EFFECT OF VERMICOMPOST APPLICATION ON MD2 PINEAPPLE PRODUCTIVITY, SOIL PROPERTIES AND BIOACTIVITY UNDER FIELD CONDITIONS

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EFFECT OF VERMICOMPOST APPLICATION ON MD2 PINEAPPLE PRODUCTIVITY, SOIL PROPERTIES AND BIOACTIVITY UNDER FIELD CONDITIONS

ABSTRACT

In the present study, a two-year field trial of pineapple, *Ananas comosus* var. MD2 plants (*in vivo* and *ex vitro*) was conducted based on a factorial randomized complete block design (RCBD) with three treatments in Jelebu, Malaysia from January 2015 until December 2016 to elucidate the effects of vermicompost (compared to supplementation with chemical fertilizer and no fertilizer) on the morphophysiology and yield performance of *in vivo* and *ex vitro* grown MD2 pineapple. The effect of vermicompost on sandy loam soil and plant nutrients of pineapple were also evaluated. Furthermore, studies were conducted to determine the effect of vermicompost on the bioactive compounds and antioxidant potential of the MD2 fruit extracts. In this study, vermicompost was applied onto the soil during transplanting, followed by a second application at seven MAP (months after planting) at the rate of 10 tan ha^{-1}. On the other hand, the chemical fertilizer was applied based on the normal conventional cultivation practice. The morphology of the plants was evaluated every month, and the fruits that were produced were subjected to quality analysis (physical, physicochemical and sensory analysis). The soil and D-leaf samples at six MAP (S1) and during red bud stage (S2; 10 MAP, *in vivo* plant; 16 MAP, *ex vitro* plant) were used to determine the soil and plant nutrient contents. For determination of antioxidant potential, three different test systems were used (ABTS, DPPH and FRAP). Data analysis revealed that there was no significant difference between plants supplied with chemical fertilizer and vermicompost in terms of plant height or the length and width of D-leaves ($P \leq 0.05$). However, based on SEM studies, pineapple plants supplemented with vermicompost showed higher stomatal density in the D-leaf. Moreover, different fertilization treatments were found to affect the yield and

physical characteristics of the resulting fruits. *In vivo* plants supplied with chemical fertilizer produced the highest fruit yield (136.97 t ha^{-1}) with the largest fruit size, followed by vermicompost (121.39 t ha^{-1}) and the control (94.93 t ha^{-1}). Similar trend was observed on *ex vitro* plants but smaller in size, contained higher total soluble solids (12.6 °Brix), titratable acidity (0.39 g kg^{-1}), total solids (20.841% wt/wt) and ascorbic acid (44.577 µg AA/g FW fruit). Soil pH was increased after a second supplementation of vermicompost and contained significantly higher total N in the soils compared to the control. The results showed that the antioxidant potential was lower in the methanolic extract of fruits harvested from *ex vitro* plants than *in vivo* plants. Based on HPLC analysis, only β-carotene was detected in the freeze-dried fruit extracts of all treatments from *in vivo* plants. However, for *ex vitro* plants supplemented with chemical fertilizer, both α-carotene and β-carotene were detected. Also, based on the cost analysis conducted, it was shown that the total cost (fertilizer and labour) for plants grown with vermicompost was lower than plants grown with chemical fertilizer.

Keywords: vermicompost, pineapple, growth productivity, nutrients, bioactivity

KESAN APLIKASI VERMIKOMPOS TERHADAP PRODUKTIVITI, SIFAT TANAH DAN BIOAKTIVITI NANAS MD2 DI BAWAH LAPANGAN

ABSTRAK

Dalam kajian ini, dua tahun kerja lapangan penanaman pokok nanas, *Ananas comosus* var. MD2 (*in vivo* dan *ex vitro*) telah dijalankan menggunakan kaedah faktorial yang disusun secara rekabentuk blok lengkap treawak (RCBD) dengan tiga rawatan di Jelebu, Malaysia bermula dari Januari 2015 sehingga Disember 2016 untuk mengenalpasti kesan vermikompos (dibandingkan dengan baja kimia dan sampel kawalan) keatas morfo-fisiologi dan prestasi hasil tanaman nanas MD2 *in vivo* dan *ex vitro*. Kesan vermikompos terhadap nutrient di dalam tanah lempung berpasir dan pokok nanas juga dikaji. Seterusnya, kajian dijalankan untuk menenetukan kesan vermikompos kepada kompaun bioaktif dan potensi antioksidan di dalam ekstrak buah MD2. Berdasarkan kajian ini, tanah dibekalkan dengan vermikompos semasa penanaman, diikuti dengan pembekalan kedua pada tujuh bulan selepas tanaman (MAP) pada kadar 10 tan ha^{-1}. Sebaliknya, baja kimia dibekalkan berdasarkan amalan penanaman konvensional. Morfologi tumbuhan dinilai setiap bulan, dan buah-buahan yang dihasilkan adalah tertakluk kepada analisis kualiti (fizikan, fizikokimia dan deria). Sampel tanah dan daun D diambil pada enam MAP (S1) dan semasa peringkat putik merah (S2; 10 MAP, pokok *in vivo*; 16 MAP, pokok *ex vitro*) digunakan untuk memnentukan kandungan nutrien di dalam tanah dan pokok. Bagi menentukan potensi antioksidan, tiga jenis sistem kajian digunakan (ABTS, DPPH dan FRAP). Hasil kajian menunjukkan tiada perbezaan ketara didapati dari segi ketinggian pokok atau panjang dan lebar daun D pokok nanas MD2 yang dibekalkan dengan vermikompos, dan pokok yang dirawat dengan baja kimia ($P \leq 0.05$). Walaubagaimanapun, berdasarkan kajian SEM, pokok nanas yang dibekalkan dengan vermikompos menunjukkan ketumpatan stomata yang tinggi pada daun D. Selain itu, jenis pembajaan yang berbeza mempengaruhi hasil dan ciri fizikal buah-buahan yang

