ecosystems with communities of organisms. Like all ecosystems they have a food web that may include bacteria, fungi, algae, protists, insects, worms, plant roots and burrowing animals all of which have the ability to either respire or exchange gases through their cellular membrane.

Biochar is produced by the pyrolysis of biomass. It can be described as a "soil conditioner" which is derived and manufactured using many types of biomass feedstock such as, wood, crop residues and manures. Biochar amendment to soil has resulted in both positive and negative effects on soil. It has been shown that biochar amendment improves soil fertility and also increases the population of microbes in soil (Gaskin *et al*., 2010) which increases the rate of soil respiration. Apart from biochar influences on soil respiration, it also affects soil microbial biomass C, soil biochemical properties, soil biological activity and the responses could be soil enzyme specific or mediated by soil enzymes as well as soil temperature (Hamdi, *et al*., 2011; Lehmann*, et al*., 2011; Wu *et al*., 2013; Lu *et al*., 2015). The aspect of soil enzyme and temperature mediation on soil CO_2 efflux processes is not the subject of this paper as soil temperature changes in the study area is of a very narrow range.

Soil respiration is the flux of carbon dioxide from soil to the atmosphere which comprises the second largest terrestrial carbon flux (Raich and Potter, 1995; IPPC, 2007; Bond-Lamberty and Thomson, 2010). Global soil respiration is estimated to be 76.5 Pg C per year which is 30-60 Pg C per year greater than global net primary production and is thus a very substantial flux in the global C cycle (Raich and Potter, 1995; Schimel, 1995). Globally, soils store at least twice as much C as is in the atmosphere (Post et al., 1982; Tarnocai *et al*., 2009). Studies have shown that the soil respiration rate for a tropical ecosystem can be as much as 1286 g C m^{-2} (Bond-Lamberty and Thomson, 2010).

Biochars are not all the same which has been one of the reasons why results on effects of biochar amendment on soil respiration have varied considerably. For example, the amendment of soil with biochar derived from switchgrass resulted in increased soil respiration (Smith *et al*., 2010) but increasing levels of maize biochar soil amendment resulted in an inverse relationship with soil respiration (Jin *et al.*,2008).

The objectives of this study were to determine the effect of rice husk biochar amendment and the influence of rainfall events on soil respiration.

MATERIALS AND METHODS

This study was conducted at the experimental field of the Faculty of Sustainable Agriculture, Universiti Malaysia Sabah, located at 6˚02' N, 116˚07' E and 37 m above sea level to measure soil respiration. The field experiment was conducted on a flat clay soil plot of land using completely randomized design. There were five biochar treatments (0, 1, 5, 10 and 20 t ha^{-1}**)** including the control, each replicated three times, resulting in 15 experimental plots, each sized 1 square meter. For each plot, the treatments were applied to the soil surface and mixed with the top 10 cm of soil. The treatments were applied evenly over each plot and then mixed well. The soil was then covered with plastic mulch and left to incubate for four weeks. At the same time, one 10 cm diameter PVC soil collar was inserted 7 cm depth into the centre of each plot. For each collar, 4 cm of the collar length was exposed at the surface to allow for attaching the automated soil CO_2 flux system (LI-Cor Inc., Lincoln, NE, USA) which was used for direct measurement of soil CO_2 flux. These collars acted as conduits for measuring soil respiration. Measurement of soil respiration commenced after the four

weeks of incubation. The readings were taken once per week at two time intervals (morning - 0600-0800 hours and evening -1700-1900 hours) for 10 weeks. Rainfall data was obtained from a nearby weather station. All the data collected were subjected to one-way analysis of variance using SPSS version 17 software followed by Tukey test for mean separation.

RESULTS AND DISCUSSION

Table 2.1 shows the overall mean soil respiration recorded between 6.00 - 8.00 a.m. and 5.00-7.00 p.m. for 10 weeks. In general, the biochar treated plots showed significantly higher soil respiration compared to the untreated (control) plots for both the morning and evening measurements. There was no significant difference in soil respiration between the four biochar treated plots for the evening measurements with the soil respiration increasing for biochar treatments ranging from 1 t ha^{-1} to 5 t ha^{-1} but either declined or remained unchanged at higher biochar application rates.

Table 2.1 Morning and evening mean soil respiration measurements over 10 weeks for the various biochar application rates

Biochar Application (t ha^{-1})	Soil Respiration (CO_2 efflux)/ µmol $m^{-2}s^{-1}$	
	0600-0800 hours	1700-1900 hours
0	0.5718^{a}	0.5397^{a}
1	0.6593^{ab}	0.7513^{b}
5	0.8808^{b}	0.8232^{b}
10	0.7709^{ab}	0.7957^{b}
20	0.8805^{b}	0.7322^{b}

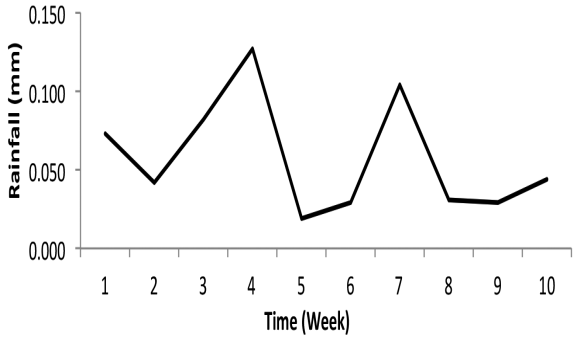
**Means with the same letter in a column are not significantly different at $p<0.05$ using Tukey test

The morning measurements for the control treatment (0 t ha^{-1}) were significantly different from that for the 5 t ha^{-1} and 10 t ha^{-1} ($p<0.05$) treatments. The 5 t ha^{-1} and 20 t ha^{-1} biochar treatments had the highest mean soil respiration rate among the five treatments with values of 0.8808 and 0.8805-µmol m^{-2} s^{-1}, respectively. For the 10 t ha^{-1} biochar treatments soil respiration decreased by 12.5% compared to that for the 5 t ha^{-1} biochar treatment. In total, the maximum soil respiration rate was about 9.45 t CO_2 ha^{-1} yr^{-1} when 5 t ha^{-1} of biochar was applied.

The results in Table 2.1 show that the 5 t ha^{-1} and 20 t ha^{-1} treatments resulted in significantly higher respiration in the morning. Based on literature, biochar amendment of between 5 t ha^{-1} to 50 t ha^{-1} have been shown to improve crop growth and encourage soil respiration in both pot and field experiments (Laird *et al.,* 2010; Husk and Major, 2010). For the rice husk biochar used in this study, the results indicate that 10 t ha-^{1} is a more practical option especially in terms of environmental concerns as this application rate seems to reduce soil respiration, which means less CO_2 released into the environment as compared to the other treatments.

Weekly rainfall during the duration of the experiment is shown in Figure 2.1(a). The results indicate that higher rainfall causes lower respiration. The maximum rainfall was in week 4 over the 10 week observation period and it coincided with the lowest rate of soil respiration. The high rainfall hampers root respiration as more water in the soil restricts oxygen uptake for the soil CO_2 efflux. Figure 2.1(b) shows that all the biochar treatments resulted in generally higher soil respiration rates compared to the control treatment, with the 5 and 10 t ha^{-1} treatments resulting in relatively higher respiration rates during the experiment. Over the entire 10 week observation period, week 5 showed the highest peak respiration rate for the 5 t ha^{-1} biochar treatment at a rate of 1.8 μmol $m^{-2}s^{-1}$.

(a)



(b)

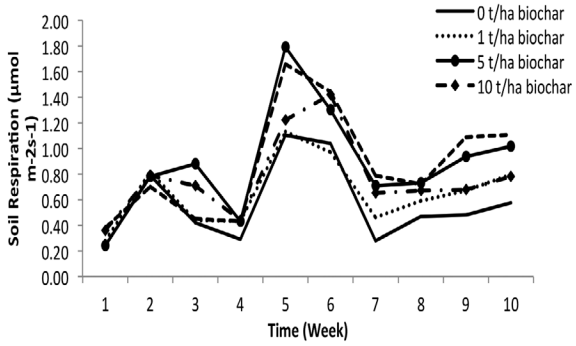


Figure 2.1(a) Weekly rainfall distribution over 10 weeks (b) Morning weekly mean soil respiration measurements over 10 weeks

The five biochar treatments showed significant differences in morning soil respiration in week 9 and week 10 (Table 2.1). The rate for the control (0 t ha^{-1}) was significantly different from that for the 5 and 20 t ha^{-1} treatments.

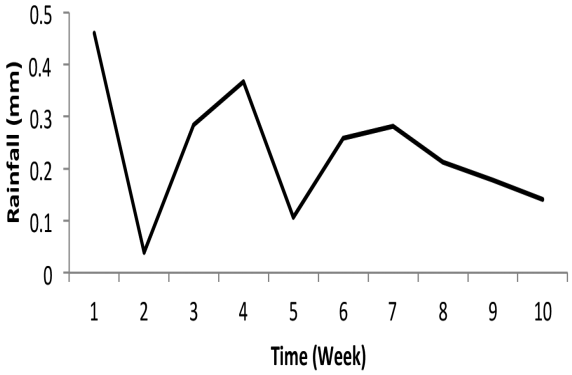
Table 2.1 Morning mean soil respiration measurements for weeks 9 and 10 for the various biochar application rates

Biochar Application (t ha^{-1})	Soil Respiration (µmol $m^{-2}s^{-1}$)	
	Week 9	Week 10
0	0.480 ^{a}	0.575 ^{a}
1	0.672 ^{ab}	0.797 ^{ab}
5	0.937 ^{bc}	1.018 ^{c}
10	0.678 ^{ab}	0.780 ^{ab}
20	1.088 ^{c}	1.107 ^{c}

**Means with the same letter in a column are not significantly different at $p<0.05$ using Tukey test

For the evening measurements, the pattern of soil respiration could be related to that of the rainfall events as was the case for the morning measurements. Rainfall events caused a reduction in soil respiration as shown in Figure 2.1. However, in general terms, it is worth noting that the untreated plots resulted in lower soil respiration in comparison to the biochar treated plots irrespective of rainfall.

(a)



(b)

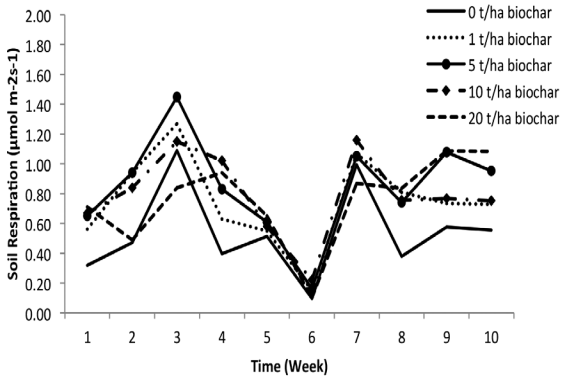


Figure 2.1 (a) Weekly rainfall distribution over 10 weeks
(b) Evening weekly mean soil respiration measurements over 10 weeks

The evening soil respiration measurements for weeks 9 and 10 are shown in Table 2.3. In week 9, there was significant difference between the control and the 5 and 20 t ha^{-1} treatments. In week 10, only the 20 t ha^{-1} treatment was significantly different from the control. Significant differences between the treatments began to manifest more clearly in week 10. This is due to the properties and characteristics of the biochar which is a long term soil conditioner and reacts with soil completely over longer

periods (Sohi *et al.*, 2009). Chen *et al.* (2015) reported that there was no immediate significant effect of biochar amendment on soil CO_2 emissions until after 6 months due to low soil temperatures (15 ^oC and below).

Table 2.3 Evening mean soil respiration measurements for weeks 9 and 10 for the various biochar application rates

Biochar Application (t ha^{-1})	Soil Respiration (µmol $m^{-2}s^{-1}$)	
	Week 9	Week 10
0	0.577 [a]	0.555 [a]
1	0.733 [ab]	0.727 [ab]
5	1.077 [b]	0.952 [ab]
10	0.768 [ab]	0.753 [ab]
20	1.087 [b]	1.085 [b]

**Means with the same letter in a column are not significantly different at $p<0.05$ using Tukey test

Soil moisture is a major factor which affects the soil respiration rate. The results of this study show that increasing soil moisture due to rainfall events cause a reduction in soil respiration. The maximum CO_2 flux occurred when the soil moisture ranged about 10% to 30%. Within this soil moisture range, the soil respiration rate was about 1.2 to 1.8 µmol $m^{-2}s^{-1}$.

This study also showed that increasing concentrations of biochar resulted in increasing soil moisture retention. This is because the biochar increases the soil organic matter which in turn increases soil water holding capacity (Glaser *et al.*, 2001). Soil moisture may limit or inhibit microbial decay of soil organic matter, both at high and low water content levels depending on the microbial species in the soil. Excessive water content or higher soil moisture will limit soil microorganism activities and reduce soil CO_2 flux (Broken *et al*., 2006). Under high soil moisture conditions, many bacteria take in too much water causing their cell membranes to lyse, or break. This can decrease the rate of soil respiration temporarily, but the lysis of bacteria causes a spike in resources for many other bacteria. This rapid increase in available labile substrates can cause short term enhancement of soil respiration (Xu *et al.*, 2004).

Lin *et al.* (2011) reported a depressive effect of soil water on a loam soil CO_2 flux in a sub-tropical monsoon climate. The short temporal water excess does not necessarily hamper respiration by roots and soil organisms. When soil moisture content temporarily exceeds field capacity, soil gaseous diffusion (such as $O_{2\ and}CO_{2)}$ is restrained, which reduces CO_2 flux (Schaefer *et al*., 1997; Dilustro *et al*., 2005). This is indicative in a study conducted in a semi-arid environment on a loamy sand soil wherein increased soil moisture content increased soil respiration (Hanpattanakit *et al*., 2009). This implies that soil water positively influences respiratory processes but if the water exceeds field capacity then there would be a reduction in diffusion of gases due to water filled soil pores. Detailed studies may be required to partition soil CO_2 flux due to heterotrophic and autotrophic respiration and how this is influenced by soil pore sizes, continuity and soil water content.

CONCLUSION

The rice husk biochar amendment enhanced soil respiration as the rate increased. This increase in soil respiration is however temporarily negated by rainfall events or high soil moisture content. Taking account of environmental concerns due to release of C into the atmosphere, a balance need to be struck in deciding the amount of biochar added to soil to improve the soil's agronomic usefuleness.

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3

Effects of Oil Palm Waste Biochar and Clinoptilolite Zeolite on Soil Nitrogen and Maize Yield

Siti Wardah Zaidun, Normah Awang Besar, Baba Musta, Osumanu Haruna Ahmed, Nik Muhamad Nik Abdul Majid and Mohamadu Boyie Jalloh

INTRODUCTION

Oxisols and Ultisols in the tropics are usually described as highly weathered, low in nutrient reserves with low pH, low CEC and poor in organic matter. Conventionally, chemical fertilisers are applied to increase the soil nutrient status. However, prolonged overuse of chemical fertilisers has an adverse effect on a soil's quality. Other soil amendments such as biochar and zeolite can be useful to counter this problem.

Biochar's history started from the *terra-preta* of the Brazillian Amazon and is a product of biomass feedstock that has undergone pyrolysis at relatively low temperature (300-700°C) producing a carbon rich material (Lehmann and Joseph, 2009). Biochar's unique properties such as high porosity and large surface area make it favourable for improving soil water holding capacity and soil structure (Vaccari *et al.,* 2011; Karhu *et al.,* 2011). Crop productivity is positively affected by adding biochar to soil as it is able to increase and retain the nutrient status of the soil due to its high CEC (Cornelissen *et al.*, 2013; Liang *et al.,* 2006). Some biochar also possess high pH, a property which is favourable especially in acidic soil as biochar helps to buffer the soil pH, substituting the use of liming to increase soil pH, and indirectly increasing nutrient availability for crop uptake (van Zwieten *et al.,* 2010; Nigussie *et al.,* 2012; Novak *et al.*, 2009). Biochar also improves soil quality by increasing soil biota abundance as it is a suitable habitat for soil micro- and macro-organisms due to its high surface area and organic matter (Lehmann *et al.,* 2011). Biochar made from a combination of empty fruit bunches and palm oil mill effluent

(EFB-POME) was used in this study. In 2010, Malaysia alone exported a total of 14.7 million tonnes of palm oil and palm oil products, contributing US$ 4500 million revenue to the country (Bazmi *et al.,* 2011). However, the biomass left over from palm oil production is as high as 90% because the oil extraction rate is only about 10 % (Basiron and Chan, 2004). Due to the abundance of EFB and POME wastes from the oil palm industry, converting these waste materials to produce biochar is an effective way to return the biomass to the soil or sequester carbon in soil.

Zeolites are hydrated aluminosilicates of alkaline and alkaline-earth minerals and their structure is made up of a framework of $[SiO_4]^{-4}$ and $[AlO_4]^{-5}$ tetrahedrons linked to each other's corners by sharing oxygen atoms forming a 3-dimensional framework (Akbar *et al*., 1999). The 3-dimensional pore structures of zeolites are interconnected and form long wide channels for easy movement of ions and molecules into and out of the structures (Polat *et al.,* 2004). The silicate (SiO_4) tetrahedron is a compromise between electrical neutrality and packing efficiency. To be electrically neutral, stable minerals require other positively charged accessory cations. This need for electrical neutrality and accessory cations leads to the important property of cation exchange capacity. Zeolites in natural conditions are combined with cations such as Na^+, K^+, Ca^{2+} and others (Dana, 1977; Navrotsky *et al.,* 1995). Zeolites are used in variety of fields worldwide. In the field of agriculture, zeolites are used as slow release fertiliser, soil amendment for pH buffering, to increase CEC and fertiliser use efficiency, act as water reservoir in the soil due its high porosity, as water filter in aquaculture systems and as animal feed additives (Polat *et al.*, 2004). This study was conducted to evaluate the effects of empty fruit bunch and palm oil mill effluent (EFB-POME) biochar and clinoptilolite zeolite on soil nitrogen and maize yield.

MATERIALS AND METHODS

This field experiment was conducted at the Faculty of Sustainable Agriculture, Universiti Malaysia Sabah, using a factorialrandomised complete block design. The three factors were: EFB POME biochar at rates of 0, 10 and 20 t ha^{-1}; clinoptilolite zeolite at rates of 0, 1.25 and 2.5 t ha^{-1} and nitrogen at rates of 60 and 120 kg ha^{-1}. Each treatment combination was replicated four times. Thai SuperSweet maize was used as the test

crop. Each plot was 1.5 m x 2.5 m in size with planting distances of 75 x 25 cm resulting in a equivalent planting density of 53,333 plants ha^{-1}. The maize was planted for two cropping cycles and harvested after reaching maturity, at 10 weeks after planting. Figure 3.1 shows the total monthly rainfall distribution during the cropping period.

The chemical properties of the soil at the study site are shown in Table 3.1. The soil is a Typic Paleudult based on USDA soil classification belonging to the Tanjung Lipat Family (Sabah soil classification), derived from sandstone and mudstone parent materials (Acres *et al.,* 1975). The clay content of the soil was around 30-40% and the soil texture was classified as clay loam. At the end of each cropping cycle soil was sampled from each plot and analysed for soil total N, NH_4^+ and NO_3^-. Soil total N was determined by the Dumas method using the LECO CHN628 Analyser (LECO Corporation) and soil NH_4^+ and NO_3^- was extracted with 2 M KCl (Tan, 1996) and the concentration was measured by SEAL AA3 Autoanalyser (SEAL Analytical). Plant height, dry matter and dry maize grain yield were measured at harvest. Maize cobs were de-husked, dried and de-shelled and the kernels were weighed for grain yield measurement. All the data were analysed with ANOVA, using SPSS version 21.

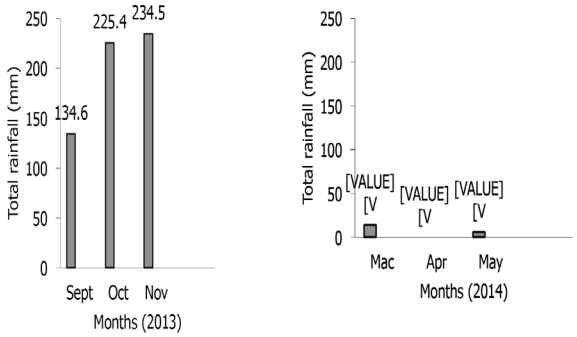

Figure 3.1 Monthly total rainfall distribution during the experiment

Table 3.1 Soil Chemical Properties for the field plot

Property	Value**
pH	4.50
CEC ($cmol^{+}$ kg^{-1})	2.10
TOC (%)	3.15
Total N (%)	0.65
NH_4^+ (mg kg^{-1})	1.09
NO_3^- (mg kg^{-1})	0.02
Available P (mg kg^{-1})	1.56
Available K (mg kg^{-1})	15.20
Available Ca (mg kg^{-1})	87.00
Available Mg (mg kg^{-1})	132.80

Based on random samples collected from the field at 0-15 cm depth

RESULTS AND DISCUSSION

Soil Total N, NH_4^+, NO_3^-

The main treatment effects and summary of significant ANOVA results ($P<0.05$) for the effects of biochar, zeolite and nitrogen on soil total N, NH_4^+ and $NO_3^-$are shown in Table 2. After the first crop, the biochar and nitrogen amendments resulted in significant interaction effects on soil total N whereas for soil NH_4^+, biochar main treatment effects was significant, while for soil NO_3^- none of the treatments had any significant effects. After the second crop, none of the factors showed significant interaction effects on soil total N, NH_4^+ and NO_3^-. There was significant biochar main treatment effects on soil total N and NH_4^+. All the factors showed no significant main effect or interaction effect on soil NO_3^-.

Table 3.2 Mean soil total nitrogen (N), ammonium (NH_4^+) and nitrate (NO_3^-) for the various biochar, zeolite and nitrogen treatments after harvest of the first and second crop

	After harvest of first crop			After harvest of second crop		
Treatment	**Total N**	**NH_4^+**	**NO_3^-**	**Total N**	**NH_4^+**	**NO_3^-**
Biochar (B)						
(t ha^{-1})						
0	0.68^c	1.25^b	0.18^a	0.69^c	1.32^b	0.15^a
10	0.96^a	1.13^b	0.13^a	0.72^b	1.38^b	0.05^a
20	0.74^b	1.86^a	0.15^a	0.75^a	1.76^a	0.11^a
P	***<0.01***	***<0.01***	*0.65*	***<0.01***	***0.03***	*0.53*
Zeolite (Z)						
(t ha^{-1})						
0	0.71^a	1.40^a	0.13^a	0.72^a	1.48^a	0.06^a
1.25	0.70^a	1.52^a	0.16^a	0.72^a	1.37^a	0.12^a
2.5	0.70^a	1.32^a	0.16^a	0.71^a	1.61^a	0.13^a
P	*0.24*	*0.55*	*0.72*	*0.61*	*0.41*	*0.69*
Nitrogen (N)						
(kg ha^{-1})						
60	0.69^b	1.53^a	0.19^a	0.72^a	1.48^a	0.10^a
120	0.72^a	1.30^a	0.12^a	0.72^a	1.50^a	0.10^a
P	***<0.01***	*0.15*	*0.06*	*0.57*	*0.93*	*0.96*
***P* (interactions)**						
B*Z						
B*N	*0.28*	*0.54*	*0.99*	*0.43*	*0.71*	*0.35*
Z*N	***<0.01***	*0.67*	*0.49*	*0.28*	*0.61*	*0.18*
B*Z*N	*0.07*	*0.55*	*0.83*	*0.06*	*0.42*	*0.21*
	0.36	*0.80*	*0.99*	*0.47*	*0.89*	*0.59*

Mean with the same letter within the columns are not significantly different ($p<0.05$). ***P*** = probability value from ANOVA results

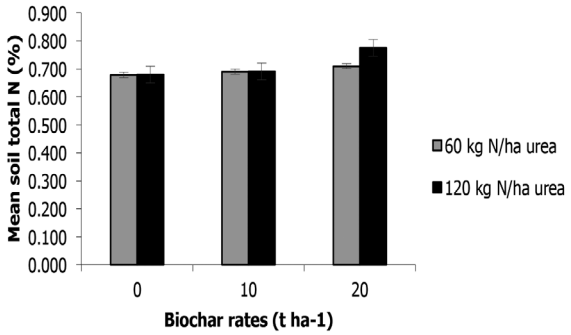

Figure 3.2 Interaction effects of biochar and urea nitrogen on mean soil total N after harvest of the first crop. Error bars represent standard error

The effects of biochar and urea nitrogen on soil total N after harvest of the first crop is shown in Figure 3.2. The 60 and 120 kg ha^{-1} N without biochar amendment resulted in the lowest soil total N (0.68%). For the 60 kg ha^{-1} N, amending the soil with 10 and 20 t ha^{-1} biochar resulted in only a slight increase in total N (0.69 and 0.71%, respectively). For the 120 kg ha^{-1} N, soil treated with 10 and 20 t ha^{-1} biochar resulted in increased soil total N of up to 0.69 and 0.78%, respectively. After harvestingthe second crop, only the biochar treatments showed significant effects ($p<0.05$) on soil total N (Table 2). The control (0 t ha^{-1}) biochar treatment resulted in the lowest soil total N (0.69%), whereas amending the soil with 10 and 20 t ha^{-1} biochar increased soil total N to 0.72 and 0.75%, respectively.

Mean separation using LSD test showed that soil treated with 20 t ha^{-1} biochar resulted in significantly higher ($p<0.05$) soil NH_4^+ (1.86 mg kg^{-1}) after the first crop but soil treated with 10 t ha^{-1} biochar resulted in the lowest soil NH_4^+ (1.13 mg kg^{-1}) which was not significantly different from the untreated soil (1.25 mg kg^{-1}). After the second crop, the untreated soil resulted in the lowest soil NH_4^+ (1.32 mg kg^{-1}). The 10 t ha^{-1} treatment slightly increased soil NH_4^+ to 1.38 mg kg^{-1} but the increase was not significant as compared to the untreated soil while the 20 t ha^{-1} biochar resulted in the highest soil NH_4^+ content with a value of 1.76 mg kg^{-1}.

After harvest of the first crop for the soil treated with lower N rate (60 kg ha^{-1}) the biochar application resulted in a slight increase in total N compared to higher N rate (120 kg ha^{-1}), which resulted in more apparent

increases in soil total N in response to the biochar amendment. This reflects the ability of biochar to retain N but not to directly add N to the soil. After the second crop, only the biochar amendments showed significant main effects on soil total N. Soil total N increased linearly with increasing biochar rates. This is probably due to the ability of biochar to immobilise organic N and consequently restrict N mineralisation which is attributed to the high C: N ration of biochar (DeLuca *et al.*, 2009; Clough *et al.,* 2013).

Based on the results of soil NH_4^+, it is possible that NH_4^+ nitrification was reduced by the biochar treatments, contributing to reduction of N losses in the form of NO_3^- despite there being no significant effects shown for soil NO_3^- between the various treatments. The restriction of NH_4^+ nitrification resulting from biochar amendment may be due to adsorption affinity of biochar for NH_4^+ (Lehmann *et al.*, 2002). There is also a possibility that the increase in N n biochar amended soil may be due to increase in biological N fixation which consequently increased N in the soil. It has been shown that biochar can increase the abundance of microbes and stimulate their activities by improving soil conditions favourable to microbes (Lehmann *et al.*, 2011). Further, previous research has provided evidence of increased biological N fixation with biochar treatments (Rondon *et al.*, 2007).

Reports have indicated increased soil total N and decrease in NH_4^+ losses due to biochar applications. Widowati *et al.*, (2012) reported that an increase in soil total N of 82% resulted from mixed biochar and N fertiliser application compared to only N fertiliser treatments. Widowati and Asnah (2014) observed an increase in total N by 39-53% due to biochar amendment in an Inceptisol. An increase in soil N by 62% due to biochar application under rice cultivation in Kerala, India was reported by Prabha *et al.,* (2013). Ding *et al.*, (2010) reported a decrease in NH_4^+ losses by 15.2% due to bamboo biochar amendment. Dempster *et al.* (2012) also reported a 25% reduction of NH_4^+ losses in biochar treated soil.

Clinoptilolite zeolite treatments did not show significant effects on all of the soil N parameters. These results are similar with that of Ahmed *et al.*, (2010) who reported that clinoptilolite zeolite did not show any significant effect on soil total N in Typic Paleudallts (Nyalau series). Tarkalson and Ippolito (2011) also reported no significant difference on the effects of clinoptilolite zeolite application on soil NH_4^+ in Wolverine sand Xeric Torripsamment treated with manure.

Plant Height, Plant Dry Matter and Grain Yield

The main treatment effects and the summary of significant ANOVA results ($P<0.05$) for the effects of biochar, zeolite and nitrogen on plant height, plant total dry matter and grain yield are shown in Table 3.3. For the first crop, there was a significant interaction effect between biochar and nitrogen on grain yield. Biochar and nitrogen showed significant main treatment effects on plant height and dry matter. For the second crop, there was no significant interaction effect between all the three parameters. Plant height was affected by biochar and N rates whereas for plant dry matter and grain yield, only biochar showed significant main treatment effects.

Table 3.3 Mean plant height, above ground plant dry matter and grain yield for the various biochar, zeolite and nitrogen treatments for the first and second crops

	First Crop			Second Crop		
Treatment	**Plant height (cm)**	**Dry matter (t ha^{-1})**	**Grain yield (t ha^{-1})**	**Plant height (cm)**	**Dry matter (t ha^{-1})**	**Grain yield (t ha^{-1})**
Biochar (B) (t ha^{-1})						
0	206.04^{b}	6.05^{c}	1.44^{b}	168.07^{b}	3.73^{c}	1.40^{c}
10	221.97^{a}	7.60^{b}	1.93^{a}	188.80^{a}	6.11^{b}	1.98^{b}
20	218.73^{a}	8.94^{a}	2.46^{a}	192.11^{a}	8.53^{a}	2.17^{a}
P	***0.01***	***<0.01***	***<0.01***	***<0.01***	***<0.01***	***<0.01***
Zeolite (Z) (t ha^{-1})						
0	209.91^{a}	6.85^{a}	1.74^{a}	179.90^{a}	6.69^{a}	1.77^{a}
1.25	214.06^{a}	7.94^{a}	2.01^{a}	185.11^{a}	4.75^{a}	1.86^{a}
2.5	222.78^{a}	7.81^{a}	2.08^{a}	183.96^{a}	6.93^{a}	1.92^{a}
P	*0.06*	*0.20*	*0.07*	*0.66*	*0.06*	*0.07*
Nitrogen (N) (kg ha^{-1})						
60	203.94^{b}	5.81^{b}	1.58^{b}	177.98^{b}	5.56^{a}	1.83^{a}
120	227.22^{a}	9.26^{a}	2.31^{a}	188.00^{a}	5.74^{a}	1.87^{a}
P	***<0.01***	***<0.01***	***<0.01***	***0.05***	*0.78*	*0.49*
***P* (interactions)**						
B*Z						
B*N	*0.29*	*0.78*	*0.17*	*0.08*	*0.23*	*0.12*
Z*N	*0.18*	*0.39*	***0.02***	*0.94*	*0.27*	*0.23*
B*Z*N	*0.52*	*0.15*	*0.62*	*0.07*	*0.71*	*0.06*
	0.98	*0.66*	*0.30*	*0.88*	*0.14*	*0.86*

Mean with the same letter within the columns are not significantly different ($p<0.05$). ***P*** = probability value from ANOVA results

For the first crop, the 10 and 20 t ha^{-1} biochar treated soil significantly increased plant height by 7.73% and 6.16%, respectively, compared to the soil without biochar amendment. Soil treated with 120 kg ha^{-1} N resulted in taller plants by 23.28% compared to the 60 kg ha^{-1} N application rate. For the second crop, 10 and 20 t ha^{-1} biochar significantly increased plant height by 11.91% and 12.12%, respectively, compared to the unamended soil. The 120 kg ha^{-1} N also increased plant height by 11.42% compared to 60 kg ha^{-1} N.

Olson and Kurtz (1982) reported that effects of N on plant performance are superior compared to other essential elements because N plays a major role in plant growth as N is a component of chlorophyll molecules, enzymes and amino acids. Further, N is essential for carbohydrate utilisation, stimulates root development and activity and enhances uptake of other nutrient elements. Therefore, it was expected that an increase in N rate would result in an increase in plant height. Findings by previous researches also showed plant height to be influenced by N fertilisation. Maqsood *et al.* (1999) reported a significant increase in wheat plant height with increasing rates of N fertiliser from 35 to 140 kg N ha^{-1}, where the height of the wheat plant increased by 9% with the increasing N fertiliser rates. Liu and Wiatrak, (2011) also reported a significant increase in plant height of maize with increasing rate of N fertiliser.

Nutrient elements, especially N, P, K, Mg and Ca, are crucial to promote rapid growth of plants and increase plant biomass (Tucker, 1999). These elements individually or in combination influence plant metabolic processes, nutrient assimilation, cell division and expansion, resistance to drought and disease and other important processes involved in making sure plants grow and reproduce at optimum levels and complete their life cycle (Tucker, 1999; Pellerin *et al.*, 2000; Raven *et al.*, 1999). Increase in plant height can be attributed to the increase in nutrient supply and plant nutrient concentration due to biochar amendment. The increase in soil pH and CEC due to biochar treatments may have contributed to the increase in soil nutrient availability for plant uptake. The results of this study are congruent with previous reported findings. van Zwieten *et al.,* (2007) reported an increase in wheat height by 30 to 40% with 10 t ha^{-1} paper mill sludge addition to acid soil. Increased plant height with biochar treatment was also reported by Hossain *et al.* (2010) in tomato cherry plants.

Plant dry matter for the first crop was significantly higher for the 20 t ha^{-1} biochar treatments with a value of8.94 t ha^{-1}. Soil treated with 10 t ha^{-1} resulted in 25.79% higher plant dry matter (7.60 t ha^{-1}) compared to the untreated soil (6.05 t ha^{-1}). In terms of nitrogen main treatment effects, soil treated with 120 kg ha^{-1} N increased plant dry matter by 59.38% compared to soil treated with 60 kg ha^{-1} N (5.81 t ha^{-1}). For the second crop, plant total dry matter was highest in plots amended with 20 t ha^{-1} biochar (8.53 t ha^{-1}). The plots without biochar amendment resulted in the lowest plant dry matter (3.73 t ha^{-1}) and the 10 t ha^{-1} biochar significantly increased plant total dry matter by 49.58% (6.11 t ha^{-1}), compared to the untreated or control plots.

According to Novoa and Loomis (1981), N improves biomass production because it promotes a faster photosynthetic rate by increasing crop radiation interception, due to higher leaf area and biomass conversion efficiency. Due to the role played by N in promoting plant growth, increase in N rates application will certainly increase the dry matter of plants. Bernardi *et al.,* (2011) reported an increase in maize dry matter production with increasing rates of N fertilisers. Arun Kumar *et al.,* (2007) recorded higher total dry matter production of maize with the application of 100% of the recommended dosage of N fertiliser compared to 50% of the recomended dosage of N into the soil.

Increase in plant dry matter with biochar treatments may be related to biochar's ability to improve nutrient availability and increase nutrient uptake. Carter *et al.*, (2013) also reported a tremendous increase in lettuce above ground biomass of 903% with 50 g kg^{-1} biochar addition to unfertilised soil, compared to untreated soil. They attributed the increase to the increase in soil pH with biochar amendments that resulted in an increase in soil nutrient availability. Rondon *et al.*, (2007) also reported an increase of *Phaseolus vilgaris* L. biomass by 39% with biochar treatment.

The interaction effects of biochar and nitrogen on grain yield for the first crop are depicted in Figure 3.3. At 60 kg ha^{-1} N, soil without biochar amendment resulted in lowest grain yield (1.06 t ha^{-1}). Adding 10 t ha^{-1} biochar increased grain yield by 67.92% (1.78 t ha^{-1}). However, doubling the amount of biochar did not result in much further improvement in the grain yield (1.89 t ha^{-1}). At 120 kg ha^{-1} N, treating the soil with 10 t ha^{-1} biochar resulted in higher maize grain yield (2.08 t ha^{-1}) compared to the unamended soil (1.83 t ha^{-1}) and increasing the biochar rate (20 t ha^{-1})

resulted in highest maize grain yield (3.03 t ha^{-1}). However, for the second crop, there was no significant interaction between the factors and only biochar showed significant main treatment effects on grain yield ($p<0.05$). Soil without biochar treatment resulted in the lowest grain yield (1.40 t ha^{-1}). Amending the soil with 10 and 20 t ha^{-1} biochar increased grain yields by 41.00% and 55.00%, respectively.

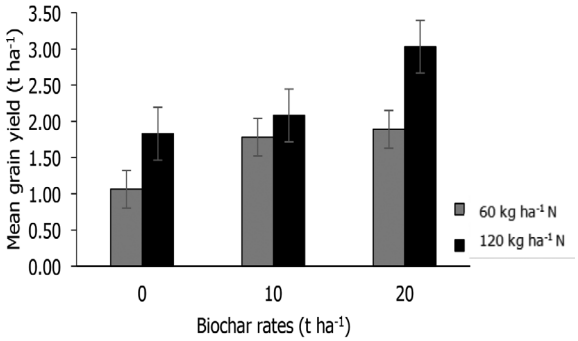

Figure 3.3 Interaction effects of biochar and nitrogen on mean grain yield after harvest of first crop. Error bars indicate standard error

The addition of 10 t ha^{-1} biochar to soil with low rate of N fertiliser almost doubled the grain yield compared to N fertiliser treatments alone. However, further increase in biochar application rates resulted in only a slight increase in maize grain yield. This indicates that biochar has the potential to increase N use efficiency and biochar application increases N retention but not directly add N to the soil. Higher increase in maize yield only resulted with biochar applied to the soil with higher rates of N. The results suggest that N was limited in soil treated with low N and given the low N content of biochar it was unable to supply enough N to the crop. Comparing the maize grain yield for 120 kg ha^{-1} N amendment with and without biochar amendment, it can be deduced that the increase in maize grain yield was not solely due to the increase in N rate. The highest recorded maize grain yield may also be the result of higher exchangeable elements supplied to the soil with biochar application when N wasis not a limiting factor, i.e., when N was applied at a higher rate.

This result corroborates that of Zhang *et al.* (2011) who reported better maize yield with biochar application under N fertilisation compared to no N fertilisation. Applying 40 t ha^{-1} biochar in the absence of N fertiliser resulted in 7.3% increase in maize yield while with N fertilisation, the increase in maize yield for the same amount of biochar applied was 12.1%. This finding is also supported by a previous study conducted by Chan *et al.*, (2007) where dry matter of radish increased with increase in biochar rates in N fertilised soil by up to 266% whereas in non N fertilised soil, increasing the rate of biochar did not increase the dry matter of the crop.

N fertiliser rate did not show significant effects for the second crop. This may partly be due reduced soil moisture (see rainfall data in Figure 1) during the second crop compared to the first crop, which was a serious limiting factor. Therefore, applying higher rates of N did not show improvement in crop yield as represented in the von Liebig model (Grimm *et al.,* 1987). However, biochar main treatment effects showed significant increase in maize grain yield. This proves that even under extreme conditions, biochar can still provide favourable soil conditions for plant growth by making nutrients available in the soil for plant uptake, thus improving crop productivity.

Hossain *et al.*, (2010) showed that improvement of cherry tomato by 64% was feasible with 10 t ha^{-1} wastewater sludge biochar applied to Alfisols. The increased production was attributed to the effects of biochar in increasing nutrient availability, especially N and P. Cumulative increases of rice and sorghum yield on a Brazillian Amazon Oxisol by up to 75% for four growing seasons (over two years) with 11 t ha^{-1} biochar treatment was reported by Steiner *et al.,* (2007).

The lower grain maize yield observed for the second crop was most likely due to insufficient rainfall during the maize's critical growth and reproductive stages. In May 2014, during the maize's reproductive stages (second crop), rainfall was 0.0 mm for the earlier month whereas in October 2013, during the reproductive stage of the first crop, the total rainfall was 225.4 mm. Insufficient water availability during the maize's reproductive silking stage, which is the most critical stage of the crop's life cycl, most likely contributed to the reduced yields. Haghihi *et al.,* (2010) and Viswakumar *et al.,* (2008) also reported very poor maize yields under dry conditions due to insufficient rainfall and little response to N application. Kiziloglu *et al.,* (2009) observed a linear relationship between

water use efficiency and maize grain yield and they found that higher water deficiency resulted in a significant reduction in water use efficiency and corn yields.

Clinoptilolite zeolite treatments did not show significant effects for all the plant parameters in both the first and second crops. It was observed that clinoptilolite zeolite did not show significant effects on soil nitrogen and thus it is predictable that clinoptilolite zeolite would not contribute to better maize growth and yield.

CONCLUSION

Biochar has the potential to increase retention of N and increase its availability in soil. Due to this quality, maize grain yield was increased with the EFB-POME biochar treatments. Biochar enhances the capacity of a soil to sustain crop growth and yield even under unfavourable conditions, as evidenced in this case during the drought season.

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4

A General Review of Arsenic Bioavailability

Wan Asrina Wan Yahaya and Scott Young

INTRODUCTION

Arsenic contamination is widely found in the geosphere but the extent and significance of contamination between countries depends on the source of As and, crucially, its solubility potential in relation to potable water supplies. The global average arsenic concentration in uncontaminated soil is 5 – 6 mg kg^{-1}. Conversely, large concentrations of As in surface soils and water are most likely to result from anthropogenic activities such as sewage sludge disposal, pesticide application, mining and smelting. However, the largest incidence of arsenic pollution in the geosphere which has significantly affected human health globally has been caused by groundwater contamination and subsequent used to irrigate agricultural land and drinking water for cattle production (Smedley *et al.*, 2002). Numerous research studies have striven to elucidate the exact nature and extent of As contamination predominantly through investigation of solubility and mobility in soil-water systems, including groundwaters, wetlands, and agricultural land, in terms of both underlying both causes and consequences for biota.