dihasilkan. Pokok *in vivo* yang dibekalkan dengan baja kimia mempunyai hasil buah tertinggi (136.97 t ha^{-1}) dengan saiz buah yang terbesar, diikuti oleh vermikompos (121.39 t ha^{-1}) dan sampel kawalan (94.93 t ha^{-1}). Trend yang sama diperhatikan pada pokok *ex vitro* tetapi dengan buah yang lebih kecil serta mengandungi lebih tinggi jumlah pepejal larut dalam pulpa buah (12.6 °Brix), jumlah keasidan (0.39 g kg^{-1}), jumlah pepejal (20.841% wt/wt) dan asid askorbik (44.577 µg AA/g buah FW). pH tanah didapati meningkat setelah ditambah dengan vermikompos kalian kedua kepada tanah dan mengandungi jumlah nutrien N tertinggi di dalam tanah berbanding dengan kawalan. Hasil kajian juga menunjukkan potensi antioksidan di dalam ekstrak metanol buah yang dituai daripada pokok *ex vitro* adalah rendah dari pokok *in vivo*. Berdasarkan analisis HPLC, hanya β-karoten dikesan di dalam ekstrak buah dari pokok *in vivo* yang diuji. Walaubagaimanapun, untuk pokok *ex vitro* yang dibekalkan dengan baja kimia, α-karoten dan β-karoten dikesan kedua-duanya.

Kata kunci: vermikompos, nanas, pertumbuhan produktiviti, nutrien, bioaktiviti

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α	:	Alpha
&	:	And
~	:	Approximately Equal
β	:	Beta
°	:	Degree
°Brix	:	Degree Brix
°C	:	Degree Celsius
γ	:	Gamma
µg	:	Microgram
µL	:	Microliter
µm	:	Micrometre
%	:	Percentage
AA	:	Ascorbic Acid
ABTS	:	3-ethyl-benzothiazoline-6-sulfonic acid
Al	:	Aluminium
ANOVA	:	Analysis of Variance
B	:	Boron
C	:	Carbon
C_2H_3NaOO	:	Sodium Acetate Anhydrous
C_2H_4O	:	Acetone
Ca	:	Calcium
CAM	:	Crassulacean Acid Metabolism
CEC	:	Cation Exchange Capacity
$(CH_3COO_2)_2Pb$	:	Lead (II) Acetate
cm	:	Centimetre
CO_2	:	Carbon Dioxide
cv.	:	Cultivar
dE	:	Dry Extract
DPPH	:	2, 2-diphenyl-1-picrylhydrazy
DW	:	Dry Weight
EDTA	:	Ethylenediaminetetraacetic Acid
et al	:	et alia (and Others)

etc.	:	et cetera.
Fe	:	Iron
$FeCl_3$	:	Iron (III) Chloride
$FeCl_3.6H_2O$	:	Ferric Chloride Hexahydrate
FE-SEM	:	Field Emission Scanning Electron Microscopy
$FeSO_4.7H_2O$	:	Ferrous Sulphate Heptahydrate
FRAP	:	Ferric Reducing Antioxidant Power
FW	:	Fresh Weight
g	:	Gram
GAE	:	Gallic Acid Equivalent
h	:	Hour
H	:	Hydrogen
HCl	:	Hydrochloric Acid
HPLC	:	High Performance Liquid Chromatography
IC_{50}	:	50% Inhibition Activity
K	:	Potassium
kg	:	Kilogram
kg f	:	Kilogram Force
kg ha^{-1}	:	Kilogram per Hectare
kV	:	Kilovolt
L	:	Litre
MAP	:	Month After Planting
mg	:	Milligram
Mg	:	Magnesium
min	:	Minute
ml	:	Millilitre
mm	:	Millimetre
mM	:	Millimolar
Mn	:	Manganese
Mo	:	Molybdenum
MPIB	:	Malaysian Pineapple Industry Board
Mt	:	Metric Ton
N	:	Nitrogen
NaOH	:	Sodium Hydroxide

ND	:	Not Determine
NH_4^+	:	Ammonium
nm	:	Nanometre
NO_3^-	:	Nitrate
NPK	:	Nitrogen, Phosphorus and Potassium
O	:	Oxygen
OD	:	Optical Density
P	:	Phosphorus
RM	:	Ringgit Malaysia
RNA	:	Ribonucleic Acid
ROS	:	Reactive Oxygen Species
rpm	:	Rotation per Minute
RSA	:	Radical Scavenging Activity
s	:	Second
S	:	Sulphur
S1	:	Sampling on 6 Months After Planting
S2	:	Sampling during Red Bud Stage
SE	:	Standard Error of Mean
SOM	:	Soil Organic Matter
SPSS	:	Statistical Package for the Social Sciences
TA	:	Titratable Acidity
t ha^{-1}	:	Tonnes per Hectare
TPTZ	:	2, 4, 6tripyridyl-s-triazine
TSS	:	Total Soluble Solids
UV-Vis	:	Ultraviolet-Visible
v/v	:	Volume per Volume
var.	:	Variety
wt/wt	:	Weight per Weight
x g	:	Gravity; measured in metres per second
Zn	:	Zinc

LIST OF APPENDICES

CHAPTER 1: INTRODUCTION

1.1 Research Background

The 'Green Revolution' in the 1950-60's was introduced when chemical fertilizer was widely used in the agronomic industry (Adhikary, 2012). The usage of chemical fertilizer dramatically increased the quantity of the food produced but at the expense of environmental degradation and society (Rajiv et al., 2010; Theunissen et al., 2010). Over the years, the quantity of chemical fertilizers needed are increasing greatly to preserve soil fertility and food yield at the same levels, thus it has functioned like a 'slow poison' for the soil with a serious 'withdrawal symptoms' (Rajiv et al., 2010). In response to this, it was thought that organic farming systems with the aid of various nutrients of biological origin such as compost or vermicompost might be the answer for the 'food safety and security' in future (Rajiv et al., 2010).

Vermicompost is the excreta of earthworm which rich in humus, macro and micronutrients, can improve soil health status and also can enhance crop production (Adhikary, 2012). The utilization of vermicompost nowadays have been widely used in the cultivation field and is well known to produce a good crop yield such as in wheat (Yousefi & Sadeghi, 2014), groundnut (Kumar et al., 2014), tomato (Zucco et al., 2015), maize (Kmeťová & Kováčik, 2014) and peppermint (Ayyobi et al., 2014). Several reports were found in literature on application of inorganic fertilizer or compost to the pineapple plants (Maeda et al., 2011; Omotoso & Akinrinde, 2013; Orluchukwu & Adedokun, 2015; Owureka-Asare et al., 2015; Teixeira et al., 2011), however no published report was found on analysis of the effects of supplementation of vermicompost towards 'MD2' pineapple plants. Moreover, the application of vermicompost was found to exert the same effect as in the case of the inorganic fertilizers administration (Singh et al., 2008), which

is a good reason to replace the application of chemical fertilizer with vermicompost for cultivation of pineapple plants.

Ananas comosus var. MD2 was used as plant material in this study. In recent years, there has been an increasing interest in MD2 pineapple around the world. This is because of its characteristics whereby it was reported to have sweeter taste, yellowish in colour, thinner skin, cylinder shape, resistant to 'physiological browning', and longer shelf life compared to other varieties (Banful et al., 2011). Therefore, MD2 pineapple is a perfect variety for canning and can perform better in long-distance shipping for fresh pineapple consumption. In Malaysia, it was identified as one of the key crops under the National Key Economic Area (NKEA) of the Economic Transformation Programme (ETP), where the objectives were to penetrate the global markets and to ensure national food security (MPIB, 2015a). Unfortunately, the quantity of suckers of MD2 pineapple was not enough and expensive to accommodate the demand for planting materials. In order to produce the plantlets in large scale, researchers have investigated the cultivation of pineapple using tissue culture technique. However, the use of MD2 pineapple plantlets generated through plant tissue culture is still not common among the farmers, especially in Malaysia. In this study, the effect of vermicompost on growth performance and fruit quality attributes of both *in vivo* and *ex vitro* MD2 pineapple plants were examined and compared to when chemical fertilizer was used.

1.2 Objectives

The objectives of this research as follows:

a) To investigate the effect of vermicompost on the morpho-physiology and yield performance of *in vivo* and *ex vitro* grown MD2 pineapple.
b) To identify the effect of vermicompost on soil and plant nutrients of MD2 pineapple plants (*in vivo* and *ex vitro*).
c) To evaluate the effect of vermicompost on bioactive compounds and antioxidant potential of the MD2 pineapple fruits extract (*in vivo* and *ex vitro*).

1.3 Scope of Study

In this study, the MD2 pineapple plants (*in vivo* and *ex vitro*) were planted in the field and supplied with vermicompost, chemical fertilizer or no fertilizer (control). Plant growth was evaluated until the fruits were harvested. The plant height, number of leaves, length and width of D-leaf and chlorophyll content (SPAD meter) were measured and recorded throughout planting until the fruits were harvested. For morphological stomatal features, the stomatal density, stomatal size, stomatal width, stomatal length, pore length and pore aperture were measured using Field Emission Scanning Electron Microscopy (FE-SEM) on nine months after planting. Fruits produced from both *in vivo* and *ex vitro* pineapple plants were evaluated for their quality based on physical, chemical, and sensory analysis. The nutrients content (nitrogen (N), phosphorus (P), potassium (K), magnesium (Mg), sulphur (S), calcium (Ca), iron (Fe), zinc (Zn), boron (B), aluminium (Al)) in the soil and D-leaf of MD2 pineapple plant was analysed on six months after planting and during the red bud stage.

Besides, the phytochemical of the methanolic fruit extract (both *in vivo* and *ex vitro*) were evaluated whereby the total phenolics, total carotenoid and chlorophyll content in

the samples were measured and compared. Furthermore, antioxidant capacities exhibited by the methanolic fruit extracts were also assessed using DPPH, $ABTS^{\bullet+}$ and ferric reducing power (FRAP) assays. The fruits extracts were also screened for common carotenoids such as α-carotene, β-carotene, neoxanthin, violaxanthin, lutein, zeaxanthin, β-cryptoxanthin and lycopene, through HPLC. A combination of quantitative and qualitative approached was used in the data analysis. Then, in order to analyse the significance of all data obtained, data analysis was performed using IBM Statistical Package for Social Sciences (SPSS) version 24.

1.4 Significance of Study

At present, the awareness on the importance of conserving natural resources and application of sustainable and green technology is increasing. It was previously thought that the application of chemical fertilizer was be the best economic practice to produce crops and produces. However, the excessive usage of chemical fertilizer has been reported to harm the environment and will directly deter soil's condition. To date, there has been no reliable evidence on the application of vermicompost to pineapple plants. Therefore, this study was constructed to investigate the effect of vermicompost application on soil quality and the nutrient status of the MD2 pineapple plants. The plant's growth performance and the yield generated by both *in vivo* and *ex vitro* pineapple plants were also investigated. The outcome of this research may be of use to increase the awareness of farmers on sustainable agriculture through the use of vermicompost as alternative nutrient supplement for the crops in the future. Furthermore, this research also aimed to garner and increase the interest of farmers, especially the pineapple growers to use *ex vitro* plants as alternative planting materials, thus welcome and embrace the importance of plant tissue culture technology a viable and important tool in plant breeding.