According to Caussay (2003), the interpretation of bioavailability can represent the percentage of the external dose of a particular element that reaches the systemic circulation of biotic (e.g. plant, animal, and human) and abiotic media (e.g. water and soil) which may be remained adsorbed the media and effects normal functions and quality. It is an addition of four components consisting of ingestion, bioaccessibility, adsorption, and first-pass effect upon exposure to a particular element, in this case is As.

Hence, this paper provides a brief view of arsenic bioavailability in plant, aquatic organisms, mammals and humans, and foodstuffs that potentially may bring harmful affect.

Epidemiology of Arsenic Contamination

A review by Ng *et al.* (2003) suggested that inorganic arsenic was regarded as the 'number one' toxin in the USEPA list of prioritised pollutants. There have been various epidemiological studies of human populations that have clearly demonstrated the carcinogenic effects of inorganic arsenic (Celik *et al.*, 2008; Liaw *et al.*, 2008; Smith *et al.*, 2006a,b; von Ehrenstein *et al.*, 2006). Sustained exposure to As has lead to chronic arsenic poisoning (arsenosis) manifested as damage to internal organs, including the respiratory, digestive, circulatory, neural, and renal systems (IPCS, 2001; ATSDR, 2000). The most significant consequence of chronic exposure to As is the occurrence of cancer in various organs especially the skin, lung, and bladder (IPCS, 2001; ATSDR, 2000). A recent report has indicated that, apart from health problems, arsenosis patients also experience severe negative social impacts such as being barred from social activities with unaffected people (Hassan *et al.*, 2005). Arsenosis problems generally occur in people who live in endemic areas which rely on groundwater (Figure 4.1) as their main source of water for drinking purposes which naturally contain high arsenic concentrations or lie within mining areas (Smedley *et al.*, 2002). Countries faced with the most severe arsenosis problems include China (Guo *et al.*, 2001), Taiwan (Liu *et al.*, 2006), Vietnam (Berg *et al.*, 2001), Bangladesh (Anawar *et al.*, 2003), United States (Smedley *et al.*, 2002), and Nepal (Pokhrel *et al.*, 2009).

Figure 4.1 Summary of the world distribution of documented problems related to arsenic in groundwater (above 50 µg L^{-1}) and the environment. Areas in blue are lakes (Source: Smedley *et al.*, 2002)

Arsenic Bioavailability

Plants

It has been established that As may cause toxic responses in plants outwith any damaging effects to the animal and human food chain. n crop plant, for example, a toxicity threshold has been established at 40 mg kg^{-1} (Sheppard, 1992); greater concentrations have lead to inhibition of photosynthesis and reduce grain yields (Rahman *et al.*, 2007; Abedin *et al.*, 2002). The distribution of As between plant organs is highly variable, with seeds and fruits generally having lower As concentrations than leaves, stems, or roots (Hartley *et al.*, 2007; Rahman *et al.*, 2007; Tlustoš *et al.*, 2002). Roots and tubers normally have the greatest As concentrations, with the skin having higher concentrations than the inner flesh (Jones, 2007). Trials have also established that the edible portions of leaf vegetables, such as lettuce, rarely accumulate large amounts of As, because most plants would be killed or severely stunted before the As concentration in the plant tissues reaches concentrations that pose a health risk to consumers (Panda *et al.*, 2010; Tišler *et al.*, 2002). Arsenic bioavailability to plants varies according to soil conditions and may increase under anaerobic conditions due to As mobilisation into solution (Xu *et al.* 2008). Thus, although it has been established that As^{V} is usually the main arsenic compound identified in

terrestrial plant tissue, As^{III} is actively transported into plants when they are grown under anaerobic conditions where arsenite is likely to be the main form present in the soil solution (Meharg *et al.*, 2002).

Studies have been undertaken to examine the influence of soil As on plant uptake and distribution in plant organs (Craw *et al.*, 2007; Rahman *et al.*, 2007a,b; Száková *et al.*, 2007). For example, fruit analysis of *Capsium annum* L. treated with As^{III} showed that As^{V} was the predominant species both in the soil solution and plant cells caused by oxidation of As^{III} (Száková *et al.*, 2007). According to Clemens *et al.* (2006), plants have specific detoxification mechanisms when growing in As-contaminated soils. Smith *et al.* (2008a,b) reported that reduction of As^{V} to As^{III} occurs rapidly inside radish's roots; the arsenite later reacted with thiolate groups to form As^{III}-sulphur compounds for transport and storage in leaves and shoots. It was proposed that complexation of As^{III} with a variety of sulphur-rich plant tissues act as phytochelatins in response to As stress. Generally, arsenic concentrations in the edible parts of crops depend on the bioavailability of soil arsenic and the ability of a crop to take up As and to translocate it to the target organs (Huang *et al.*, 2006; Quaghebeur *et al.*, 2005). Hence, soil applications such as lime and iron-bearing materials from industrial by-products were recommended as a way to immobilise As in garden soils (Lidelöw *et al.*, 2007).

Aquatic Organisms

In response to anthropogenic introduction of As in aquatic environments, accumulation of As has been noted in marine and freshwater organisms such as phytoplankton, sea anemones, duckweed, water spinach, and macrophytes. The As is usually reported to be present as hexafluoroarsenate, tetramethylarsonium and arsenobetaine (Daus *et al.*, 2009; Mkandawire *et al.*, 2005; Neff, 1997). Rooted aquatic macrophytes for example were presumed to have an important function in moderating the cycling and toxicity of As because these plants are closely associated with other aquatic consumers (Caussy, 2003). It was suggested that rooted macrophytes accumulate more As than other submerged plant species since they are located within anaerobic sediments where the As is mobilised. Thus As uptake by rooted macrophytes led to increased uptake of As by Greylag geese which feed on *Scirpus maritimus* rhizome and bulb material (Taggart

et al., 2009). The use of *L. gibba* (duckweed) has been suggested as a preliminary bioindicator for As transfer from substrate to plants and a means of monitoring the transfer of arsenic from lower to higher trophic levels in abandoned mine sites (Mkandawire *et al.*, 2005). Similarly assimilation of As by sea anemones, which are commonly found on the sea bed, can produce large concentration of TEMA (tetramethylarsonium). It has been suggested that these non-edible marine animals are an important animal group in arsenic cycling through transference of TEMA to marine predators (Ninh *et al.*, 2008). However, since accumulation of As by aquatic organism is mostly in the form of organic As species this therefore poses less of a toxic problem for aquatic consumers. In general, bioavailability of As to marine and freshwater organisms would be influenced by alkalinity, hardness, pH, dissolved oxygen, temperature, turbidity, carbon dioxide, magnesium salts, phosphates, and chelating agents (Neff, 1997).

Mammals and Humans

Exposure to arsenic among humans (Figure 4.2) may occur through the air (inhalation) and intake of food and soil material (oral) (Wang *et al.*, 2006; Smedley *et al.*, 2002). Most arsenic poisoning cases in humans have been related to drinking arsenic-contaminated water (Smedley *et al.*, 2002). About 50% of As uptake is expelled in urine 3 to 4 days after consumption (Hindwood *et al.*, 2004), with small portions deposited in hairs, nails, lungs and skin (Ng *et al.*, 2003). Results from a simple regression model by Hindwood *et al.* (2004) suggested that soil As concentration was the most significant predictor of increased urinary As concentration in residents living in old mining areas of Australia. Hence, human urine has frequently been tested and results are used as an immediate indicator of the As hazard to the population (Mandal *et al.*, 2002).

In the human body, inorganic As consumed in drinking water will be methylated to MMAs and DMAs before being rapidly excreted in urine (Vahter, 1999). The reported proportions of the four As compounds in human urine, irrespective of the type and extent of exposure, are 10 - 30%, 60 - 80% and 10 - 20% for inorganic As, DMAs, and MMAs respectively (Shraim *et al.*, 2003). Generally, the As concentration of urine is reported to vary from 5 - 40 μg day^{-1} whereas an As level that would indicate acute or sub-acute As poisoning is > 100 μg day^{-1} (Duker *et al.*, 2005). Total As intake also varies by gender, age, socioeconomic class and level of As

concentration in local soil (Tseng *et al.*, 2009). A major transfer route for As into the human body is via seafood consumption. The daily intakes of As in Spanish (Falćo *et al.*, 2006) and Korean (Lee *et al.*, 2006) populations through seafood consumption are estimated as 217 and 38.5 μg person^{-1} day^{-1}. However, this source of intake is regarded as safe because the As is present as organic species rather than arsenate or arsenite.

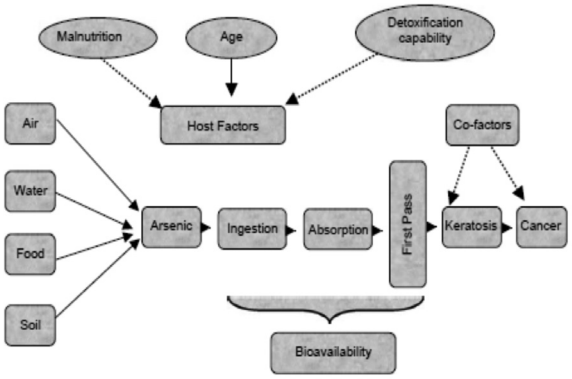

Figure 4.2 A schematic diagram of proposed model of arsenicosis (Source: Caussay 2003)

Limited established reports are available on bioaccumulation of As in mammals or small animals (Rahman *et al.*, 2008). Most arsenic studies have used mice (Ichikawa *et al.*, 2010; Matos *et al.*, 2010) and primates (Roberts *et al.*, 2007, 2002) to evaluate bioavailability and toxicity of As to humans. Recent studies by Sigrist *et al.* (2010) showed that only small As concentrations were found in raw bovine milk (< 2.2 μg L^{-1}) following consumption of As contaminated water which suggested that transfer to milk following bovine ingestion is relatively minor. Nevertheless, Rahman *et al.* (2008) suggested that deposition of As in cattle may be substantial when consuming rice straw and husk grown in As contaminated soil and this possibly transferable to human bodies.

Foodstuffs

A review by Borak *et al.* (2007) on the dietary As in humans dealt with two aspects: (i) type of arsenic consumed (i.e. organic vs. inorganic) and (ii) source of As (i.e. seafood products vs. grains and rice). According to Roychowdhury *et al.* (2002) the bulk of As intake by humans is from seafood products (fish, shellfish, seaweed and algae). Speciation analysis of seafood products indicated that arsenobetaine was the predominant species present, while other species including As^{V}, MMAs, DMAs, arsenocholine, arsenosugars, trimethyl arsine oxide (TMAO) and tetramethyl arsonium ion (TETRA) mainly exist at low concentrations (Devesa *et al.*, 2005; Li *et al.*, 2003). Global arsenic-food contamination may occur as foodstuffs imported from arsenic-contaminated countries (Al Rmalli *et al.*, 2005). However, because of the extremely limited database available on organic As toxicity to humans no Provisional Tolerable Weekly Intake (PTWI) has been adopted for organic As; there is only the general assumption that it has a low toxicity compared to inorganic species (COT, 2003).

Analysis of As speciation in fish, shellfish, and algae has indicated that arsenobetaine and arsenosugars are the predominant species found in many seafood products while inorganic As species are < 3% of total As (Borak *et al.*, 2007; Li *et al.*, 2003; de Gieter *et al.*, 2002). It has even been suggested that the presence of As at low concentrations in marine organism might be essential for their growth (Neff, 1997). Nevertheless, the presence of As in seafood products has been related to local contamination. In Cuba, for example (Fattorini *et al.*, 2004), China (Li *et al.*, 2003) and Spain (Muñoz *et al.*, 2000) where concentrations may vary according to geographic location and As concentration in the sea. It was reported that about 27 – 43 and 218 – 228 mg kg^{-1} of As (wet weight) was measured in seaweeds and clams in Canada with (human) bioaccessible fractions of 34 - 46% and 63 – 81% (Koch *et al.*, 2007). Those concentrations were larger than typical concentrations found in clams (3.5 to 10.4 mg kg^{-1}, wet weight) and marine algae (0.8 to 14.5 mg kg^{-1} wet weight) as reported by Borak *et al.* (2007). In another survey by Muñoz *et al.* (2000) 66% of 153 seafood products (fresh, canned, and salted fish) contained total As concentrations that exceeded the maximum permitted level imposed by the Australian, Malaysian and Singapore legislation for seafood products, 1 μg L^{-1} wet weight (w/w). The transformation of organoarsenical species during cooking has been shown in fish samples (Devesa *et al.*, 2005; Hanaoka *et al.*, 2001). Devesa *et al.*

(2005) measured transformation of arsenic species during cooking which resulted in increased DMA and TMA^+ in 64 cooked seafood products. These transformations may therefore produce a change in the toxicity of seafood and therefore risk assessment should consider the organoarsenical species present in cooked seafood components of a diet.

The importance of arsenic-contaminated soil and rice has recently been recognized in south-east Asia, especially because rice is a staple food for the region (Meharg, 2004). Results for As concentration in different rice types have indicated greater inorganic As and DMAs concentrations in brown rice than in white rice. Surprisingly, this may cause greater problem to consumers in developed countries who prefer brown rice in their diet (Meharg *et al.*, 2008a, b). Increased As concentration in rice and other foods has also been seen when contaminated water has been used for cooking purposes (Díaz *et al.*, 2004; Misbahuddin, 2003). In Japan, substantial amounts of inorganic arsenic (75 - 90%) were also measured in rice products such as rice milks, cereals, crackers and rice condiments which are commercially manufactured (Sun *et al.*, 2009; Meharg *et al.*, 2008a). An earlier market survey in the United States indicated that other sources of As enriched foodstuffs included grape juice and cooked spinach which previously received organic As-pesticides during plant growth (Schoof *et al.*, 1999). It was reported that washing As-contaminated rice might remove 57% of total As present in rice grains and so reduce dietary exposure (Sengupta *et al.*, 2006). For foodstuffs a level of 1 mg kg^{-1} total As in rice is regarded as a standard maximum permissible concentration; this equates to the 1959 UK standard (The Stationary Office, 1959). It has been accepted that As in rice is wholly bioavailable (Smith *et al.*, 2006a,b).

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5

Influence of Reducing Conditions towards Lead Solubility in Soil Amended with Biosolids

Wan Asrina Wan Yahaya and Scott Young

INTRODUCTION

Research interest in trace metal such as Pb, Co, and Ni solubility in is mostly motivated by environmental concern related to remediation programs arising from anthropogenic contamination. Many investigations have been conducted to understand trace metal solubility in soil as influenced by changes in soil conditions such as pH, organic carbon, redox potential and source of contamination (Christensen *et al*. 1996; Cornu *et al*. 2007; Du Laing *et al*. 2009; and Gray and McLaren 2006).

Understanding the multiple effects of reducing conditions on Pb solubility solubility is important. These are likely to include variations in pH, redox state (valence), competition from competing ions, complexation with ligands and even the surface area of adsorbents. Grybos *et al.* (2007) concluded that under reducing conditions observed in wetland soil samples, there were four mechanisms responsible for Pb, Co and Ni solubilisation interrelated to metal/metalloid-adsorbing soil constituents association: (1) DOM mobilisation which responsible for trace elements increases, (2) mixed effects of DOM released and Fe^{III} reduction reactions toward Pb and Ni solubility, (3) enhanced of Co concentrations solely by Fe^{III} reduction, and (4) unclear source of mechanisms for Cr, Cu and Th since mechanism would involve species transformation and/or solution speciation. Pareuil *et al.* (2008) stated that increased concentration of Pb, Ni, Cd, and Cr was related to the reduction and dissolution of oxides surfaces.

Those suggested mechanisms might be responsible for enhanced metal solubility as Eh declines and the relative importance of those mechanisms may vary between soils and metals. As a consequence of reducing conditions, rice and rice products, fish and vegetables have all been reported to contain elevated concentration of Pb and other trace elements (Meharg 2004; Das *et al.* 2004). Hence, the main objective of this study was to examine the influence of reducing conditions towards Pb solubility in soil amended with biosolids.

MATERIALS AND METHODS

Site Selection and Sampling

Soil sample for microcosm experiments was collected from sewage treatment (SF) and disposal farms in the East Midlands, Nottingham, U.K. (Grid reference: c. 52°57'35" N; 1°02'30" W). This site is characterised as having different levels of contaminants (i.e. Pb and Cd) following decades of biosolids amendment.

Batch Incubation

Batch incubations were undertaken in 1 L polyethylene bottles: sub-samples of 200 g (dry-weight basis) of field-moist soil samples (< 2 mm sieved) were suspended in 600 mL deionised water (w/v ratio, 1:3). Half the suspensions were treated with 0.5% (w/w) of powdered straw intended to act as a carbon source to increase microbial activity. All treatments were undertaken in triplicate. The suspensions were incubated in a dark controlled-temperature room (25ºC) for six weeks with daily hand-shaking, end-over-end.

Weekly, soil Eh (Jenway 3010 voltmeter) and pH (Orion 720A meter) were measured and a 20 mL aliquot was withdrawn, centrifuged (2,200 rpm for 10 min), filtered (0.2 µm) and further divided for analyses. For Fe, Mn, and Pb measurement, an aliquot of 5 mL was preserved with 0.2 mL of 50% HNO_3 and 2 mL of 0.001 M Na-EDTA prior to analysis by ICP-MS (Thermo-Fisher Scientific X-Series^{II}, Germany). While, an aliquot for anion analysis (i.e. SO_4^{2-}, Cl^-, CO_3^{2-}, NO_3^-, and PO_4^{3-}) by Ion Chromatography (Dionex DX500 with IonPac AS-14, 4 x 250 mm anion exchange column) and dissolved organic carbon (Shimadzu TOC-V CPH/

CPN Total Organic Carbon Analyser, Model TNM-1) was preserved, without treatment, by freezing and storing at -20ºC.

RESULTS AND DISCUSSION

Soil Characterisation

The chemical composition of sewage farm soil (SF) under investigation is shown in Table 5.1. The soil pH value was 6.55 with the LOI results was 17.9%. The elevated loss of ignition (LOI) percentage for SF soil was originated from historical amendment of biosolid on the surface. Concentration of Pb in SF soil was elevated than the concentration found in background soil (10-50 mg/kg) as resulted by almost 100 years of sewage sludge amendment in the area (Heaven and Delve, 1997).

Table 5.1 Selected physico-chemical characteristics of sewage farm soil (SF)

Metal (mg kg^{-1})	Soil
pH (H_2O)	6.55
SOM (LOI, %)	17.9
Fe	21,000
Mn	589
Pb	400

Effect of Anaerobic Incubation on Eh, Ph, and Dissolved Organic Carbon

Figure 5.1 shows the effect of anaerobic incubation on Eh, pH and dissolved organic carbon (DOC) for the sewage farm soil with, and without, amendment with powdered straw. In general, for both soil-treatment combinations, anaerobic incubation caused an increase in DOC concentrations and soil pH and a reduction in Eh. Ongoing anaerobism in soil suspensions of SF ultimately caused a decline in soil Eh from oxidizing to reducing conditions with an average Eh value of 100 mV at the end of the experiment (Figure 5.1a).

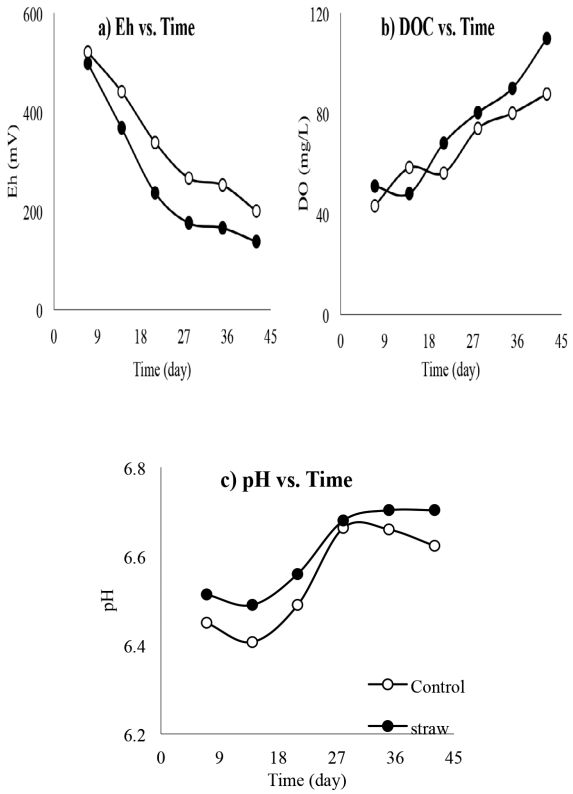

Figure 5.1 Suspension of Eh (Fig. 1a), DOC (Fig. 1b), and pH (Fig. 1c) values for Sewage Farm soil as a function of time during anaerobic incubation at 25°C

Decomposition of soil organic matter during anaerobic incubation increased DOC (Figure 1b) concentrations in straw-amended treatments relative to unamended suspensions. This may have arisen: (i) directly from straw decomposition products and partly from, (ii) enhanced decomposition of humus and (iii) release of adsorbed humic or fulvic acid from dissolving Fe/Mn hydrous oxides. Generally, for both soil treatments between 80 to 110 mg L^{-1} DOC was dissolved after 45 days of incubation.

The effect of straw-amendment on the pH of the soil suspensions (Figure 5.1c) had caused a consistent increase for SFH soil; 6.7 to 6.6 for amended and unamended soil suspensions. A change in soil pH under

progressively anaerobic conditions may occur as the net result of several biochemical reactions in soil suspensions. These include a build-up of CO_2 which hydrolyses to carbonic acid and, at circum-neutral conditions, produces HCO_3^- ($+H^+$). Moreover, decomposition of added straw may lead to release of organic acids (e.g. acetic acid; pK_a 4.7) which will release virtually all weak acid protons where pKa << pH. However, under anaerobic conditions reduction reactions invariably consume H^+ ions and would therefore elevate the pH of soil suspensions - e.g. dissolution of ferric hydrous oxides:

$$Fe(OH)_3 + 3H^+ + e^- = Fe^{2+} + 3H_2O$$

Regardless of the straw treatment imposed on the sewage farm soil, anaerobism consistently elevated soil pH: results generally ranged from 6.45 initially to ~6.7 toward the end of incubation. However, the range of pH values shown between unamended and amended suspensions soils was very small (≤0.1 units) and both samples displayed a general shift toward neutrality as anaerobism progressed. From the observation, suggestion can be made that under reduced conditions with soil pH showed circum-neutral values, roles of pH was irrelevant to explain metals bioavailability. Similar observation was reported as well by Grybos *et al.* (2007) and Ma and Dong (2004) after assessing effect of several pH values (pH 5 to 6) towards trace metals solubility.

Effect of Anaerobic Incubation on Iron and Manganese Solubility

During anaerobic incubation, dissolved Fe^{2+} and Mn^{2+} concentrations in the solution phase of SF suspensions generally increased (Figure 5.2). All straw-amended suspensions of SH showed greater solubility of Fe^{2+} and Mn^{2+} than unamended suspensions (Figure 5.2a-b). However, the dissolved Fe^{2+} concentrations between SF treatments were insignificantly different during anaerobism with an average of 0.7 mg L^{-1} released into solution. The Mn^{2+} dissolution range of SF soils was in the range of 0.5 to 0.8 mg L^{-1} with small differences as resulted by treatment received.

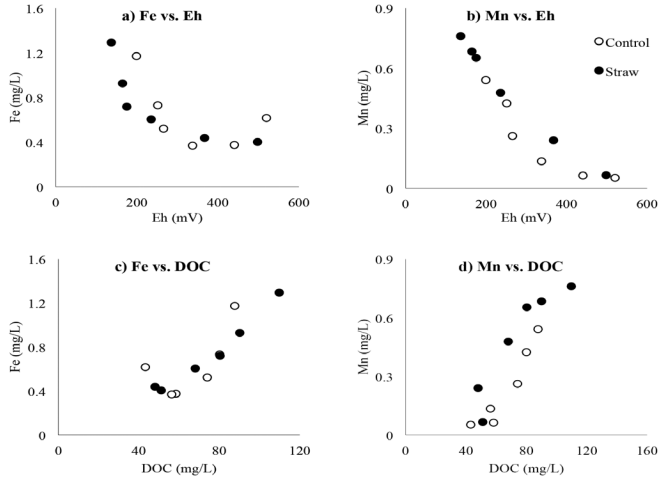

Figure 5.2 Iron and manganese concentrations of Sewage Farm soil as a function of DOC (2a-b), and Eh (1e-f) during anaerobic incubation at 25°C

Redox potential (Figure 2a-b) is likely to be the key factor controlling Fe and Mn solubility under reducing conditions, particularly if pH range is restricted (Figure 1c). With a decrease of Eh generally from 500 mV to 150 mV, Fe^{2+} and Mn^{2+} concentrations in SFH soil suspension evidently increased due to reduction and dissolution of amorphous Fe^{III} and Mn^{IV} hydrous oxides. Indeed, it has been reported in earlier studies that transformation of ferric and manganic compounds occurs within the Eh ranges of 300 to 100 mV and 300 to 200 mV for soil pH values of 6 – 7 under anaerobic conditions (Onken and Hossner, 1996; Gotoh and Patrick, 1974).

Under reducing conditions with soil pH close to alkaline conditions, increased Fe and Mn solubility in SF suspension may also be related to DOC concentrations through the formation of Fe/Mn-dissolved organic complexes (Figure 2c-d). Thus, DOC concentration may be elevated because of release of adsorbed humic and fulvic acid (FA) during the reduction of Fe/Mn oxides but DOC will also form complexes with Fe^{2+} and Mn^{2+} and thereby retain those cations in solution under anaerobic conditions. This relationship is suggested in Figure 2c-d, where DOC and Fe/Mn concentration are highly correlated and had been observed as well

by Christensen *et al.* (1999). Hence, increasing Fe^{2+} and Mn^{2+} eventually will subsequently releases absorbed trace elements into solution which beneficial for plant uptakes and/or can cause land-water contamination.

Effect of Reducing Conditions on the Solubility of Lead

Figure 5.3 shows the effects of continuous reducing conditions towards Pb solubility in soil amended with biosolids. General solubility trend for Pb has indicates that the concentration was increases as Eh declined from 550 to 150 mV. This releases was caused by the combined effects of reductive dissolution of Fe/Mn oxides and organic matter released, as previously reported by Grybos *et al.* (2007) and Pareuil *et al.* (2008). Reducing conditions lead to increased Pb solubility in SF soil suspensions with an average proportion of total Pb releases was 0.02%. Low Pb solubility in the sewage-sludged soil is probably related to the high phosphate availability from decades of sewage addition (Alloway, 1990). The Pb in these soils may be precipitated as chloropyromorphite ($Pb_5(PO_4)_3Cl$).

Anion analysis during the incubation indicated that the sewage farm soils contained larger soluble phosphate with concentration ranged from 2 mg L^{-1} to 5 mg L^{-1}. The relationship between Pb and Fe does not necessarily imply that the Pb is adsorbed on Fe oxides. The trend shown may arise from Fe^{2+} competition with Pb^{2+} for adsorption sites on humus in the CM soil (Charlatchka *et al.*, 2000). In all soil suspensions dissolved Pb fell above the WHO drinking water permissible level of 10 µg L^{-1}. Amendment of straw (70 µg L^{-1}) into incubation soil relatively affects Pb concentration compare to unamended soil (55 µg L^{-1}).

Observations from incubation experiment confirmed the important role of Eh in enhancing Pb solubility through reduction reactions involving Fe and Mn hydrous oxides. However, the solubility trends for metals were observed to be vary qualitatively as anaerobism progressed. These differences were attributed to different solubility mechanisms involving each metal, the emergence of competition between dissolved anions and cations to form complexes, and metal-specific adsorption-desorption strength. Pb solubility did not seem to be enhanced in the straw-amended suspensions although straw-amendment significantly accelerated reduction ($p < 0.05\%$). Similarly, the trend in Fe^{II} and Mn^{II} concentrations in unamended and straw-amended suspensions were not

significantly different. Furthermore, straw-amendment did increase DOC concentrations thus did not seem to influence the formation of metal-organo complexes. Metals solubility in all six soil suspensions seemed to be strongly related to the reduction of hydrous oxide surfaces.

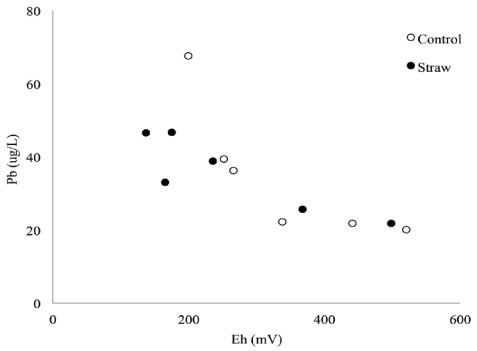

Figure 5.3 Influence of redox potential on (a) lead concentrations in Sewage Farm suspension during anaerobic incubation at 25°C (error bars are SEM values)

One of the issues that clearly need further investigation is the solubility mechanism of Pb as well for other trace elements under reducing conditions. Central to this is understanding the fate of dissolved Fe(II) and Mn(II) and minor cations (e.g. Pb^{2+}) which may compete for DOC complexes. It is still unclear how Fe and Mn hydrous oxides influence trace element solubility under reducing conditions. Solubility results from the batch incubation experiment demonstrated that Pb solubility had produced various responses when compared against Fe(II) and Mn(II). However, solubility calculation for Fe and Mn oxides suggested that Fe oxides ($Fe(OH)_3$ and $Fe_3(OH)_8$) were the major hydrous oxides controlling release of associated metal as anaerobism started.

Floodplain areas are often sites of high soil fertility which then encourages the area to be intensively used for agriculture. However, flooding is common, and hence gives rise to concern over the potential for trace elements contamination in relation to UK and WHO drinking water standards (WHO 1993). Results had suggest this is a seriously considered at Eh values below 400 mV (Figure 3) which is easily found in many floodplains and yet such redox potentials (500 to 400 mV) would

be categorized as oxidizing conditions. Results from the batch incubation suggest that Pb (15 μg L^{-1}) in sewage farm soils almost exceeded UK (Pb = 25 μg L^{-1}) and WHO (Pb = 10 μg L^{-1}) drinking water standards. The potential for mobilisation of Pb may require consideration when drawing up risk assessments for floodplain agricultural soils.

CONCLUSION

In conclusion, anaerobic conditions significantly enhance Pb solubility and by means of Fe and Mn hydrous oxides dissolution and DOC complex formation. Hazardous levels of Pb concentration were reached within a range of redox potentials typically prevailing in wet topsoils in Autumn and Spring in the UK. The metal concentrations were considerably greater than permissible levels proposed by the WHO (1993) as drinking water standards.

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6

Important Hotspot of *Aquilaria* Species Producing Higher Grade of Gaharu in Belaga Sarawak: A Collectors View

Phillip Lepun and Ribka Alan

INTRODUCTION

The rainforests of South-East Asia are unique because of its richness and most valuable of world heritage in socio-economic and environmental value in the world. Once of the valuable tree species occurred in tropical forest is *Aquilaria* species which also called as the Wood of God in Asia. There are three species distributed in Sarawak namely *A. malaccensis* (Gaharu Nibong/Beringin), *A. beccariana* (Gaharu Air/Babi/Buaya) and *A. microcarpa* (Gaharu Bukit/Lala) (Donovan and Puri, 2003; Tawan, 2003). For centuries Gaharu has been traded internationally for the resinous wood infected by fungi, called agarwood or gaharu amongst other things. It is used as incense, perfume and in traditional medicine. The increase in levels of trade over the past decade has resulted in over exploitation throughout its ecological range.

Historically, for both local people as well as their governments, the sale of such forest products has been an important source of cash income and means of access to foreign goods. As with many non-timber forest products, *gaharu* has a history of boom and bust markets (Gianno, 1990). The most recent expansion of commercial activity began in the late 1970s. Lately, prices for the best quality *gaharu* have been quoted at about twenty times that rate, with prices to buyers across the straits in Singapore at ten to twenty times again this price (Hansen, 2000; Clear 2000). With much of the best quality material smuggled out of Sarawak, trade records for this product are admittedly poor. As a result, the true point of origin

and even the species of much of the traded material may be difficult to ascertain with the naked eye. Malaysia and Indonesia, however, appear to be the world's major exporters of this product, which trades in the form of wood chunks, chips, and dust as well as processed goods such as essential oil, perfume, incense, and medicinal preparations (Chung and Purwaningsih, 1999; Barden *et al.*, 2000). Global demand for gaharu was reported currently exceeds the available supply and this has contributed to the hike of price. In 2004, the price of super quality gaharu wood or chips of *Aquilaria* species from the collector in Belaga was in the range of RM 4,000.00 – RM 6,000.00 per kilogram and in 2007, the price for good grade B and above was doubled.

Belaga district in the 40 years ago is an isolated area from the development and the only access by Rejang river. Most of the communities were depending on the natural resources for butter trade in Bazaar Belaga. Belaga district area is one of the best areas of gaharu produce and form the best quality of gaharu in Sarawak. Most of the collectors are the Orang Ulu (Kayan, Kenyah, and Penan). Gaharu is the most valuable good trade for the Orang Ulu since late 1960s in Belaga. When Malaysia government launched its own National Policy on Biological Diversity in 1998, to prevent the loss of forest habitats and its species in the country and since then, the collectors slowly refused to hunting for gaharu. Three *Aquilaria* species namely *A. malaccensis, A. beccariana* and *A. microcarpa* were included in the list of protected plant in Sarawak (Faridah-Hanum *et al.*, 2009). Convention on International Trade in Endangered Species (CITES) latest meeting in October, 2004 held in Bangkok highlighted that the genus of *Aquilaria* spp. and *Gyrinops* spp. to be regulated under Appendix II with annotation one that covers all products. Follow-up to that CITES on 17 February 2005 listed all *Aquilaria* sp. and *Gyrinops* sp. in Appendix II (Lim *et al.*, 2007).

In Sarawak, not much documentation work was done on gaharu except for some taxonomic and ecology studies. This present study proposes to conduct a specialist study of the distribution of *Aquilaria* species in Belaga Sarawak based on the collection historical of the best quality of gaharu product, in order to determine if their occurrence is in recognizable their needs correlated to geographical, geological or ecological features.

The practical aspects would be chiefly to assess and map the distribution of Sarawak *Aquilaria* species in order to;

1. Assess the relative restriction of their ranges; and to
2. Pinpoint special site for gaharu plantation needs in the future.

MATERIALS AND METHODS

Data of the gaharu distribution had been mapped based on the total of 292 specimens during the visits to the herbaria at Universiti Kebangsaan Malaysia (UKMB), Botanic Garden Singapore (SING), Forest Research Institute of Malaysia (KEP), University of Malaya (KLU), Sarawak Forestry Department (SAR), and Sabah Forestry Department (SAN). The data were also obtained from many field trips undertaken during few surveys on gaharu distribution with collectors and the traders in Belaga and Baram areas in Sarawak. The ecology andenvironmental factors which influenced the formation of gaharu of the different species were also noted down from these sources. The exact localities were determined from the gazetteers and global positioning system (GPS). With ArcGIS 9.1 software, a map on the distribution of *Aquilaria* spp. in Belaga distric was generated.

RESULTS AND DISCUSSION

Eventually the distribution of gaharu could be found in most areas in Sarawak, but not all areas could produce gaharu or good quality of gaharu. The results of the interviewed which involved of 38 respondents from various villages and group of the rural communities who involved in gaharu hunting since 40 years ago had figured up the locality of gaharu areas produced good quality in Belaga district as shown in Table 1. Peliran-Danum areas which also known as the Usun Apau is identify as the best area for gaharu. The formation of good resin in *Aquilaria* species visually related with climatic, Soil types, geology, rainfall distribution, vegetation, elevation, and association plants species and the hotspot of gaharu in Belaga area as shown in Table 6.1 and Figure 6.1.

Table 6.1 Hotspot areas and environmental factors need for gaharu formation

Area	Species	Ecological factors	Vegetation Characteristics	Soil & Geology
Belaga-Koyan	*A. malaccensis* and *A. microcarpa*	Light intensity 2,000-2,500 LUX; Humidity 25 RH; Daily Temperature 20-33°c; Rainfall 3,000-5,000 mm/yr	Lowland to hill MDF up to 1,000 m a.s.l.; Found in Merurong range and Dulit range. Association plants species are *Arenga brevipes, Oncosperma horridum, Pholidocarpus macrocarpus*, and *Koompassia excelsea*,	Soil type Alluvial. Upper miocene – Lower Eocene sedimentary basins & associated igneous rocks. Mainly quartz and lithic sandstones, mudstone and shale conglomerate.
Peliran-Danum	*A. beccariana* and *A. microcarpa*	Light intensity 2,000-2,500 LUX; Humidity 25 RH; Daily Temperature 16-30°c; Rainfall 3,000-5,000 mm/yr	Lowland to hill MDF up to 1,000 m a.s.l.; Found in Sg. Luar, Sg. Singu-Kenaban, Sg. Metalun, Sg. Tiyut and Ulu Sg. Tekulang. Association plants species are *Licuala valida*, *Pholidocarpus macrocarpus, Iguanura sp, Motleya borneensis, Ocharus* spp., *Xanthophyllum* spp. and varieties species of mosses.	Soil type Alluvial and Gleysols. Cenozoic sedimentary basins and associated igneous rocks. Mainly basalt & andesite lava, breccia and tuff.
Balui-Linau	*A. beccariana*	Light intensity 2,000-2,500 LUX; Humidity 24 RH; Daily Temperature 24-31°c; Rainfall 4,000-5,000 mm/yr	Lowland to hill MDF on yellowish sandy loam soil up to 1,000m a.s.l. and mainly riverine area. Found in Ulu Sg. Keluan, Sg. Urak, Sg. Ema and Sg. Bahau. Association plants species are *Koompassia excelsea*, *Pholidocarpus macrocarpus*, and *Xanthophyllum* spp.	Soil type Acrisols. Oligocene-Eocene sedimentary basins & associated igneous rocks with oceanic crustal rocks & overlying deep water sediments. Mainly terrestrial sediments, carbonaceous and rare limestone.

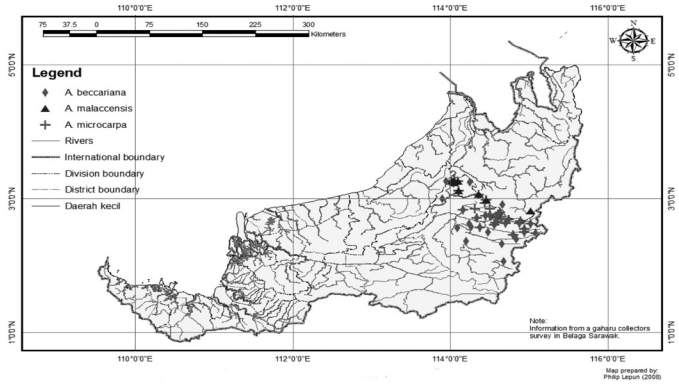

Figure 6.1 Gaharu Productive areas in Belaga, Sarawak

A shade-tolerant tree, *Aquilaria* is an understorey tree of mature evergreen and semi evergreen forest occurring at low to medium altitudes, generally up to about 1000 m.a.s.l. The occurrence of the gaharu tree itself does not guarantee the presence of the resin. Scientists estimate that only 10% of the *Aquilaria* trees in the forest may contain *gaharu* (Gibson, 1977). But, according to the experiences gaharu collectors in Belaga, the famous areas producing the good quality or considered of 10% of gaharu trees found to be producing gaharu depending what species as shown in Table 1. The resinous agarwood product is only found naturally in 10-20% of trees of these species, with a still imprecisely understood combination of wounding, vectors of infection (bacterial infection, fungus) and resinous response (*i.e.* to external and internal causal factors) producing the formation of agarwood (Compton and Ishihara, 2003). The resin forms in response to wounding and subsequent fungal infection, and is found in many parts of the tree, according to some sources in the bark and the roots as well as the heartwood (Jalaluddin, 1977). Donovan and Puri (2004) cited in Sadgopol (1959), under natural conditions, the resin is more commonly found in trees of about 20 years or older, with trees more than 50 years old reportedly having the highest concentration. But, this is view was contradicted with the experiences collector knowledge where the formation of gaharu is not related with the age of the trees. A productive tree may yield several kilograms of the valuable dark, heavy resinous wood with the characteristic honey-like scent. Little detailed information exists

on the scientific need especially on the gaharu productive area, however, on its distribution and its exploitation or use in the rural in Sarawak was well understood.

Penan recognizes that microclimate, soils, and plants differ at different elevations. The Penan recognized that trees containing *gaharu* tend to be located on the steep banks of usually dry streambeds high in the headwaters of small streams (Puri 1992). Similarly, Yamada (1995), working with Penan informants, located a greater abundance of *Aquilaria* on steep slopes. According to the collectors and the Penan informants (Donovan and Puri,2004), *Aquilaria* can be found throughout the forest, whereas *gaharu* tends to be found in trees located on the steep banks of usually dry streambeds high in the headwaters of small streams. Paoli *et al.* (2001) also reported finding that trees at higher elevation contained more than those found at lower elevations. There could be several factors associated with this location that predispose *Aquilaria* trees in this habitat to produce the aromatic resin. Shallower soils may mean more stress on the plant. Location on the ridge may provide a less sheltered environment than riverine forest. Such circumstances may expose these trees to storms and associated wind damage, the wounding necessary for the entry of the fungi. Alternatively, such sites could also provide better habitat for the pathogen or the vector associated with its transmission.