CHAPTER 2: LITERATURE REVIEW

2.1 Introduction to Vermicompost

In agriculture industry, the use of organic matter such as food wastes, animal manures sewage sludge and composts have been known to give a positive effect on plant growth, yield and for maintenance of soil fertility (Khaliq et al., 2017; Yang et al., 2014). There are various processes related to composting which can improve the beneficial utilization of organic wastes, such as by vermicomposting. Vermicompost is the excreta of earthworm, whereby certain species of earthworm (especially *Eisenia fetida* or *Eudriculus eugeniae*) feed on the biodegradable wastes such as agro-wastes (Chaudhuri et al., 2016), sewage sludge (Ludibeth et al., 2012), food waste (Majlessi et al., 2012) etc. and these are then converted while passing through of earthworm's gut to produce a nutrient rich vermicompost (Adhikary, 2012). By the time organic waste is excreted by the earthworms as vermicasts, it will be rich in humus and contain plant macronutrients as well as trace elements depending on the feedstock types used (Adhikary, 2012; Ray, 2016).

2.1.1 Production of Vermicompost

Vermicomposting which involves the composting of organic wastes through earthworm activity, has been proven to be successful in processing sewage sludge and solids from wastewater, materials from breweries, paper waste, urban residues, food and animal waste, as well as horticultural residues from processed potatoes, dead plants and mushroom industry (Amouei et al., 2017; Domínguez et al., 2000; Li et al., 2016; Ray, 2016). Various earthworms have been used for vermicomposting and these include *Eisenia fetida* (Agarwal et al., 2010; Aksakal et al., 2015; Amouei et al., 2017), *Eudrilus eugeniae* (Agarwal et al., 2010; Oo et al., 2013), *Eisenia Andrei* (Domínguez et al., 2000). However, *E. eugeniae* has been noted as the earthworm of choice for vermicomposting

because of its voracious appetite, high rate of growth and reproductivity ability (Adhikary, 2012). The other epigeic species used in large-scale vermin culture is *E. fetida* which able to adapt to changing conditions and has lower chances of compromising the vermicompost process (Ray, 2016). Epigeic earthworm are smaller in size, with uniformly pigmented body, short life cycle, high reproduction rate and regeneration, rarely ingest soil, contain active gizzard (aid in rapid conversion of organic matter into vermicompost), tolerant to disturbances, efficient bio-degrader and nutrient releasers (Pathma & Sakthivel, 2012).

There are several environmental factors that may influence the survival and growth of earthworm in the pit such as moisture content, temperature, pH and substrates. In vermicomposting systems, the optimum range of moisture contents has been stated to be between 50 to 90% (Domínguez et al., 2000). Adequate moisture should be maintained during the process, either stagnant water or lack of moisture could kill the earthworms (Adhikary, 2012). Vermicomposting also uses a mesophilic process that utilizes microorganisms and earthworms that are active at 10 °C to 32 °C (not ambient temperature but temperature within the pile of moist organic material) whereby at higher temperature, the worms are found to aestivate and at lower temperature, they will hibernate (Adhikary, 2012; Ray, 2016).

Furthermore, although earthworms preferred more acidic materials, prolonged exposure in acid soils with pH less than 4.5 must be avoided as this could have lethal effects (Ray, 2016). Earthworms are very sensitive to anaerobic conditions and their respiration rates are depressed in low oxygen concentrations. As a result, feeding activity might be reduced under these sub-optimal conditions (Ray, 2016). Moreover, earthworms cannot survive in organic wastes containing high levels of ammonia such as fresh poultry litter. They also die in organic wastes with large amounts of inorganic salts. The

earthworms also should be protected against birds, termites, ants and rats (Adhikary, 2012).

Adhikary (2012) reported the process of vermicomposting using a farm waste. Firstly, the pit was prepared with size of 2.5 m length, one m breadth and 0.3 m depth. The bottom and sides of the pit are made by compacting with a wooden mallet. Then, at the bottom of the pit a layer of coconut husk is spread and moistened with the concave side upward to ensure drainage of excess water and for proper aeration. Bio-waste mixed with cow dung in the ratio of 8:1 is added on the top and spread up to a height of 30 cm above the ground level and water is sprinkled daily. After seven to 10 days, 500 to 1000 worms/pit are introduced into the partial decomposition of wastes, then covered with a jute bag. Temperature is maintained at 20 °C to 30 °C and moisture at 40 to 50 per cent population density by sprinkling water over the bed. When the compost is ready, all compost is removed from the pit and heaped in shade with ample light. After one or two days, worms will be at the bottom of heap, the compost from the top of the heap is removed. The undecomposed residues are put back to the pit with worms for further composting (Adhikary, 2012).

2.1.2 Benefits of Vermicompost

Numerous studies have shown that vermicompost amendment could promote soil quality, by improving soil structure, increasing plant available nutrients, and promoting microbial activity, thereby increasing plant production relative to conventional chemical fertilization (Song et al., 2015). Studies also confirm that vermicompost is at least four times more nutritive than conventional cattle dung compost (Suhane, 2007). This is mainly due to “humus” content in vermicompost excreted by earthworms which otherwise takes very long time to form humus in conventional composting system through slow decay of organic matter (Rajiv et al., 2010). It also makes vermicompost to have

very high porosity, aeration, drainage and water holding capacity than conventional compost (Suhane, 2007). Therefore, the requirement for irrigation was reduced by 30-40% compared to the usage of chemical fertilizers (Rajiv et al., 2010).

Vermicompost is a 'slow-release organic-fertilizer'. The nutrients are release slowly and gradually into the soil solution rather than allowing immediate nutrient leaching (Adhikary, 2012; Theunissen et al., 2010). However, it retains nutrients for a longer time than the conventional compost and while the latter fails to deliver the required amount of macro and micronutrients including the vital NPK to plants in shorter time, the vermicompost does (Rajiv et al., 2010). Vermicompost contains nutrients in forms that are readily taken up by the plants such as nitrates, exchangeable P, and soluble K, Ca and Mg (Atiyeh et al., 2000). Moreover, the application of humic acids derived from vermicompost also shows a significant accumulation of N, P, K, Ca and Mg in the roots and shoots of *ex vitro* 'Victoria' pineapple plants compared to control (Baldotto et al., 2010).

Vermicompost contains plant nutrients including N, P, K, Ca, Mg, S, Fe, manganese (Mn), Zn, Copper (Cu) and B, which improves the nutrient content of different plant components such as roots, shoots and the fruits (Theunissen et al., 2010). Trough supplementation of vermicompost, its enhances size, colour, smell, taste, flavour and keeping quality of flowers, fruits, vegetables and food grains (Rajiv et al., 2010). There are a positive effect on yield parameters of wheat (Gopinath et al., 2008; Yousefi & Sadeghi, 2014), tomatoes (Alidadi et al., 2014; Azarmi et al., 2008; Lazcano et al., 2009), peppermint (Ayyobi et al., 2014), strawberry (Singh et al., 2008) and maize (Oo et al., 2013) when vermicompost is applied. Based on the experiment, strawberry plant supplied with vermicompost (10 t ha^{-1}) took only 86.8 days to flower compared to 93.1 days when plants received inorganic fertilizers only (Singh et al., 2008). They also reported that fruit

per plant (27.5), individual berry weight (14.2 g) and total fruit yield (396.2 g/plant) at maximum when supplied with vermicompost (10 t ha^{-1}), whereas plants supplied with inorganic fertilizer has minimum fruit per plant (25.5), lowest berry weight (11.7 g) and total fruit yield (298.4 g/plant). However, the benefits that plants receive from vermicompost depend on the plant's ability to extract from the fertilizing substrate, the substances needed for the growth and development (Lazar et al., 2014).

Furthermore, this earthworm vermicast is rich in micronutrients, beneficial soil microbes like 'nitrogen-fixing bacteria' and 'mycorrhizal fungi' (Sinha, 2009). It also increases 'biological resistance' in plants (due to Actinomycetes) and protect them against pest and diseases either by repelling or by suppressing them (Sinha, 2009). Studies report statistically significant decrease in arthropods (mealy bug, spider mite, aphids, buds) populations, and subsequent reduction in plant damage in tomato, pepper and cabbage trials with 20% and 40% vermicompost supplementation (Adhikary, 2012; Arancon & Edward, 2005). Vermicompost possess the ability to fight soil-borne plant diseases such as root rot. One study reported that mean root disease was reduced from 82% to 18% in tomato and from 98% to 26% in capsicum in soils amended with compost (Adhikary, 2012).

The plant defence against disease attack and environmental stressors are as results from secondary metabolites produced by plants (Yusof et al., 2018). Based on the previous study, peppermint plant treated with cow manure vermicompost (7 Mt ha^{-1}), vermiwash prepared from seven Mt ha^{-1} vermicompost, and seven Mt ha^{-1} vermicompost leachate + vermiwash had the highest levels of chlorophyll a, chlorophyll b, total chlorophyll and carotenoids compared to chemical fertilizer (NPK) and control (no fertilization) (Ayyobi et al., 2014). In addition, vermicompost application also increase the levels of enzymatic (superoxide dismutase, catalse, glutathione peroxide) and non-

ezymatic antioxidants (gluthione, vitamin E, vitamin C, β-carotene) in the medicinal plant, *Coleus aromaticus* and *Andrographis paniculata* when compared to the control and inorganic fertilizer application (Lamma & Moftah, 2016; Suneetha et al., 2011). The food waste vermicompost positively influenced total phenolic, and highest antioxidant activity (81%) was obtained in methanolic extract of sweet pepper (*Capsicum annum* L.) plant treated with 10 t ha^{-1} vermicompost while their lowest value was recorded in the control plants (Aminifard & Bayat, 2016). In all the studies reviewed here, vermicompost is recognised as great supplements to the soils which give a positive affect to various types of plants.

2.2 *Ananas comosus*

Pineapple (*Ananas comosus* (L.) Merr) ranks second in the four major fresh tropical fruit in terms of importance in global productions after mango, followed by papaya and avocado (FAO, 2017). The world production of pineapple increases by 30% between 2007 (20 million tons) to 2017 (25.9 million tons) with Costa Rica, Brazil and Philippines as the top three pineapple producers in the world (FAO, 2017). Several other tropical countries such as Hawaii, Indonesia, Thailand, India, China, Mexico and Malaysia are also growing pineapples for locally consumes or import to international market either as fresh fruits or processed food (canned, juice, syrup, etc).

2.2.1 Botany of *Ananas comosus*

Ananas comosus is the most economically important in the family *Bromeliaceae*, which is divided into three subfamilies: *Pitcarnioideae*, *Tillandsioideae* and *Bromilioideae* (Malezieux & Bartholomew, 2003). The family *Bromeliaceae* consists approximately about 55 genera and 2,600 known species (d'Eeckenbrugge et al., 2011). The taxonomy of *Ananas comosus* as following (NRCS, 2018):

Kingdom	: Plantae – Plants
Subkingdom	: Tracheobionta – Vascular plants
Superdivision	: Spermatophyta – Seed plants
Division	: Magnoliophyta – Flowering plants
Class	: Liliopsida – Monocotyledons
Subclass	: Zingiberidae
Order	: *Bromeliales*
Family	: *Bromeliaceae*
Subfamily	: *Bromelioideae*
Genus	: *Ananas*
Species	: *comosus*

The family *Bromeliaceae* is able to adapt to a wide range of habitats ranging from terrestrial, to epiphytic, shady to full sun and from hot humid tropics to cold dry subtropics. They also can grow in moist to extremely dry situations and at varying altitudes from sea level to alpine conditions (Malezieux & Bartholomew, 2003). Members of this family are characterized by a short stem, narrow stiff leaves arranged in a circular cluster, terminal inflorescences, hermaphroditic and actinomorphic trimerous flowers. Fruits are capsules or berries that contain small naked, winged or plumose seeds, with a reduced endosperm and a small embryo (Malezieux & Bartholomew, 2003).