Despite its long trade history, the enduring interest of consumers on was made gaharu hunters in Belaga areas (as indicated by three distinctions of quality of gaharu) where they could found the good quality and sale their gaharu with high prices (Table 1). In several decades of research, no one yet has succeeded in producing high quality commercial *gaharu* from plantations (Barden *et al.* 2000; Soehartono and Newton 2001a; Chang *et al.* 2002; Tabata *et al.*, 2003). In an attempt to address this dilemma, we examine traditional knowledge of collectors could gave us a new prospect on gaharu plantation-the accumulated knowledge of a group of people who have been collecting and trading *gaharu* for several centuries-compared with scientific knowledge published in historical records and scientific journals on the species *Aquilaria* and its product *gaharu*. Dawend *et al.* (2005) has had examined for information on the Sarawak ethnic groups that have been identified as *gaharu* collectors or traders. Locating *gaharu* in the forest is a complex and uncertain task, even for expert collectors.

The experiences collectors such as Penan and Kenyah Badeng noted

that several species, especially palms, were often found near *gaharu*-producing trees. Accordingly, they look for other plant species that might signal the presence of *Aquilaria* likely to contain *gaharu*. One of these local ecological indicators is a species of tree palm known as Talang/*boh* (*Arenga brevipes*) more related to *A. beccariana*, which grows in similar habitats and may be harvested for its edible leaf buds (palm hearts) and starchy pith. Another indicator species is the tree palm called *nyivung* (*Oncospermae horridum*), found in areas below where someone is likely to find *A. malaccensis*. Thus, encountering several of these trees, easily recognized by their black spines, encourages the collectors to pursue their search upstream. This perceived association might be why local Kenyah and Penan call *A. malaccensis* trees *sekau nyivung*. Finally, the palm, *silat* (*Licuala valida*), Anau (*Pholidocarpus macrocarpus*) and a small stemless palm known as *lotup* (*Iguanura* sp) that often grow in the general area of *A. microcarpa* trees containing *gaharu* are also considered indicator species. Other endemic species (*Motleya borneensis*) and *Ocharus* sp. (Jerangau) always been found occurred with the areas known as gaharu area (Pers. Comm. with Okang Lepun, 2008). There has been a lot of interest in the extent to which discrete species association, or consociations, are correlated with site factors in the tropical forest (Wyatt-Smith, 1995). Data collected in Brunei revealed that species associations were correlated with complex soil factors not clearly defined (Brunig, 1970). Although Ashton (1967) found species diversity correlated with total soil phosphorus, especially at low concentrations, plant-available phosphorus is difficult to correlate with total phosphorus. Most research has shown, however, that consociations result from the influence of several factors, not just soil conditions (Whitmore, 1985). Thus, it may be the synergistic effect of the confluence of environmental factors that determines species composition in the *gaharu* habitat.

Others additional factors that could influence the quantity or quality of gaharu produced include the age of the tree, edaphic conditions, and the season of the attack (Donovan and Puri, 2004). *Gaharu* is found in trees as young as 20 years old, but the best quality resin reportedly comes from specimens of 50 years or more (Beniwal, 1989). There are none scientific research has actually been done on the influence of age on the quantity and quality of resin production. Competition among organisms is intense and much influenced by environmental factors, such as temperature and

moisture (Shigo, 1967). The Penan's noting of the location of better gaharu producing trees on just such poorer sites, up toward the ridges, may be an indication that edaphic conditions, perhaps water stress, are important in enabling the infection to take hold and promoting resin formation. Many collectors believe that the fundamental cause of gaharu formation because of many diseases is to be found in the state of the soil. Bakshi (1954) noted, the soil harbors a large number of parasites including ant, leeches and cicadas, which are normally harmless under good conditions of growth but become pathogenic when plants are grown under adverse conditions.

CONCLUSION

Commercial cultivation of gaharu producing *Aquilaria* species in Sarawak and particularly in Belaga district area is need to be studied deeply because of it different in term of natural species distribution attributes of price appreciation and agroecological requirement. The gaharu species also had been proven to be a potential source of new genes as well as new products particularly drugs and thus are valuable to the international agriculture and pharmaceutical industries. Thus, genecological zonation should be done as a tool in conservation of the genetic resources of gaharu species in Sarawak. By packaging the ethno-knowledge of the local communities and scientific approach, the gaharu future in Sarawak will be given a new look and brighter future.

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7

A Study on *Aquilaria Microcarpa* Baill. Growth Performance using Different Fertilizers in Belaga, Sarawak

Phillip Lepun, Nor Azizah Jamil and Ribka Alan

INTRODUCTION

Gaharu, also known as Karas locally or agarwood, aloeswood and eaglewood elsewhere is from the genus *Aquilaria* Lam. (Family: Thymelaeaceae). Gaharu is the fragrant resin produced from *Aquilaria* trees and is formed when an injured or wounded tree is attacked by a fungus or certain insects (I. Faridah-Hanum *et al.*, 2009). Gaharu serves as raw material for the production of many aromatic medicinal products, stimulants and tonics. The essential oil extracted from the wood also serves as constituents for perfumes and cosmetics while the scented wood is highly sorted for incense. Agriculture sectors especially in the rural areas have many alternative crops which they are really understood could giving them higher return in near future such as Gaharu wood. *Aquilaria* spp. is a resinous woody plant from the forest grows in the tropical rainforests of Asia and produce agarwood inside the tree. For centuries, trees of *Aquilaria* spp. have been harvested from the wild for the purpose of collecting gaharu, a highly commercial resinous wood use as incense (Soehartono *et al.,* 2001).

Aquilaria cultivation is suggested to use of the proper and right fertilizer application to give higher outcome of the yield. However, there are still no specific studies about which fertilizer is best in term of promoting faster growth of *Aquilaria* tree. But there is some suggestion of which fertilizer should be use, Tarigan (2004) suggests the use of NPK fertilizer together with organic fertilizers such as compost and manure. Dawend Jiwan (2008) also suggested that in order to be accorded organic farming and environment-friendly gaharu farming system, the choice of growth

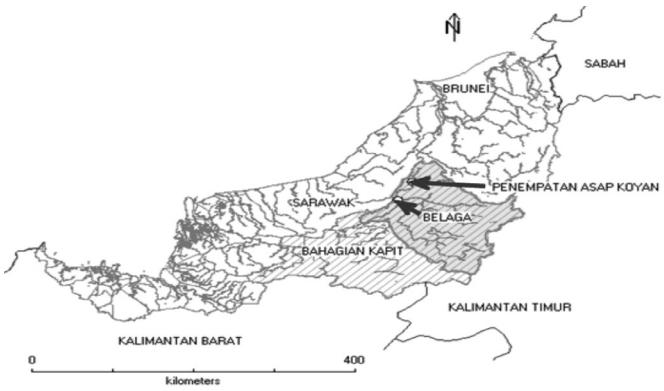
booster should be organic base and a combination of good microbes. Despite that, further research needs to be conducted to investigate the type of the most supportive fertilizers to encourage the growth performance of *Aquilaria* plant. The study was specifying the best fertilizer could be use at the suitable amount and giving the suggestion for the best fertilizer application for gaining *Aquilaria* spp. optimum growth. The growth is the biological phenomenon of increasing in size with time. Growth involves the formation, differentiation and expansion of new cells, tissues or organs. Growth causes trees to change in weight, volume or size and form or shape. It is determined by measurements of mass, length or height (Gardner *et al.*, 1985).

According to Blanchette *et al.,* (2005), the *Aquilaria* tree is an evergreen that grows up to 40 m high and 60 cm in diameter. While according to Tarigan (2004), the growth of *Aquilaria microcarpa* can reach 40 m height and the diameter of 80 cm. In an effort to conduct growth study reported by Dawend Jiwan *et al.*, (2007), planting trial of 1.2 hectare was done at Sabal Agroforestry Centre in April, 2005. Mixed planting of *Aquilaria microcarpa* with *Calophyllum tesymanii* var. *inophylloide* (anti-HIV Bintangor species) was planted in 3 x 3 m spacing under a 22 years old *Acacia mangium* and old secondary forest vegetation. The possibilities of factors affecting mortality and growth were high mortality for planted seedling below one foot, too shady areas may cause slow growth, and too open an area will cause high mortality.

One of the normal silvicultural practices to improve the growth of planted tree crops is fertilizer application. Continuous fertilizing for a specified period from young to intermediate age will improve the growth performance.

MATERIALS AND METHODS

This study was conducted at a smallholder of *Aquilaria* cultivation site at Resettlement Asap Koyan Belaga, Sarawak (Figure 7.1). The aged of the *Aquilaria* plant was about eight months and 600 *Aquilaria* seedlings were cultivated at the area by Kenyah local people. Asap Koyan Belaga is located near Asap and Koyan river, Belaga.



The study plot at one hectare area was planted with 600 seedlings of *Aquilaria* spp. at Asap Koyan, Belaga which consist of *Aquilaria microcarpa, Aquilaria malaccensis,* and *Aquilaria beccariana.* Only the *Aquilaria microcarpa* tree was selected for the study. The selected plot was established in area at 50 m x 50 m where more than 100 of *A. microcarpa* trees were planted and 50 trees of them were randomly selected for this study.

NPK fertilizer, organic compost, slow-release fertilizer, ribbon, weighing apparatus, measuring tape and callipers. NPK fertilizers, organic compost, slow-release fertilizer, are used as the treatments for *Aquilaria microcarpa* trees. The ribbon was used to labelled all replication and treatments. Ordinary weighing apparatus is used for weighing fertilizers. The growth of *A. microcarpa* trees is measured with measuring tape (for height) and callipers (for DBH).

The experiment was laid out randomly; T1 for control, T2 for NPK blue fertilizer, T3 for organic compost and T4 for slow-release fertilizer for four blocks. Sixty selected young *A. microcarpa* plants (eight months planted) ranging from two to four feet height at the site was tested using the treatments. Each fertilizer treatment was applied at three different amounts that are 100 gram, 200 gram, and 300 gram with five replicates for each treatment (15 trees for each fertilizer). There is a total of 50 replicates combination in the experimental design.

Two parameters of height and DBH (diameter at breast height) measurement on all trees in the plot were performed every month started at November 2008 until the next three month. The change in height of each

tree was measured by using measurement tape in centimetre (cm), and the change at DBH was measured using calliper in millimetre (mm).

Data Analysis

The height and DBH increments were calculated of the three months. One-way analysis of variance (ANOVA) was employed to assess the effects of fertilizers on height and diameter (DBH) growth. Duncan's New Multiple Range Test (DMRT) was used to compare mean values of all the treatments at 0.05 level of probability.

RESULTS AND DISCUSSION

The three months observation of the growth on *Aquilaria microcarpa* in Asap Koyan, Belaga, is show in Table 4. Before fertilizers application, the highest *A. microcarpa* tree height and DBH recorded from total 50 replicates is 188.3 cm and 30.4 mm respectively. While, the lowest *A. microcarpa* tree height and DBH recorded is 104.5 cm and 5.9 mm. The highest tree height and DBH is recorded from different tree, meaning that the tallest tree does not represent the highest DBH or otherwise.

The study observed the growth of *A. microcarpa* tree at eight months old after planted, treated with four treatments of control, NPK fertilizer, organic compost and slow-release fertilizer for three months period. For fertilizer treatments, the different amounts of fertilizer (100 g, 200 g, and 300 g) effect on the growth of *A. microcarpa* are compared. In the third month (February), Table 1 show that the growth of *A. microcarpa* tree achieved the tallest height at 260.2 cm and the highest DBH recorded is 48 mm. While, the shortest tree recorded is 140.3 cm and the lowest DBH is 15.4 mm. For control treatment observation, the initially tallest or widest (highest diameter) of *A. microcarpa* tree, still performed to be the tallest and widest at the end of experiment compared with other replicates.

Table 7.1 Detailed of *Aquilaria microcarpa* growth over a period of three months for different fertilizers application

Treatment	Amount	Rep.	Initial growth		1st month		2nd month		3rd month	
			Height (cm)	DBH (mm)	Height (cm)	DBH (mm)	Height (cm)	DBH (mm)	Height (cm)	DBH (mm)
T1 Control		1	168.5	15.9	174.5	18.9	180.4	21.7	189.4	22.6
		2	121.0	11.5	134.2	13.3	140.5	17.9	160.3	20.9
		3	151.0	10.6	162.3	11.5	166.4	**15.6**	170.6	18.2
		4	131.6	13.5	148.3	14.8	160.8	22.2	170.7	25.1
		5	121.7	11.8	136.0	11.8	150.7	15.6	160.7	18.6
		Average	138.76	13.46	151.06	14.06	159.76	18.6	170.34	21.08
T2 NPK fertilizer	100 g	1	165.0	**30.4**	174.9	31.0	190.8	39.4	210.5	**48**
		2	113.2	9.0	138.1	12.1	160.5	16.4	189.7	19.7
		3	162.0	13.1	174.4	13.9	190.7	15.7	220.7	24.5
		4	146.9	10.0	162.5	11.4	190.5	20	220.9	20.1
		5	119.8	8.3	126.1	8.7	140.4	10.9	170.9	22.8
		Average	141.38	14.2	155.20	15.4	174.58	20.5	202.54	27.02
	200 g	1	121.8	7.7	125.9	8.4	140.6	13.4	180.4	18.9
		2	117.0	7.1	127.2	7.5	138.8	10.3	140.7	23.8
		3	**188.3**	19.7	203.3	20.8	220.9	25	250.4	38.7
		4	137.7	12.9	141.5	13.5	145.4	14.2	150.3	29.5
		5	144.5	7.8	153.4	8.4	180.5	13.7	201.4	2.25
		Average	141.86	11	150.26	11.7	171.85	15.3	184.64	22.63
	300 g	1	120.3	9.1	129.7	9.4	140.7	13.7	170.6	20.5
		2	138.1	10.6	148.7	11.9	150.5	17.3	180.5	21.9
		3	120.2	10.2	133.0	10.6	140.7	11.9	190.6	21.7
		4	137.5	9.5	157.2	10.3	160.7	16.6	200.4	25.3
		5	137.0	12.2	140.5	12.9	148.5	16.4	160.7	18.4
		Average	130.62	10.3	141.82	11	148.22	15.2	180.56	21.56

Cont'd Table 7.1

	100 g	1	163.5	14.3	189.3	17.4	230.1	20.2	250.2	28.6
		2	163.3	7.1	167.8	8.8	180.7	10.9	186.8	16.9
		3	146.5	9.1	163.5	10.4	190.6	14.9	209.3	18.4
		4	136.4	10.7	153.8	11.9	170.6	21.7	190.4	26.6
		5	158.8	18.1	169.2	22.6	180.9	30.8	200.2	33.9
		Average	153.70	11.9	168.72	14.2	190.58	19.7	207.38	24.88
	200 g	1	101.5	9.8	109.0	10.2	120.9	11.5	130.6	**15.4**
		2	114.9	9.5	126.1	9.9	160.1	15.6	180.7	20
		3	185.0	14.6	199.5	15.5	230.9	19.0	**260.2**	28
T3 Organic compost		4	110.5	8.0	121.3	4.5	139.8	9.6	150.7	15.2
		5	144.4	7.1	155.7	7.8	170.5	12.3	190.8	24.4
		Average	131.26	9.8	142.32	9.6	170.60	14.6	182.6	20.6
	300 g	1	136.6	8.1	149.6	8.4	170.0	12.7	170.7	21.6
		2	**104.5**	6.7	117.1	7.5	170.4	9.6	172.6	17
		3	123.1	9.1	136.6	9.8	140.4	16.2	152.6	23.5
		4	171.2	14.1	179.5	18.0	190.7	25.2	230.4	30
		5	142.2	13.2	153.3	13.7	170.6	18.4	180.8	26.7
		Average	135.52	10.2	147.22	11.5	168.42	16.4	181.42	23.76
	100 g	1	169.5	20.3	173.4	20.7	180.3	26.8	207.7	31.2
		2	120.6	9.1	130.5	9.7	160.7	12.4	200.4	16
		3	143.7	8.3	149.2	8.6	170.6	13.5	190.5	19.9
T4 Slow-release fertilizer		4	114.2	9.7	134.2	10.5	160.8	20.5	200.5	20.7
		5	104.5	8.9	125.3	9.4	130.4	14.1	**140.3**	19.6
		Average	130.50	11.3	142.52	11.8	160.56	17.5	187.88	21.48

Cont'd Table 7.1

	200 g	1	110.6	9.2	118.0	9.4	140.8	11.7	160.7	22.7
		2	127.0	**5.9**	140.4	6.4	146.7	11.9	170.3	15.4
		3	149.5	21.6	157.9	25.8	170.7	30.2	192.4	33.9
		4	165.0	23.1	174.9	23.8	180.3	32.2	190.6	34.7
		5	175.4	13.7	192.5	14.5	220.1	26.2	250	30.7
		Average	145.50	14.7	156.45	16	171.72	22.4	192.8	27.48
T4 Slow-release fertilizer	300 g	1	99.10	7.3	107.6	7.8	110.4	9.6	120.7	14.4
		2	126.5	11.3	133.0	11.8	160.0	32.7	180.5	32.7
		3	111.3	8.3	118.3	8.7	120.4	12.9	140.8	15
		4	150.0	11.5	165.8	20.7	170.5	29.8	185.1	30.2
		5	106.7	7.1	113.9	7.9	120.3	10.1	120.8	11.9
		Average	118.72	9.1	127.72	11.4	136.32	19	149.58	20.84

The growth performance of *Aquilaria microcarpa* in height show that the highest tree height recorded was in T3 (organic compost) while the lowest was in T4 (slow-release fertilizer) for every month (Table 7.2). The data explain the tallest (153.7 cm) and the shortest (118.72 cm) height of *A. microcarpa* tree measured at initial experiment still maintained their status over the period of study. This indicates that the early growth of *A. microcarpa* seedling is essential to be well promoted so their growths are faster.

The overall height increment of *A. microcarpa* treated with different fertilizers at different amounts is shown at Figure 7.2. Averagely, the *A. microcarpa* tree shows the highest height increment (61.16 cm) by NPK fertilizer treatment at 100 g application amount, followed by 200 g application amount of organic compost (51.34 cm). While the lowest height increment (30.86 cm) is show by slow-release fertilizer treatment with 300 g application amount. All fertilizer types show the best height increment at 100 g application amount. This indicates that the 100 g fertilizer amount is appropriate for application to one year old *A. microcarpa* tree.

Table 7.2 Average of *Aquilaria microcarpa* height increment over a period of three months treatments application

Treatments	Amounts (g)	Height (cm)				Total increment (cm)
		Initial height	1st month	2nd month	3rd month	
T1: Control		138.76	151.06	159.76	170.34	31.58
T2: NPK Blue	100	141.38	155.2	174.58	202.54	**61.16**
	200	141.86	150.26	171.85	184.64	42.78
	300	130.62	141.82	148.22	180.56	49.94
T3: Organic Compost	100	**153.7**	**168.72**	**190.58**	**207.38**	53.68
	200	131.26	142.32	170.6	182.6	51.34
	300	135.52	147.22	168.42	181.42	45.9
T4: Slow-release	100	130.5	142.52	160.56	187.88	57.38
	200	145.5	156.45	171.72	192.8	47.3
	300	**118.72**	**127.72**	**136.32**	**149.58**	**30.86**

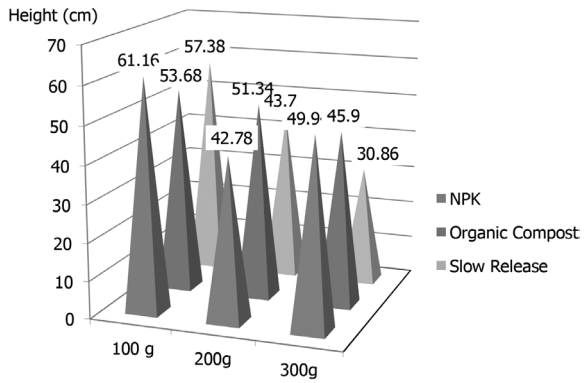


Figure 7.2 Total height increment of *Aquilaria microcarpa* after treated with different fertilizers at different amounts

The DBH performance of *Aquilaria microcarpa* in Table 7.6 show that the highest tree growth recorded was in T4 (slow-release fertilizer) while the lowest was in T3 (organic compost) for every month. The data explain that the tallest and the shortest DBH of *A. microcarpa* tree measured at initial experiment still maintained their status over the period of study.

Table 7.3 Average of of *Aquilaria microcarpa* DBH increment recorded over a period of three month after fertilizers application

Treatments	Amounts (g)	DBH (mm)				Total increment (mm)
		Initial growth	1st month	2nd month	3rd month	
T1: Control		13.46	14.06	18.6	21.08	8.62
T2: NPK Blue	100	14.2	15.4	20.5	27.02	12.82
	200	11	11.7	15.3	22.63	11.63
	300	10.3	11	15.2	21.56	11.26
T3: Organic Compost	100	11.9	14.2	19.7	24.88	12.98
	200	**9.26**	**9.6**	**14.6**	**20.6**	**10.8**
	300	10.2	11.5	16.4	23.76	**13.56**
T4: Slow-release	100	11.3	11.8	17.5	21.48	10.18
	200	**14.7**	**16**	**22.4**	**27.48**	13.14
	300	9.1	11.4	19	20.84	11.74

The statistical analysis of variance (ANOVA) was performed based on the obtained result, to compare the effect of different fertilizer amounts in different treatments on the growth of Aquilaria microcarpa (Table 7.9). The result show there is no significant differences between all means at p≤0.05. All fertilizer treatments at different amounts give the same effect on the growth of A. microcarpa. Therefore, the growth means of 100 g amount of all fertilizer treatments (NPK, organic compost and slow-release) is used for treatments comparison with control treatment in Table 10.

Table 7.4 The mean comparison of Aquilaria microcarpa height and DBH at different amounts indifferent fertilizer treatments

		Mean parameters	
Treatments	**Amounts**	**Height (cm)**	**DBH (mm)**
NPK	100 g	61.16 a	12.82 a
	200 g	49.94 a	11.63 a
	300 g	42.78 a	11.26 a
Organic Compost	100 g	53.68 a	13.56 a
	200 g	51.34 a	12.98 a
	300 g	45.90 a	10.8 a
Slow-release	100 g	57.38 a	10.18 a
	200 g	47.30 a	13.14 a
	300 g	30.86 a	11.74 a

**Means with the same alphabet was not significantly different at p≤0.05 (DMRT)

The Duncan Multiple Range Test show that there is a significant difference between T1 (control treatment) correspond to T2, T3, and T4 (fertilizer treatments) in both height and DBH growth parameters of *Aquilaria microcarpa.* The application of fertilizer to *A. microcarpa* tree do increase its growth if compare with non-fertilized tree.

Table 7.5 The mean comparison of treatments on the height and DBH of *Aquilaria microcarpa*

	Mean parameters	
Treatments	**Height (cm)**	**DBH (mm)**
T1	31.58 b	8.62 b
T2	61.16 a	12.82 a
T3	53.68 ab	13.56 a
T4	57.38 ab	10.18 ab

**Means with the same alphabet was not significantly different at p≤0.05 (DMRT)

CONCLUSION

Based on the growth performance of the first year planted *Aquilaria microcarpa*, the suitable amount of fertilizer application is 100 g of NPK, organic compost and slow-release fertilizer. The growth rate of *A. microcarpa* in this study was not significantly different for the different type of fertilizers. However, the fertilizer application is significantly increased the growth rate of *A. microcarpa* tree at Asap Koyan, Belaga compared with non-fertilized tree. Therefore, karas cultivator can decide the type of fertilizer to apply based on their choice and ability because all types of fertilizer gives the same effect on the growth of *A. microcarpa*.

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8

Growth Performance and Sucrose Accumulation in Lycopersicum Esculentum Cultivated in Lemba (*Molineria rubriclavata*) Fibre Based Media

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INTRODUCTION

It is a common practice to use soil as growing media but due to its poor drainage, poor water holding capacity and do not provide good aeration, new type of planting media are often developed to amend planting media for greenhouse crops. With the merging of science and innovative technologies, various methods and techniques has been developed for growing plant without using soil and all these methods are called as soilless method for plant culture. Growing medium are comprised of two or three components which are mixture of organic and inorganic substrates. Some of the list of materials for making soilless media materials are includes peat moss, bark (composted), compost, pumice, worm castings, vermiculite, perlite, sand, rice hulls, sheep wool, and coconut coir. Materials such as vermiculite, perlite, pumice and sand are the example of inorganic components meanwhile the others are organic components. The light weight and high porosity of growing medium made of peat and perlite ensures good water retention, allows good roots penetration deeper and increases length of stems. In some country, due to its good chemical and physical properties, peat is either being used alone or mixed with inorganic coarse materials and is commonly used for ornamental nursery as growing media. This shows that soilless media has become a new trend in horticulture. In cultivation with soilless media was shown to be the most intensive production method that can also affect fruit quality and nutrient composition. In areas with low quality growing condition this system indicates high yield of production.

Lemba or *Molineria* spp. colla is a native perennial herb that has a remarkable sweet protein named neoculin or curculin. *Molineria latifolia* is a plant that can found in tropical Asia and can be categorized as a wild plant. This perennial herb with specialized underground stems is also locally known as 'lembah', 'pinang puyoh' or 'kelapa puyoh'. *Molineria rubriclavata* was described as a new species which can be found throughout Malaysia (Rozilawati, 2015). The unique feature that distinguishes this species from other species is that petiole that is green with maroon stripe. Petioles of *Molineria rubriclavata* are 35- 140cm long and hairy with upper surface. Meanwhile, leaves are often 3-12, simple, elliptic to widely elliptic, green and clasping at the base of the base of plant to form a false stem. Among the seven species and two varieties compared, *M. rubiclavata* has the largest fibre cells (Duratul Ain, 2014).

In the fruits of *Molineria spp.* colla, there are sweet proteins which are namely curculin and neoculin which accumulate during fruit ripening (Babaei *et al*. 2014). Both proteins exhibit taste modifying capability where any sour solution, water and substances will become sweet after the fruits are consumed. Highest protein concentration was found in the ripening fruits but proteins can also be found in the entire plants. The sweetness of curculin is believed to be 500 times sweeter than sucrose (Okubo *et al.* 2008). High numbers of fruits are difficult to obtain, therefore it is more economical to use their vegetative part due to its availability and also to promote zero waste in agriculture.

It was found that *M*. *rubiclavata* contained the highest number of fibre cell and having the best fibre quality. Therefore, this study aim at proving that their vegetative parts will be suitable for the development of fibre based media. Crop response to this new media will be reported. Tomato was chosen to determine the effect of this media on sugar concentration in their fruits. Organic grown tomatoes are known to be sweeter. However, organic products are still not popular among Malaysian consumer due to its high price. This study, attempts to produce sweeter tomatoes grown in lemba based fibre media. Sweet protein in the lemba fibre released by application of effective microorganism (EM) is expected to increase the sugar content in tomatoes.

OBJECTIVES

The objectives of this study are to (1) to determine the effect of different ratio of lemba fibre based media on the growth of *Lycopersicum esculentum* and (2) to determine the concentration of sucrose in *Lycopersicum esculentum* planted in lemba fibre based media.

SIGNIFICANCE OF STUDY

It was hypothesized that the sucrose content in cherry tomato was able to be increased when planted in lemba fibre based planting media. The approach may also improve the growth performance of the plant.

MATERIALS AND METHODS

This study was carried out at Fertigation Unit, Agriculture Park and Soil Science Laboratory, Universiti Putra Malaysia Bintulu Campus. *Molineria rubiclavata* were collected from the field area at University Putra Malaysia Bintulu Campus and Sebauh area in Bintulu. The leaves and petiole were cut into small pieces and was oven dried at 50^{o}C for at least 48 hours. After that the leaves and petiole were grind into smaller pieces.

A total of 14 treatments were compared in this experiment. Each treatment was consisted of different ratio of lemba fibre, cocopeat and organic matter. Seven different media ratio compared were 1:1:1, 1:2:1, 1:3:1, 2:1:1, 2:3:1, 3:1:1 and 3:2:1 ratio (lemba: cocopeat: organic matter). These treatments were repeated with the application of Effective Microorganism (EM) (*MY EM ENTERPRISE*, Alor Gajah, Melaka). Planting media treated with EM were prepared earlier before experiments were conducted by spraying the media with 250 ml of EM on two weeks interval for a month. The standard media with a ratio of 3:2:1 were used as control where the ratio was consisted of 3 top soil: 2 cocopeat: 1 organic matter. Each treatment was replicate five times.

Seeds of cherry tomato varieties Season Red were germinated in germinating tray containing peat moss to ensure uniform growth of seedlings. The seedlings were raised in a greenhouse and watered twice daily. After 21 days, when the seedlings were about 10 cm in height with 3-4 true leaves, the plants were transplanted into 15''X 18'' polybag containing the respective growing media. Watering activities

were provided daily according to the cherry tomato growth requirement. Agronomic practices for tomato were applied with fertilizing following the standard protocol (Table 8.1), watering twice a day and weeding when needed.

Table 8.1 Standard fertilizer requirement for *Lycopersicum esculentum* (recommended by Ministry of Agriculture and Agro-Based Industry Malaysia)

Plant age (days after transplanting)	Fertilizer	Rate (per plant)
-7	Chicken dung	500 g
14	NPK Blue 12:12:17:2	30 g
28	NPK Blue 12:12:17:2	80 g
56	NPK Blue 12:12:17:2	60 g

The treatments were arranged in randomize completely block design layout and experiment were carried out for four month. Assessment of *Lycopersicum esculentum* growth performance planted in different media ratio was monitored into two ways, based on their parameter and nutrient properties. The growth parameters consisted of measurement of plant height, leave width, and leave length, number of flower, days to flower, number of fruit, fruit fresh weight and sucrose content in fruit. Related data was collected during vegetative and reproductive growth phase at an interval of seven days until the end of the experiment. While, determination on nutrient content in plants and media were done by collecting data of their total N, P and K content. Soil analysis was also carried out. Sucrose concentration in the *Lycopersicum esculentum* fruits were measured by using a hand refractometer. The fruit was cut into sections from the centre of the fruit. Each section was drained by using cheesecloth for the extract. The extract was dropped on the prism and flattens to the attached cover plate. The chart of brix was obtained and recorded.

The nutrient content in the media and leaves were measured following the pH (H_2O Method). A 5 g of air dried samples were weighted in plastic vial. Then 50 ml distilled water were added, stop and shake by using orbital shaker model 720 at 180 rpm for 15 minute. The sample was left for 24 hours. The pH was measured with pH meter model AB 15. The pH was read in the scale of pH. Electrode was rinsed with distilled

water and calibrated in the buffer solution of pH. The determination of total N followed the Kjedahl (1883) method, total P followed the Single Dry Ashing and Blue Method by Crossland (1955) and total K and Mg followed the method by Knudson *et al*. (1982).

RESULTS

Nutrient Content in Media

This study showed that there was a significant difference of % N content in lemba fibre among treatments but not among blocks (Table 8.2). This was also obtained for % of P (Table 3), % of K (4) and % of Mg (Table 8.5). This data was obtained before planting.

Table 8.2 Anova table for total N (%) content in lemba fibre media before harvesting

Source	DF	Anova SS	Mean Square	F value	Pr>F
Treatment	13	3.51	0.27	5.91	0.0001
Block	2	0.02	0.01	0.19	0.8260
Error	26	1.19	0.05		
Corrected Total	41	4.72			

**Values are not significantly different at ($p< 0.05$) level

Table 8.3 Anova table for total P (%) content in lemba fibre media before harvesting

Source	DF	Anova SS	Mean Square	F value	Pr>F
Treatment	13	0.09597	0.00738	19.89	0.0001
Block	2	0.00005	0.00003	0.07	0.9317
Error	26	0.00965	0.00037		
Corrected Total	41	0.10567			

**Values are not significantly different at ($p< 0.05$) level

Table 8.4 Anova table for total K (%) content in lemba fibre media before harvesting

Source	DF	Anova SS	Mean Square	F value	Pr>F
Treatment	13	2.94	0.23	17.00	0.0001
Block	2	0.01	0.02	1.55	0.2310
Error	26	0.35	0.01		
Corrected Total	41	3.32			

**Values are not significantly differences at ($p < 0.05$) level

Table 8.5 Anova table for total Mg (%) content in lemba fibre media before harvesting

Source	DF	Anova SS	Mean Square	F value	Pr>F
Treatment	13	0.24	0.018	8.01	0.0001
Block	2	0.01	0.003	1.24	0.3067
Error	26	0.06	0.002		
Corrected Total	41	0.30			

**Values are not significantly different at ($p < 0.05$) level

Before harvesting in T1, when there is an equal amount of lemba fibre, cocopeat, organic matter, the N content was 0.47% (Table 8.6). When cocopeat was increased the % of N increased but too much cocopeat will reduce the % of N again. In T4, the amount of lemba fibre increases which sees increment of %N by two told. Too many lemba fibres in T6 were not as efficient as T4 in providing %N. However, when EM was added %N was made more available. T1 and T7 have the same ratio but N% was higher in T7 because it was supplied with EM. In planting media added with EM, T12 has the highest % of N and Mg but it was not significant different to T8. However T12 has higher % of Mg as compared to T8. When cocopeat was increased from 3:1:1 (T12) to 3:2:1 (T13), the % of N decreased.

Table 8.6 The total available N (%), total P (%), total K (%), total Mg (%) of lemba fibre media for before and after harvesting

Treatments	Total N (%)		Total P (%)		Total K (%)		Total Mg (%)	
	Before	After	Before	After	Before	After	Before	After
(T1)	0.47±0.03^{c}	0.45±0.07^{abc}	0.17±0.004^{bcde}	0.81±0.24^{a}	1.06±0.01^{abc}	1.63±0.25^{a}	0.32±0.02^{abc}	0.41±0.04^{b}
(T2)	0.73±0.09^{bc}	0.45±0.07^{abc}	0.18±0.01^{bcde}	0.65±0.01^{ab}	1.23±0.05^{a}	0.15±0.02^{b}	0.33±0.02^{abc}	0.47±0.04^{ab}
(T3)	0.60±0.12^{c}	0.31±0.05^{bc}	0.16±0.01^{cde}	0.14±0.04^{b}	0.97±0.02^{abcd}	0.19±0.02 ^{b}	0.36±0.01^{abc}	0.52±0.01^{ab}
(T4)	1.03±0.03^{abc}	0.50±0.07^{abc}	0.24±0.02^{a}	0.75±0.12^{ab}	0.88±0.15^{bcd}	0.22±0.03 ^{b}	0.30±0.01^{abcde}	0.52±0.03^{ab}
(T5)	0.63±0.09^{c}	0.56±0.09^{ab}	0.13±0.01^{ef}	0.74±0.22^{ab}	1.02±0.05^{abc}	0.33±0.06 ^{b}	0.25±0.02^{bcde}	0.52±0.02^{ab}
(T6)	0.70±0.10^{c}	0.76±0.11^{a}	0.16±0.01^{cde}	0.69±0.17^{ab}	1.15±0.04^{ab}	0.33±0.07 ^{b}	0.22±0.01^{cde}	0.51±0.03^{ab}
(T7)	1.07±0.07^{abc}	0.53±0.10^{ab}	0.23±0.02^{ab}	0.94±0.17^{a}	0.96±0.03^{abcd}	0.39±0.12 ^{b}	0.24±0.001^{cde}	0.54±0.02^{a}
(T8)	1.37±0.17^{ab}	0.45±0.09^{abc}	0.20±0.01^{abcd}	0.86±0.14^{a}	0.99±0.08^{abc}	0.32±0.09 ^{b}	0.26±0.01^{bcde}	0.54±0.02^{a}
(T9)	0.63±0.13^{c}	0.42±0.04^{abc}	0.14±0.01^{def}	0.82±0.08^{a}	0.72±0.04^{cde}	0.26±0.04 ^{b}	0.31±0.06^{abcd}	0.52±0.03^{ab}
(T10)	1.07±0.15^{abc}	0.64±0.07^{ab}	0.21±0.01^{abc}	0.72±0.07^{ab}	0.71±0.09^{cde}	0.22±0.01 ^{b}	0.43±0.01^{a}	0.52±0.06^{ab}
(T11)	0.73±0.17^{bc}	0.62±0.11^{ab}	0.06±0.004^{g}	0.97±0.07^{a}	0.46±0.07^{ef}	0.33±0.02 ^{b}	0.17±0.002^{de}	0.55±0.02^{a}
(T12)	1.40±0.10^{a}	0.59+0.08^{ab}	0.20±0.01^{abcd}	0.50±0.07^{ab}	0.84±0.03^{bcd}	0.30±0.06 ^{b}	0.39±0.01^{ab}	0.59±0.02^{a}
(T13)	0.83±0.09^{abc}	0.70±0.06^{ab}	0.15±0.01^{cde}	0.69±0.10^{ab}	0.65±0.11^{de}	0.31±0.02 ^{b}	0.26±0.07^{bcde}	0.58±0.02^{a}
(T14)	0.50±0.21^{c}	0.11±0.01^{c}	0.08±0.02^{fg}	0.10±0.04^{b}	0.23±0.04^{f}	0.21±0.02 ^{b}	0.16±0.02^{e}	0.26±0.04^{c}

**Values in column with same letter did not differ significantly at ($p< 0.05$) level by ANOVA (Tukey Test)

T1 - 1:1:1 T2 - 1:2:1 T3 - 1:3:1 T4 - 2:1: T5 - 2:3:1
T6 - 3:1:1 T7 - 1:1:1 + EM T8 - 1:2:1 + EM T9 - 1:3:1 + EM T10 - 2:1:1 + EM
T11 - 2:3:1 + EM T12 - 3:1:1 + EM T13 - 3:2:1 T14 - 3:2:1(control) (lemba: cocopeat: organic matter)

The pH for all treatments (table 8.7) ranged between pH5.95 - 6.6, whereas the highest recorded was in T10 and lowest was in T14.

Table 8.7 pH values of lemba fibre based media before planting

Treatment	pH values
1 lemba: 1 cocopeat: 1 organic matter (T_1)	6.00
1 lemba: 2 cocopeat: 1 organic matter (T_2)	6.18
1 lemba: 3 cocopeat: 1 organic matter (T_3)	6.19
2 lemba: 1 cocopeat: 1 organic matter (T_4)	6.15
2 lemba: 3 cocopeat: 1 organic matter (T_5)	6.27
3 lemba: 1 cocopeat: 1 organic matter (T_6)	6.24
1 lemba: 1 cocopeat: 1 organic matter + EM (T_7)	6.35
1 lemba: 2 cocopeat: 1 organic matter + EM (T_8)	6.20
1 lemba: 3 cocopeat: 1 organic matter + EM (T_9)	6.44
2 lemba:1 cocopeat: 1 organic matter +EM (T_{10})	6.61
2 lemba: 3 cocopeat: 1 organic matter +EM (T_{11})	6.56
3 lemba: 1 cocopeat: 1 organic matter + EM (T_{12})	6.55
3 lemba: 2 cocopeat: 1 organic matter (T_{13})	6.19
3 top soil :2 cocopeat: 1 organic matter (T_{14})	5.95

Nutrient Content in Leaves of *Lycopersicum Esculentum* Cultivated in Lemba Fibre Based Media after Harvesting

Table 8.8 showed that there was significant different of total N (%) between treatments but there is no significance different of total N (%) between blocks. The same trend was also seen in % of P (table 9), % of K (table 8.10) and % of Mg (table 8.11).

Table 8.8 Anova table for total N (%) content in leaves of *Lycopersicum esculentum*

Source	DF	Anova SS	Mean Square	F value	Pr>F
Treatment	13	3.17	0.24	3.14	0.0017
Block	4	0.26	0.07	0.84	0.5075
Error	52	4.05	0.08		
Corrected Total	69	7.48			

**Values are not significantly different at ($p< 0.05$) level

Table 8.9 Anova table for total P (%) content in leaves of *Lycopersicum esculentum*

Source	DF	Anova SS	Mean Square	F value	Pr>F
Treatment	13	1.07	0.08	4.68	0.0001
Block	4	0.18	0.05	2.60	0.0564
Error	52	0.92	0.02		
Corrected Total	69	2.17			

**Values are not significantly different at (p< 0.05) level

Table 8.10 Anova table for total K (%) content in leaves of *Lycopersicum esculentum*

Source	DF	Anova SS	Mean Square	F value	Pr>F
Treatment	13	14.99	1.15	11.00	0.0001
Block	4	0.71	0.18	1.69	0.1654
Error	52	5.45	0.10		
Corrected Total	69	21.15			

**Values are not significantly different at (p< 0.05) level

Table 8.11 Anova table for total Mg (%) content in leaves of *Lycopersicum esculentum*

Source	DF	Anova SS	Mean Square	F value	Pr>F
Treatment	13	0.197	0.015	5.22	0.0001
Block	4	0.018	0.004	1.53	0.2070
Error	52	0.150	0.003		
Corrected Total	69	0.365			

**Values are not significantly different at (p< 0.05) level

The nutrient content (Table 8.12) of % N was highest in leaves when grown in media ratio of 3.1.1 (T6) and 3.2.2 (T13). As for % of P content, leaves from plant grown in media ratio of 3.2.2 (T13) is the highest and % of K was highest in the leaves of plant grown in media ratio 3.2.1 (T14). There was no significant difference in % Mg among the treatments.