The subfamily *Bromelioideae*, is the most diverse and consists of the largest number of genera but the lowest number of species. Most members are epiphytes characterised by a rosette like form, with spiny leaves and berry-like fruit containing wet seeds (d'Eeckenbrugge et al., 2011). The genus *Ananas* is recognised among *Bromeliaceae* by the characteristic inflorescence, which is fused into a syncarp, a unique dense rosette of scape-wide leaves and medium to large fruits (Australia, Department of Health and

Ageing, 2008). Pineapple plants are set apart from other monocots by the unique characteristics star-shaped, scale-like multicellular hairs and the unusual conduplicate, spiral stigmas, which fold together lengthwise (Australia, Department of Health and Ageing, 2008; d'Eeckenbrugge & Leal, 2003; Gilmartin & Brown, 1987).

Pineapple can be grown once (a single cycle) or in one or more additional ratoon cycles (Souza & Reinhardt, 2007). The main morphological structures of pineapple plant involve the stem, leaves, peduncle, the multiple fruit, crown, shoots and roots (Figure 2.1). The plant has a short, thick stem around which grow narrow, rigid trough-shaped leaves and together with aerial roots (d'Eeckenbrugge et al., 2011). The root system is superficial and fibrous and generally grows no deeper than 30 cm and is rarely more than 60 cm from the soil surface (Souza & Reinhardt, 2007). Inserted into the stem is the peduncle which supports the flowers and the fruit. Vegetative propagules develop from auxiliary buds located in the earthed part of the stem (suckers) and the peduncle (slips), which are most used as planting material. The crown can also be used for planting when the fruit is processed (d'Eeckenbrugge & Leal, 2003).

The adult plant is 1-2 m high and wide, the leaf number is variable between cultivars but generally around 40-80 (d'Eeckenbrugge & Leal, 2003). The leaves are classified according to their position on the plant, as A, B, C, D, E, F, from the oldest on the outside, to the youngest towards the centre (Figure 2.2). The leaves originates from the mother plant (A) are smaller (5-20 cm) compared to younger ones (D) which can reach more than 1.6 m in length and seven cm in width depending on cultivars and ecological conditions (d'Eeckenbrugge & Leal, 2003). The most physiologically active is the 'D' leaf which is used to evaluate the growth and nutritional state of the plant (Souza & Reinhardt, 2007). It grows at 45^{o} to the soil surface and it presents its lower borders perpendicular to the base. The D leaf is also easily separated from the plant. Moreover, the D leaf can be

identified by gathering all the leaves in the hands to form a vertical "bundle" in the centre of the plant. The D leaves are the longest ones (Souza & Reinhardt, 2007).

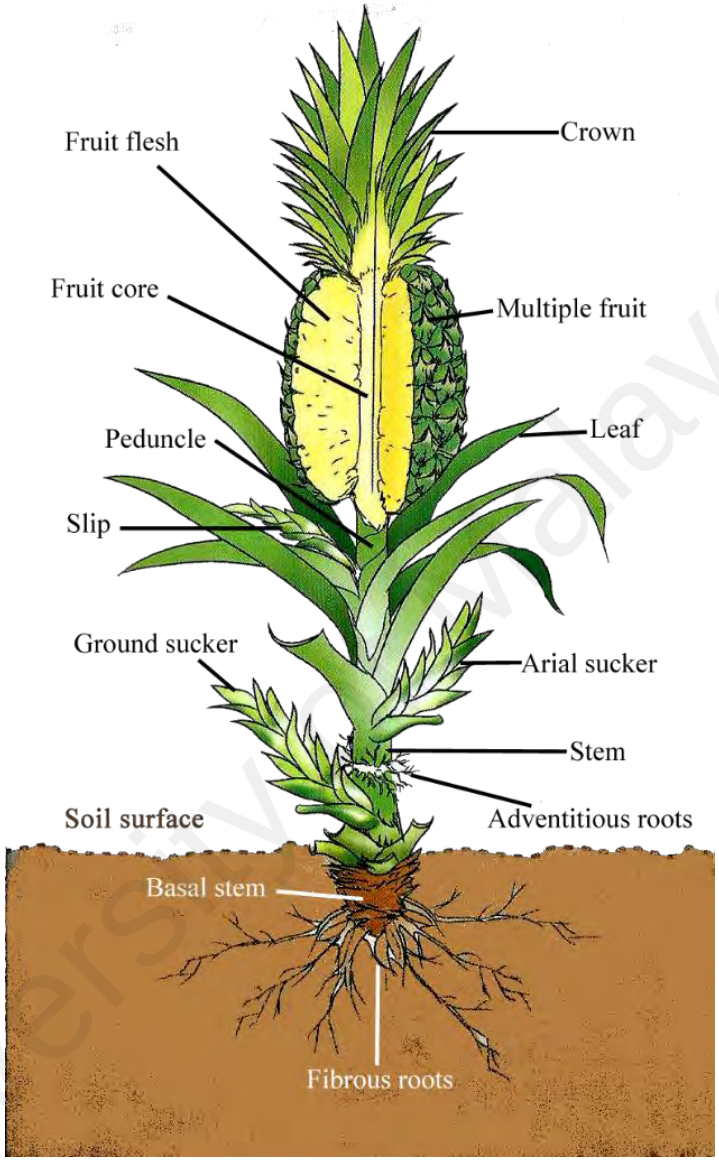

Figure 2.1: Morphological structure of pineapple. (MPIB, 2015b).

Leaves are semi-rigid, the adaxial side is concave while the abaxial side is convex, both sides are covered by peltate trichomes particularly the abaxial one, and leaf margins are usually thorny, however certain cultivars are partially or totally enormous (d'Eeckenbrugge & Leal, 2003). Pineapple plant also exhibit the crassulacean acid metabolism (CAM), characterized by assimilation of CO_2 and stomata opening

predominantly at night. Generally, the leaves rosette structure with thick cuticle, the water-storage tissue, the location of the stomata in furrows, the trichomes and the crassulacean acid metabolism (CAM) are important role in the water economy of pineapple plants (d'Eeckenbrugge & Leal, 2003).

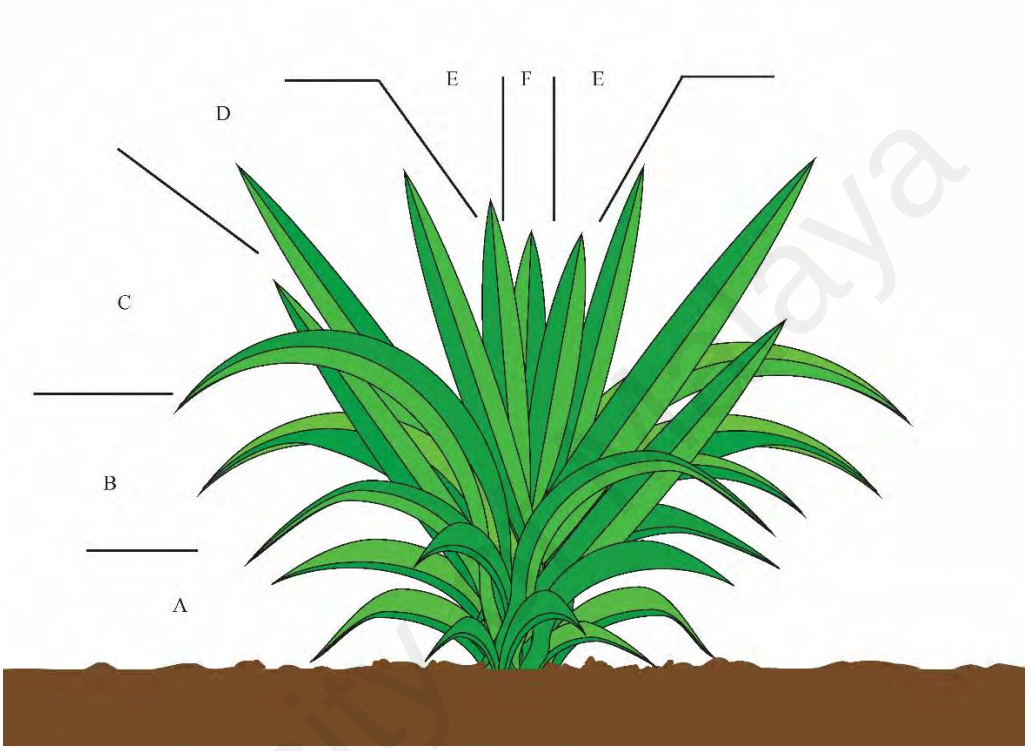

Figure 2.2: Classification of the pineapple plant leaves according to their age from A (oldest) to F (youngest).

In the apex of the stem, pineapple flowers are formed by the same meristem that initiates the leaves (Cunha, 2005). The stage of inflorescence emergence is called 'red heart' or 'red bud' because of the five to seven reddish peduncle bracts at its base (Figure 2.3A). These bracts are shorter and narrower than the ordinary leaves and connected with peduncle which elongates after flower formation. In many cultivars, a variable number of slips grow along the axis of the peduncle bracts or grouped just beneath the fruit (d'Eeckenbrugge & Leal, 2003). For instance, Yan Kee pineapple plants as shows in the Figure 2.3B. The pineapple inflorescence is formed by a group of sessile flowers that are connected with their subtending bracts and among each other around an axis – the core,

aligned in eight spiral rows, presenting an 8/21 phyllotaxy in large-fruited cultivated pineapple (Cunha, 2005; d'Eeckenbrugge & Leal, 2003).

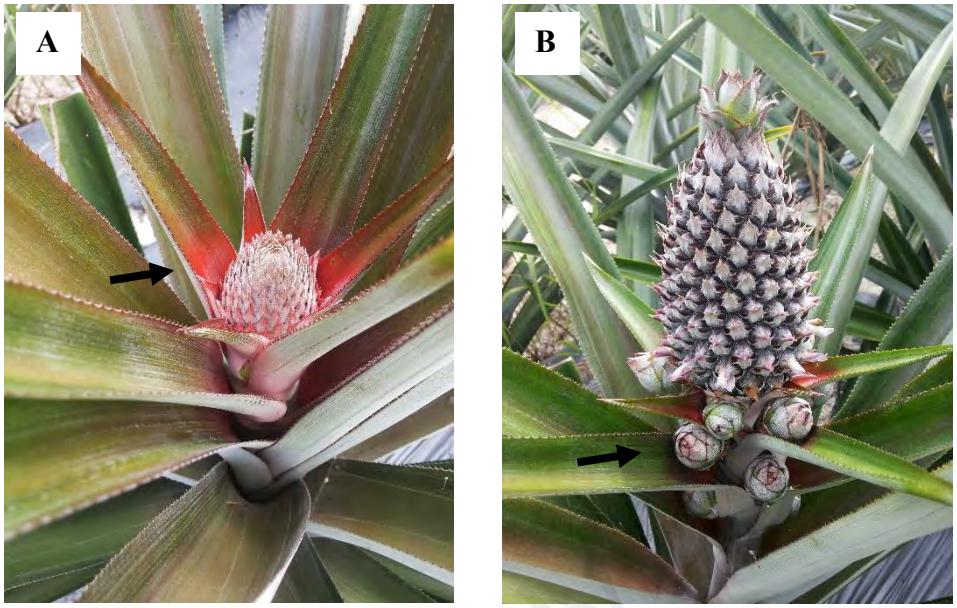

Figure 2.3: The arrow shows (A) reddish bracts and (B) slips of Yan Kee pineapple.