Table 8.12 Variation of nutrient content in leaves of *Lycopersicum esculentum* in lemba fibre based media after harvesting

Treatment	**Total N (%)**	**Total P (%)**	**Total K (%)**	**Total Mg(%)**
(T_1)	0.92 ± 0.16^{ab}	0.39 ± 0.05^{bc}	0.88 ± 0.12^{cde}	0.53 ± 0.05^{b}
(T_2)	1.09 ± 0.08^{ab}	0.31 ± 0.03^{c}	1.63 ± 0.20^{ab}	0.54 ± 0.02^{b}
(T_3)	0.50 ± 0.06^{b}	0.42 ± 0.05^{bc}	1.41 ± 0.17^{bc}	0.55 ± 0.01^{b}
(T_4)	0.87 ± 0.12^{ab}	0.44 ± 0.06^{abc}	1.52 ± 0.26^{abc}	0.54 ± 0.09^{b}
(T_5)	0.84 ± 0.13^{ab}	0.57 ± 0.05^{abc}	1.18 ± 0.17^{bcde}	0.55 ± 0.03^{b}
(T_6)	1.21 ± 0.20^{a}	0.54 ± 0.04^{abc}	1.26 ± 0.15^{bcd}	0.48 ± 0.03^{b}
(T_7)	0.84 ± 0.09^{ab}	0.48 ± 0.12^{abc}	0.81 ± 0.10^{cde}	0.49 ± 0.03^{b}
(T_8)	0.78 ± 0.11^{ab}	0.30 ± 0.02^{c}	0.82 ± 0.02^{cde}	0.56 ± 0.02^{ab}
(T_9)	0.62 ± 0.11^{ab}	0.50 ± 0.06^{abc}	0.58 ± 0.06^{de}	0.51 ± 0.09^{b}
(T_{10})	0.73 ± 0.16^{ab}	0.53 ± 0.11^{abc}	0.53 ± 0.08^{e}	0.54 ± 0.03^{b}
(T_{11})	0.72 ± 0.15^{ab}	0.58 ± 0.03^{bc}	0.54 ± 0.06^{e}	0.47 ± 0.02^{b}
(T_{12})	0.78 ± 0.15^{ab}	0.66 ± 0.08^{ab}	1.46 ± 0.08^{abc}	0.47 ± 0.03^{b}
(T_{13})	1.23 ± 0.13^{a}	0.73 ± 0.08^{a}	0.90 ± 0.25^{cde}	0.49 ± 0.23^{b}
(T_{14})	0.56 ± 0.11^{b}	0.32 ± 0.03^{c}	2.16 ± 0.11^{a}	0.68 ± 0.04^{a}

**Values in column with same letter did not differ significantly at ($p< 0.05$) level by ANOVA (Tukey Test)

Effect of Different Ratio of Lemba Fibre Based Media on Growth Variables of *Lycopersicum Esculentum*

The figure below showed that plant grown in T8 (1 lemba fibre: 2 cocopeat: 1 organic matter) has the highest plant height as compared to other treatments. At 70 days, the plant height reached up to 1265.2mm (figure 8.1). The measurements for each plant for all treatments were recorded at every 14 days interval. After 14 days of planting, the highest plant height was T8 at 464 mm meanwhile the lowest plant height was T6 at 125.2mm (figure 8.1). Treatment six possessed the lowest plant height comparable to the T14 from the beginning to the end of planting.

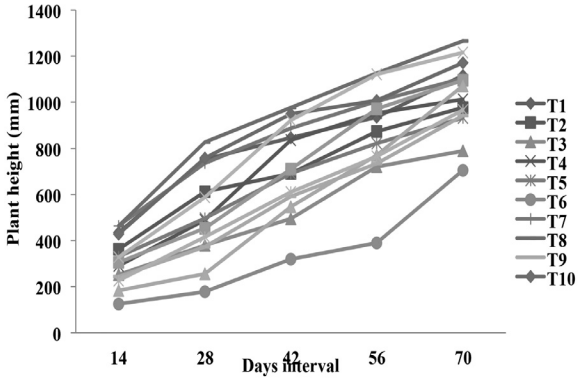

Figure 8.1 Height of tomato plants grown in different ratio of lemba fibre based media

Leaves Blade Length

It was found that plant grown in treatment eight was significantly had longer leaves blade length as compared to the plant grown in treatment 14 where the highest value was on 70 days with 283.3 mm (figure 8.2). The lowest value of leaves blade length was that of plant grown in treatment 14 on 14 days where the leaves blade length was 181.7 mm (figure 8.2). Although plant from treatment 14 had the lowest leaves blade length value on 14 days but at the last 70 days it was plant grown in treatment six that has the lowest value of leaves blade length.

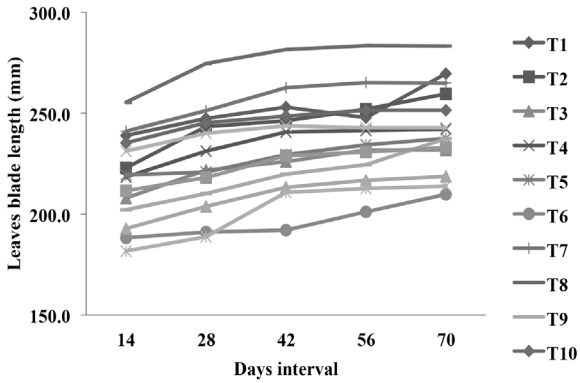

Figure 8.2 Leaf blade length of tomato plants grown in different ratio of lemba fibre based media

The figure below showed that plant grown in treatment eight has significantly wider leaves width as compared to the rest of the treatment where the highest value was on 42 days with 226.9 mm (Figure 3) and leaves width reduces slowly until 70 days of planting. Plant grown in treatment 14 had the lowest leaves width value on 14 days where the width was 174.8 (Figure 8.3) mm until 28 days but at the last 42 days to the end of planting days it was plant grown in treatment six that has the lowest value of leaves width.

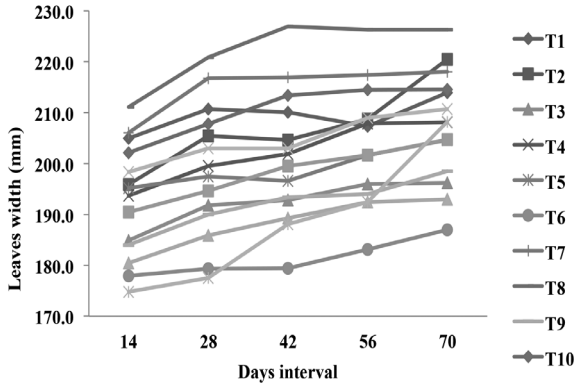

Figure 8.3 Leaf width of tomato plants grown in different ratio of lemba fibre based media

The figure below showed the effect of growing tomato in different ratio of lemba fibre based media on number of flowers. It showed that at 14 days of planting, all treatments did not produce any flowers yet. On the 28th days of planting, flowers were produced first in treatment seven and the number increased steadily where plants in this treatment produced the highest number of flower at 56th days of planting. Four month after the first flower initiation, the highest number of flower produce was 28 flowers (figure 8.4) by plants grown in treatment 10. This showed that treatment eight has significantly higher number of flowers as compared to treatment 14 and treatment nine has the lowest number of flowers.

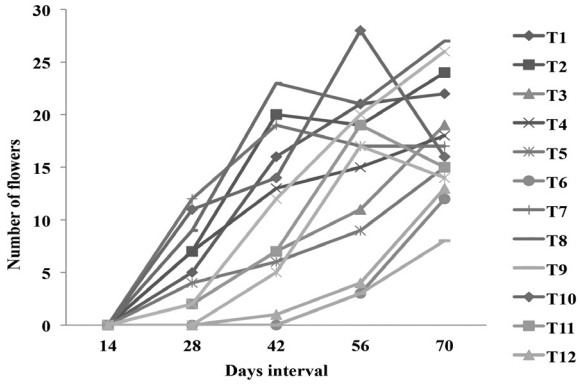

Figure 8.4 Number of flowers 70 days after planting in different ratio of lemba fibre based media

The highest number of fruits recorded was 44 fruits (figure 8.5) by plant grown in T8 after 70 days of planting which was significantly higher than T14 (control). Most of the treatments produced fruits after 42 days of planting where the lowest number of fruits produced of two fruits was by T3, T11 and T3 whereas treatment 10 possessed the highest number of fruits by producing 14 fruits (figure 8.5). Comparable to T4, treatment six was significantly lower in producing fruits. Treatment six only produces one fruit (figure 8.5) at 56 days of planting.

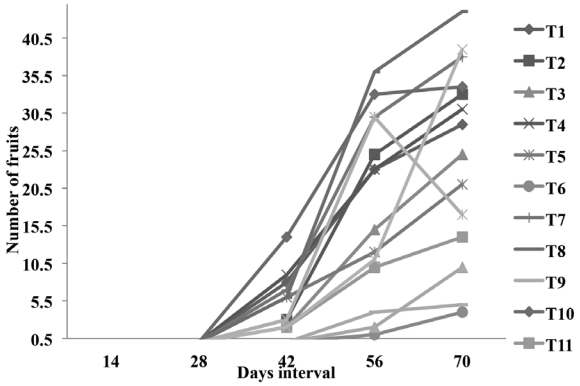

Figure 8.5 Number of fruit 70 days after planting in different ratio of lemba fibre based media

Effect of different ratio of lemba fibre based media on days of flowering of *Lycopersicum esculentum*

Table below showed that there is significant different between treatments for days of flowering but there is no significance different between blocks.

Tables 8.13 Anova table for days of flowering

Source	DF	Anova SS	Mean Square	F value	Pr>F
Treatment	13	6619.09	509.16	6.18	0.0001
Block	4	391.06	97.76	1.19	0.3280
Error	52	4287.34	82.45		
Corrected Total	69	11297.49			

Values are not significantly different at ($p < 0.05$) level

Figure 8.6 showed the means values of days to flowering among treatments. Case shows values that are different at 5% level. It was found that T6 has significant different in days of flowering as compared to T8 and T9.

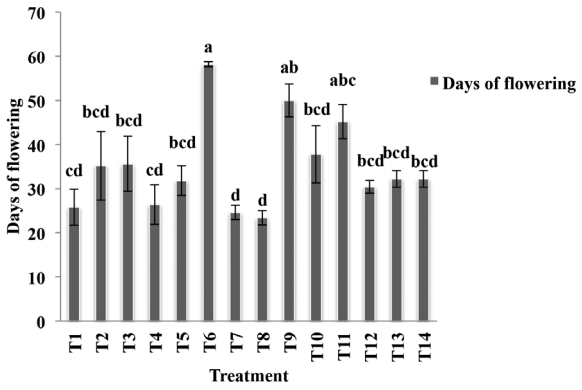

Figure 8.6 Days of flowering after planting in different ratio of lemba fibre based media

Effect of Different Ratio of Lemba Based Fibre Media on Days of Fruiting of *Lycopersicum esculentum*

Table below showed that there is significant different between treatments for days of fruiting but there is no significance different between blocks.

Table 8.14 Anova table for days of fruiting

Source	DF	Anova SS	Mean Square	F value	Pr>F
Treatment	13	5909.89	454.61	5.21	0.0001
Block	4	426.20	106.55	1.22	0.3136
Error	52	4541.20	87.33		
Corrected Total	69	10877.49			

**Values are not significantly different at ($p< 0.05$) level

Variation on Days of Fruiting

Figure 8.7 showed the means values of days of fruiting among treatments. Case shows values that are different at 5% level. It was found that T6 has significant different in days of flowering as compared to T8, T9, T1 and T10.

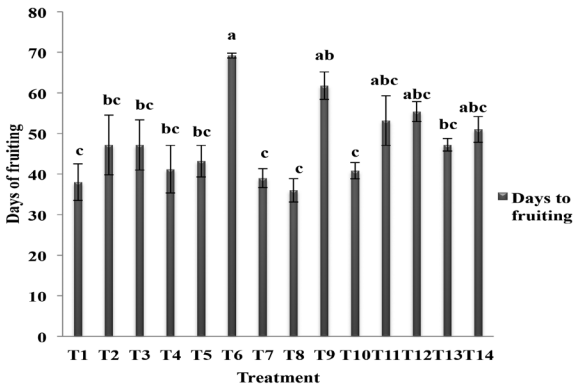

Figure 8.7 Days of fruiting after planting in different ratio of lemba fibre based media

Effect of Different Ratio of Lemba Based Fibre Media on Fruit Fresh Weight of *Lycopersicum esculentum*

Table below showed that there is significant different between treatments for yield but there is no significance different between blocks.

Table 8.15 Anova table for fruit fresh weight

Source	DF	Anova SS	Mean Square	F value	Pr>F
Treatment	13	1058397.51	81338.27	3.40	0.0008
Block	4	51278.61	12819.65	0.54	0.7098
Error	52	1243333.18	23910.25		
Corrected Total	69	2352009.31			

**Values are not significantly different at ($p< 0.05$) level

Figure 8.8 showed the means values of fruits fresh weight among treatments. Case shows values that are different at 5% level. It was found that treatment eight and T10 has significant different as compared to T6 and T9. Whereas the highest fruit fresh weight was recorded in T8.

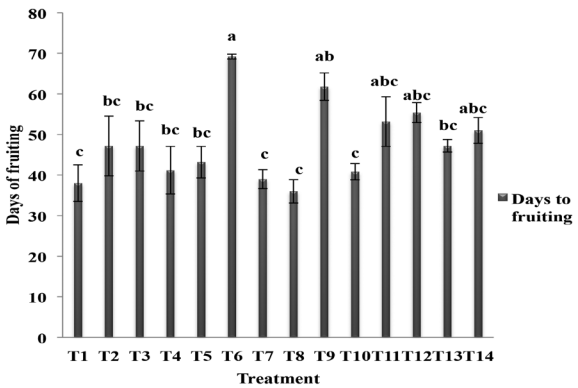

Figure 8.8 Fruits fresh weight after planting in different ratio of lemba fibre based media

Effect of Different Ratio of Lemba Fibre Based Media on Sucrose Content in Fruits of *Lycopersicum esculentum*

Table below showed that there is significant different between treatments for sucrose content in fruits but there is no significance different between blocks.

Table 8.16 ANOVA for sucrose content in fruit

Source	DF	Anova SS	Mean Square	F value	Pr>F
Treatment	13	8.18	0.63	2.97	0.0026
Block	4	1.37	0.34	1.62	0.1840
Error	52	11.03	0.21		
Corrected Total	69	20.58			

**Values are not significantly different at ($p< 0.05$) level

The figure 8.9 showed the means values of fruits fresh weight among treatments. Case shows values that are different at 5% level. It showed that there was significant different of sucrose content in all treatments.

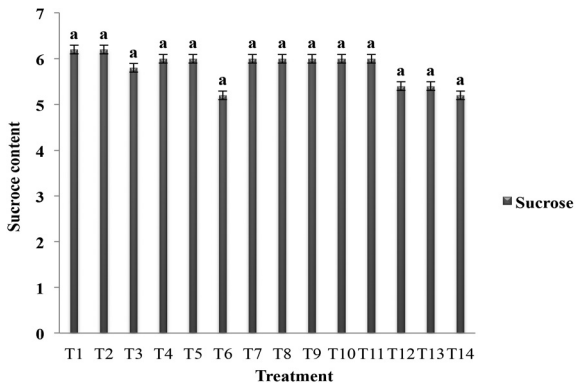

Figure 8.9 Fruits fresh weight after planting in different ratio of lemba fibre based media

Profit of Fresh Yield of Tomato Fruits

The cost in producing tomato grown in different media of lemba, cocopeat and organic matter was obtained in order to calculate the nett profit for each treatment (Table 17). From our calculations based on fruit weight (yield), the most profitable treatment was seen in treatment eight because treatment eight produced the highest yield. Although the cost or production was seen in treatment six but the yield was lower as compared to treatment eight. In treatment six, the net profit was the lowest and this is due to the very low yield of fruits. Low yield may be due to higher ratio of lemba fibre.

Table 8.17 The profit earned from tomato fruits produced after 70 days of planting in different ratio of lemba, cocopeat and organic matter

Treatment	Fruits Weight (g)	Price Per 100 g	Cost Per Treatment (RM) (B)	Profit (RM) (A)	A-B Nett Profit
(T_1)	1798.09	9.90	13.75	178.01	164.26
(T_2)	1252.97	9.90	14.38	124.04	109.66
(T_3)	717.06	9.90	14.75	70.99	56.24
(T_4)	1065.62	9.90	11.55	105.50	93.95
(T_5)	624.99	9.90	13.15	61.87	48.72
(T_6)	159.17	9.90	10.25	15.76	5.51
(T_7)	1713.09	9.90	14.45	169.60	155.15
(T_8)	2026.28	9.90	15.08	200.60	185.52
(T_9)	91.87	9.90	15.45	9.10	-6.35
(T_{10})	1915.37	9.90	12.25	189.62	177.37
(T_{11})	806.44	9.90	13.85	79.84	65.99
(T_{12})	426.51	9.90	10.95	42.22	31.27
(T_{13})	1140.30	9.90	11.25	112.89	101.64
(T_{14})	782.87	9.90	13.80	77.50	63.7

DISCUSSION

Differences in media ratio significantly affected the growth, flowering and fruiting performance of *Lycopersicum esculentum.* From the result, using lemba as part of the growing media for tomato did not gives much effect on the growth of *Lycopersicum esculentum*. This is because higher amount of lemba caused the decrement of yield and slow growth. From the nutrient

analysis, the % of N, P, K and Mg was always higher in media that content more cocopeat as compared to lemba. This study showed that treatment eight (1:2:1 + EM) produced the highest fruit weight. Although the cost for using treatment eight was more as compared to treatment with higher lemba, the yield obtained from treatment was very high that the net profit was able to cover the cost. In the end, it was more profitable to use media from treatment eight. When treatment eight was compared with control, yield from treatment eight was significantly different. In a way by using lemba in replacing soil can increase the yield of has significant and positive effects on the growth of *Lycopersicum esculentum* plant height, leaves blade length and leaves width. The usage of EM in breaking down fibre to release the nutritive value from organic matter is well known.

From the result (table 6), both T8 and T12 has the highest N (%) content and Mg (%) content. Magnesium plays a vital role in photosynthesis, as it is in the central atom in the chlorophyll molecule (Schwartzkopfp 1972). Nitrogen is more related with vegetative growth than with fruit growth (Castro *et al.* 2006: Filgueira 2000). Nitrogen promotes rapid growth, increases leaf size and quality, hastens crop maturity, and promotes fruit and seed development because nitrogen is a constituent of amino acids, which are required to synthesize proteins and other related compounds, it plays a role in almost all plant metabolic processes (Tucker 1999). On the 14 days after planting, plants from treatment eight have the highest plant height and leaf size. This is because the %N and %Mg content in this media was the highest prior to planting. Higher ratio of lemba in T12 seems to reduce the availability of %N and %Mg resulting in the poor growth performance of tomato plant as compared to treatment eight.

Treatment 14 (control) has higher Mg but high content of Mg does did not give much effect on yield. From the result, low in N and P content has significantly negative effect treatment 14. Low in total N and total P that reduces the plant growth thus reduces the yield. Qiu *et al*. (2008) reported that with low in nitrogen or no nitrogen nutrient medium, microorganisms did not affect the plants growth obviously and the statistical interaction intensity of plant-microorganism approached zero.

According to Nazari *et al.* (2011) physiological characters such as photosynthetic rate and water use efficiency were also improved by cocopeat due to its acceptable pH, electrical conductivity and other chemical attributes. However, cocopeat has been recognized to have high

water holding capacity which causes poor air-water relationship, leading to low aeration within the medium, thus affecting the oxygen diffusion to the roots (Awang *et al*. 2009). From the result, media contain higher ratio of cocopeat which are in treatment three and treatment nine had led to a poor growth performances *Lycopersicum esculentum.* The main aim of this study was to observe if lemba fibre was able to replace cocopeat or be as comparable to cocopeat. Although it showed some negative effect on growth of tomato in term of vegetative growth and also yield, further studies are needed to characterized their physical properties and its effects on plant growth.

Treatment two and T8 possessed the same ratio of media which are 1 lemba: 2 cocopeat: 1 organic matter. From table 6, it also showed that T2 has higher total K content (%) as compared to treatment eight but, treatment eight recorded the highest number of fruits fresh weight. According to (Jiffon and Lester 2011), Potassium is well recognized as the essential plant nutrient with the strongest influence on many quality parameters of fruits and vegetables it is involved in numerous biochemical and physiological processes vital to plant growth, yield, and quality. Addition of EM into treatment eight influence the growth of *Lycopersicum esculentum* positively which led to fast flower production, fruits production and greater yield as compared to T2. As shown by Xu *et al.* (2000) (cited in Ncube *et al*. 2011) where application of EM intensified fruit yield and plant growth of a tomato crop. Apparently, the application of EM into the soil is usually related with an increase in soil microbial biomass which increases the rate of symbiotic biological nitrogen fixation through increases in Azotobacter bacteria (Ncube *et al.* 2011: Hussain *et al*. 1994). As shown by Sharma and Trlakur (2001) (cited in Martinez *et al.* 1993: Pandey and Kumar 1989) reported that higher yield of tomato with the application of Azotobacter. Inoculation of effective microorganism can increase the available nutrition for plant roots and enhanced photosynthesis (Muthaura *et al.* 2010) These results suggest that EM introduction into a high total K content media initially can stimulate better flowering and fruiting. Although K is not an integral part of cell structure, potassium regulates many metabolic processes required for growth, fruit and seed development (Tucker 1999).

The results obtained in this study showed that treatment six and treatment 13 which does not have EM with higher ratio of lemba and cocopeat have negative effects on growth of *Lycopersicum esculentum* growth. Increasing ratio of lemba in media was significantly reduce the growth of *Lycopersicum esculentum* that lead to longer days for the plant to produce flower and fruiting. This suggests the possible existence of some synergistic activities between higher ratio of lemba and cocopeat that could lead to decrease fruiting in tomato thus decrease in yield.

From the result of leaf nutrient, it shows that treatment that have higher ratio of cocopeat and lemba have high in nutrient content especially total N (%) and total P (%) and this can be related to the lemba itself that are high in nutrient content of total N (%) and P (%). However, as noted by Parker *et al*. (1995) cited by Shenker *et al.* (2004) even though initial compositions of traditional nutrient are well defined, depletion occurs upon nutrient uptake by the plant, and the actual concentrations that are related to deficiency or toxicity are neither controlled nor easily identified and the study were still underground. Anti-nutrient content in lemba that can affects the growth of plant also were still not in detailed. Thus we still cannot determine the actual potential of lemba in promoting plant growth.

From the result, it showed that control treatment 14 was significantly low in total N (%) and Total P (%) compared to other treatments. This can be related to low performance of *Lycopersicum esculentum* plant in treatment 14. This can be due to lack of microbial activity. The level of nitrogen seems to play an important role in the maintenance of the balance in the interaction between microorganisms and plants (Walker *et al.* 2001). Interaction of microorganism will promote plant as according to Muthaura *et al.* (2010), interaction of microorganism will increase in the number of leaves and leaf area because are provided with proper nutrition and this can increase the photosynthetic activity of the plants thus increase in leaf area and number of leaves should result to higher rates of photosynthesis hence increased plant growth.

From the result, different in ratio of lemba based fibre media does positively affects the sucrose content in *Lycopersicum esculentum*. There was no significance different in sucrose content both in control treatment that use chemical fertilizer and treatments without chemical fertilizer. This could be due to no significance different in total K (%) in each *Lycopersicum esculentum.* Different in other nutrient content might

influence the yield of but does not significantly affect the sucrose content in *Lycopersicum esculentum*. The effects of nutrient in content in different ratio of lemba based fibre cannot be used as only factor that can influenced the sucrose content in *Lycopersicum esculentum.* According to Dorris *et al*. (2008) and especially more recent author Beckles (2012) cultural practices will also affects the sweetness of *Lycopersicum esculentum* as pre-harvest environment, including solar radiation, temperature, day-length, water availability, soil mineral content, irrigation, fertilization regime and pruning techniques, can all influence fruit sugar levels. Time of harvesting also might affect the sucrose content as determining the best time to harvest fruit from an eating quality perspective while reducing physical damage is not easy and varies by cultivar (Beckles, 2012: Posada and Avenda, 2008). Wang *et al.* (1993) proved that young fruit had the highest level of and presumably, they were the major sinks as 60 days after germination, fruit showed the highest levels of both sucrose synthase activity and starch in the plants.

CONCLUSION

This study was able to determine the effect of different ratio of lemba based fibre media on the growth of *Lycopersicum esculentum*. Good growth performance of cherry tomato was observed on media incorporated with lemba fibre as compared to the standard 3:2:1 media. Amongst the treatments, treatment eight with the ratio of 1:2:1 was the best planting media. Fruits from this treatment also had the highest sucrose content. Further studies are required especially to determine the water holding capacity and the physical properties of media when using lemba fibre as one of its component.

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9

Review on Nutritional Value of Indigenous Leafy Vegetables in Sarawak

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INTRODUCTION

A large diverse plant species were exists with many benefits to human and animals. Human for decades adopted varies plant species as their food sources. People in Sarawak especially in rural communities obtain indigenous leafy vegetables supply from their nearest rainforest. Sarawak with a vast tropical rainforest area covering about 8.7 million ha provides diverse indigenous leafy vegetables to the local people (Voon and Kueh, 1999; Shaffiq *et al*. 2013). People include indigenous leafy vegetables in their daily diet as relish or commonly known in Malay as *ulam* (Samy *et al*. 2009). Indigenous leafy vegetables can be defined as native plant species which originate from a particular region, or were introduced from other place from a long time through natural processes or farmer selection (van Rensburg *et al*. 2007).

The term of leafy vegetables was adopted to refer to leafy part of plant which may include young shoots, succulent stems, petioles and young tender stems used as vegetables while old and hard stems are discarded (Vorster *et al*. 2002; van Ransbergs *et al.* 2007). By considering the medicinal value, some of the indigenous leafy vegetables believed to have potential to relieved particular ailments such as diabetes, high blood pressure, fever, coughs, skin rashes, preserving a youthful complexion and so on (Samy *et al*. 2009). This paper aims to review the current studies of indigenous leafy vegetables in Sarawak and provide the information to the people on nutritional value status.

PREVIOUS RESEARCH

The studies on leafy vegetables have been carried out worldwide such as in Ghana (Glew *et al*. 2010; Kwenin *et al*. 2011; Darkwa and Darkwa, 2013; Nyadanu and Lowor, 2015), India (Gupta *et al.* 2005; Misra *et al*. 2008; Chauhan *et al*. 2014; Sibangini and Malaya, 2014), Africa (Vorster and van Ransberg 2005; Uusiku *et al*. 2010; Njume *et al*. 2014), Nigeria (Sadiat, 2003; Aworh, 2015) and other developing nations. These studies mainly focused on the role of the leafy vegetables in combating hunger and malnutrition as inhabitants in this region experienced food insecurity. The importance of indigenous leafy vegetables commonly neglected and underestimated, as the result of inadequate research and development. The need for research and baseline information on indigenous leafy vegetables was highlighted by van Rensburg *et al*. (2005) to increase food security in this region.

In Southeast Asia, the study of leafy vegetables has been documented by PROSEA (1994); Bates *et al*. (2012); Chavasit (2012); Hiroyuki (2012) and Srivinasan (2012). Some of the indigenous leafy vegetables in a particular country in Southeast Asia have been potentially recognized for economic aspects (PROSEA, 1994). Similar as in Africa region, indigenous and wild leafy vegetables also played an important role in nutrition as recorded by many Southeast Asia countries such as Vietnam (Vuong, 2000; Ogle 2001; Ogle *et al.* 2001), Thailand (Ratchanee *et al*. 2005; Panpipat *et al*. 2010; Plernchai and Gassinee, 2014) and Philliphines (Bacho *et al.* 2012; Tolentino, 2014). Other than foods, indigenous leafy vegetables were considered to be able to prevent chronic disease especially plants that are rich in natural antioxidants. This study was recorded in Southern Thailand by Ratchanee *et al*. (2015).

Research on leafy vegetables in Malaysia has been developed as they are important for foods and economic aspects. The studies on vegetables have been recorded by Zainal Abidin (1990), PROSEA (1994), Ismail (2000), MARDI (2000), Rukayah (2000), Chooi (2004), Rukayah (2006), Normiadilah and Noriah (2012) and Shafiei (2015). Generally, the studies of indigenous leafy vegetables in Malaysia have been focused in the remote area as recorded in 2010 statistics which showed that 27.8 % of its people living in rural area (Peter 2012). Researches in ethno botany showed the used of various types of plants by villagers and indigenous

people in Malaysia as reported by Kueh (2000), Lin (2005), Bharati and John (2013), Rashidi *et al*. (2014) and Norsuhaila *et al.* (2014).

The use of indigenous leafy vegetables in Sarawak commonly practiced among people in rural areas because of their difficult access to the market and potential of indigenous leafy vegetables to generate income (Voon and Kueh, 1999). The studies on ethno botany in Sarawak showed the used of various types of wild and indigenous plants to the different ethnic such as Iban and Kelabit (Hanne, 2002), Penan (Miyako, 2005), Melanau (Latifah, 2009) and Orang Ulu (Noraini, 2009) and Kedayan (Effazura, 2010). Otherwise, the nutritional value of wild and indigenous leafy vegetables have been reported by Voon *et al*. (1992), Voon and Kueh (1999), Kueh (2000), Lee (2004), Shaffiq (2013), Noorasmah *et al*. (2014).

Nutritional Value

Proximate Composition

The data of proximate composition of indigenous leafy vegetables in Sarawak and its comparison with other studies in Peninsular Malaysia was summarized in Table 2. Generally, the moisture content recorded by various studies ranged from 59.0-92.5% where the lowest was recorded in *P.alternifolium* and the highest was recorded in *E.valerinofolia* (Voon *et al*. 1992). Water is the major component constitutes about 80-90% in plants and 70% in young tissue as stated by Geraldo and Henrique, (2012) but moisture content in *P.alternifolium* was slightly low. The values recorded by the other studies were similar to latest studies recorded by Ratchanee *et al*. (2015). Water is required in many plant growth activities such as deposition of protein, carbohydrate, and other metabolites as well as maintaining rigidity of soft plant tissue (Geraldo and Henrique, 2012).

The highest concentration of protein recorded in *S.grandifolia* and this result were supported by the studies in Peninsular Malaysia which showed the similar result. The highest amount of carbohydrate and crude fiber were observed in *P.alternifolium* and *S.polustris* respectively. The intake of fiber is important to colon and human digestive system and it is recommended for the adult to eat 20-35 g of dietary fiber each day (Devinder *et al.* 2012; Darkwa and Darkwa 2013). Generally, the composition of fat and energy found in the studies generally low because a fibre rich diet is commonly lower in energy density, low fat content, and rich in micronutrient (Devinder *et al.* 2012).

Table 9.1 Proximate composition of selected indigenous leafy vegetables for 100g edible portions

Local Name	Scientific Name	Family	Energy (kcal)	Moisture (g)	Ash (g)	Protein (g)	Carbohydrate (g)	Crude Fibre (g)	Fat (g)	Location	References
Keremak	*Althernanthera sessilis*	Amaranthaceae	46	82.3	3.8	0.3	8.9	2.7	0.3	Sarawak	Voon *et al.* (1992)
			39	88.0	2.2	2.3	5.5	1.1	0.9	Peninsular	Rukayah (2000)
			-	-	-	2.0	8.9	2.7	0.3	Peninsular	Ismail (2000)
Anak Mambong	*Erechtites valerianifolia*	Compositae	22	92.5	2.0	1.6	2.4	0.8	0.7	Sarawak	Voon *et al.* (1992)
			-	79.3	7.4	2.9	27.8	3.8	-	Peninsular	Norsuhaila *et al.*(2014)
Bungar	*Cyrtosperma lasiodes*	Araceae	10.7	92.3	1.03	2.15	0.11	1.03	0.18	Sarawak	Voon *et al.* (1992)
			-	-	-	-	-	-	-	Peninsular	-
Sawi rusa	*Erechtites hieracifolia*	Compositae	30	89.7	2.0	0.4	4.4	1.3	0.4	Sarawak	Voon *et al.* (1992)
			-	-	-	-	-	-	-	Peninsular	-
Sabong	*Gnetum gnemon*	Gnetaceae	71.5	80.9	1.6	1.7	17.3	5.4	0.07	Sarawak	Shaffiq (2013)
			-	81.7	-	4.2	6.6	4.7	-	Peninsular	Ismail (2000)
Turi	*Sesbania grandiflora*	Leguminosae	85	78.2	2.0	6.0	10.9	2.1	0.9	Sarawak	Voon *et al.* (1992)
			-	77.2	-	8.4	9.7	1.8	-	Peninsular	Ismail (2000)
Simpuh	*Dillenia suffruticosa*	Dilleniaceae	106	81.1	1.6	2.6	10.7	2.8	1.2	Sarawak	Voon *et al.* (1992)
			-	-	-	-	-	-	-	Peninsular	-
Paku ikan	*Athyrium esculentum*	Athyriaceae	71.5	92.3	1.33	1.03	7.4	1.13	0.07	Sarawak	Shaffiq (2013)
			30	90.5	1.1	2.5	4.6	1.1	-	Peninsular	Rukayah (2000)
Setawar hutan	*Costus speciosus*	Costaceae	69	80.7	2.2	2.3	10.6	2.3	1.9	Sarawak	Voon *et al.* (1992)
			-	-	-	-	-	-	-	Peninsular	-
Jinjir	*Limnocharis flava*	Butomaceae	-	90.63	0.76	0.17	-	1.07	0.22	Sarawak	Noorasmah (2014)
			-	-	-	1.0	0.5	-	-	Peninsular	Ismail (2000)
Paku midin	*Stenochlaena palustris*	Polypodiaceae	30.8	91.52	1.02	0.89	7.7	7.59	0.89	Sarawak	Shaffiq (2013)
			-	91.6	1.4	2.4	-	2.5	-	Peninsular	Norsuhaila *et al.*(2014)
Tangki	*Neptunia oleracea*	Leguminosae	-	83.75	1.05	3.01	-	2.30	0.25	Sarawak	Noorasmah (2014)
			40.0	81.0	1.76	5.37	1.75	8.79	1.31	Sarawak	Voon *et al.* (1992)
			-	89.4	-	-	-	-	-	Peninsular	Ismail (2000)
Somah	*Ploiarium alternifolium*	Bonnetiaceae	128	59.0	2.21	2.54	27.95	7.85	0.48	Sarawak	Voon *et al.* (1992)
			-	-	-	-	-	-	-	Peninsular	-

(-) =Not determined

Mineral Composition

The data of mineral composition of indigenous leafy vegetables in Sarawak reported from different studies and its comparison with other studies in Peninsular Malaysia were summarized in Table 9.1. It showed that most selected vegetables contain highest concentration of K compared to other elements where *E. valerianifolia* recorded by Voon *et al.* (1992) showed the highest value while in contrast, data recorded by Noorsuhaila *et al.* (2014) showed lower amount of K. There is variation existed in the concentration of elements among the studies recorded in different places might be due to different agro-climatic conditions, and different age and stages of the plants (Gupta *et al*. 2005). According to WHO (2012) unrefined food such as fruit and vegetables was a good sources of K and this elements is essential to the human bodies for maintenance body fluid volume, acid and electrolyte balance, and normal cell function.

The highest concentration of P was observed in *E.hieracifolia* recorded by Voon *et al*. (1992) while there is no data recorded from Peninsular Malaysia to support this result. According to Ismail (2000), vegetables are rich sources of P and it might be helpful in metabolism, nutrient absorption and formation of bone and teeth. *P.alternifolium* and *D.suffuroticosa* recorded the highest concentration of Ca while the highest Mg was recorded in *P.alternifolium.* Overall, most of the indigenous leafy vegetables selected contain low concentration of Fe, Mn, and Cu except for *E.valerianifolia* which contains high amount of Fe and Zn (Norsuhaila *et al.* 2014). This might be beneficial to health but if excessive amount of intake can lead to tissue damage (Nazanin, 2014).

Table 9.2 Mineral compositions of selected indigenous leafy vegetables (mg/100g)

Local Name	Scientific Name	Family	P	K	Ca	Mg	Fe	Mn	Zn	Cu	Location	References
Keremak	*Althernanthera sessilis*	Amaranthaceae	27	474	65	104	42.4	8.3	4.56	0.85	Sarawak	Voon *et al.* (1992)
			27	474	65	-	-	-	-	-	Peninsular	Ismail (2000)
			10	0	195	-	3.3	-	-	-	Peninsular	Rukayah (2000)
Anak Mambong	*Erechtites valerianifolia*	Compositae	32	586	58	16	2.7	1.6	0.88	0.63	Sarawak	Voon *et al.* (1992)
			-	114	0.7	2.5	214	-	171	-	Peninsular	Norsuhaila *et al.* (2014)
Bungar	*Cyrtosperma lasiodes*	Araceae	43	349	87	26	2	-	-	-	Sarawak	Voon *et al.* (1992)
			-	-	-	-	-	-	-	-	Peninsular	-
Sawi rusa	*Erechtites hieracifolia*	Compositae	129	417	78	45	1.3	1.4	0.68	0.32	Sarawak	Voon *et al.* (1992)
			-	-	-	-	-	-	-	-	Peninsular	-
Sabong	*Gnetum gnemon*	Gnetaceae	68	419	94	37	3.8	4.1	1.21	0.15	Sarawak	Voon *et al.* (1992)
			15	-	44	-	0.1	-	-	-	Peninsular	Ismail (2000)
Turi	*Sesbania grandiflora*	Leguminosae	79	308	96	65	16.4	3.3	0.66	0.27	Sarawak	Voon *et al.* (1992)
			29	356	181	-	0.3	-	-	-	Peninsular	Ismail (2000), Rukayah (2000)
Simpuh	*Dillenia suffruticosa*	Dilleniaceae	53	480	195	38	2.6	1.3	0.87	0.43	Sarawak	Voon *et al.* (1992)
			-	-	-	-	-	-	-	-	Peninsular	-
Paku ikan	*Athyrium esculentum*	Athyriaceae	83	410	14	19	1.8	3	14.5	2.1	Sarawak	Voon *et al.* (1992)
			100	389	94	-	4.8	-	-	-	Peninsular	Rukayah (2000)
Setawar hutan	*Costus speciosus*	Costaceae	15	587	114	44	2.6	15.1	0.43	0.21	Sarawak	Voon *et al.* (1992)
			-	-	-	-	-	-	-	-	Peninsular	-
Jinjir	*Limnocharis flava*	Alismataceae	56.3	389	13	21	-	0.4	0.7	0.4	Sarawak	Noorasmah (2014)
			63	252	23	17	2.1	0.2	0.08	0.06	Sarawak	Voon *et al.* (1992)
			-	-	-	-	-	-	-	-	Peninsular	-
Paku midin	*Stenochlaena palustris*	Polypodiaceae	56	295	12	21	3.1	6	3.3	1.2	Sarawak	Voon and Kueh (1999)
			56	-	12	-	-	-	-	-	Peninsular	Ismail (2000)
			21.2	-	6.8	2.2	77.1	-	12.7	-	Peninsular	Norsuhaila *et al.*(2014)
Tangki	*Neptunia oleracea*	Leguminosae	76	467	112	57	19	5	1	0	Sarawak	Voon *et al.* (1992)
			64	524	56	30	-	2	1.6	0.4	Sarawak	Noorasmah (2014)
			7	-	387	-	5.3	-	-	-	Peninsular	Ismail (2000)
Somah	*Ploiarium alternifolium*	Bonnetiaceae	13	279	574	111	2	2	1	1	Sarawak	Voon *et al.* (1992)
			-	-	-	-	-	-	-	-	Peninsular	-

(-) =Not determined

Vitamins and Antioxidant Contents

Generally, there are less detailed findings in vitamins and antioxidant content of the vegetables recorded (Table 9.3). The reports on those nutrient studies of *A.sessilis, C.lasiodes, E.hieracifolia, G.gnemon, D.suffuroticosa, C.speciosus* and *P.alternifolium* are not available. According to Nursuhaila *et al*. (2014), currently there is limited information on the antioxidants properties of some indigenous vegetables. Hence, more research and development needed in future to determine the vitamins and antioxidant content in these vegetables.

Table 9.3 Vitamins and antioxidant content of selected indigenous leafy vegetables for 100 g edible portion

Local Name	Scientific Name	Family	Vitamin A (µg)	Vitamin B1 (mg)	Vitamin B2 (mg)	Vitamin B3 (mg)	Vitamin C (mg)	Flavonoids (mg)	Phenolic (mg)	Location	References
Keremak	*Althernanthera sessilis*	Amaranthaceae	1850	0	0	0	37	-	-	Peninsular	Rukayah (2000)
			-	-	-	-	-	-	-	Sarawak	-
Anak Mambong	*Erechtites valerianifolia*	Compositae	-	-	-	-	-	21.86	140	Peninsular	Norsuhaila *et al.* (2014)
			-	-	-	-	1.2	-	-	Sarawak	Voon *et al.* (1992)
Bungar	*Cyrtosperma lasiodes*	Araceae	-	-	-	-	-	-	-	Peninsular	-
			-	-	-	-	-	-	-	Sarawak	-
Sawi rusa	*Erechtites hieracifolia*	Compositae	-	-	-	-	-	-	-	Peninsular	-
			-	-	-	-	-	-	-	Sarawak	-
Sabong	*Gnetum gnemon*	Gnetaceae	-	-	-	-	-	-	-	Peninsular	Ismail (2000)
			-	-	-	-	-	-	-	Sarawak	-
Turi	*Sesbania grandiflora*	Leguminosae	837	0.6	0.71	2.4	114	-	-	Peninsular	Ismail (2000)
			-	-	-	-	15.5	-	-	Sarawak	Voon *et al.* (1992)
Simpuh	*Dillenia suffruticosa*	Dilleniaceae	-	-	-	-	-	-	-	Peninsular	-
			-	-	-	-	-	-	-	Sarawak	-
Paku ikan	*Athyrium esculentum*	Athyriaceae	220	0.04	0.14	0.7	34	-	-	Peninsular	Ismail (2000)
			-	-	-	-	0	161	45.29	Sarawak	Voon *et al.* (1992)
Setawar hutan	*Costus speciosus*	Costaceae	-	-	-	-	-	-	-	Peninsular	-
			-	-	-	-	-	-	-	Sarawak	-
Jinjir	*Limnocharis flava*	Butomaceae	5000	-	-	-	-	-	-	Peninsular	Ismail (2000)
			-	-	-	-	0.3	-	-	Sarawak	Voon *et al.* (1992)
Paku midin	*Stenochlaena palustris*	Polypodiaceae	-	-	-	-	1.8	-	-	Peninsular	Ismail (2000)
			-	-	-	-	1.8	-	-	Sarawak	Voon *et al.* (1992)
Tangki	*Neptunia oleracea*	Leguminosae	5155	-	-	-	1.8	-	-	Peninsular	Ismail (2000)
			-	-	-	-	-	-	-	Sarawak	-
Somah	*Ploiarium alternifolium*	Bonnetiaceae	-	-	-	-	-	-	-	Peninsular	-
			-	-	-	-	-	-	-	Sarawak	-

(-) =Not determined

CONCLUSION

Indigenous leafy vegetables have potential to be commercialized as food crops as they contain great sources of nutritional value and pesticide free. The studies on agronomic factor required for the cultivation of indigenous leafy vegetables should be carried out in future to optimize its production for the domestic purpose. Further studies that not only focused on nutritional value but also stressed on vitamins and antioxidants was required. The studies on anti-nutritional value also have potential to be explored to give information to people about its effect and to optimize the nutrients intake. Knowledge on the importance of indigenous leafy vegetables should be passed to young generation to sustain their use over generation.

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10

Review of Aquatic Weed, *Neptunia Oleracea* Lour (Daun Tangki) as a Leafy Vegetable in Sarawak, Malaysia

Noorasmah Saupi, Muta Harah Zakaria, Japar Sidik Bujang and Aziz Arshad

INTRODUCTION

Aquatic macrophytes are considered as those large plants which grow in excessive amounts in a continuous supply of water or at least present in soils which are covered with water during a major part of the growing season and interfered with the intended usage of particular area (Weldon *et al.,* 1973; Edwards, 1980). They are divided into four categories based on their life form, namely floating, submerged, emergent and marginal (Edwards, 1980; Mashhor, 1988; Said *et al.*, 1991; Muta Harah *et al.,* 2005; Closs *et al.*, 2006; Singh, 2008).

The high density and population of aquatic macrophytes and their ability for vegetative growth in drainage systems, ponds, reservoirs and paddy fields can cause problems to the humans in terms of in agriculture, health, hydroelectricity dams, reduced water quality and recreational purposes. For that reason, many researchers considered aquatic macrophytes as invasive or unwanted and refer to them as "aquatic weeds" (Weldon *et al.,* 1973; Edwards, 1980; Said *et al.,* 1991; Mashhor, 1994). One of the best controls of weeds is by utilizing them (Mashhor, 1988). Various reports have been documented on their uses mainly in waste treatment management, agriculture, foods and paper pulps. As for example, in West Malaysia, 56 species had been used as medicinal plant, 32 species for feed plants, 27 species for vegetables, 16 species for aquarium and 14 species for green manure (Said *et al.,* 1991).

The major pathways involving aquatic macrophytes for food production in South East Asia is shown in Figure 10.1. Basically, aquatic macrophytes provided three types of foods to human being, the foliage for green vegetables, grain or seeds for protein, starch and oil and swollen fleshy roots for starch (National Academy of Sciences, 1976).

Previous studies on the uses of aquatic macrophytes in Sarawak showed that 31 species are edibles, 11 species are used for medicinal, 12 species for fodder, 19 species for ornamental, 2 species for food wrapper, 3 species for paper and mat and 2 species for bio-filter (Muta Harah *et al.,* 2005). Twenty-three of freshwater macrophytes are reported utilized for food (Suzalina Akma, 2008; Dayangku Alifah, 2009). Local peoples consumed them either as raw or cooked vegetables. However, most of these aquatic macrophytes in Sarawak are harvested from the wild, and there has been no cultivation for these edible species on a commercial scale (Muta Harah *et al.,* 2005; Suzalina Akma, 2008; Mohd Syahrul, 2009; Dayangku Alifah, 2009). From observations in Sarawak, at least in several places, e.g., Sibu and Bintulu, the common aquatic macrophyte, *Neptunia oleracea* Lour. are consumed locally as leafy vegetable. Peoples gathered this plant from several places e.g., irrigation and drainage systems for own consumption and also offered for sale in native markets.

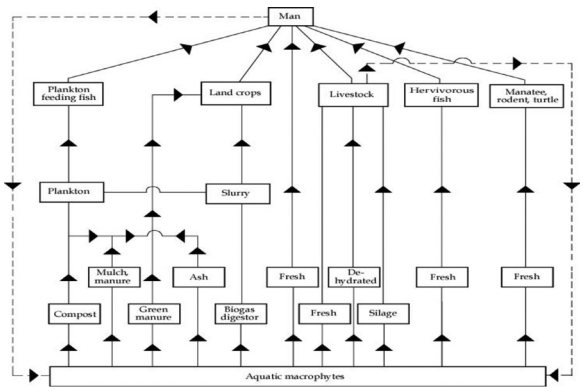

Figure 10.1 The major pathways involving aquatic macrophytes in food productions in South East Asia (Source: Edwards, 1980)

Aquatic macrophytes commonly consumed as green leafy vegetable is water mimosa, *N. oleracea.* It is synonym to *N. prostrata* (Lamk) Baillon and *N. natans* (L.f) Druce. It is known by various names as keman air, keman gajah, kangkung puteri in West Malaysia and daun tangki in Sarawak (Rukayah, 2002; Halimatul Saadiah, 2003). A plant has bipinnate leaves and stems made buoyant by their spongy white covering. The young shoot comprising of leaves, spongy stems and young seedpods can be eaten raw and cooked as green vegetables (National Academy of Sciences, 1976; Edwards, 1980; Paisooksantivatana, 1994; Rukayah, 2002; Halimatul Saadiah, 2003; Muta Harah *et al*., 2005; Samy *et al*., 2005; Mohd Syahrul, 2009, Dayangku Alifah, 2009; Jain *et al.,* 2011). Subjects pertaining to *N. oleracea* include its description and morphology (Windler, 1966; Ridley, 1967; Shah and James, 1968; Holtum, 1969; Henderson, 1974; Pancho and Soerjani, 1978; Paisooksantivatana, 1994; Kamarudin and Latiff, 2002; Holtum and Ivan, 2002), distribution and ecology (Windler, 1966; Ridley, 1967) and propagations (Paisooksantivatana, 1994).

It can be found floating or prostrate near the edge of water reservoir, water channel and ditches with stagnant to slow moving water of pH 5.4 – 6.0 (Cook *et al.,* 1974; Paisooksantivatana, 1994). In Thailand, it is commonly used as a vegetable and cultivated in inundated fields or in canals (National Academy of Sciences, 1976; Edwards, 1980; Paisooksantivatana, 1994). *Neptunia oleracea* can be harvested after 3 – 4 weeks after planting and 250 shoots are gathered into bunch then traded in local market. About 30,000 – 50,000 shoots can be harvested for each harvest in one hectare area (Paisooksantivatana, 1994). In Sarawak *N. oleracea* is sold in Sibu central market and Tamu Nyelong Sarikei depending on the availability at RM 1.00 – 2.00 per bunch (Muta Harah *et al*., 2005).

Generally in Malaysia, most of the studies of wild or indigenous fruits and vegetables are recorded in West Malaysia e.g., research on wild fruit plants (Chung *et al.,* 2004), salad and vegetables (Rukayah, 2002), and herbs (Samy *et al.,* 2005). In Sarawak, reports pertaining on utilization and marketable of indigenous fruits and vegetables were documented by Voon *et al.* (1990) and Mohd Syahrul (2009). Several studies were focusing on aquatic macrophytes distribution and utilization (Muta Harah *et al.,* 2005; Suzalina Akma, 2008) and marketable species (Dayangku Alifah, 2009). Those studies reported on the occurrences of *N. oleracea* in Sarawak native markets with data observation on its availability throughout the year.

Distribution and Environmental Condition of Neptunia oleracea

The geographical distribution of *N. oleracea* are recorded mostly in tropical region, i.e., the geographical distribution of *N. oleracea*: Malaysia (Ridley, 1967; Kamarudin and Latiff, 2002), Singapore (Holtum and Ivan, 2002), Thailand (Cruz-Garcia and Price, 2011), India (Cook*,* 1996), Australia (Brooks *et al.,* 2008), Namibia (Burke, 2000), Burkina-Paso (Wittig, 2005), Guinea-Bissau (Catarino *et al.,* 2001), Nigeria (Abulude, 2005), Colombia (Núñez *et al.,* 2011), Taiwan (Chou *et al.*, 2007), Sri Lanka (Kotawala, 1976,) Madagascar (Cook, 1996), and Vietnam (Windler, 1966; Ogle *et al.,* 2003).

The various habitats of *N. oleracea* are given in Table 10.2. The species inhabited the ditches, paddy fields, irrigation channel and swamps. *Neptunia oleracea* can be found mostly in both paddy fields and swampy areas.

The chemical and physical factors such as amount of oxygen, hydrogen ion concentration, available inorganic mineral salts, temperature, light and water movements are important in the limitation of the distribution of organism in water bodies (Singh, 2008). The chemistry status of water in some freshwater habitats are summarized in Table 10.3. Various factors contribute to the soil quality such as physical conditions of soil and nutrient availability, root growth and extension, organic matter, cropping system and quality of irrigation water (Ashman and Puri, 2002; Tolanur, 2006; Singh, 2008). Emergent macrophytes, *Lunorium natans (*L.*)* Raf. inhabited at Rhône and Ain river flood plain, France where the percentage of N and concentration of P were 1.70% and 2600 ppm respectively. Nutrient concentration in water bodies is environmental variables that significantly affected plant community composition (Sharip *et al.,* 2011) where the nitrate, phosphorous and ammonia are of great economic important macronutrients that can cause eutrophication (Lomoljo *et al.,* 2009). Potassium, phosphorous, calcium, magnesium and sodium in *Potamogetum pectinatus* L.*, P. crispus* L.*, Aponogeton natans* (L.) Engl. & K. Krause and *Hydrilla verticillata* (L.f.) Royle were strongly correlated to the nutrients in waters as reported by Shardendu and Ambasht (1991). The communities of freshwater macrophytes and their associations are important where diverse species of emergent, submerged, floating and half submerged (marginal) can co-exist in an area (Suzalina Akma, 2008; Singh, 2008).

Table 10.1 A summary compiled on the type of habitats of *N. oleracea*

Habitat	Location	References
Ditches	**Thailand**	Edwards (1980); Paisooksantivatana (1994)
	Malaysia	Ridley (1967)
	Australia	
	Queensland	DPI (2009)
Paddy fields	**Malaysia**	
	Muda, Kedah	Mashhor (1988)
	Thailand	Paisooksantivatana (1994)
	Guinea-Bissau	Catarino *et al.* (2001)
Irrigation channels	**India**	Cook *et al.* (1974)
	Australia	
	Queensland	DPI (2009)
Swamps and marshes	Colombia	
	Ayapel, Betanci and Lorica wetlands, Córdoba	Núñez *et al.* (2011)
	Thailand	
	Kalasin Province	Cruz-Garcia and Price (2011)
	India	
	Keoladeo National Park	Prusty *et al.* (2007)
	Manipur state	Jain *et al.* (2011)
	Samaspur Wetlands, Uttar Pradesh	Behera *et al.* (2012)
	Malaysia	
	West Malaysia	Keng (1983); Ivan (1988)
Dams	**Burkina-Paso**	
	Sahelian seasonal lakes	Wittig (2005); Müller (2005)
	Nigeria	Abulude (2005)

Table 10.2 Water chemistry of N. oleracea habitats

Location	pH	NO_3^- (ppm)	PO_4^{3-} (ppm)	References
Thailand				
Pichet Farm, Prachinburi Province	7.70 - 8.10	0.01 - 1.03	-	Suppadit *et al.* (2005)
India				
Keoladeo National Park, Bharatpur	7.00 - 8.80	-	0.10 - 3.50	Prusty *et al.* (2007)

Morphological Characteristic of Neptunia oleracea

Subjects focusing on *N. oleracea* include its description and morphology (Windler, 1966; Ridley, 1967; Henderson, 1974; Pancho and Soerjani, 1978; Soepadmo, 1986; Holtum and Ivan, 2002; Paisooksantivatana, 1994; Kamarudin and Latiff, 2002; Samy *et al., 2005*), and the illustration of *N. oleracea* is shown in Figure 2 and the detail descriptions are summarized in Table 10.4.

Neptunia oleracea is an aquatic perennial legume with ascending or floating habits. Stems are cylindrical rarely branched, can elongate up to 1.5 m with ascending or floating horizontal. This plant has long internodes that branches and roots arise at the mature nodal regions (Shah and James, 1968). The older parts of stem are enveloped by thick spongy layers or white internodal aerenchyma tissue which helps the plant in its flotation. Adventitious roots of *N. oleracea* are formed from the nodes. The roots are also reported nodulated symbiotically by *Rhizobium* (Rivas *et al.,* 2003; Subba-Rao *et al.*, 1995), *Aeschynomene* spp. Schaede (1940: In Alazard, 1985) and *Labrys neptuniae* (Chou *et al.,* 2007*).*

The leaves are generally bipinate compound with 2 to 3 pairs of pinnae that formed 1 to 16 pairs of assymetrical oblong leaflets. However, Windler (1966) recorded that *N. oleracea* in Vietnam having 2 to 4 pairs of pinnae and formed 8 to 20 pairs of leaflets.

Neptunia oleracea possesses spike typed inflorescence on long peduncle. The spike is a dimorphic flower with lower part was yellow petal-like absent of androecium and gynoecium. Upper part spike is a perfect flower having pistil surrounded with 10 exserted stamens. Fruit is pod type marginally dehiscent and contained 4 to 8 ovoid brown seeds.

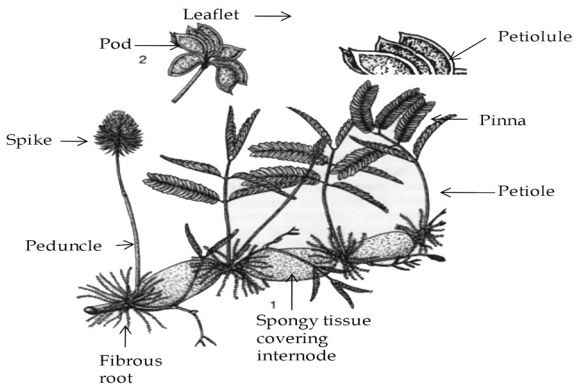

Figure 10.2 The morphology of *N. oleracea* (Source: Paisooksantivatana, 1994)

Table 10.3 A summary compiled on description of *N. oleracea* morphology

	Location	Description	References
Root	West Malaysia	Fibrous adventitious roots.	Soepadmo (1986)
	West Malaysia	Fibrous adventitious roots.	Kamarudin and Latiff (2002)
	Indonesia	Fascicles from the nodes.	Pancho and Soerjani (1978)
	Taiwan	Nodulated symbiotically by *Labrys neptuniae.*	Chou *et al.* (2007)
	Vietnam	Fibrous adventitious roots.	Windler (1966)
Habit and stem	West Malaysia	Cylindrical either ascending or horizontal.	Ridley (1967)
	West Malaysia	White tissue is much greater in bulk that the rest of stems.	Henderson (1974)
	West Malaysia	The older parts of stem are enveloped by thick spongy layers or white internodal aerenchyma.	Soepadmo (1986)
	West Malaysia	Cylindrical either ascending or horizontal and the older parts of stem are enveloped by thick spongy layers or white internodal aerenchyma.	Kamarudin and Latiff (2002)

cont'd Table 10.3

	West Malaysia	The older parts of stem are enveloped by thick spongy layers.	Samy *et al*. (2005)
	Singapore	With white tissue forming the bulk around the stems.	Holtum and Ivan (2002)
	Indonesia	The older parts of stem are enveloped by thick spongy layers or white internodal aerenchyma.	Pancho and Soerjani (1978)
	Thailand	Rarely branch and terete, becoming detached from the primary root system and forming spongy-fibrous swollen internodes.	Paisooksantivatana (1994)
	Vietnam	Rarely branch	Windler (1966)
	India Gujarat	Long internodes that branches and roots arise at the mature nodal.	Shah and James (1968)
	India	Cylindrical either ascending or horizontal stem with a mass of a white tissue with many air spaces between cells.	Cook (1996)
Leaf	West Malaysia	Bipinnate compound, 2 - 3 pairs of pinnae and consist of 8 - 15 leaflet pairs. Leaflets are 8.75 - 18.75 mm long and 5.0 mm wide.	Ridley (1967)
	West Malaysia	Bipinnate compound, 2 - 3 pairs of pinnae and consist of 8 - 18 leaflet pairs. Leaflets are 12.50 mm long and blunt shape.	Henderson (1974)
	West Malaysia	Bipinnate compound.	Soepadmo (1986)
	West Malaysia	Bipinnate compound and leaflets are 8 - 12 mm long, 5.0 mm wide, asymmetrical, oblong, obtuse to broadly acute and rounded at end.	Kamarudin and Latiff (2002)
	West Malaysia	Bipinnate compound and consist of 8 - 18 leaflet pairs.	Samy *et al*. (2005)

cont'd Table 10.3

	Singapore	Bipinnate compound.	Holtum and Ivan (2002)
	Indonesia	Bipinnate compound, 2 - 3 pairs of pinnae and consist of 1 - 16 leaflet pairs. Leaflets are 25 - 45 mm long, asymmetrical, oblong and obtuse to broadly acute.	Pancho and Soerjani (1978)
	Thailand	Alternate bipinnate, 2 - 4 pairs of pinnae and consist of 8 - 20 leaflet pairs. Leaflet are 5 - 18 mm long, 1.5 - 3.5 mm wide, assymetrical and glabrous or with sparsely ciliate margins.	Paisooksantivatana (1994)
	India	Alternate arrangement.	Cook (1996)
	Vietnam	Bipinnate compound with 2 - 4 pairs of pinnae and consist of 8 - 20 leaflet pairs. Leaflet are 5 - 18 mm long, 1.5 - 3.5 mm wide, broad, oblong occasionally mucrunulate, assymetrical, glabrous and ciliate at margin.	Windler (1966)
Inflorescence	West Malaysia	Peduncle 7.5 - 30.0 cm long.	Ridley (1967)
	West Malaysia	Slender and glabrous peduncle 1.25 - 2.50 cm long rising out of the water.	Henderson (1974)
	West Malaysia	Peduncle more than 25 cm long.	Soepadmo (1986)
	West Malaysia	Slender and glabrous 1.25 - 2.50 cm long peduncle rising out of the water. Spike consisted of 30 - 50 flowers and 3.0 - 11.0 mm long.	Kamarudin and Latiff (2002)
	West Malaysia	Peduncle 7.5 - 30.0 cm long.	Samy *et al*. (2005)
	Indonesia	30 - 50 flowers in each ovoid spike with 2 bracts subtending the spike and 2.0 - 3.0 mm long.	Pancho and Soerjani (1978)

cont'd Table 10.3

	Thailand	Peduncle 5.0 - 30.0 cm long with slightly solitary spike consist of 30 - 50 flowers.	Paisooksantivatana (1994)
	India	Spike, erect or slightly nodding, pedunculate, borne solitary in the axils of the leaves and long peduncle 7.5 - 30.0 cm.	Cook (1996)
	Vietnam	Spike is ovoid, consists of 30 - 50 flowers with two subtended bracts, erect or slightly nodding, borne solitary in the axils of the leaves, 5.0 - 30.0 cm long peduncle.	Windler (1966)
Flower	West Malaysia	Dimorphic and upper flowers is perfect, sessile, calyx is minute, campanulate or bell shape. Lower flower is sterile, sessile, campunulate calyx, 5 lobed yellow petal-like and absent of gynoecium and androecium. Petal is 5 toothed, regular, strap shaped and connate at base clubbed.	Ridley (1967)
	West Malaysia	Bisexual, small, sessile and bright yellow in globosehoods	Henderson (1974)
	West Malaysia	Dimorphic and upper flowers are perfect, sessile, minute calyx, campanulate or bell shape and petal is green and elliptic.	Kamarudin and Latiff (2002)
	Singapore	Bisexual, small, sessile and bright yellow in globosehoods.	Holtum and Ivan (1990)
	Thailand	Bisexual flowers with campunulate calyx. Petal is 5 toothed and regular.	Paisooksantivatana (1994)
	India	Bisexual, small, sessile and bright yellow in globosehoods. Lower flower is sterile, sessile, campunulate calyx, 5 lobed yellow petal-like and absent gynoecium and androecium. Petal is 5 toothed and regular	Cook (1996)

cont'd Table 10.3

	Vietnam	Sessile and each subtended by a single bract. Lower flower is sterile, sessile, campunulate calyx, 5 lobed yellow petal-like and absent of the gynoecium and androecium. Petal is 5 toothed, regular, broadly acute, entire margin, green and 2.0 - 3.0 mm long.	Windler (1966)
Stamen	Vietnam	10 with filaments slender, flattened, white, 5.1 - 8.2 mm long, anther exserted, bilocular, yellow, 0.7 - 0.9 mm long and lacking of terminal stalked gland.	Windler (1966)
	Indonesia	10.	Pancho and Soerjani (1978)
	India	10.	Cook *et al.* (1974)
	Thailand	10.	Paisooksantivatana (1994)
Ovary	West Malaysia	Sessile, glabrous and 1.2 - 2.0 mm long.	Kamarudin and Latiff (2002)
	Thailand	Pistil up to 9 mm long, usually exserted beyond stamens.	Paisooksantivatana (1994)
	Vietnam	Stipitate, style slender, elongate, glabrous, stigma truncate, concave and 1.2 - 2.0 mm long.	Windler (1966)
Fruit	West Malaysia	Pod long 12.5 - 25.0 mm.	Ridley (1967)
	West Malaysia	Pod typed, oblong to oblongoid, flat, membranous-coriaceous and marginally dehiscent.	Henderson (1974)
	West Malaysia	Pod long 15.0 - 25.0 mm.	Kamarudin and Latiff (2002)
	Indonesia	Pod types, oblong to oblongoid, flat, membranous-coriaceous and marginally dehiscent with 4 - 8 seeds.	Pancho and Soerjani (1978)

cont'd Table 10.3

	Thailand	Legume, broadly oblongoid and flat, dehisced along both sutures, 20.0 - 30.0 mm long, 10 mm wide and containde 4 - 8 seeds.	Paisooksantivatana (1994)
	India	Pod typed, oblong to oblongoid, flat, membranous-coriaceous and marginally dehiscent.	Cook (1996)
	Vietnam	Broadly oblong, flat, membranous-coriaceous, glabrous, marginally dehisced, 19.0 - 28.0 mm long, 0.8 - 1.0 mm wide and contained 4 - 8 seeds.	Windler (1966)
Seed	Thailand	Compressed ovoid, brown, 4.0 - 5.1 mm long and 2.7 - 3.5 mm wide.	Paisooksantivatana (1994)
	India	Compressed, ovoid and brown.	Cook (1996)
	Vietnam	Compressed, ovoid, brown, 4.0 - 5.1 mm long and 2.7 - 3.5 mm wide	Windler (1966)

Propagation of Neptunia oleracea

Neptunia oleracea can be propagated via sexual and vegetative by seed and stem cutting respectively (Paisooksantivatana, 1994; Holtum and Ivan, 2002). In Thailand, this species is conventionally cultivated by 50 to 150 cm stem cuttings in undated rice fields as an intercrop with paddy (Paisooksantivatana, 1994).

Availability and Nutrient Content Of Neptunia oleracea in Markets

Availability of Neptunia oleracea in Markets

Various studies have been documented on the wild food plants from various parts of the world to maintain a balance between population growth and agricultural activity such as in Spain (Tardio *et al.,* 2006), Bosnia-Herzegovina (Redzic, 2006), Turkey (Yildrim *et al.,* 2001), Himalaya

(Sundriyal and Sundriyal, 2001), Vietnam (Ogle *et al.,* 2003), Nigeria (Ezebilo, 2010), Kenya (Orech *et al.,* 2007), Bostwana (Flyman and Afoloyan, 2006; 2007), West Malaysia (Mohd Shahwahid, 1990; Rukayah, 2002; Samy *et al.,* 2005) and Sarawak, East Malaysia (Voon and Kueh, 1999).

Furthermore, wild leafy plants played an important role in complementing staple foods by providing a balanced diet supplying minerals, fiber, vitamins and essential fatty acids to enhance taste and color (Yildrim *et al.,* 2001; Lyimo *et al.*, 2003; Tardio *et al.*, 2006). Wild plants naturally grown, clean, environmentally friendly and pesticide-free, e.g., *Anthyrium esculentum, Stenochlaena palustris* and *Leucosyke capitellata* have better taste, richer in minerals, greater in nutritional and have medicinal properties than in some cultivated vegetables (Voon and Kueh, 1999).

In South East Asia about 100 species of the 225 vegetables are weeds or wild plants including *N. oleracea* (Grubben *et al.,* 1994). Local leafy vegetables in northern Nigeria commonly traded in markets were also being collected by the traders from the wild (Barminas *et al.,* 1998). The known wild and indigenous edible aquatic macrophytes such as *L. flava, Centella asiatica, Althernantherea sessilis* and *Portulaca oleracea* can be found easily in local markets in Sarawak mainly in Sibu, Sri Aman and Kapit Districts (Voon and Kueh, 1999). However, no report had been done on the availability of this species throughout the year in Sarawak.

Proximate Compositions and Mineral Contents of Neptunia oleracea

Analytic chemical analysis of a food is the nutritional composition of that food and it is the estimation of the nutritive value of human food in its chemical form (Alli Smith, 2009). In food, fat serves as a concentrated source of energy (Fleck, 1981). The main components of fats are fatty acids which can be grouped as saturated, monosaturated, polysaturated and trans. Plants basically contains mainly unsaturated fat (Ong, 2008). Crude fiber is defined as the residue remaining as well as lignin, hemicelluloses and cellulose after acid and alkaline extraction of a defatted sample (deMan, 1999). Fibers are established mainly in fruits, leafy vegetables, legumes and

cereals and can reduce blood cholesterol, slow down the rate of digestion and increases enzymes activities (Kadam and Salunkhe, 1998; Ong, 2008). Another important composition in food is the ash content that is an index of inorganic minerals in biota (Fleck, 1981; Hassan and Umar, 2006). The energy values of leafy vegetables are usually low (Voon and Kueh, 1999). Umar *et al.* (2007) stated that the calorific values of most vegetables are low within the range of 125.00 - 209.00 kJ/100 g.

Minerals play a major role in the functioning of the physiological activities and reproduction in inorganic elements (Fleck, 1981; Hazra and Som, 2005; Ong, 2008). The inorganic elements are resulting from ash content (Fleck, 1981). Fresh vegetables are one of the major dietary sources of potassium (K) and it facilitated reactions of enzymes in protein and carbohydrate metabolism (Insel *et al.,* 2002). It is required for regulating body fluids, circulation of ions, regulating heartbeat, helps body to excrete excess sodium and relieve raised blood pressure (Ong, 2008). Plants generally have higher content of K than Na (deMan, 1999).

Sodium (Na) required in small amounts for regulating body fluid and helps in muscles and nerves functions (Ong, 2008). deMan (1999) and Hassan and Umar (2006) noted that K/Na in diet is an important factor in prevention of hypertension and arteriosclerosis, with K depresses and Na enhances blood pressure. Calcium (Ca) is the essential of bones and teethes, blood clotting, contraction of muscles, control of nerves and regulation heartbeat (Fleck, 1981; Hazra and Som, 2005; Ong 2008).

Phosphorous (P) generally found with calcium in the body contribute to the supportive structures of the body, energy and minerals metabolism and even platelet aggregation (Fleck, 1981; Hazra and Som, 2005; Ong, 2008; Borah *et al.,* 2009). Ca/P ratio played an important role on development of human and its ratio of at least below 1 is for good absorption of both minerals (Raigón *et al.*, 2008).

Although responsible to the formation of chlorophyll or deep green color in vegetables, primary function of magnesium (Smgt Macfarlane, Macfarlane, & Cummings) to human is as an activator of many enzyme systems, functioning of muscles and nerves and for normal calcium function (Fleck, 1981; Insel *et al*., 2002; Hazra and Som 2005; Ong, 2008; Borah *et al.,* 2009). Green leafy vegetables and legume products are good sources of Mg (Fleck, 1981). Copper (Cu) is another trace element, essential in human body where it exists as an integral part of copper

proteins ceruplasmin, the enzyme that catalyzes the oxidation of ion iron, production of melanin pigments and energy release from food (Fleck, 1981; Insel *et al*., 2002; Ong 2008).

Despite being functional as K, zinc (Zn) element is needed during pregnancy particularly in the development of fetus central nervous system (Fleck, 1981). Manganese (Rumney & Rowland) is also important as an essential component for bone structure, reproductive and normal function in nervous system (Fleck, 1981; Ong, 2008). Above ground organs (young shoots and leaves) are rich in P, Fe, Mg, vitamin C, carotene and oxalic acid that can converts Ca from soluble to insoluble forms (Kadam and Salunkha, 1998; Redzic, 2006).

For that reason, the dieticians recommend a daily consumption of at least 116 g of vegetables for a balanced diet (Singhal and Kulkarni, 1998). Table 10.5 and Table 10.6 show the proximate composition and mineral content of some aquatic leafy vegetables including *N. oleracea*. However, the nutritive value of vegetables varies widely as a result of environmental factors, varietal differences, cultural practices, harvesting stage of plant, methods of storage, processing, cooking and preparation, but is usually greatest in those eaten raw or salad (Grubben *et al.,* 1994, Guil-Guerrero *et al.*, 1998).

Table 10.4 Proximate composition in *N. oleracea*

Location	Moisture (%)	Ash (%)	Crude protein (%)	Crude fat (%)	Crude fiber (%)	Source
Thailand	89.4	1.2	6.4	0.4	1.8	Paisooksantivatana (1994)

Table 10.5 Mineral contents in *N. oleracea* (mg/100g)

Location	K	Na	Ca	Mg	P	Cu	Zn	K/Na	Ca/P	Source
Nigeria	820.0	320.0	320.0	308.0	185.0	1.1	1.4	2.7	1.7	Abulude (2005)
Thailand	-	-	387.0	-	7.0	-	-	-	55.3	Paisooksantivatana (1994)

CONCLUSION

Despite the uses of *N. oleracea* as food by the local people in Sarawak, Malaysia, the plant have not been given due attention in terms of its propagation modes, availability and nutritional content. For this reason, the future study need to be carried out to complement those studies mentioned above focusing on the plants' biology, availability in native markets, proximate and minerals compositions, and also its propagation modes. It is hope that the information would be used for advocating its increased utilization as vegetable crop.

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11

Termite Species and Their Ecological Significance in Oil Palm on Peat

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INTRODUCTION

Termites, which are the most dominant invertebrate living socially in tropical ecosystem, belong to order Isoptera. This particular group of soil organism is part of the natural ecosystem, and presence of termites is not, by itself, evidence of a pest problem. Of more than 2800 described species of termite in the world, only about 185 species are pests which constituted about 6.6% of the total discovered termite species (Verma *et al*., 2009). Termites are considered as pest when economic damage is caused by termite activities. It is their diets which are mainly of wood and cellulotic plant materials, which lead them to be recognized as a destructive pest when they damage the wooden structure of building, wood furniture, paper, cloth, tree plantation like oil palm and rubber, fruit trees and growing crops, causing a steady loss of property and amenity. Global damage caused by termites are in the billions (USD) each year (Lewis, 2006). Latest estimates of termite damage in Malaysia are hardly available in published literatures. However, Lee (2002) had reported that the cost for termite control has accounted for 50% of the pesticide industry's business, hitting a total of USD10 million in year 2000. The lack of knowledge on biology and behaviour of termite species has made the oil palm industry rely heavily on chemical insecticide to control and eliminate termites.

There have been many ecological studies concerning termite in Malaysia and most of the studies were conducted in Peninsular Malaysia primarily in rainforests. In East Malaysia, termite study can be traced back to late 1970s and early 1980s in Sabah where Thapa (1981) had published

a book entitled '*Termite of Sabah*' from which the termite samples were collected within the state of Sabah and some parts in Sarawak. This book together with the book '*Termite of Peninsular Malaysia*' by Tho (1992) had become the major source of references by local termite researchers for termite identification. Nevertheless, the available data do not represent the entire environment notably in the state of Sarawak that is favourable for termite activity. Hence, this study was intended to provide a better understanding of termite occurring in peat soil that will eventually contribute towards effective oil palm management strategies as well as biodiversity conservation of fauna in peat area plantation as asserted by Kon *et al.* (2012).

As biodiversity and conservation publication particularly in oil palm plantation was mostly on mammals and birds but less on insects (Turner *et al.*, 2008), this chapter was, therefore, initiated with the objective to introduce the species of termites that are found in oil palm plantation on peat in the central region of Sarawak, Malaysia. The distribution and ecological significance of these termites are also discussed.

Sampling of Termites in Oil Palm on Peat

The studies were conducted at six sites, two sites at each of three oil palm plantations on peat soil selected in the central region of Sarawak, Malaysia, namely Semanok plantation (Coordinate: N03°01'0.6", E112°52'51.7") (hereafter referred to as SM), Setuan plantation (Coordinate: N02°52'936'', E112°36'152'') (hereafter referred to as ST) and Sessang plantation (Coordinate: N03°00'41.7", E112°52'47.2") (hereafter referred to as SS). The sampling plot characteristics and existing environment are shown in Table 11.1.

Table 11.1 Sampling plot characteristics and existing environment

Site	Palm age (years)	Estate management intensity	Water table (cm)	Soil pH (KCl)	Soil pH (water)	Soil moisture (%)	Nearest distance to peat forest (km)	Nearest distance to mineral soil forest (km)
SM	5-7	high	60-70	2.76-3.65	3.63-4.78	62.71-80.52	2.10	3.30
ST	6-8	low	>80	2.39-2.79	3.37-3.73	83.54-85.49	6.10	6.70
SS	13-15	medium	40-50	2.93-3.47	3.82-4.30	62.83-80.66	0.85	9.10

**Note: SM- Semanok, ST- Setuan, SS- Sessang

A modified transect from standard transect method measuring 100 (length) × 2 m (width) (Jones and Eggleton, 2000) was designed to sample termites in between two rows of oil palm trees measuring 50 (length) × 6 m (width). Jones and Eggleton (2000) reported that one transect is sufficient with required level of sampling efficiency. Two sampling sites were randomly selected by rolling a dice for each plantation resulting 6 transects in 3 sites. The transect was then subdivided into 15 grids measuring 10 × 2 m. The investigation was carried out from 30 cm below the ground until 2 metre up the oil palm trunk. Each grid was thoroughly searched for any termite mud trails on the tree trunk, infested fallen oil palm rachis, buried rotting wood logs, tree stump and rooting zone of ground vegetations. All termites were hand sorted according to different castes and were preserved in 70% alcohol for further identification. Feeding information of collected species was recorded from field observation and confirmed by referring to Thapa (1981) and Tho (1992).

A total of 18 termite species from 2 families (Rhinotermitidae and Termitidae), 5 subfamilies (Coptotermitinae, Rhinotermitinae, Termitinae, Macrotermitinae and Nasutitermitinae), 11 genera (*Coptotermes, Schedorhinotermes, Termes, Macrotermes, Nasutitermes, Globitermes, Amitermes, Parrhinotermes, Pericapritermes, Havilanditermes,* and *Prohamitermes*) were collected from site SM, site ST and site SS oil palm plantations by modified transect sampling (Table 11.2). Lower termites of subfamily Termitinae dominated the assemblage (6 species, 33%), followed by subfamily Rhinotermitinae and Nasutitermitinae (4 species, 22% respectively), subfamily Coptotermitinae (3 species, 17%),

and subfamily Macrotermitinae (1 species, 6%). Highest species richness of termite was recorded from SM (13 species), followed by 12 species from SS, and ST showed the lowest species richness (10 species) among the sampling sites. Species accumulation curves indicate sampling was relatively complete at ST site as the curve showed trend of approaching an asymptote (Figure 11.1). However the increasing curves for SM and SS sites indicated more samplings are needed to produce a significantly increased species count. The work done by Vaessen *et al*. (2011) provides the only comprehensive comparison of termite assemblage structures in peat soil for Sarawak. Their study collected 6 species in the 26 months old oil palm plantation in peat, showing clear reduction of species after forest clearance compared to near peat swamp forest which has 11 species. Comparatively, the present study recorded 13, 10, and 12 species at sites SM, ST, and SS respectively, which have been established for a relatively longer period of time. The termite species richness in SM site is comparable to that of Bong *et al*. (2012) from the same plantation although with slight difference in species composition.

Table 11.2 Termite species collected from various sites using modified transect method

Termite species	Sampling sites		
	SM	**ST**	**SS**
Family : Rhinotermitidae			
Coptotermes curvignathus Holmgren, 1913	x	x	
Coptotermes sepangensis Krishna, 1956	x	x	x
Coptotermes borneensis Oshima, 1914	x	x	x
Parrhinotermes aequalis (Haviland, 1898)	x	x	x
Schedorhinotermes sarawakensis (Holmgren, 1913)	x	x	
Schedorhinotermes medioobscurus (Holmgren, 1913)	x	x	
Schedorhinotermes javanicus Kemner, 1934	x		x
Family : Termitidae			
Sub-family : Macrotermitinae			
Macrotermes gilvus (Hagen, 1858)			x
Sub-family : Termitinae			
Prohamitermes mirabilis (Haviland, 1898)			x
Globitermes globosus (Haviland, 1898)			x

cont'd Table 11.2

Termes propinquus (Holmgren, 1913)			x
Amitermes dentatus (Haviland, 1898)			x
Pericapritermes nitobei (Shiraki, 1909)	x	x	x
Pericapritermes latignathus (Holmgren, 1914)	x	x	
Sub-family : Nasutitermitinae			
Nasutitermes sp. A	x		x
Nasutitermes havilandi (Desneux, 1908)	x		
Nasutitermes matangensiformis (Holmgren, 1913)	x	x	x
Havilanditermes atripennis (Haviland, 1898)	x	x	
Totals	13	10	12

**Note: SM- Semanok; ST-Setuan; SS- Sessang; x- termite present

Termites collected in present study comprised of only two families: Rhinotermitidae (lower termites) and Termitidae (higher termites), out of three families available in Malaysia which include family Kalotermitidae. This finding was in agreement with Vaessen *et al*. (2011) and Bong *et al*. (2012). Rhinotermitidae constituted the majority of the termite species in sites SM and SS, whereas Termitidae was the major termite species in SS site.

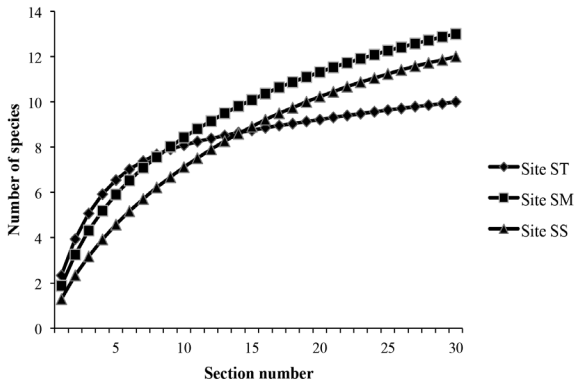
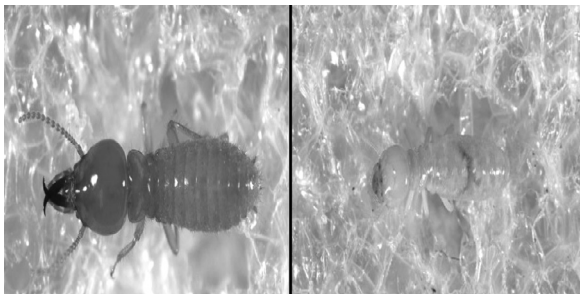


Figure 11.1 Sample-based species accumulation curves for termite sampling at Site ST (Setuan), Site SM (Semanok) and Site SS (Sessang) against the unit sampling effort (per section) by pooling 30 sections per site and each section measures 10 × 2 mMorphological Identification of Termites Species in Oil Palm

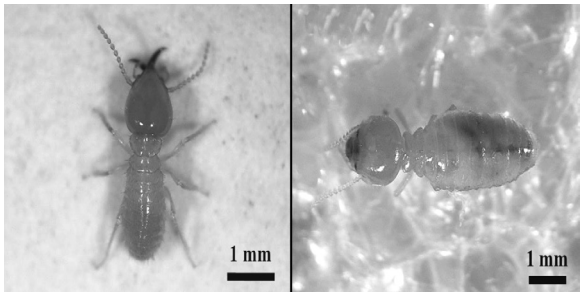
Morphological Identification of Termites Species in Oil Palm

The termites were examined and measured under a real-time imaging dissecting microscope (Nikon SMZ800) with an installed photo analysing programme (NIS-Element D2.30). Identification of termites were based on Thapa (1981) and Tho (1992). Termite colony consists of several castes, i.e. workers, soldiers, nymphs, winged reproductives (alate), king, and queen. However, the identification of termites is generally based on the soldier caste. Therefore, features of termite soldier include (i) pro- and mesonotum length and width, postmentum length and width, (iii) body length, (iv) head length, width, and height, (v) fontanelle width and length, (vi) labrum length and width, (vii) rostrum length, and (viii) segment number of antenna, were recorded and shown in Tables 11.3 and 4). Between 5 and 15 termite soldier specimens were used in morphological study. These termite morphology measurements provide additional valuable information for future termite identification. Plates of soldier and worker castes for diversified forms of termite species collected from oil palm plantations are exhibited in Figure 11.2. The reproductive pairs (king and queen) some of which are rarely seen in the field are revealed in Figure 11.3.

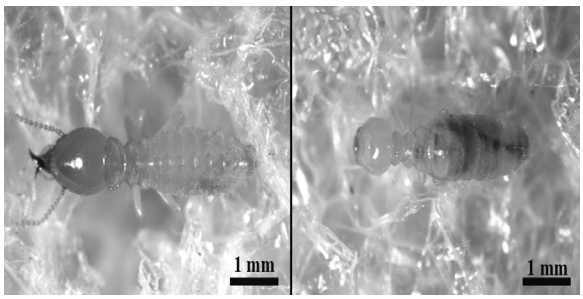
***Coptotermes* Wasmann**



a) *Coptotermes curvignathus Holmgren*



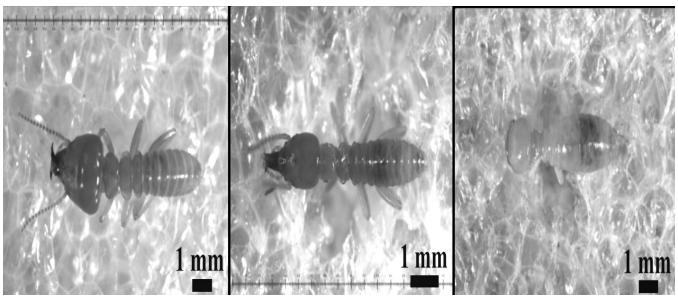
b) *Coptotermes sepangensis Krishna*



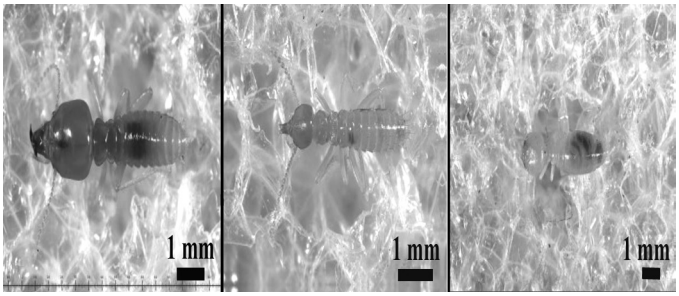
c) *Coptotermes borneensis Oshima*

Figure 11.2 Termite species of oil palm on peat (left: soldier, right: worker)

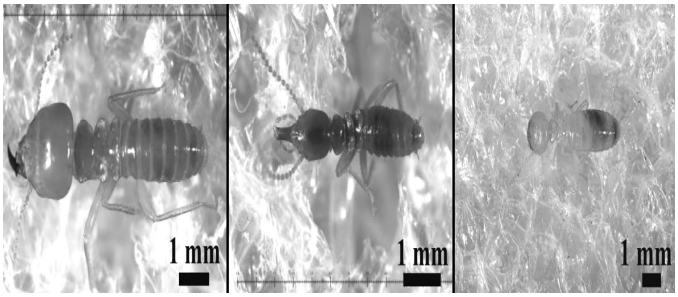
***Schedorhinotermes* Silvestri**



d) *Schedorhinotermes sarawakensis (Holmgren)*

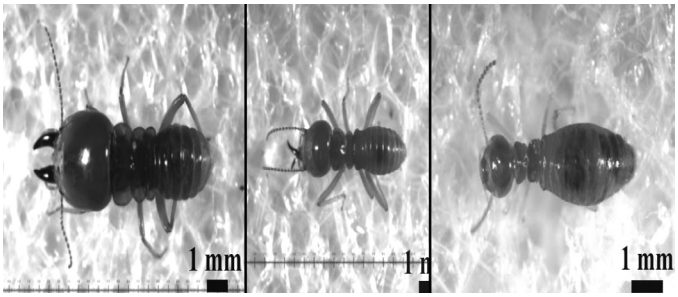


e) *Schedorhinotermes medioobscurus (Holmgren)*



f) *Schedorhinotermes javanicus Kemner*

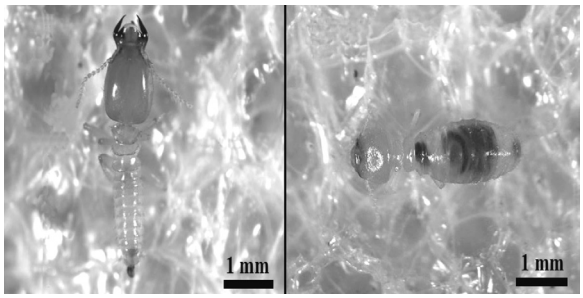
***Macrotermes* Holmgren**



g) *Macrotermes gilvus (Hagen)*

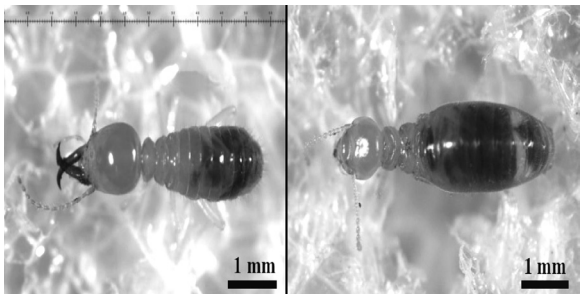
Figure 11.2 (*Continued*) (left: Major soldier, middle: Minor soldier, right: worker)

***Parrhinotermes* Holmgren**



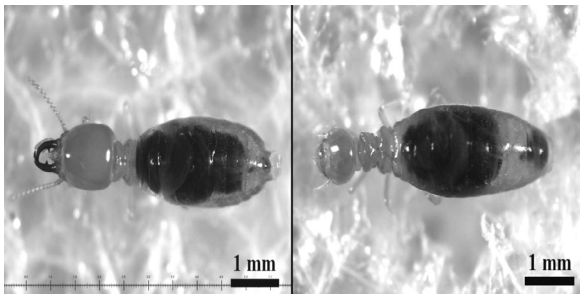
h) *Parrhinotermes aequalis (Haviland)*

***Prohamitermes* Holmgren**



i) *Prohamitermes mirabilis (Haviland)*

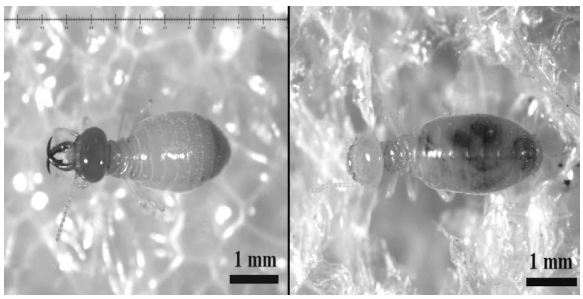
***Amitermes* Silvestri**



j) *Amitermes dentatus (Haviland)*

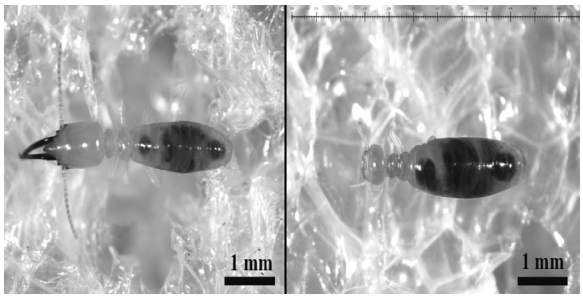
Figure 11.2 (*Continued*) (left: soldier, right: worker)

***Globitermes* Holmgren**



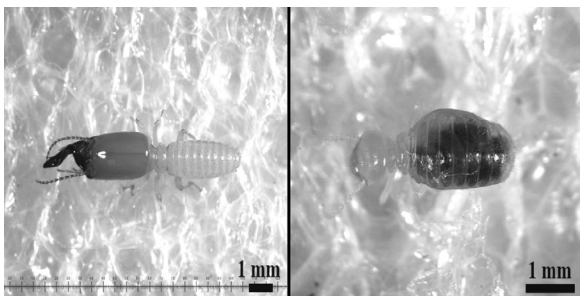
k) *Globitermes globosus (Haviland)*

***Termes* Linnaeus**



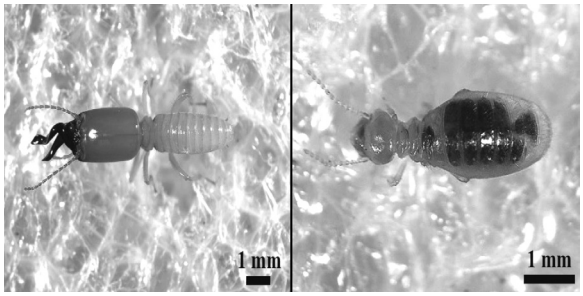
l) *Termes propinquus (Holmgren)*

***Pericapritermes* Silvestri**



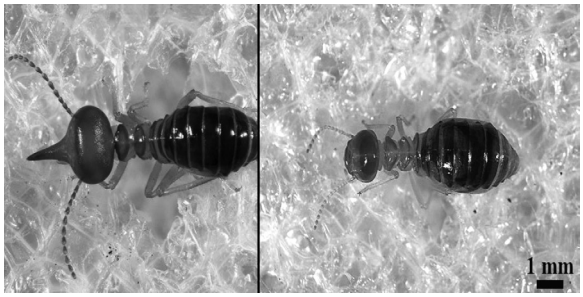
m) *Pericapritermes nitobei (Shiraki)*

Figure 11.2 (*Continued*) (left: soldier, right: worker)



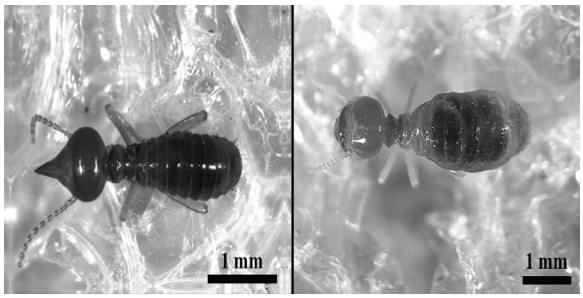
n) *Pericapritermes latignathus (Holmgren)*

***Havilanditermes* Light**



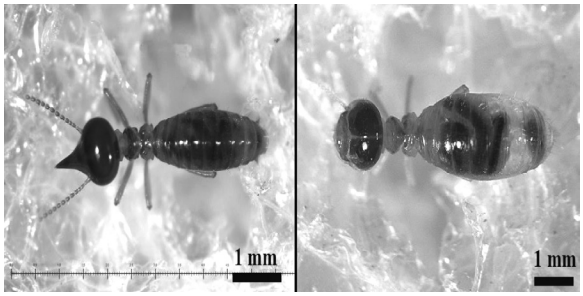
o) *Havilanditermes proatripennis (Haviland)*

***Nasutitermes* Dudley**

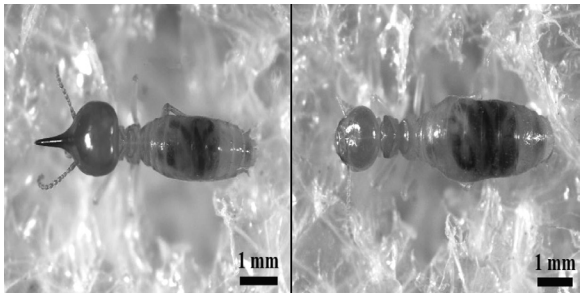


p) *Nasutitermes havilandi (Desneux)*

Figure 11.2 (*Continued*) (left: soldier, right: worker)



q) *Nasutitermes matangensiformis (Holmgren)*



r) *Nasutitermes sp. A*

Figure 11.2 (*Continued*) (left: soldier, right: worker)

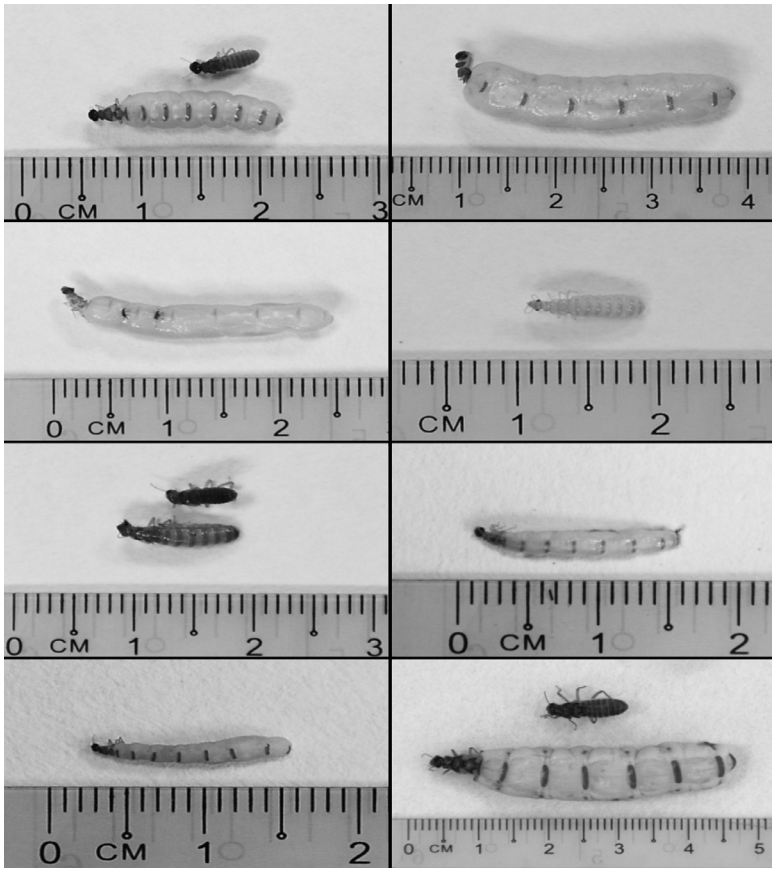


Figure 11.3 Termite queen (a) and king (b) collected from oil palm plantation in peat soil. 1) Nasutitermes havilandi; 2) Coptotermes sepangensis; 3) Pericapritermes nitobei; 4) Prohamitermes mirabilis (secondary queen); 5) Schedorhinotermes medioobscurus; 6) Parrhinotermes aequalis; 7) Termes propinquus; 8) Macrotermes gilvus

Table 11.3 Mean (range) (in millimetre) of morphometric measurement of different termite soldiers (Family Rhinotermitidae)

Character	Termite species (Family Rhinotermitidae)									
	C. curvignathus	*C. sepangensis*	*C. borneensis*	*S. sarawakensis*		*S. medioobscurus*		*S. javanicus*		*P. aequalis*
				Major	Minor	Major	Minor	Major	Minor	
Pronotum length	0.65 (0.48-0.69)	0.40 (0.38-0.42)	0.44 (0.41-0.48)	0.77 (0.66-0.85)	0.59 (0.53-0.63)	0.73 (0.69-0.75)	0.41 (0.38-0.42)	0.60 (0.57-0.62)	0.40 (0.39-0.42)	0.31 (0.29-0.32)
Pronotum width	1.10 (0.82-1.17)	0.71 (0.68-0.74)	0.81 (0.76-0.87)	0.87 (0.34-1.45)	0.87 (0.83-0.90)	1.29 (1.22-1.33)	0.61 (0.57-0.63)	1.00 (0.93-1.03)	0.56 (0.53-0.58)	0.59 (0.57-0.60)
Mesonotum width	1.00 (0.72-1.07)	0.69 (0.64-0.71)	0.72 (0.70-0.76)	1.38 (1.27-1.49)	0.90 (0.85-0.97)	1.33 (1.26-1.40)	0.62 (0.59-0.65)	0.95 (0.88-0.99)	0.57 (0.53-0.59)	0.54 (0.52-0.57)
Postmentum length	1.14 (1.09-1.19)	0.74 (0.70-0.79)	0.90 (0.86-0.94)	1.71 (1.61-1.78)	0.98 (0.90-1.04)	1.37 (1.30-1.44)	0.63 (0.59-0.65)	1.20 (1.16-1.26)	0.59 (0.55-0.62)	0.84 (0.79-0.89)
Postmentum width (Max.)	0.44 (0.42-0.47)	0.33 (0.31-0.35)	0.40 (0.38-0.42)	0.64 (0.60-0.67)	0.41 (0.39-0.43)	0.57 (0.56-0.59)	0.31 (0.29-0.32)	0.44 (0.42-0.46)	0.29 (0.25-0.31)	0.31 (0.30-0.32)
Postmentum width (Min.)	0.27 (0.24-0.29)	0.22 (0.20-0.23)	0.21 (0.19-0.23)	0.32 (0.29-0.35)	0.28 (0.25-0.30)	0.30 (0.28-0.31)	0.23 (0.21-0.25)	0.26 (0.23-0.28)	0.23 (0.19-0.24)	0.14 (0.13-0.15)
Body length	6.18 (5.23-6.98)	3.51 (3.10-3.80)	4.56 (4.15-4.94)	8.32 (7.34-9.10)	5.59 (4.81-6.45)	7.18 (6.63-7.75)	3.95 (3.44-4.52)	5.43 (4.64-6.12)	3.93 (3.30-4.34)	3.56 (3.16-3.84)
Head length	1.59 (1.53-1.66)	1.06 (0.99-0.11)	1.26 (1.17-1.35)	2.34 (2.23-2.41)	1.35 (1.28-1.41)	1.93 (1.80-2.15)	0.91 (0.87-0.96)	1.66 (1.54-1.76)	0.88 (0.83-0.92)	1.04 (0.98-1.11)
Head width	1.51 (1.47-1.58)	1.03 (0.99-1.08)	1.08 (1.05-1.10)	2.52 (2.36-2.58)	1.21 (1.15-1.25)	2.00 (1.95-2.06)	0.83 (0.81-0.85)	1.66 (1.61-1.76)	0.77 (0.69-0.80)	0.99 (0.83-1.04)
Head height	1.00 (0.93-1.08)	0.66 (0.39-0.74)	0.73 (0.70-0.77)	1.44 (1.17-1.53)	0.84 (0.80-0.88)	1.14 (1.08-1.17)	0.64 (0.62-0.67)	1.04 (1.00-1.11)	0.60 (0.59-0.62)	0.58 (0.53-0.62)
Fontanelle length	0.18 (0.16-0.19)	-	0.12 (0.11-0.12)	0.07 (0.06-0.09)	0.07 (0.05-0.07)	0.07 (0.06-0.08)	0.05 (0.04-0.06)	0.07 (0.06-0.08)	0.05 (0.04-0.05)	0.03 (0.03-0.04)
Fontanelle width	0.23 (0.21-0.25)	-	0.13 (0.11-0.14)	0.07 (0.06-0.09)	0.06 (0.05-0.07)	0.07 (0.06-0.08)	0.05 (0.04-0.06)	0.07 (0.06-0.08)	0.05 (0.04-0.06)	0.03 (0.03-0.04)
Labrum length	0.42 (0.37-0.45)	0.26 (0.21-0.37)	0.32 (0.29-0.36)	0.58 (0.50-0.64)	0.61 (0.53-0.66)	0.45 (0.38-0.53)	0.49 (0.45-0.52)	0.50 (0.42-0.54)	0.48 (0.44-0.50)	0.31 (0.28-0.33)
Labrum width	0.36 (0.33-0.38)	0.26 (0.22-0.36)	0.30 (0.27-0.34)	0.75 (0.72-0.77)	0.41 (0.36-0.44)	0.59 (0.56-0.66)	0.23 (0.21-0.26)	0.51 (0.49-0.53)	-	0.31 (0.29-0.33)
Rostrum length	-	-	-	-	-	-	-	-	-	-
Antenna segments	16	13	13	16	16	18	16	16	16	13

Table 11.4 Mean (range) (in millimetre) of morphometric measurement of different termite soldiers (Family Termitidae)

Character	Termite species (Family Termitidae											
	P. mirabilis	*A. dentatus*	*G. globosus*	*T. propinquus*	*P. nitobei*	*P. latignathus*	*M. gilvus*		*N. havilandi*	*N. matangensi-formis*	*N. sp. A*	*H. atripennis*
							Major	Minor				
Pronotum length	0.39 (0.35-0.43)	0.31 (0.28-0.35)	0.29 (0.25-0.32)	0.25 (0.23-0.29)	0.40 (0.27-0.42)	0.42 (0.39-0.44)	1.31 (1.22-1.42)	0.85 (0.76-0.88)	0.17 (0.14-0.22)	0.20 (0.16-0.21)	0.21 (0.20-0.25)	0.37 (0.29-0.42)
Pronotum width	0.71 (0.68-0.75)	0.67 (0.63-0.70)	0.55 (0.52-0.58)	0.47 (0.45-0.49)	0.84 (0.61-0.90)	0.88 (0.80-0.97)	2.53 (2.39-2.70)	1.34 (1.29-1.40)	0.41 (0.35-0.49)	0.47 (0.43-0.50)	0.53 (0.49-0.58)	0.77 (0.63-0.83)
Mesonotum width	0.71 (0.66-0.74)	0.63 (0.59-0.66)	0.55 (0.52-0.58)	0.44 (0.40-0.46)	0.79 (0.73-0.84)	0.82 (0.72-0.86)	2.48 (2.24-2.61)	1.24 (1.12-1.39)	0.35 (0.30-0.40)	0.38 (0.35-0.41)	0.44 (0.43-0.47)	0.66 (0.55-0.71)
Postmentum length	0.73 (0.69-0.76)	0.71 (0.64-0.78)	0.32 (0.26-0.35)	0.60 (0.52-0.68)	1.85 (1.71-1.96)	1.65 (1.55-1.85)	2.63 (2.49-2.80)	1.57 (1.50-1.67)	0.29 (0.25-0.32)	0.32 (0.28-0.36)	0.37 (0.35-0.39)	0.36 (0.33-0.41)
Postmentum width (Max.)	0.39 (0.36-0.42)	0.32 (0.31-0.34)	0.24 (0.20-0.26)	0.30 (0.28-0.31)	0.50 (0.47-0.54)	0.50 (0.47-0.52)	0.87 (0.82-0.91)	0.58 (0.55-0.60)	0.31 (0.29-0.36)	0.33 (0.28-0.40)	0.32 (0.31-0.34)	0.42 (0.40-0.46)
Postmentum width (Min.)	0.28 (0.26-0.31)	0.27 (0.25-0.29)	-	0.19 (0.17-0.23)	0.20 (0.17-0.23)	0.24 (0.20-0.26)	0.56 (0.53-0.61)	0.49 (0.46-0.51)	-	-	0.28 (0.27-0.30)	-
Body length	4.52 (3.95-5.24)	4.81 (4.28-5.52)	3.89 (3.37-4.56)	4.10 (3.73-4.75)	8.45 (7.25-9.22)	7.43 (6.67-8.62)	9.43 (8.57-10.31)	5.98 (5.33-6.38)	3.60 (3.06-4.01)	4.37 (4.07-4.52)	4.55 (4.12-4.90)	6.60 (5.77-7.19)
Head length	1.23 (1.15-1.36)	1.23 (1.16-1.38)	0.72 (0.66-0.76)	1.08 (0.99-1.18)	2.70 (2.52-2.87)	2.70 (2.64-2.78)	3.62 (3.34-3.82)	2.13 (2.03-2.23)	0.88 (0.84-0.96)	0.98 (0.88-1.04)	1.18 (1.12-1.27)	1.43 (1.32-1.56)
Head width	1.18 (1.10-1.23)	1.08 (1.02-1.21)	0.88 (0.80-0.93)	0.73 (0.70-0.75)	1.46 (1.38-1.53)	1.58 (1.42-1.65)	3.27 (3.12-3.46)	1.78 (1.22-1.88)	0.87 (0.78-0.94)	1.04 (0.93-1.11)	1.12 (1.07-1.16)	1.53 (1.16-1.70)
Head height	0.85 (0.80-0.89)	0.90 (0.86-0.99)	0.74 (0.72-0.78)	0.71 (0.67-0.74)	1.20 (1.12-1.47)	1.22 (1.15-1.33)	2.11 (1.97-2.28)	1.23 (1.19-1.30)	0.71 (0.67-0.82)	0.68 (0.62-0.72)	0.87 (0.82-0.91)	1.13 (1.04-1.21)
Fontanelle length	-	0.03 (0.02-0.04)	-	-	0.03 (0.02-0.05)	0.03 (0.03-0.04)	0.06 (0.05-0.08)	0.03 (0.02-0.04)	-	-	-	-
Fontanelle width	-	0.03 (0.03-0.04)	-	-	0.03 (0.02-0.05)	0.03 (0.03-0.04)	0.07 (0.06-0.08)	0.03 (0.03-0.04)	-	-	-	-
Labrum length	0.22 (0.19-0.25)	0.19 (0.17-0.22)	0.21 (0.18-0.26)	-	0.45 (0.21-0.58)	0.37 (0.29-0.41)	0.70 (0.60-0.80)	0.67 (0.57-0.75)	-	-	-	-
Labrum width	0.30 (0.26-0.32)	0.23 (0.21-0.28)	0.26 (0.22-0.34)	-	0.30 (0.24-0.34)	0.30 (0.28-0.32)	0.70 (0.62-0.75)	0.48 (0.45-0.51)	-	-	-	-
Rostrum length	-	-	-	-	-	-	-	-	0.53 (0.49-0.56)	0.58 (0.49-0.67)	0.73 (0.69-0.77)	1.30 (1.22-1.40)
Antenna segments	14	14	14	14	14	14	17	17	13	13	13	14

Ecological Significance and Distribution of Termite Species in Oil Palm on Peat

Termites live in nests called as termitaria which they constructed. The nest has several functions as to protect the colony, store food, and maintain an optimum environment of survival and reproduction. Emerson (1938) had made a very extensive and impressive study of the phylogeny behaviour of termite nesting habits in all families under Isoptera. Termite nesting can be classified into 6 types as first described by Abe (1984): a) Drywood termites, which are mainly of termites from Kalotermitidae where they live in and consume the dry hard wood; b) Damp wood termites, which are mainly of termites from Archotermopsidae and some Rhinotermitidae, consume wet decaying wood and live in it; c) Intermediate termites, in which the termites feed on other wood sources besides the wood they nest in and are generally of termites from Mastotermitidae, some Kalotermitidae, most Rhinotermitidae, and some Termitidae; d) Arboreal termites, where the termites build nest on the tree trunk but forage away from the nest, which are commonly seen in many Termitidae; e) Subterranean termites, where the termites build their nest underground (hypogean) and forage away from the nest, are commonly seen from termites of Hodotermitidae, some Rhinotermitidae, Serritermitidae, and many Termitidae; f) Humus feeding termites, which feed on humus and nesting underground as seen in many Termitidae. Three general categories of termite nesting can be drawn from these 6 types of nesting sites which are one-piece, intermediate, and separate type (Abe, 1987).

Termite's feeding habits are basically on cellulosic plant materials. Termites are able to digest the cellulose with the aid of enzymes produced by symbiotic microbes in their gut, with exception of higher termites, where they live in mutualistic relationship with other microorganisms such as fungi and bacteria, or bacteria alone, together with enzyme cellulase to digest their food (Slaytor, 1992). It is well known that termite eat wood as their food. Nonetheless, their food materials also includes manure, lichen, grass, plant debris, soil, humus, fungi, termites of their own colony (cannibalism), and their eggs (oophagy) (Lee and Wood, 2001). Donovan *et al*. (2001) have classified the feeding group of termites into 4 groups based on the food consumed, together with the morphology and anatomy of worker termites. Group I feeds on wet and dry wood, grass and detritus with one-piece or intermediate nest types. Group II feeds on wood, fungus,

grass, detritus, litter, and microepiphyte with intermediate or separate nest types. Group III and IV both feed on soil and soil-wood interface with separate nest types that occasionally feed on the nest too.

Termites are most well-known for their pest status to the human activities and compete with beneficial organism (Sorokin and Whitaker, 2008). Economically important termites particularly in the tropics have caused extensive losses to agriculture and infrastructures. Among these, the most destructive termite species (from family Rhinotermitidae), notably of genus *Coptotermes*, are usually subterranean and have been reported to attack wooden structures in urban area and also feed on living trees such as rubber, *Acacia mangium*, mango, oil palm, and coconut (Harris, 1971; Masijan *et al*., 2006). Without adequate employment of protection and control towards termite infestation, farmers may suffer from severe loss of crop yield which will directly affect their income, and homeowners are charged for high repair cost. However, termites also play an important role in ecosystem by decomposing woods and leaf litter (Wood and Sands, 1978; Matsumoto and Abe, 1979). The soil carbon dynamics are affected as termite feeds and digests the organic matters and returns the carbon into the environment. In present study, the distribution and location of termite species in oil palm on peat are shown in Figure 11.4. The nesting, feeding, and ecological role of each termite species are also discussed and summarised in Table 11.5.

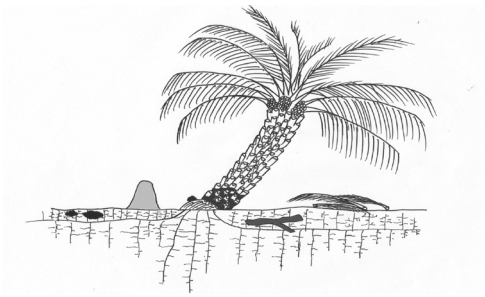


Figure 11.4 Distribution of different termite species in peatland planted with oil palms

***Coptotermes curvignathus*:** This species of termite was encountered infesting the oil palm and rubber wood logs in baiting stations. The infested oil palm trunks were generally covered with mud works from ground until the shoot. These termites are very fierce and aggressive. Milky white secretion is secreted from frontella on the head upon provocation. Serious attack was represented by snapping oil palm shoot, which lead to yellowing of leaves and eventually the palm toppled to the ground as the termites have hollowed out the centre part of the tree and weakened the structure. Chan *et al*. (2011) gave a detailed description of the damage to oil palm caused by this species.

***Coptotermes sepangensis*:** This species was encountered foraging and nesting in the rotten wood log buried in the peat soil. They were occasionally being observed infesting the living oil palm tree with mud works around the spear, fruit bunches and trunk in ST site outside the sampling transect. However, the degree of damage is unknown and study is needed in the future to assess damage. The species is less aggressive compared to *C. curvignathus*. They secrete milky white secretion upon provocation. The queen termite which measured 330 mm in length (Figure 11.2-2) was observed moving among spaces created by termite feeding in the rotten wood log upon dissecting of the log.

***Coptotermes borneensis*:** Termites were encountered devouring the rotten wood log buried underground. Species is easily mistakenly identified as *C. sepangensis* by naked eye due to their similarity in size. As in the case of *C. sepangensis*, this species was infesting some of the oil palm trees outside the transect region at ST site. Mud works were observed on trunk, fruit bunches and young shoot. Actual damage cause by this species is unknown as palm status is healthy during the observation. *C. borneensis* was not found in Peninsular Malaysia (Tho, 1992) but was reported in Sabah, Malaysia, in the swamp forest and virgin jungle reserve (Thapa, 1981).

***Schedorhinotermes sarawakensis*:** Species was found foraging on the rotten wood log buried in the peat soil. However, it was found occasionally foraging in the rotten inter-row stacking of dead oil palm fronds. All castes walked quite slowly. A pungent smell was detected upon digging out the colony.

***Schedorhinotermes medioobscurus*:** Species was found foraging in the rotten wood log buried in the peat soil. Minor soldier was fast moving. Pungent smell was released from the colony as in *S. sarawakensis*. Soldier caste was less aggressive. A pair of reproductive (Figure 11.2-5) was also seen establishing their colony in a rotting frond on the ground.

***Schedorhinotermes javanicus*:** This species was found foraging on the rotten wood log buried in the peat soil. It released the same pungent smell as with the two *Schedorhinotermes* sp. discussed earlier.

***Parrhinotermes aequalis*:** Species was found foraging on the rotten wood log buried in the peat soil. It released a pungent smell as with the *Schedorhinotermes* spp. All castes moved slowly. The nest was build in the soil and seen as a lump on the ground. Lamina was yellowish brown in colour. A queen of this species which measured 150 mm in length was found in a compartment or space created in the soil guarded by some soldiers (Figure 11.2-6).

***Prohamitermes mirabilis*:** This species was only found in SS site. Their nest is blackish with scattered white spots. Oil palm tree roots were extended all over the nest. The nests were found buried in the peat soil and near to ground surface, measured between 240–290 mm in length, 150–190 mm in width, and 125–135 mm in height. Nest surface was lumpy. Each lump is a space with tiny circular hole connected to other space. A tiny spherical soil granule was found in each of the space, which serves as a defensive strategy to plug the hole after escaped (Tho and Maschwitz, 1988), to keep the predator away. Fifteen secondary reproductives (Figure 11.2-4) were collected within a nest.

***Amitermes dentatus*:** Species was encountered dwelling in the mound of *Macrotermes* sp. Soldier is not fierce and all castes are moving fairly slowly. Alates were also being found, and were swarming out and up into the sky upon the breaking open of the soil despite the hot day. Swiflets were observed feeding on the swarming termite alates. This species was fairly hard to distinguish from *P. mirabilis* by naked eye, as they both shared quite similar morphological characteristics.

***Globitermes globosus*:** Species was encountered foraging on the rotten wood log buried in the peat soil. Termite was easily distinguished by their whitish abdomen. Their movement was slow.

Termes propinquus: Species was encountered in the raised blackish round nest constructed on the exposed roots of oil palm tree base. Oil palm trees looked healthy with no visible root damage by this species. Species having this nesting behaviour is thought to use oil palm roots as structural support and to avoid flooding. All castes move very slow. Mandibles of soldier are very fine and hardly visible by naked eyes. A queen is discovered from the nest with a body length of 105 mm (Figure 11.2-7). Spaces within the nest were flat oval–shaped from side-view and connected with each other by a small circular hole.

***Pericapritermes nitobei*:** Species was discovered in the soil with no clear nest structure. Castes were seen squeezed in crowds with underground passageway to other locations. Occasionally it was seen below the root zone of mosses and rotten wood log buried in the soil. Workers were the most common caste encountered. Species was moving very slowly. Disturbed soldier was seen to be propelled 8-10 cm away by the flicking motion of its mandibles. The queen (Figure 11.2-3) was also being discovered in the soil.

***Pericapritermes latignathus*:** Species shared characteristic much the same with *P. nitobei* except it is quite difficult to distinguish between them visually, as they only differ in the head width.

***Macrotermes gilvus*:** Species was encountered in the mound built on peat soil using whitish clayey substratum soil as building material. Species was occasionally encountered on the rotten wood log buried in the peat soil. Mounds were built quite close to the oil palm tree. Reproductive castes (Figure 11.2-8) were found in the royal cell situated at the base of the mound, with macro soldiers guarding the royal cell. Oil palm roots were seen interweaving inside the mound with no visible damage caused by the termite foraging activity although several reports stated its pest status in the oil palm plantation.

***Nasutitermes havilandi*:** Species was encountered constructing nest in the frond base on the oil palm tree trunk, with covered single-tract passageways connecting each other up to fruit bunches. However the fruits were not being harmed by the termites while foraging on the organic debris trapped in the crown. The queen termite (Figure 11.2-1) was also being discovered within the frond base.

***Nasutitermes matangensiformis*:** Species was mostly encountered on the tree trunk with covered runways from tree base up to the fruit bunches and fronds. They are occasionally found in the rotting inter-row fronds.

***Nasutitermes* sp. A:** New species with close relationship with *N. regularis*. It was encountered foraging in the rotting inter-row oil palm fronds as well as on the tree trunk with covered single-tract runways. Soldier caste is as much seen as worker caste.

***Havilanditermes proatripennis*:** Species was encountered foraging on the rotten inter-row dead fronds. Soldier is very fierce, squirting sticky fluid to a fairly long distance upon provocation. The colony has a pungent smell.

Table 11.5 Termite species collected from oil palm on peat: summary of feeding, nesting and ecological role

Termite species	Feeding group	Nesting group	Ecological role
Family : Rhinotermitidae			
Coptotermes curvignathus	w	w	Pest
Coptotermes sepangensis	w	w	Pest
Coptotermes borneensis	w	w	Pest
Parrhinotermes aequalis	w	w	Scavenger
Schedorhinotermes sarawakensis	w	w	Scavenger
Schedorhinotermes medioobscurus	w	w	Scavenger
Schedorhinotermes javanicus	w	w	Scavenger
Family : Termitidae			
Sub-family : Macrotermitinae			
Macrotermes gilvus	w/l (f)	e	Scavenger
Sub-family : Termitinae			
Prohamitermes mirabilis	s/w	h	Scavenger
Globitermes globosus	w	h	Scavenger
Termes propinquus	s/w	e	Scavenger
Amitermes dentatus	s/w	h	Scavenger
Pericapritermes nitobei	s	h	Scavenger
Pericapritermes latignathus	s	h	Scavenger
Sub-family : Nasutitermitinae			
Nasutitermes sp. A	w	a	Scavenger
Nasutitermes havilandi	w	a	Scavenger
Nasutitermes matangensiformis	w	a	Scavenger
Havilanditermes atripennis	w/l	h	Scavenger

*For feeding group, w/l = wood/litter interface feeders, s = soil feeders, s/w = soil/wood interface feeders, w = wood feeders, (f) = fungus growers. For nesting groups, a = arboreal, e = epigean, h = hypogean, w = in dead wood

CONCLUSION

Being an important soil macrofauna which has been regarded as ecosystem engineer, termites, however, have been receiving strong attention by oil palm plantation owner as one of the major pest. Present research is therefore, initiated to study the diversity of termite occurring in oil palm plantation especially in peat soil, with respect to their functional groups and also their ecological roles, with the aid of morphological identification methods. This study hopes to contribute to a better understanding of termite species, which will allow a better decision to be made in oil palm management strategies and species conservation.

A total of eighteen termite species were collected from oil palm plantations in peat soil. Termite species collected in present study were generally beneficial to the crops, as evidenced by high encounter rates of rotten wood feeder, soil-wood interface and soil feeder species. Exception is particularly made to *Coptotermes curvignathus* species which damages the oil palm trees, followed by *C. sepangensis* and *C. borneensis* which infest but with no obvious damage to the palms. From this study, it is evident that not all termites that exist in the peat soil oil palm plantation are pest to the crop. Pest control measures should be carefully planned so as not to harm the beneficial termites. It is inevitable that diversity of termite species was disturbed after the land clearing for oil palm plantation. However, with a proper plantation management practice which will pose lower impact to the beneficial termites or other insects exist in plantation, a balance could be achieved between profit-wise and environment healthiness for a sustainable crop plantation. More study should be carried out in more oil palm estates as well as other agricultural crops that also utilize the peat soil, to provide more information of termite assemblage in different crops area in peat soil.

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12

Nasutitermes Termite of Oil Palm on Peat

Jing Ee Yii and Choon Fah Joseph Bong

INTRODUCTION

Termitidae is the largest family, consisting of four subfamilies: Macrotermitinae, Apicotermitinae, Termitinae and Nasutitermitinae (Tho, 1992; Kambhampati and Eggleton, 2000; Eggleton, 2001). Nasutiterminae is the most diversified group in Isoptera (Kambhampati and Eggleton, 2000) and highly specialized form of higher termite. *Nasutitermes* (Figure 12.1) is a genus broadly distributed in the tropics and can build nests in trees, roots, or soil (Noirot and Darlington, 2000).

Figure 12.1 *Nasutitermes* sp. foraging in oil palm on peat

The soldier caste of the subfamily Nasutitermitinae is distinct from the worker termites in the colony in having reduced and generally vestigial mandibles which consist of a forwardly-directed rostrum (nasus) with the large frontal gland at its tip while the workers have no special weapons. The soldiers squirt out a sticky and odorous secretion from their defensive gland through the rostrum when they are disturbed (Nutting *et al.*, 1974). The secretion can assist in overcoming arthropods much larger than themselves by mechanical immobilization, obstruction of the spiracles, and chemical irritation (Eisner *et al*., 1976). Whereas the distinct character of worker *Nasutitermes* is that the worker mandibles are very much adapted for feeding on wood which have long, straight cutting edges and flat molar plates with many transverse ridges (Tho, 1992).

Despite of the most destructive subterranean *Coptotermes* species (Chan *et al*., 2011; Kon *et al*., 2012), Malaysian *Nasutitermes* are omnipresent and widespread termite species that inhabit oil palm. However, the information of the *Nasutitermes* spp. in oil palm plantation is still lacking. Furthermore, their behaviour and role in oil palm plantation are not known. Nevertheless, the misconception or "termiphobia" of most oil palm plantation owners lead to high investments in chemicals to control and eliminate the termites. Therefore, more detail studies of termite *Nasutitermes* spp. in oil palm would be discussed in this book chapter.

Distribution of *Nasutitermes* in Oil Palm

The study was conducted in two locations with different soil types namely, Semanok (Woodman) oil palm estate on peat soil and the oil palm estate in Universiti Putra Malaysia Bintulu Sarawak Campus (UPMKB) on mineral soil. Two plots were selected in each of the study site. Transect sampling method with 100 m long and 2 m wide transects (modified from Jones and Eggleton, 2000) was carried out. Four transect lines without contiguous sections were implemented within a plot for the presence or absence of the *Nasutitermes* spp. Oil palms infested by *Nasutitermes* spp. were assessed carefully. For each termitarium, the presence or absence of the termites was recorded and the distribution of galleries and covered runways of the tree and the activities of termites were observed. T-test was used for data analysis. Besides, soil was sampled randomly from the bottom area of palms.

Soil condition around palms with presence or absence of *Nasutitermes* spp. were tested for pH and moisture at 0-15 cm soil depth in order to determine the condition which was suitable for *Nasutitermes* spp. living or foraging. Gravimetric method was used to determine soil moisture. While the pH of the soil samples was determined using the potentiometric method in a 1:2.5 or 1:10 of soil: distilled water suspension. The data was analysed using ANOVA contrast and the means were separated using Duncan's New Multiple Range Test (DNMRT) at $p \leq 0.05$.

The transect sampling data of Table 12.1 showed that infestation of *Nasutitermes* spp. in peat soil (51.71%) was significantly higher than in mineral soil (10%). While the results in Table 12.2 displayed that soil moisture in peat (74%) was significantly higher than in mineral soil (25-34%). There was no significant difference between soil moisture *Nasutitermes* spp. infested and uninfested area in peat soil. However, significant difference was found between soil moisture *Nasutitermes* spp. infested and uninfested area in mineral soil. Moreover, the soil pH of *Nasutitermes* spp. infested area was slightly acidic than uninfested area in both peat and mineral soils.

Table 12.1 Comparison of *Nasutitermes* spp. infestation in peat and mineral soil

Site	Mean of infestation (%)
Semanok (peat soil)	51.71 ± 11.92 a
UPMKB (mineral soil)	10.00 ± 0.00 b

*Means with same letters within the column are not significantly different at $p \leq 0.05$ (t-test)

Table 12.2 Soil moisture content and soil pH of *Nasutitermes* spp. infested and uninfested area in both peat and mineral soils

Treatment	Mean moisture content (%) of soil	Range of soil pH
Peat (Infested area), T1	74.17 ± 1.33 a	4.06 – 4.63
Peat (Uninfested area), T2	74.91 ± 1.27 a	4.08 – 5.02
Mineral (Infested area), T3	34.58 ± 2.05 b	4.42 – 4.92
Mineral (Uninfested area), T4	25.71 ± 2.11 c	4.49 – 5.07

**Means with same letters are not significantly different at $p \leq 0.05$ (DNMRT)

In oil palm plantation of UPMKB on mineral soil, there was less infestation of *Nasutitermes* spp. compared to peat soil in Semanok estate. The oil palms were almost 24 years old (in year 2010) and lacked maintenance. The drainage system was poor and fewer fronds heaps in the area. Meanwhile, there was less frond bases on the oil palm trunk for the termites to build nests in. As opposed to UPMKB oil palm plantation, the oil palms in Semanok estate were younger at nearly 6 years old. With high moisture in peat, it indirectly gives insights into the fact that *Nasutitermes* spp. prefers to live in moist condition. Besides, there were more organic matter content such as frond heaps and debris around the infested area which was available food sources for them.

Nasutitermes spp. was wide spread in the oil palm estate. Hence, it is noteworthy to check the incidence rate of *Nasutitermes* spp. in oil palm on peat, whereby 2 plots were selected and 4 replications were carried out in Semanok estate. Around 120 palms were assessed per plot based on three conditions, which included the presence of *Nasutitermes* spp., the absence of *Nasutitermes* spp., and the presence of mud trail but with no termites were found.

Table 12.3 Comparison between *Nasutitermes* spp. incidence in Semanok (Woodman) oil palm estate site

Site	Absence of *Nasutitermes* spp. (%)	Presence of *Nasutitermes* spp. (%)	Presence of trail but no *Nasutitermes* spp. (%)
Site 1	44.17 ± 4.59 a	37.50 ± 9.47 a	18.33 ± 5.00 a
Site 2	81.75 ± 6.26 b	5.79 ± 1.51 b	12.46 ± 5.78 a

*Means with same letters within the same column are not significantly different at $p \leq 0.05$ (t-test)

Table 12.4 Soil moisture content and soil pH of *Nasutitermes* spp. infested and uninfested area in Site 1 and 2 in Semanok estate

Treatment	Mean moisture content (%) of soil	Range of soil pH
Site 1 (Infested area), S1	71.86 ± 3.76 ab	4.18 – 5.08
Site 1 (Uninfested area), S2	68.90 ± 2.14 b	4.26 – 5.29
Site 2 (Infested area), S3	75.37 ± 1.33 ab	3.51 – 4.36
Site 2 (Uninfested area), S4	77.18 ± 0.97 a	3.73 – 4.48

*Means with same letters are not significantly different at $p \leq 0.05$ (DNMRT)

Table 12.3 shows that *Nasutitermes* spp. incidence of Site 1 and Site 2 in Semanok estate was significantly different except the observation where trails were present with no *Nasutitermes* spp. The average occurrence of *Nasutitermes* spp. found in Semanok estate consisted of 21.6% per hectare. However, the occurrence of *Nasutitermes* spp. in Site 2 was significantly less than Site 1. For soil condition tests, the result of soil moisture content showed no variation between Site 1 and 2. Whereas in terms of soil pH, that of Site 2 was more acidic than Site 1. In addition, infested area was slightly more acidic than uninfested area as shown in Table 12.4.

Figure 12.2 Field conditions of Site 1 (left) and Site 2 (right) at Semanok estate

As shown in Figure 12.2, some discrepancies were manifested between Site 1 and Site 2 in Semanok estate. In Site 1, peat soil had proper and good drainage system, where there were a number of small drains built across the site, but there was only one drain in Site 2. Drainage and effective water system played a vital role in oil palm plantation to provide adequate drainage for optimum crop yield (Kamal and Abdullah, 2001) and also to maintain the water-table at an optimum depth to prolong the life of the organic soil. Subsidence would occur due to uncontrolled drainage system that further led to serious leaning effect as indicated in leaning oil palm of Site 2.

From the results, *Nasutitermes* spp. was more abundant in Site 1 which was well drained and full of vegetation with no leaning oil palms. However, there were no significant differences between moisture content of infested and uninfested areas. Hence, soil moisture was probably not the main factor attributable to *Nasutitermes* infestation. According to Lee and Wood (1971), vegetation played a vital role in determining their

distribution and abundance of termites as plant tissue was either directly or indirectly the source of food for termites. The structure of the vegetation was also essential as it would affect the amount and distribution of shade. From field observation, Site 1 was full of vegetation compared to Site 2. Furthermore, the nests were most often being found at the base of the oil palms which had more shade. It implies that *Nasutitermes* spp. prefers shaded condition. These factors might contribute to infestation incidence of *Nasutitermes* in oil palm.

Simultaneously, there was a condition whereby trails were present but no *Nasutitermes* spp. was found inside the mud trails. The trails that no termites were found inside were dry and broken, while there were moist, connected and ramified from the ground up to the frond in the trails in which termites were present. This abscondment of termites might be due to the spraying of pesticide by the plantation owners. This indiscriminate killing of non-pest termite species should be avoided.

The distribution pattern of *Nasutitermes* spp. in oil palm plantation was clustered. Generally, when one oil palm was infested, the neighbouring palms would eventually be infested too either through ground or overlapping frond leaves. Infestation by *Nasutitermes* spp. was moderately serious in oil palm plantation on peat. However, the oil palms were still in healthy condition even though infested by *Nasutitermes* spp. Thus, the presence of *Nasutitermes* spp. on oil palm would not bring serious harmful impacts to the oil palm.

Identification of *Nasutitermes* spp. in Oil Palm

Termite samples were collected from the field and preserved in 70% ethanol for further identification. Soldier termite samples were randomly selected for morphological and phylogenetic studies. Identification of termites species were conducted primarily on the basis morphological criteria of termites soldiers presented by Thapa (1981) and Tho (1992) by using dissecting microscope Nikon SMZ800 with Software NIS element D2.30, then verified using molecular techniques and phylogenetic analyses.

To extract the genomic DNA from the termite samples, termites' head segment was ground into fine powder using liquid nitrogen with a pestle. Vivantis GF-1 Tissue DNA Extraction Kit was used according to the manufacturer's protocol with slightly modification. The Vivantis DNA

Amplification Kit was used to amplify the DNA samples. The reaction mixture in 25 µL contained 10X Buffer A, 2 mM dNTP mix, 50 mM $MgCl_2$, 5 µ/µL Taq DNA Polymerase and 0.25 µL of each primer. The PCR was conducted using the primers TL2-J-3037 (5'-ATG GCA GAT TAG TGC AAT GG-3') and TK-N-3785 (5'-TTT AAG AGA CCA GTA CTT G-3') (Liu and Beckenbach, 1992; Simon *et al*., 1994; Jenkins *et al*. 1999). These PCR primers were amplified an approximately 800-bp fragment of the COII gene from the samples.

According to Lee *et al.* (2005), the conditions for amplication were as follow: a initial denaturation step at 94 ˚C for 2 min, a final extension at 70 ˚C for 5 min, and 35 cycles of a standard three-step PCR (i) denaturation at 94 ˚C for 1 min, annealing at 50 ˚C for 1 min, and (iii) extending at 70 ˚C for 2 min. The PCR products were subjected to electrophoresis on a 1% TBE-agarose gel. The gel was stained with ethidium bromide (EtBr) and visualized under UV light. The purified PCR product was sent out for sequencing. The sequences were compared to those available in the GenBank database.

The morphological measurements of *Nasutitermes* spp. found in Semanok estate was determined based on 14 parameters with 20 termite specimens each. Morphometric characters indicated that there were three species of *Nasutitermes* in the study area, namely *N. havilandi, N. matangensis* and *N. matangensiformis* (Figure 12.3). Several parameters of morphological measurements indicated significant differences among the three *Nasutitermes* spp. (Table 12.5) especially the size of head was the most prominent part in differentiating them. These three species of *Nasutitermes* were significantly different in most of the parameters except number of antennal segments.

N. havilandi found in the Semanok estate had brownish head with reddish brown tip of rostrum. There were 13 segments of the yellow and brownish tinge antennae. The shape of the head without rostrum was sub-circular with a pair of bristles on it. The rostrum was short and cone-shaped with two pairs of bristles around the base of rostral hump. The pronotum was emarginated shallowly on the anterior part and there were bristles along the margins. Both *N. matangensiformis* and *N. matangensis* had reddish brown to dark brown subcircular head. Their pronotum were saddle-shaped and emarginated shallowly on the anterior margin. They also had bristles on the tips and head. The major differences between these three species

were the size of the head and the length of rostrum. *N. matangensis* was the largest among three species based on morphological characteristics, followed by *N. matangensiformis* and *N. havilandi*.

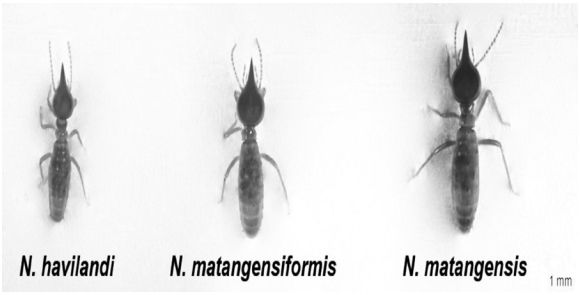

Figure 12.3 Three different species of *Nasutitermes* of oil palm: *N. havilandi*, *N. matangensiformis* and *N. matangensis*

Table 12.5 Comparison between *Nasutitermes* species with 14 parameters

Parameter (mm)	*N. havilandi*	*N. matangensiformis*	*N. matangensis*	Standard error
Maximum width of head	0.9695 c	1.1610 b	1.3275 a	± 0.0198
Height of head	0.6555 c	0.7822 b	0.9075 a	± 0.0147
Length of postmentum	0.1915 b	0.2375 a	0.2480 a	± 0.0050
Maximum width of postmentum	0.3200 c	0.3705 b	0.3940 a	± 0.0044
Maximum length of pronotum	0.2185 c	0.2455 b	0.2620 a	± 0.0035
Median length of pronotum	0.1560 b	0.2185 a	0.2040 a	± 0.0048
Width of pronotum	0.4835 c	0.5470 b	0.6055 a	± 0.0073
Number of antennal segments	13.000 a	13.000 a	13.000 a	± 0.0167
Length of head with rostrum	1.4920 c	1.6810 b	1.9480 a	± 0.0252
Length of head without rostrum	0.8550 c	1.0040 b	1.1920 a	± 0.0187
Length of rostrum	0.6365 c	0.6770 b	0.7540 a	± 0.0080
Rostrum-head index	0.7480 a	0.6745 b	0.6335 c	± 0.0094
Width of mesonotum	0.3720 c	0.4380 b	0.4800 a	± 0.0062
Width of metanotum	0.4945 c	0.6055 b	0.6480 a	± 0.0088

*Means with same letters across same row are not significantly different at $p \leq 0.05$ (Tukey)

Table 12.6 Sequencing results of termite DNA based on COII genes

Morphology studies	DNA sequencing (Similarity)
N. havilandi	98% similar *N. matangensis*
N. matangensiformis	99% similar *N. matangensiformis*
N. matangensis	99% similar *N. matangensiformis*

Cytochrome oxydase subunit II (COII) was a good marker to analyze relative relationships, such as species relationships within a genus, including termites (Miura *et al*., 1998, 2000). Based on morphology identification (Table 12.5), there was discrepancy in termite identification between DNA sequencing and morphometric results, whereby three termite species namely *N. havilandi*, *N. matangensiformis* and *N. matangensis* were clearly confirmed via morphometric results. Sequencing based on COII gene at this moment, however, indicated that there were two species, i.e. *N. matangensis* and *N. matangensiformis* from the three distinct samples analysed. This implied that this DNA marker resolution can be used for identification up to genus level only. Hence, further research work is needed in identification of *Nasutitemes* up to species level based on phylogeny studies.

Role and Behaviour of *Nasutitermes* in Oil Palm

Nasutitermes is an ordinary termite species found in agriculture. Arboreal *Nasutitermes* nests were conspicuous in forested areas, especially in neotropics and Southeast Asia. These species were usually found in leaf litter or rotten wood on the forest floor (Miura *et al*., 2000). Sudharto *et al*. (1991) had stated that *Nasutitermes* was not harmful to the palms. They fed on dry male inflorescences or the fragments of leaves or flowers accumulated at the axils of the fronds. Light trails with runways covered with crumbly soil were built by *N. havilandi* from the base to the crown. Hoe and Bong (2008) also stated that termite species that commonly found in oil palm, for instance, *Nasutitermes havilandi*, *Globitermes globusus*, *Odontotermes grandiceps*, *Schedorhinotermes sarawakensis* and *Pericapritermes* spp. act as scavenger species that perform beneficial ecological function of breaking down dead woody materials and converting them into organic matter which can be reused as nutrient by the palms. However, the mindset that all termites are pests is still trapped in most of the plantation people.

Such prejudiced views and blinkered thinking against termites could have negative influence to our environment and human health in future. Therefore, more evidences would be needed to verify if *Nasutitermes* were beneficial termites. Herein the role and behaviour of *Nasutitermes* spp. in the oil palm plantation were studied in details based on the parameters as follow: presence of *Nasutitermes*, location of nest, condition of tree and environment, interaction with other insects, foraging territory, feeding/ food source and defensive behaviour.

Nasutitermes species was commonly encountered foraging on the dead plant matter around the frond base or frond heaps on the ground of oil palm estate (Kon *et al*., 2012). Nests and galleries were often built at the base of the fronds that were already dried or pruned some time ago (Figure 12.4-B, J, K). They penetrated at the axils of the frond at all levels. Mostly, the penetration generally began at the axils of the lower frond bases which were normally partially rotten. Dissection of several infested oil palm fronds indicated that the main entry point of termites into the fronds was on the partially rotten frond base and the axils of frond. In addition, the galleries built in the partially decomposed fronds were very moist (Figure 12.4-C, E). Generally, the mud shelter of *Nasutitermes* spp. was in single-tract runways connecting each other from the palm base up to fruit bunches and fronds (Figure 12.4-A, G). However, a large mudwork patch built by *N. matangensis* had been observed on the oil palm in rare condition (Figure 4-H). Yet, the mudwork built by *Nasutitermes* spp. were slightly dry, crispy and have intense smell as compared to mudwork of *Coptotermes curvignathus*. Red ants such as *Anoplolepis gracilipes* and *Oecophylla* spp. (Lee and Tan, 2004) were the predators of *Nasutitermes* species (Figure 12.4-I). As a higher termite, soldiers of termite *Nasutitermes* were observed to carry out social work other than protecting the colony and queen. When attacked by predators, soldiers of *Nasutitermes* would assist the workers in carrying the eggs or nymphs (Figure 12.4-P).

On the other hand, it had previously been reported that when termites from different colonies were placed together, they usually fight (Thorne, 1982). This agonistic interaction of conspecific termite had been reported in several studies on *C. formosanus* and *Reticulitermes* spp. (Su and Haverty, 1991; Getty *et al*., 1999; Haverty *et al*., 1999; Cornelius and Osbrink, 2003). This agonistic behaviour also occurred in termite

Nasutitermes when the workers or soldiers from different colonies or various species mixed together where they would attack each other. When a colony of *Nasutitermes* termites was disturbed, the workers, which act as nest building and other quotidian tasks of the colony, would rush into depths of the nest while the soldiers would run out and began to position themselves for attack. They would fire a gluey secretion (Chuah *et al*., 1989) out from the tips of the frontal organs with great accuracy, striking the head, antennae and legs of the predators (Figure 12.4-N). This action impeded the predators and it was unpalatable to vertebrate predators such as anteaters (Wilson, 1976). Among these three species of *Nasutitermes*, the largest *Nasutitermes matangensis* was the most aggressive compared to the other two species when disturbed.

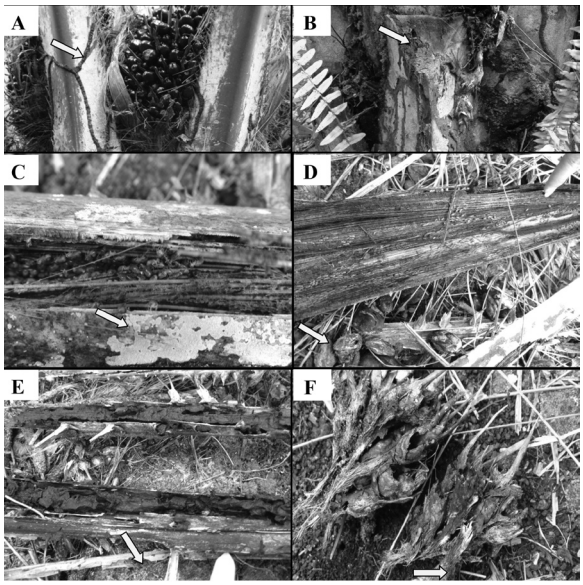

Figure 12.4 A) Infestation of Nasutitermes on oil palm by building trails (arrow). B) Nasutitermes species penetrated (arrow) at the axils of the frond at all levels. C) Nasutitermes species (arrow) foraged at moist partially decomposed frond. D) N. havilandi (arrow) foraged on the decomposed fronds. E) The galleries (arrow) were built in the partially decomposed fronds. F) Nasutitermes species foraging (arrow) on the decomposed inflorescences

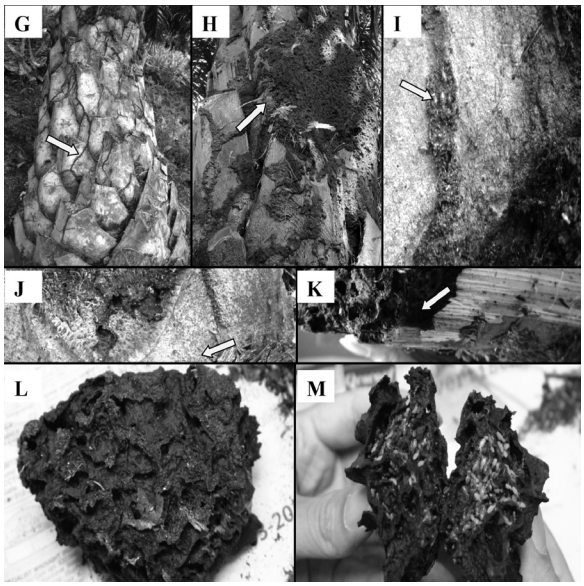

Figure 12.4 G) Mud shelter tube (arrow) of Nasutitermes spp. on oil palm trunk. H) Mudwork patch (arrow) built by N. matangensis in rare case. I) Soldiers (arrow) of Nasutitermes spp. defended the colony when attacked by predators. J) The main entry point of termites into the fronds was at the partially rotten frond base (arrow). K) Nest (arrow) was built on upper part of partially decomposed frond base. L) The nest of N. havilandi in moist condition. M) The inner part of the nest

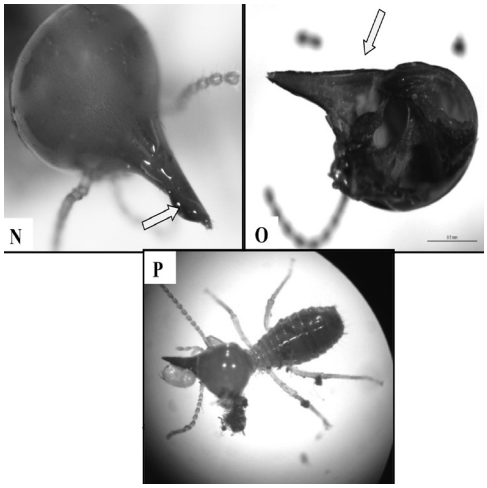

Figure 12.4 N) Soldiers defend using the frontal gland - secretes terpenes (arrow). O) Frontal gland (arrow) in dissected termite head capsule. P) Soldiers N. havilandi also carried out social work, carrying the egg (arrow)

Feeding Preference of *Nasutitermes havilandi*

The most common species *Nasutitermes havilandi*'s feeding preference was investigated in this study. The colony of *N. havilandi* was collected from infested oil palms. The termites were cultured inside large PVC container or harbourage, and rubber woods were used as food source. Laboratory temperature was maintained at 28±1˚C with relative humidity at more than 85% in dark condition.

A multiple choice feeding test using fresh and partially decomposed food sources were set up to investigate the food preference by *Nasutitermes*. The treatments include oil palm apical meristematic tissue, fruit, frond and rubber wood. Each food choices measuring approximately (1 × 1 × 2 cm) were cut and 20 pieces of food choices were randomly selected and weighed before oven-dried at 105 ˚C for 1 day or 60 ˚C for 4 days until the weight differences was negligible to determine the total moisture content in each food choices. Initial fresh food weight was also recorded.

The design of the experiment apparatus (Figure 12.5) was modified from Chan and Bong (2008). Autoclaved river sands and vermiculite were distributed evenly within the Petri dishes. The food sources were put into the feeding chamber (FC) after the initial weight had been taken. The FC were arranged randomly around and connected to the harbourage via a bridge (plastic drinking straw) 7 cm long with 0.5 cm diameter (Figure 1). A total of 90 workers and 10 soldiers were placed in each harbourage for simultaneous foraging activities. The apparatus was arranged in a Completely Randomized Design (CRD). All apparatus were incubated in the dark for 3 days at 28±1˚C and more than 85% RH. Each treatment was replicated 3 times. The food materials were oven-dried until constant weight to determine mass loss (consumption) due to feeding by the termite. The data in milligrams was subjected to ANOVA and the means were separated using Duncan's New Multiple Range Test (DNMRT) at $p \leq 0.05$. The consumption was calculated by the formula below:

Consumption= Initial fresh food weight – total moisture content (%) – the weight of the feeding food after drying

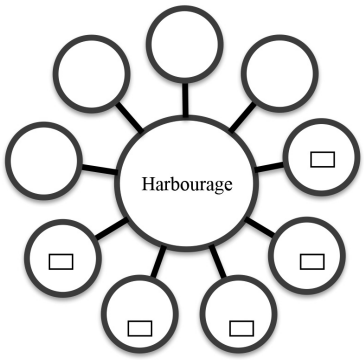

Figure 12.5 Multiple choices feeding test apparatus setting

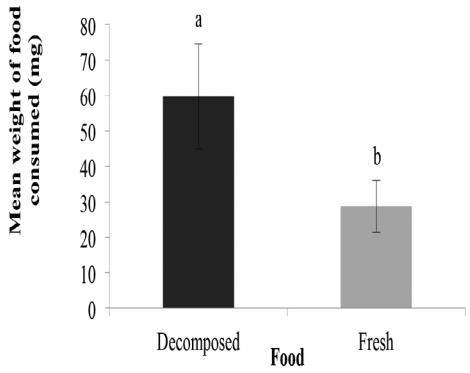

Figure 12.6 Comparison between freshness of food consumed by *N. havilandi*. Bars with different letters are significantly different at $p \leq 0.05$ (DNMRT)

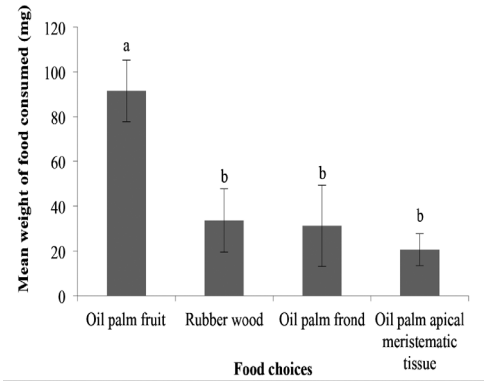

Figure 12.7 Comparison between food preferences of *N. havilandi*. Bars with different letters are significantly different at $p \leq 0.05$ (DNMRT)

The food preferences of *N. havilandi* were shown in the result of multiple food choice feeding test. In the multiple choice food tests on *N. havilandi,* decomposed food was generally preferred with a mean consumption of 59.73 mg to the fresh food (Figure 12.6). Specifically, oil palm fruit was the favourite food of *N. havilandi* with a mean consumption

of 91.51 mg (Figure 12.7). This implied that *Nasutitermes* spp. would not attack the oil palms living tissue that might cause loss of production or death.

After feeding from an available food source, workers would transfer the cellulose from one colony member to another via trophallaxis which involves the exchange of secretions or liquid food between individuals during feeding. Stomodeal trophallaxis, or mouth to mouth feeding, occurs when a worker passed the partially digested semi-liquid food from the crop or secretions by mouth (Pearce, 1997). Proctodeal feeding occurs when the termites receive secretions from the anus of another termite and it was more important in immature termite (Krishna and Weesner, 1969). However, stomodeal feeding was practiced among termite *N. havilandi* whereby workers fed the soldiers during food choice test observation period.

CONCLUSION

Nasutitermes spp. plays an important role in decomposition process in the ecological system. However, the uncertainty in the environment condition would affect the roles and behaviour of termites. Morphometric analyses showed that there were three species of *Nasutitermes* in the study area, namely *N. havilandi, N. matangensis* and *N. matangensiformis.* However, DNA sequencing using COII genes was unable to verify the species. As a higher termite, *Nasutitermes* had special defense weapon, in the form of secretion to protect themselves from predators. The soldiers of *Nasutitermes* also carried out social works other than protecting their colony. The food preferred by termite *Nasutitermes* was the decomposed food with consumption of 59.73 mg and oil palm fruit recorded the highest consumption of 91.51 mg of in the multiple food choice tests. In the oil palm plantations, they were found foraging on the dead plant matter around the frond base or frond heaps on the ground. Mostly, nest was built in the partially decomposed frond bases on the trunk. *Nasutitermes* spp. prefer moist condition with plenty of vegetation. Trails were built from the ground up to the crown of oil palm. They were observed to feed on partially decomposed food such as rotting fronds and inflorescences of oil palms in nature. No direct damage on the palm by this termite was observed. As a result, *Nasutitermes* spp. was harmless to the oil palms. Hence, *Nasutitermes* spp. was not a pest in the oil palm plantation.

ACKNOWLEGEMENT

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13

In Vitro Fermentation of Resistant Starch Portion from Dabai by Human Faecal Microbiota

Hanif Rawi, Dayang Marshitah Abang Bajury, Siti Aisyah Zaman, Morven Mundi and Shahrul Razid Sarbini

INTRODUCTION

Resistant starch (RS) is a starch component that goes into large intestine of human elementary canal after resisting the physical and biochemical processes of digestion of mouth, stomach and small intestine. It may be delivered to the lower gut for further microbial anaerobic fermentation. There are up to five types of RS that may occur naturally or through chemical modification, cooking and cooling of food, and physical food processes. Type I RS refers to starch that is physically inaccessible, such as partially milled grains and seeds. Type II RS are native starches with highly packed structure inside starch granules, such as potato starch, bean starch, and high amylose cornstarch. Type III RS are retrograded starches, which undergo heating and cooling procedures, creating a crystalline structure resistant to amylase. Type IV RS are chemically modified starches with etherized, or cross-bonded sugars to decrease digestibility. Type V RS are fatty acid modified starches that have recently been proposed. Recognition of RS can be tracked back to the 1980s with their potential in improving health and preventing chronic diseases (Li, 2010).

A consumption of RS meal has been shown to decrease glycemic index, for example blood glucose and insulin levels, showing the potential role of RS in preventing and improving type II diabetes and obesity. Long term consumption of RS has been shown to decrease colon carcinogenesis and improve inflammatory damages caused by colitis (Li, 2010). Human feeding trials, animal models, and in vitro fermentation models have been extensively used to discover physiological significances of resistant starch.

RS showed potential as a prebiotic, but knowledge of its other interactions with the microbiota is limited.

Fermentation of RS by lower gut microbiota is one of the most important aspects of RS in maintaining colon health. Human gut microbiota ferment the RS to produce short-chain fatty acids (SCFA), mainly acetate, propionate, and butyrate with other end products. Studies have shown that SCFA can improve human health by increasing the population of good microorganism in the large bowel. Increased fecal bulking, decreasing colonic pH, increased short chain fatty acid (SCFA), as well as gas production are major fermentation outcomes observed during starch anaerobic fermentation (Zaman & Sarbini, 2016). These SCFA, especially butyrate, have drew attention in the past decades because of a cumulation of in vitro evidence showing multi-function of butyrate in supporting colon health.

The microbial activities in the gut are mainly using substrate from SCFA. To study health effects of different foodstuffs and nutrients on the large intestine, samples that resemble contents of the gut after digestion of the respective foodstuffs is needed. The in vitro fermentation is a closed anaerobic system containing faecal suspension, growth medium and dietary component, RS in a reactor or sealed bottle. The simplest form of these in vitro models is the batch style fermentation. Other models involve multistage systems simulating the whole gastrointestinal tract.

The objective of this study is to undergo fermentation of RS in human colon model, to determine the growth population of *Lactobacillus* sp. in relation with time towards the fermentation of different resistant starch, mainly dabai (*Canarium odontophyllum*) starch.

MATERIALS AND METHODS

Faecal Inocula

Experiments were carried out using fresh faecal samples from four healthy volunteers, aged 22-26, had no history of gastrointestinal disorder, had avoided intake of pro- or prebiotics for at least 1 month prior to the study, and had not taken antibiotics for 3 months before the experiment. Samples were diluted 1:10 (w/v) in sterile phosphate-buffered saline (pH 7.3) and homogenized in a stomacher for 2 min at normal speed.

Medium Preparation

Fermentation vessels are obtained from Soham Scientific, United Kingdom, which is customly made for colon model fermentation. Sterile stirred batch culture fermentation vessels were prepared and aseptically filled with 50 ml of sterile basal nutrient medium. The medium comprised of peptone water (2 g/l), yeast extract (2 g/l), NaCl (0.1 g/l), KH2PO4 (0.04 g/l), MgSO4•7H2O (0.01 g/l), CaCl2•6H2O (0.01 g/l), NaHCO3 (2 g/l), Tween 80 (2 ml/l), haemin (0.05 g/l), vitamin K (10 µl/l), L-cysteine hydrochloride (0.5 g/l) and bile salts (0.5 g/l). The basal medium was adjusted to pH 7.0 and then 4 ml of 0.025 % (w/v) resazurin solution was added per liter before autoclaving. Once in the vessels, the sterile medium was purged overnight with O2-free N2 (15 ml/min) to maintain anaerobic conditions.

In Vitro Fermentation

Batch culture fermentation was done as described by Sarbini *et al.* (2013). Each vessel was inoculated with 5 ml of fresh faecal slurry. The RS assayed were added at a concentration of 1 % (w/v) to each vessel just prior to the addition of the faecal slurry. The temperature of the fermentation vessels was held at 37 °C using a circulating water bath. The pH was maintained in the range of 6.8 via pH controllers (Fermac 260, Electrolab) and adjusted by the addition of 0.25 mmol/l NaOH and HCl to the vessels when required. Anaerobic conditions were maintained throughout the fermentation by purging the vessels with O2-free N2 (15 ml/min). Batch culture fermentations were ran for 24 h and samples were taken at 0, 6, 12 and 24 h for bacterial enumeration. The substrates used are dried extract of dabai and Hi-maize® starch (commercial high amylose resistant starch from maize).

Bacterial Enumeration

An amount of 5 ml sample from each vessel were transferred into individual sterilin tubes. The samples were dispensed 1 ml in other sterilin tubes for serial dilution of 1 X 10^{-2} to 10^{-6}. Spread plate technique was performed to culture the bacteria in the sample. The plate was incubated at 43ºC for 24 h or longer. The same methods applied for samples taken at 6h, 12h and 24h of sampling.

Statistical Analysis

Statistical analysis of bacterial count was performed by ANOVA in the form of completely randomized design (CRD) for samples from each colon model. Duncan's test was performed to determine the significant difference between substrates used on bacterial group population at 5% significant level.

RESULTS

The experiment was conducted with 4 vessels, which consists of three vessels of dabai, and one with positive control of Hi-maize®. The samples of faecal slurry were taken from each vessel at 0h, 6h, 12, and 24h. It was then analysed by using total count of viable bacteria in agar plating. Table 1 shows the data for each sampling time for every vessel. It should be noted that each vessel contains different inoculum from different volunteers.

Bacterial count for each substrate is presented in Table 13.1. Bacterial growth in Vessel 1, which contains Hi-maize® as the substrate shows the highest mean value, whereas the lowest mean value is in Vessel 4. Statistically, V1, V2 and V3 have no significant difference. The same pattern are shown between V2, V3 and V4.Only V1 and V4 showed a significant difference The growth of bacteria in V1 seems to have a consistent growth from theoretical bacteria growth curve (Zwietering, 1990) with lag, exponential and stationary phases plotted. This is shown in Figure 13.2. The other vessels do not have particular pattern of bacteria growth along the 24h period of fermentation. Figure 1 shows the growth curve of Lactobacillus. The graph of V2, V3, and V4 was plotted to show the growth curve of *Lactobacillus* during the fermentation. From the statistical analysis, between those dabai substrates, there is no significant difference. The graph shows that the treatments do not have consistent effect on growth of bacteria.

Table 13.1 Number of bacteria per mL for every sampling time in Vessel 1: Hi-maize® as a control, Vessel 2: Dabai, Vessel 3: Dabai, and Vessel 4: Dabai with different inoculum in every vessel

Vessel	Number of bacteria for respected time period (bacteria unit/ml)				
	0h	6h	12h	24h	Mean
V1: Hi-maize® (control)	3.0 $X10^{6}$	1.2 $X10^{7}$	8.5 $X10^{7}$	1.38 $X10^{8}$	5.95 $X10^{7a}$
V2: Dabai	1.6 $X10^{7}$	5.4 $X10^{6}$	1.01 $X10^{7}$	3.0 $X10^{7}$	1.54 $X10^{7ab}$
V3: Dabai	3.2 $X10^{6}$	1.52 $X10^{7}$	1.7 $X10^{6}$	3.0 $X10^{7}$	1.25 $X10^{7ab}$
V4: Dabai	3.0 $X10^{6}$	1.0 $X10^{6}$	1.1 $X10^{6}$	2.6 $X10^{6}$	1.93 $X10^{6b}$

[a,b] Mean with same letter showed no significant difference at ($p \leq 0.05$) by using ANOVA (Duncan Multiple Test Range)

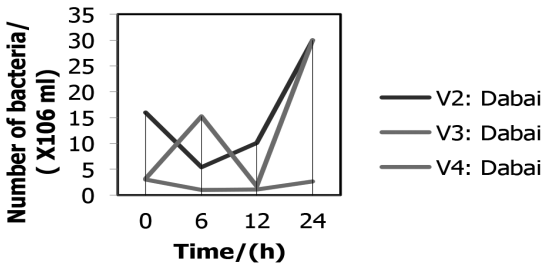

Figure 13.1 Growth curve of *Lactobacillus* in all fermentation vessels over a period of time

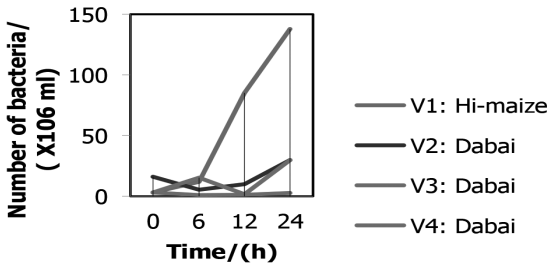

Figure 13.2 Bacterial growth curve between V2, V3 and V4 over 24 hour of fermentation

DISCUSSION

Possible Interaction of Probiotic Growth with Dabai Substrate

The data were compared in SAS system between the control and the treatments to determine if there is any different between the treatments. The data from V1 the growth pattern consistent with the growth theory of positive promotion of bacteria growth by RS. This is expected as Hi-maize® is a commercial resistant starch (Brown *et al.*, 1995). This shows that *Lactobacillus* feeds on Hi-maize® substrate throughout the fermentation process for their replication and metabolism. From the analysis, two out of three comparison combination between each dabai treatment with Hi-maize® (V1 vs V2 and V1 vs V3) showed no significant difference which suggest that dabai may work as good as Hi-maize® to support the growth of Lactobacillus, while only V4 showing the difference with the control, V1.

Looking at the growth curve of all dabai treatments there is no particular pattern followed as they opposed the typical exponential growth curve of bacteria. The increase in the cell size and cell mass during the development of an organism is called as growth. The growth of the organism is affected by both physical and nutritional factors (Zwietering *et al.*, 1990). The physical factors include the pH, temperature, osmotic pressure, hydrostatic pressure, and moisture content of the medium in which the organism is growing which in this case is the *Lactobacillus*. The nutritional factors include the amount of carbon, nitrogen, sulphur, phosphorous, and other trace elements provided in the growth medium. Bacteria are unicellular organisms. When the bacteria reach a certain size, they divide by binary fission, in which the one cell divides into two, two into four and continue the process in a geometric fashion. The bacterium is then known to be in an actively growing phase (Cappuccino and Sherman, 2008). The bacterium starts utilising the components of the media and it will increase in its size and cellular mass.

Bacteria Growth Curve

When a microorganism is introduced into the fresh medium, it takes some time to adjust with the new environment. This phase is termed as Lag phase, in which cellular metabolism is accelerated, cells are increasing in size,

but the bacteria are not able to replicate and therefore no increase in cell mass. When the microorganism growing in a rich medium is inoculated into nutritionally poor medium, the organism will take more time to adapt with the new environment (Rolfe *et al*., 2012). As in the vessel containing the dabai substrate, which may not provide preferable carbon molecule, the organism will start synthesising the necessary proteins, co-enzymes and vitamins needed for their growth and hence there will be a subsequent longer lag phase. Similarly, when an organism from a nutritionally poor medium is added to a nutritionally rich medium, the organism can easily adapt to the environment. It can start the cell division without any delay, and therefore will have shorter lag phase. This seemed consistent with the positive control Hi-maize®.

The microorganisms are in a rapidly growing and dividing state. Their metabolic activity increases and the organism begin the DNA replication by binary fission at a constant rate. The growth medium is exploited at the maximal rate, the culture reaches the maximum growth rate and the number of bacteria increases exponentially and finally the single cell divide into two, which replicate into four, eight, and sixteen and so on. This will result in a balanced growth. The time taken by the bacteria to double in number during a specified time period is known as the generation time (Powell, 1956). The generation time tends to vary with different organisms.

From the present study there were unbalanced growth occurred among the vessels containing dabai substrate. This may occur under a variety of condition such as the change in nutrient levels as explained above when microorganism is transferred from rich to poor medium nutrient. Usually, we also could examine the shift-up or shift-down of the curve when the microorganism moves from poor medium to rich medium or rich medium to poor medium respectively. The difference in nutrient availability will then effects the adaptation of Lactobacillus from the changes of environmental conditions from the colon into the fermentation vessels.

The graph also at some point gradually becomes horizontal as the bacteria started going into stationary phase. The possible reasons for this to happen maybe due to the nutrient limitation, toxic waste accumulation and also population density. As the bacterial population continues to grow, the microorganism uses up all the nutrients in the growth medium for their rapid multiplication, which results in the accumulation of waste materials, toxic metabolites and inhibitory compounds such as antibiotics

in the medium. This shifts the conditions of the medium such as pH and temperature, thereby creating an unfavourable environment for the bacterial growth (Pelczar *et al.*, 1996). The reproduction rate will slow down, the cells undergoing division is equal to the number of cell death, and finally bacterium stops its division completely. The cell number is not increased and thus the growth rate is stabilised. If a cell taken from the stationary phase is introduced into a fresh medium, the cell can easily move on the exponential phase and is able to perform its metabolic activities as usual.

The depletion of nutrients and the subsequent accumulation of metabolic waste products and other toxic materials in the media will facilitates the bacterium to move on to the Death phase. The bacterium completely loses its ability to reproduce. Individual bacteria begin to die due to the unfavourable conditions and the death is rapid and at uniform rate. The number of dead cells exceeds the number of live cells.

CONCLUSION

In summary, the growth population of *Lactobacillus* in *in vitro* fermentation that are observed suggest the ability of dabai substrate as the sole carbon source for probiotic growth. Statistically, V2 and V3, both containing dabai as the substrate, showed no statistical difference from V1 (control) in term of bacterial growth curve trend. This however could not confirm that dabai have clear potential as prebiotic since the data generated from vessel V4 which also contain dabai substrate is not consistent with the control and other replicates (V2 and V3). Furthermore, in the present study, the effect of dabai towards pathogenic bacteria was not analysed.

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14

Evaluation of Short Chain Fatty Acids Production from in Vitro Fermentation of Resistant Starch and Inulin

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INTRODUCTION

In the human gastrointestinal tract, there is colonization of beneficial microbes known as probiotics such as *Lactobacilli* and *Bifidobacteria* living in our colon ((Bouhnik *et al.* (2004); Gibson and Roberfroid, 1999; Goldin and Gorbach, 1992). These probiotics feed on prebiotics, the non-digestible fibre as their food source, promoting colonic health, metabolism, immune system, nervous system and many more. Colonic anaerobic fermentation of non-digestible fibres formed metabolites called short-chain fatty acids (SCFAs) within the colon (Hijova and Chmelarova, 2007). Acetic acid, propionic acid and butyric acids are the major SCFAs produced (J H Cummings, 1995; Flint, 2006; Levitt, Gibson, and Cristl, 1995; S. Macfarlane and Macfarlane, 2003). Previous studies has proven that SCFAs contribute in colonic activities, metabolism and even preventing pathogenic attacks (Hijova & Chmelarova, 2007). These SCFAs eventually promote better colonic health, thus reducing the risk of colonic diseases such as colonitis, colon cancer, diarrhoea, constipation and gastric problems (Floch and Hong-Curtiss, 2002; Jenkins, Kendall, and Vuksan, 1999; Roediger, 1980). It is also proven that SCFAs not only play an important role in the gastrointestinal tract (GIT), but it is also being absorbed into the bloodstream and utilised by other parts of the body (Hijova & Chmelarova, 2007). Acetate is always readily transported by bloodstream to be utilised by muscle, adipose tissue and peripheral tissue as a source of energy. Even as energy source in the liver, acetate aids in production of cholesterol, long chain fatty acids, glutamine and glutamate (Bloemen *et al*., 2009; Knowles,

Jarrett, Filsell, & Ballard, 1974; Pomare, Branch, & Cummings, 1985). Butyrate can also be produced from the anaerobic colonic fermentation as well as conversion from SCFA, acetate itself (Duncan, Barcenilla, Stewart, Pryde, and Flint, 2002). They play an important role of main energy source for colon tissue that eventually promote intestinal epithelial cell growth and production of thick mucosa barrier for defence purposes (Campbell, Fahey, and Wolf, 1997; Roediger, 1982). Propionate is mostly metabolised in the liver and has been shown to inhibit the process of gluconeogenesis and increase glycolysis (Anderson and Bridges, 1984). Therefore, propionate has a way to lower and inhibit hepatic cholesterol synthesis (Cheng and Lai, 2000; Hijova and Chmelarova, 2007; Wolever, Fernandes, and Rao, 1996). In short, these SCFAs works together in various ways, thus promoting better daily metabolism processes, body immunity and cholesterol level. Prebiotics are non-digestible ingredients that beneficially affect the host by selectively stimulating probiotics growth and metabolic activity within the colon, thus improving host health (Gibson and Roberfroid, 1999). The major source of prebiotics includes high fibre food that composed of component such as non-starch polysaccharides, resistant starch and inulin that comes from plant (Burkitt, Walker, amd Painter, 1974; Painter and Burkitt, 1975; Trowell, 1972, 1974; Tungland and Meyer, 2002). Resistant starch is starch and starch degradation products that are able to resist enzymatic hydrolysis process in the ileum of human digestive system (Englyst and Cummings, 1990). Whereas for inulin, they are naturally occurring polysaccharides produced in edible plants that is used mainly for energy storage in the carbohydrate form (Roberfroid, 2005).

However, the insufficient information of these SCFA-producing foods as well as their contributions towards colonic health is limited. Therefore, present study aims to evaluate and compare the SCFA profile generated through *in vitro* fermentation of resistant starch and inulin. This rapid evaluation and comparative study of SCFAs is done via aerobic fermentation by using isolated lactobacilli from cultured milk with substrates resistant starch and inulin. The SCFAs were then separate and quantified into acetate, propionate and butyrate by using high-performance liquid chromatography (HPLC).

METHODOLOGY

Materials

Inulin (from chicory), ultra pure water, acetonitrile and sulphuric acid are from *Sigma-Aldrich*. The Ultra pure water, acetonitrile and sulphuric acid was degassed prior to HPLC-UV analysis of SCFAs to prevent gas bubbles interruption. Acetate standard solution, propionate standard solution, butyrate standard solution is diluted with ultra-water at the concentration of 25, 50, 75 and 100 mM for calibration purposes.

Preparation of Resistant Starch (RS)

RS used in this study is of RS type III, a retrograded sago starch. The native sago starch was debranched with enzyme pullulanase for eight hours of incubation and then autoclaved to obtain fully gelatinised starch. The RS was then dried; grinded and sieved through a 175μm sieve to obtain a uniform particles sized RS.

Isolation and Preparation of Inoculum

Bacterial strain *Lactobacillus casei Shirota* (*LcS*) from *Yakult* cultured milk was used in this study. *Yakult* milk sample was drawn and serially diluted into 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6} and 10^{-7}. The diluted samples from the dilution series of 10^{-5}, 10^{-6} and 10^{-7} is plated onto the MRS agar plates. The petri dish is incubated for 48 hours at 37°C. Purification of the cultured strains is repeated for 2 to 3 times to ensure pure single colony are obtained. Lastly, a single colony is streaked and then incubated on slant agars for bacterial stock. Upon fermentation process, a *LcS* seed inoculums is prepared by inoculating a single colony into 100 mL of sterile nutrient broth for 24 hours incubation period.

In Vitro Fermentation of RS and Inulin

A batch-cultured fermentation system with 40 mL working volume is set up by mixing a 36 mL of sterile nutrient broth, 1% (w/v) of substrates (0.4g) and 10% (4 mL) of test suspension from the seed inoculum into a 50 mL sterilized conical flask. The mixture is then incubated at 37°C for 24 hours. Sampling of the fermentation slurry is performed at the period of time at 0, 6, 12 and 24 hours for analysis of SCFAs.

HPLC-UV Analysis of SCFAs

Samples of the fermentation slurry was centrifuged at 13000 rpm for 10 minutes to obtain supernatant. The supernatant (1.5 mL) was filtered through a 0.22μm filter unit (Milllipore), followed by dilution at a ratio of 1:10 with ultra-water and homogenised with vortex mixer. Lastly, 1.5 mL of the filtrate was transferred into the HPLC vial. The analysis of SCFA was performed by using reverse phase High Performance Liquid Chromatography (HPLC) system. The identification, separation, qualification and quantification of SCFAs were done with reverse phased HPLC, with C18 silica column, mobile phase of 10% acetonitrile and 90% ultrapure water, and a UV detector. An amount of 20μl of the sample is injected into the HPLC, operating at a flow rate of 0.5 mL/min with a heated column 37.0°C. The entire program for SCFAs analysis will be run in an isocratic elution for 30 to 40 minutes. Sample quantification will be carried out using calibration curves for acetate, butyrate and propionate at concentration of 25, 50, 75 and 100mM.

Statistical Analysis

Data is analysed statistically by using analysis of variance (ANOVA) to detect treatment effect and fermentation period effect. Means of treatment and fermentation period were compared by using Duncan's Test ($p \leq 0.05$). The statistical software used was Statistical Analysis System (SAS) version 9.3.

RESULTS

Short chain fatty acids concentration is shown in Table 14.1. There is no significant difference between the total SCFAs produced from fermentation of resistant starch and inulin at all hour of sampling/. BThe fermentation of both substrate showed a significant highest production of SCFAs at 0 hours and a significant lowest production of SCFAs at 24 hours.

Table 14.1 SCFAs concentration (mM) in batch culture fermentation of *Lactobacillus casei Shirota* with substrate resistant starch (Treatment 1) and substrate inulin (Treatment 2) at fermentation period of 0, 6, 12, 24 hours

Type of Short Chain Fatty Acids (SCFAs)	Fermentation Period (t/hr)	Concentration of SCFAs (mM)			
		Type of Substrate			
		Resistant Starch (Treatment 1)		Inulin (Treatment 2)	
		Mean	SD	Mean	SD
Acetate	0	191.666^{ax}	± 11.619	185.006^{ax}	± 11.236
	6	139.630^{abx}	± 36.606	157.400^{abx}	± 24.723
	12	139.760^{abx}	± 38.197	125.595^{bx}	± 20.678
	24	101.335^{bx}	± 7.822	79.950^{cx}	± 10.285
Propionate	0	0.000^{bx}	± 0.000	16.119^{ay}	± 2.103
	6	0.000^{bx}	± 0.000	6.910^{ay}	± 0.508
	12	9.150^{ax}	± 1.799	10.496^{ay}	± 3.246
	24	9.596^{ax}	± 2.975	8.047^{ay}	± 2.530
Butyrate	0	12.195^{ax}	± 3.500	17.368^{ax}	± 1.739
	6	10.770^{ax}	± 0.841	14.200^{cx}	± 0.201
	12	13.560^{ax}	± 2.303	12.246^{bx}	± 1.116
	24	13.512^{ax}	± 1.971	11.718^{cx}	± 0.481
Total SCFAs	0	203.861^{ax}	± 10.153	218.492^{ax}	± 10.942
	6	151.030^{abx}	± 36.969	178.510^{bx}	± 25.343
	12	162.470^{abx}	± 41.039	148.340^{bx}	± 24.337
	24	124.443^{bx}	± 6.521	99.715^{cx}	± 11.203

(Mean values and standard deviation, n=3)

^{a,b,c} Mean value with unlike superscript letters were significantly higher/lower in comparison among fermentation periods in the same substrate fermentation system ($p \leq 0.05$)

^{x,y} Mean value with unlike superscript letters were significantly difference in comparison between substrate fermentation system of RS and inulin in the same fermentation period ($p \leq 0.05$)

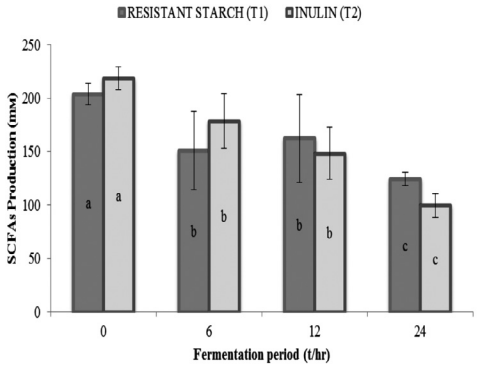

Figure 14.1 Comparison of total SCFAs production (mM) from 24 hours non-pH controlled batch culture fermentation of inoculation of *Lactobacillus casei Shirota* with substrate resistant starch (T1) and substrate inulin (T2)

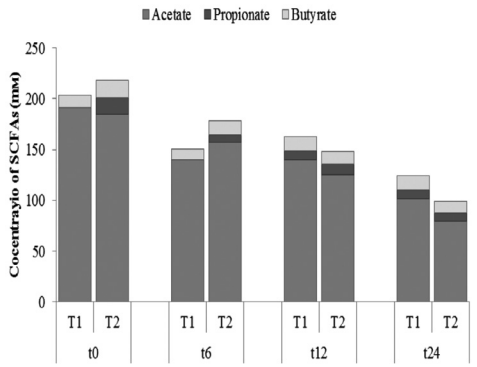

Figure 14.2 The composition of acetate, propionate and butyrate in each batch culture fermentation of inoculation of Lactobacillus casei Shirota with substrate resistant starch (Treatment 1) and substrate inulin (Treatment 2) at fermentation period of 0, 6, 12, 24 hours

In terms of type of SCFAs, the result shows no significant difference between the acetate and butyrate production between both substrate treatments, except for propionate production. Comparing acetate, propionate and butyrate among all fermentation period, the result shows that the production of acetate is the highest, whereas propionate and

butyrate shows a lower production. The least significant SCFA among all SCFAs is propionate which even shows a zero production at the fermentation period of 0 hours in resistant starch fermentation system.

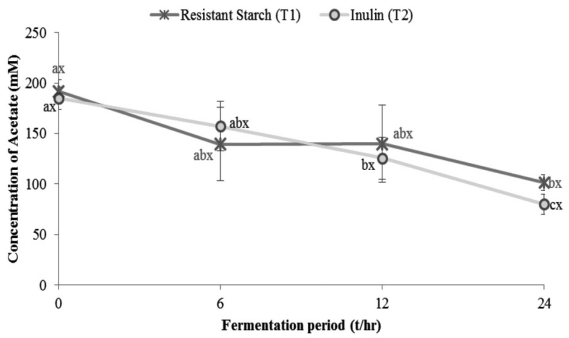

Figure 14.3 Acetate production pattern (mM) of 24 hours batch culture fermentation of inoculation of *Lactobacillus casei Shirota* with substrate resistant starch (Treatment 1) and substrate inulin (Treatment 2)

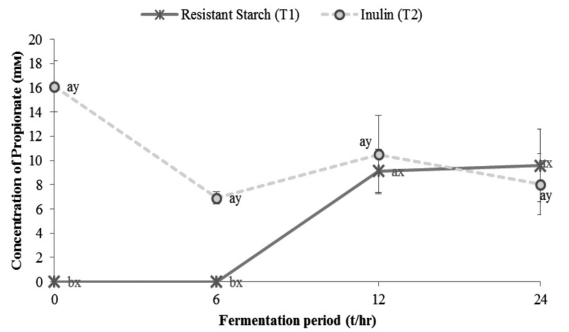

Figure 14.4 Butyrate production pattern (mM) from 24 hours batch culture fermentation of inoculation of *Lactobacillus casei Shirota* with substrate resistant starch (T1) and substrate inulin (T2)

The acetate production pattern (Figure 14.3) shows a decreasing trend for both resistant starch and inulin fermentation system. Along the fermentation period, acetate production shows a significant higher

concentration at0 hour and a significant lower concentration at the fermentation period of 24 hours. The same pattern applied to butyrate production with inulin treatment (Figure 14.4). Whereas for fermentation, the butyrate production shows an insignificant increasing trend along the 24 hours fermentation process (Figure 14.4).

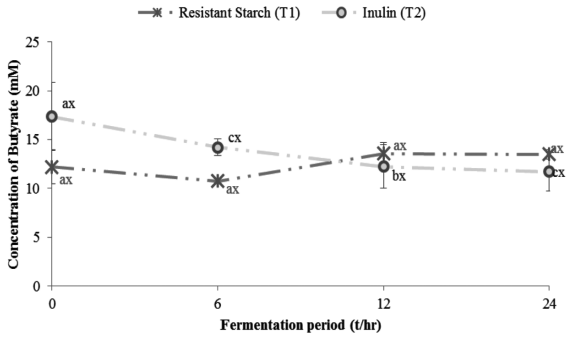

Figure 14.5 Propionate production pattern (mM) from 24 hours batch culture fermentation of inoculation of Lactobacillus casei Shirota with substrate resistant starch (T1) and substrate inulin (T2)

The statistical analysis of propionate production (Figure 14.5) shows a significant difference between resistant starch and inulin fermentation. In inulin fermentation, it shows a decreasing trend along the fermentation period with 0 hour showing a slightly higher but insignificant production and a slightly lower but insignificant production at as opposed to 24 hours fermentation. Resistant starch fermentation shows an increasing pattern along the fermentation period with a significantly higher production at fermentation period of 24 hours and a significantly lower production at fermentation period of 0 and 12 hours.

DISCUSSION

This study aimed to investigate the production of SCFAs from different substrates fibre through anaerobic fermentation with bacterial strain *Lactobacillus casei Shirota* (*LcS*). The production of SCFAs from the fermentation of RS and inulin give rise to no significant difference in SCFA profile. This may be related to the prebiotic potential of RS and inulin in the

aspect of solubility and fermentability (Henningsson, Björck, and Nyman, 2001). In other words, the production of SCFAs is directly proportional to the solubility and fermentability of substrates. The insoluble and partially fermentable resistant starch contributes the "slower-to be-digested" property compared to the soluble and fully fermentable inulin. This has leads to the total SCFAs production from fermentation of RS to be lower at the beginning of fermentation period and then slowly increase throughout the fermentation period. Whereas, the SCFAs production of soluble and fermentable inulin has a contrary production trend compared with RS. This indicates that *LcS* is able to ferment inulin easily; and also utilise RS as source of carbohydrate, yet with a longer time consumption. In the end, both substrates are able to produce similar quantity of SCFAs.

In addition, prebiotic substrates with low molecular weight are more preferable to be fermented by bacterial *Lactobacillus sp* (Gibson, Probert, Van Loo, Rastall, & Roberfroid, 2004). According to the study, substrate inulin has a degree of polymerisation (Painter & Burkitt, 1975) from 2 to 60 with average DP of 12. While substrate RS (type III) has a DP of 10 to100. As both treatments have a similar production of SCFAs, this suggest that both inulin and RS (type III) have a similar molecular weight and degree of polymerisation.

From the result, the production trend of SCFAs also showed a decreasing pattern for both inulin and RS treatment (Figure 1). According to Henningsson *et al.* (2001), the production of SCFAs is highly dependent on the type, quantity and digestibility of substrates, and also the type and population of probiotics.

Probiotics in majority including *LcS* uses glycolytic pathway to process carbohydrate source and generate various SCFAs at the same time (G. T. Macfarlane and Gibson, 1996). Inulin and RS as among the carbohydrate source for probiotic, *LcS*; these substrates eventually undergone the same pathway in Figure 6 and thus contributes in the production of SCFAs. According to S. Macfarlane and Macfarlane (2003), excess nutrient substrates condition promotes high growth rate and increases the production of lactate. Lactate is later converted into various SCFAs (Sarbini, Kolida, Gibson, and Rastall, 2013). This implemented that the bacterial substrate availability has a direct effect on the production of SCFAs (J. Cummings, Hill, Bone, Branch, and Jenkins, 1979; John H Cummings, 1978; John H Cummings, Bingham, Heaton, and Eastwood, 1992). In other words,

the depletion of substrates availability may result in the reducing trend of SCFAs production as the fermentation of both substrate treatments have been carried out for 24 hours.

According to S. Macfarlane and Macfarlane (2003), the production of SCFAs is related to types and population of bacterial, environmental-related factors such as stress and secretions. Commonly, the population of bacterial is directly proportional to the competition among bacteria for similar nutrients requirement (Hibbing, Fuqua, Parsek, and Peterson, 2010). In the beginning of the fermentation, the population of *LcS* is lower and less competitive for nutrients substrates compared to the end of fermentation period. There are less stress and less secretion of toxic by-product accumulation at the beginning of the fermentation. Substrates are in excess in the beginning and limited at the end of the fermentation. As a result, a higher production of total SCFAs in the beginning of fermentation creates a decreasing trend in 24 hours fermentation due to depletion of substrates, increasing population, competition stress and toxic by-product.

Among the produced SCFAs, acetate stands the most abundant portion followed by butyrate and lastly propionate (Figure 14.2). Acetate production in the fermentation of both substrates, RS and inulin, shows no significance difference between two treatments (Figure 14.3). Acetate production decreased in 24 hours fermentation system. According to the study of Duncan *et al*. (2002), utilisation of acetate includes conversion and production of butyrate. Additionally, other factors which includes increase of population size and competition, depletion of substrates availability, stress and toxic accumulation, leads to the decreasing trend of acetate.

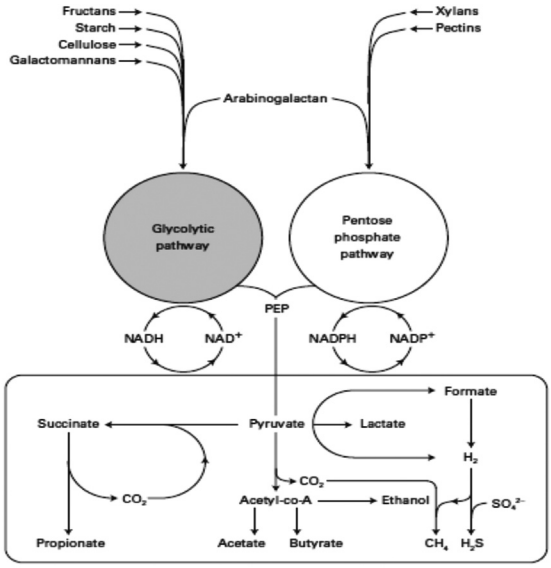

Figure 14.6 Simplified probiotics pathway in breaking down polysaccharides and carbohydrate fermentation (S. Macfarlane and Macfarlane, 2003)

In detail, the decreasing pattern of acetate in inulin fermentation system is linear whileRS fermentation system has a sudden decreased in the beginning, followed by a slight increase and then decreasing pattern. This phenomenon is related to the solubility and fermentability of substrates. The partially fementable and insoluble RS causes a sudden decrease of acetate production due to inadaptation of *LcS* towards RS as source of carbohydrate, followed by an increasing trend of acetate due to increasing adaptations towards substrate in fermentation of RS and decreasing due to depletion of subtrate availability. Addionally, the end period of fermentation with decreasing of acetate are the effect of re-utilisation of acetate and lactate by the microbes under high competition condition and the occurance of cross feeding in the fermentation, thus a low acetate condition is observed (Belenguer *et al.*, 2006; Sarbini and Rastall, 2011).

Owing to the solubility and fermentability of substrates, propionate production of highly fermentable inulin is similar to the decreasing pattern of acetate production in the same fermentation system (Figure 14.5). Whereas propionate production of insoluble and partially fermentable RS

has lead to a slow production of proprionate due to inadaptation towards RS substrate. Regarding to the study of Chen, Anderson, and Jennings (1984), propionate may inhibit the process of cholesterol synthesis while for acetate, it act as the precursor for cholesterol synthesis. In the fermentation system of RS, the decreasing production of acetate and increasing production pattern of propionate in 24 hours fementation indicates a low acetate:propionate ration. This indicates that RS may have the ability of regulating cholesterol level. Unlike RS, inulin fermentation system with both acetate and propionate in decreasing pattern may have a less capability in this matter.

Butyrate production in the fermentation of both RS and inulin shows no significant difference between treatments (Figure 14.4). This may be related to the solubility and fermentability of substrates (Henningsson *et al*., 2001). In which, inadaptation towards insoluble and partially fermentable RS contributes a slow increasing butyrate production in 24 hours and contrary result for inulin that is soluble and highly fermentable. Furthermore, the result showed is also similar to the study of Topping and Clifton (2001) whereby the production of butyrate may be related to the reason that RS is in favourites of butyrate production compared to inulin. Thus, unlike RS, the readily and highly fermentable inulin contributes to a fast yet decreasing butyrate production. However, it is undeniable that the decreasing in butyrate production for inulin fermentation may be related to depletion of substrate nutrients, increased bacterial population, competition, stress and toxic accumulation at the end of fermentation.

Several investigations involving *in vitro* fermentation suggested that SCFAs is produced by fermentation in an anaerobic environment similar to the gut environment. Probiotics *LcS* are facultative anaerobe which grow optimumly in human body temperarure of 37ºC. According to Hogg (2005), facultative anaerobe like *LcS* are microbes that are able to carry out aerobic respiration for energy metabolism with the presence of oxygen and switches into anaerobic fermentation under the absence of oxygen. The production of SCFAs in this study has a lower concentration compare to other studies. This may be due to the incomplete anaerobic condition during *in vitro* fermentation. *LcS* may have undergone respiration instead of fermentation due to the presence of oxygen and thus resulting in lower SCFAs production.

In short, the production of SCFAs are similar between fermentation of RS and inulin. This suggest that RS from sago starch has the similar properties as inulin in their prebiotic potential for human health purposes. However, the issue of uncomfortable gastro condition with consumption of high amount prebiotics is a concern for consumers (Tuohy, Probert, Smejkal, and Gibson, 2003). The rapid fermentation of inulin substrates with probiotics in the gut may causes flatulence and also bloating symptoms (Eswaran, Muir, and Chey, 2013; Stephen and Cummings, 1980). Therefore, comparing rapid fermented inulin, the slowly fermented RS may be a better choice of prebiotic functional food. However, further studies including cllinical studies should be carried out for confirmation.

CONCLUSION

Based on the result obtained, resistant starch (type III) retrograded from sago starch has a similar prebiotic potential with inulin. The production of major short chain fatty acids, acetate, propionate and butyrate from the fermentation of resistant starch with *Lactobacillus casei Shirota* are similar to inulin substrate. However, the slight differences of short chain fatty acids production between resistant starch and inulin is that resistant starch has a slower rate and increasing production of short chain fatty acids production compared to inulin with a rapid rate yet decreasing production of short chain fatty acids production due to the differences in their solubility and fermentability. Besides, it is also found out that butyrate production that is beneficial towards intestinal health is favourable in fermentation of resistant starch than inulin. Furthermore, the production of short chain fatty acids from resistant starch create an interest towards lowering cholesterol level potential in human due to the low acetate: propionate ratio.

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15

Some Characteristics of Broiler Meat during Refrigerated Storage After Marinated with Selected Spice Extracts

Nor Fadhliana Jamaluddin, Masnindah Malahubban and Zakry Fitri Abdul Aziz

INTRODUCTION

The use of synthetic preservatives in food nowadays is increasing due to the market demand for products that can last longer for storage purposes. However, rising consciousness and concern about the safety and quality of meat have led to numerous developments in meat preservation. Synthetic preservatives have long been used, but their application has been generally rejected by consumers, in some cases because of the suspected potential carcinogen and changing liver enzyme activities (Anand and Sati, 2013).

There is an urgent need to search for an alternative that much preferably derive from natural and probably safer sources of preservatives. One of the potential natural resources is spices or medicinal spices that have been traditionally used as food additive for centuries, not only to improve the sensory characteristics of food, but also to extend their shelf life (Tajkarimi *et al*., 2010; Babuskin *et al*., 2014). They can extend shelf life of food by reducing or eliminating survival of pathogenic bacteria and enhance overall quality through inhibition of oxidative rancidity (Tajkarimi *et al*., 2010). The ability of spices to improve meat quality might be related to their antimicrobial and antioxidant properties. Phenolic and flavonoid compound that contained in spices are possibly responsible to retard microbial growth and inhibit lipid oxidation.

Various spices have been documented as potential sources of antimicrobial and antioxidant such as black pepper, cumin, and curry leaves (Zarai *et al*., 2013; Raeisi *et al*., 2015; Najeeb *et al.,* 2015). However, the information is still limited especially on broiler meat, one of

the important food products. In the present study, three species of spices have been selected to evaluate their ability to retard bacterial growth especially *Escherichia coli,* a medical importance, and also to observe pH, moisture, and crude fat of broiler meat after marinated with respective spices and stored at about 4°C.

MATERIALS AND METHODS

Plant Materials

Three spices namely black pepper (*Piper nigrum*), cumin (*Cuminum cyminum*) and curry leaves (*Murraya koenigii*) were used in this study and were obtained from retail market around town of Bintulu, Sarawak, Malaysia.

Chemicals and Reagents

Eosin methylene blue agar medium (Merck), sodium chloride, petroleum ether, sodium hydroxide (NaOH), sulphuric acid (H_2SO_4), boric acid, hydrochloric acid (HCl), bromocresol green solution, methyl red solution, urea, methanol, borax and catalyst tablets.

Sample Preparation

Black pepper and cumin were ground and sieved through 1 mm. Curry leaves was oven-dried at 60°C for 48 h. The dried curry leaves was ground and then passing through 1 mm sieve for fine powder. Table 15.1 shows the content of moisture, crude fibre and ash in cumin, black pepper and curry leaves.

Fresh boneless and skinless chicken breast meat was obtained from fresh market around Bintulu town. Using a sterile knife, obvious fat and blood spots were removed and the chicken breast meat was cut into several cubes with 10g each.

Table 15.1 Composition of moisture, crude fibre, and ash in cumin, black pepper and curry leaves

Percent	Cumin	Black Pepper	Curry Leaves
Moisture	13.61	11.97	12.72
Crude Fibre	25.20	13.25	17.27
Ash	12.60	10.67	14.72

Extraction of Spices

A total of 50 g of each spice was added to 500 ml of water and placed in a water bath at 40°C for 8 h to allow liquid adsorption before transferring to a water bath shaker for orbital shaking at 40°C for another 16 h. Subsequently, all extracts were then filtered by using Whatman No. 1. The filtrates were then evaporated using a rotary evaporator at 40ºC for 1 h. The concentrated extracts were then transferred to glass petri dishes and dried in an oven at 40°C. Dried concentrated extracts were then kept in 4ºC before use (Ho *et al*., 2010).

Treatments

Broiler breast meat of 10 g each was marinated with black pepper, cumin, curry leaves extracts, and unmarinated broiler breast meat was served as control. The treatment was repeated five times. After 1 h, all treated and untreated breast meat samples were dried at room temperature to prevent contamination. All air-dried breast meat samples were aerobically packed in plastic bags and stored at 4°C for 9 days.

Data Collection

Determination of pH, moisture, and crude fat were determined according to AOAC method (1975; 1995). A total of 10 g of meat cube was homogenized in 100 ml of distilled water and the mixture was filtered. The filtrate was then measured for pH at ambient temperature.

A serial dilution was conducted to determine the presence and population of *Escherichia coli* using Eosin methylene blue (EMB) agar medium. A total of 10 g of meat samples of each treatment was added with saline solution (0.9% NaCl) and make up to a volume 100 ml. Then, 1 ml

of a 1:10 diluted saline-meat mixtures was serially transferred up to five-fold. A 0.1 ml of each dilution was withdrawn and seeded on EMB agar medium by spread plate technique. All the inoculated EMB plates were then incubated in universal oven for 24 h at 37°C. The *E. coli* colonies on EMB agar medium will show as green metallic sheen. The data were transformed and expressed as log colony forming unit (CFU) per gram of breast meat (log CFU/g breast meat).

For determination of moisture of broiler breast meat, a cleaned petri dish was kept in a hot air oven drying for about 7 h. After that, the petri dish was put into a desiccator for about 15 to 20 min. The petri dish was then removed from desiccator and weighed by using an electronic balance. Initial weight of empty petri dish was recorded. Two g of meat sample were then put into the petri dish and weighed to get the weight of petri dish and meat sample (W1). The meat sample was then dried together with petri dish in the oven at 135°C for 24 h. The sample was covered and kept in desiccator to cool. The final weight of petri dish and meat sample was then weighed and recorded immediately (W2). The moisture content was calculated by subtracting the dried weight of meat sample from the wet weight of the meat sample. Moisture percentage was calculated according to the formula:

$$\text{Moisture (\%)} = (W_1 - W_2) / W_1 \times 100$$

Note : W_1 = Weight of petri dish and meat sample before oven-dried

W_2 = Weight of petri dish and meat sample after oven-dried

Soxtec method used to determine crude fat by extracting meat sample with petroleum ether. A one g of meat sample was weighed into an extraction thimble and covered with absorbent cotton. A total of 50 ml of petroleum ether was added to aluminium container or extraction cup. Both thimble and aluminium container were then attached to the extraction unit. The sample was subjected to extraction with solvent (petroleum ether) by boiling for 15 min, followed by rinsing for 30 min, and recovery for 10

min. The thimble with lipid was placed in the oven at 103ºC for 4 h. The crude fat was calculated after sample cooled using the following formula:

Crude fat (%) = (Extracted fat / Sample weight) x 100

Statistical Analysis

All data was analyzed by analysis of variance (ANOVA) to detect treatment effects. Means of treatments were compared using Duncan New Multiple Range Test (DNMRT) at p= 0.05. The statistical software used was Statistical Analysis System (SAS) version 9.3 (SAS 2011).

RESULTS

pH Changes

Figure 15.1 shows the effect of spice extracts on the pH of broiler breast meat during refrigerated storage at 4°C for 9 days. The initial pH values of the broiler breast meat in all treatments were found to be around 5.86 to 6.01. The pH value of broiler breast meat treated with cumin was the highest (pH 6.01) during the first day but turned out to be the lowest at the end of storage period, day 9 (pH 5.98). However, the pH values of control and breast meat treated with black pepper and curry leaves were trending upward from 5.97 ± 0.01 to 6.76 ± 0.02, 5.86 ± 0.02 to 6.45 ± 0.06 and 5.92 ± 0.04 to 6.38 ± 0.02, respectively.

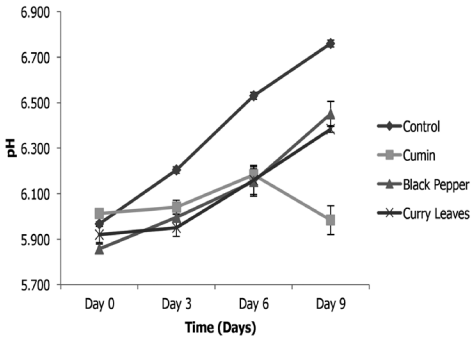

Figure 15.1 pH changes of broiler breast meat treated with cumin, black pepper and curry leaves during refrigerated storage at 4°C for 9 day

Population of Escherichia coli in Broiler Meat

The *E. coli* counts on broiler breast meat samples with or without spice extracts in refrigerated storage at 4°C were shown in Figure 2.

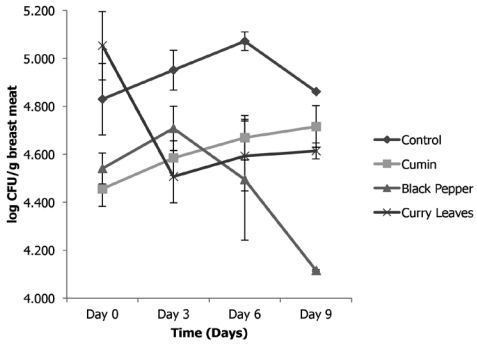

Figure 15.2 *Escherichia coli* bacteria population (log CFU/g of meat) of broiler breast meat treated with cumin, black pepper and curry leaves during refrigerated storage at 4°C for 9 days

During refrigerated storage from day 0 to day 9, *E. coli* number was ranged from 4.12 to 5.07 log CFU/g of breast meat (Figure 15.3).

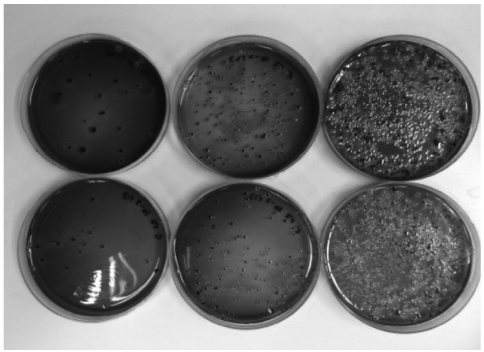

Figure 15.3 *E. coli* colonies appear as green metallic sheen on EMB agar

In general, all spice treatments were found not statistically different ($p>0.05$) during refrigerated storage up to 9 days, except, black pepper treatment. Broiler breast meat marinated with black pepper was only found significantly decreased ($p<0.05$) in *E. coli* number at day 9 of refrigerated storage at 4.12 log CFU/g of breast meat as compared to breast meat treated with curry leaves, cumin, and untreated control at 4.61, 4.72, 4.86 log CFU/g of breast meat, respectively.

Moisture and Crude Fat in Broiler Breast Meat Treated with Selected Spices

The moisture and crude fat in broiler breast meat during refrigerated storage at 4°C were shown in Table 15.1.

Table 15.1 Moisture and crude fat composition in broiler breast meat during refrigerated storage

Treatment	Moisture		
	Day 3	**Day 6**	**Day 9**
Control	$73.960^{b} \pm 0.476$	$74.703^{b} \pm 0.237$	74.950 ± 0.07
Cumin	$74.880^{ax} \pm 0.101$	$74.250^{bcy} \pm 0.179$	$74.690^{xy} \pm 0.096$
Black Pepper	$74.903^{ay} \pm 0.067$	$75.647^{ax} \pm 0.166$	$75.147^{y} \pm 0.088$
Curry Leaves	$74.017^{by} \pm 0.138$	$74.003^{cy} \pm 0.043$	$74.913^{x} \pm 0.355$
	Crude fat		
	Day 3	**Day 6**	**Day 9**
Control	0.108 ± 0.052	0.038 ± 0.023	0.307 ± 0.242
Cumin	$0.083^{x} \pm 0.003$	$0.042^{y} \pm 0.007$	$0.062^{xy} \pm 0.014$
Black Pepper	0.083 ± 0.019	0.082 ± 0.019	0.040 ± 0.013
Curry Leaves	0.052 ± 0.004	0.090 ± 0.013	0.058 ± 0.016

**Note: Treatment means ± standard errors are presented. Treatment means with different superscript letters (a,b) within the same column differ significantly ($p<0.05$). Treatment means with different superscript letters (x,y) within the same row differ significantly ($p<0.05$)

The moisture content in broiler breast meat with cumin extract was found significantly decreased ($p<0.05$) from day 3 to day 6 of storage period, but then increased insignificantly ($p>0.05$) when reaching day 9. The moisture content was increased significantly ($p<0.05$) from 74.9% to 75.7% during day 3 to day 6 in the broiler breast meat treated with black pepper extract. However, it was decreased significantly ($p<0.05$) to 75.2% when reaching day 9. In the broiler breast meat treated with curry leaves extract, there was no significant different ($p>0.05$) during day 3 until day 6, which were flat at 74.0%, but it was increased significantly ($p<0.05$) to 74.9% at day 9. There was no significant different ($p>0.05$) in moisture content in control breast meat throughout refrigerated storage (day 0 to day 9). Highest moisture content (significant at $p<0.05$) was demonstrated in breast meat treated with black pepper extract during day 3 as compared to untreated control breast meat. The moisture content in broiler breast meat treated with black pepper extract remained the highest during day 6 up to day 9 of refrigerated storage.

Crude fat in broiler breast meat treated with black pepper, curry leaves, and also untreated control were found no significant different ($p>0.05$) during nine days of refrigerated storage. The crude fat content in the breast meat treated with cumin extract was significantly decreased ($p<0.05$) from day 3 to day 6, and then was flat up to day 9 (not significant at $p>0.05$).

DISCUSSION

Present study demonstrates the effect of spices on some characteristics of broiler breast meat during refrigerated storage. Characteristics of broiler breast meat observed and evaluated in the present study were pH, number of *E. coli*, moisture and crude fat. Present study found that pH values of breast meat treated with black pepper and curry leaves were trending upward during refrigerated storage (day 0 to day 9). This phenomenon may provide much conducive environment for bacteria to grow or multiply. Kanjee and Houry (2013) reported that the increasing in pH values may be due to the utilization of amino acids by bacteria, released during the protein break down as the stored glucose had depleted. Accumulation of ammonia and the product of amino acid degradation will lead in the rise of pH. However, there was a twist in the breast meat treated with cumin extract, where the pH value was decreased after day 6 of refrigerated storage. According to

Rathore *et al*. (2012), the decreased in pH could probably due to the lactic acid released by lactic acid bacteria.

Among three spice treatments, black pepper extract was the most promising anti-*E. coli* agent after showed significantly decreasing number of *E. coli* at the end of refrigerated storage period. This activity could possibly due to the antimicrobial properties of black pepper that may associated with composition of phenols and phenolic acids, terpenoids and alkaloids (Kaefer and Milner, 2008). Several researchers have reported that phenolic compounds from different plant sources could inhibit various food-borne pathogens, and the total phenolic content have been highly correlated with antibacterial activity (Sethi *et al*., 2013; Witkowska *et al*., 2013; Sivarajan *et al*., 2016). According to Cowan (1999), membrane disruption by phenolics and metal chelation by flavonoids are considered to inhibit the growth of microorganisms. The mechanisms for the antibacterial actions of spices include the interference with the phospholipid bilayer of the bacterial membrane resulting in greater permeability, loss of cellular components, impaired enzyme systems needed for production of energy and structural components and inactivation or destruction of genetic material (Kaefer and Milner, 2008). Present study suggests that based on the effectiveness of spices on *E. coli* number at the end of refrigerated storage period, generally, the type of spices may be arranged as the following order, where from the most effective to the less effective: black pepper>curry leaves>cumin.

Present study found that the moisture content of broiler breast meat treated with black pepper extract was significantly higher ($p<0.05$) than untreated control breast meat during day 3 and day 6 of refrigerated storage, but failed to maintain significant moisture content at day 9. The ability of black pepper extract to maintain moisture content of broiler breast meat during refrigerated storage longer than other spices indicates that the meat able to retain its tenderness and juiciness.

Broiler breast meat marinated with cumin was only spice showed decreasing crude fat over time, at day 3 to day 6, and was flat up to day 9. The decreased values of crude fat in broiler breast meat treated with cumin indicated that the ability of cumin to reduce the deterioration of meat caused by lipid oxidation. Fats or lipids are susceptible to oxidative process in the presence of catalytic system such as light, heat, enzymes, metals, metalloproteins, and microorganisms, giving rise to the

development of off-flavours and loss of essential amino acids, fat-soluble vitamins and other bioactives (Shahidi and Zhong, 2005). Autoxidation of lipids and the production of free radicals are natural processes which affect fatty acids and lead to oxidative deterioration of meat and off-flavours development (Schwarz *et al*., 2001). According to Xu (2012), naturally occurring phenolics in plants have been reported to possess the capability of inhibiting lipid oxidation.

As health concern are arisen nowadays, people started to looking for an alternative way of using natural preservatives instead of using synthetic preservatives such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) which give negative effects on human health. Therefore, spices such as black pepper and cumin have something to explore further in future, and substitute the use of synthetic preservatives.

CONCLUSION

In conclusion, black pepper and cumin indicate potential for further investigation after showed promising results on number of *E. coli* presence, moisture, and crude fat of broiler breast meat during refrigerated storage. Black pepper extract was good at lowering the number of *E. coli* and maintaining the moisture content in broiler breast meat during refrigerated storage. Cumin was only performed best at lowering crude fat content in broiler breast meat after six days of refrigerated storage. Therefore, black pepper and cumin as a natural source of antimicrobial and antioxidant have the potential to replace the use of synthetic preservatives in meat processing industry.

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