2.2.2 Cultivars of *Ananas comosus*

There are approximately 30 cultivars of *A. comosus* that are commercially grown around the world. The numerous pineapple cultivars are grouped in four main classes despite much variation in the types of each class for convenience in global trade which are 'Smooth Cayenne', 'Red Spanish', 'Queen', and 'Pernambuco' (Abacaxi) (d'Eeckenbrugge et al., 2011). 'Smooth Cayenne' with non-spiny leaves is the most widely grown before 'MD2' is introduced. 'Queen' is grown in South Africa and Australia, 'Cabezona' in Puerto Rico, and 'Sugar Loaf' in the Bahamas (Fernandez & Pomilio, 2003). In Malaysia, pineapple also is one of the major commercial fruits after banana. Some of the varieties of pineapple that cultivated in Malaysia are described in the following section.

2.2.2.1 Golden ripe

The hybrid MD2 (Figure 2.4) were developed by Del Monte Fresh Produced Hawaii Inc. from a cross made between the PRI hybrids 58-1184 and 59-443 for the fresh-fruit market. MD2 pineapple gives a medium to large (1.3 to 2.5 kg), cylindrical, square-shouldered fruit, with large flat eyes and an intense orange-yellow colour. The clear yellow pulp is sweet, compact and fibrous. It has high in sugar (15-17 °Brix) and ascorbic acid. The core is edible, tender and thinner compared to Smooth Cayenne. However, the crown is larger and must be broken at harvest. The leaves are mostly spineless and yellow-green in colour with a reddish tip (Malezieux & Bartholomew, 2003).

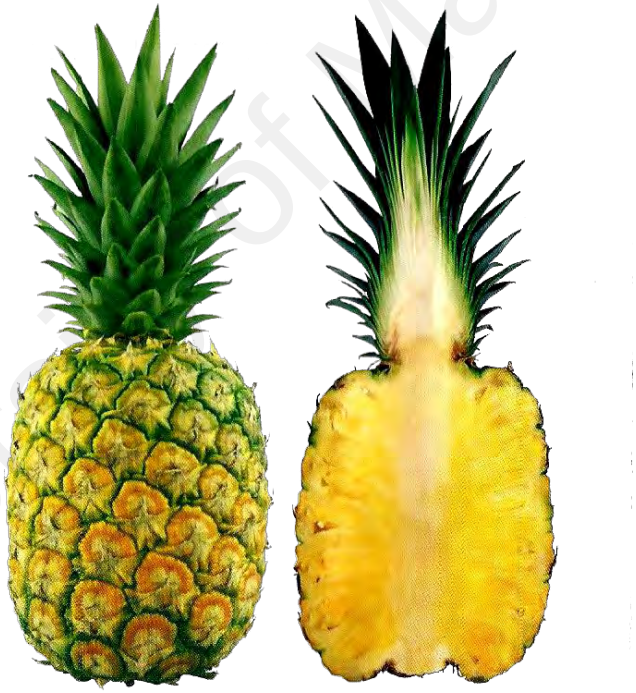

Figure 2.4: MD2 pineapple (MPIB, 2015b).

In the study by Wardy et al. (2009), it was shown that MD2 variety has a great potential in the horticultural industry compared to Sugar Loaf and Smooth Cayenne. The MD2 variety is in high demand due to its good quality characteristics which are blemish-free flesh, uniform fruit size with golden yellow pulp and a very pleasant aroma when ripe

with a longer shelf life. Furthermore, it has low acidity with 0.4-0.45% (Malezieux & Bartholomew, 2003).

2.2.2.2 Sarawak

Sarawak pineapple (Figure 2.5) is also known as the Smooth Cayenne. The fruits weigh about 1.5-2.5 kg with cylindrical shape and 100-160 shallow eyes. The flesh is yellow and juicy, and when totally ripen, it is rich in sugars (13-19% Brix), with acidity level higher than other cultivars (Maeda et al., 2011).

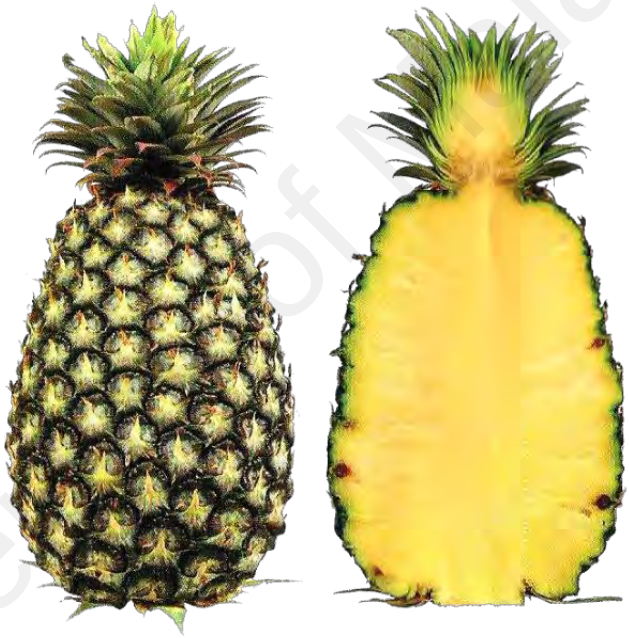

Figure 2.5: Sarawak pineapple (MPIB, 2015b).

2.2.2.3 Josapine

Josapine pineapple (Figure 2.6) is a hybrid pineapple between Johor (Singaporean Spanish x Smooth Cayenne) and Sarawak (Smooth Cayenne) that was developed by the Malaysian Agricultural Research and Development Institute (MARDI), Malaysia in August 1996 (Shamsudin et al., 2009). It fruits very early (120 days after flowering was induced) and this allows it to cultivate annually. The plant is vigorous and produces two

to three shoots. Leaves are lightly purple-tinged, usually with spineless margins except for the leaf tip. Crowns are medium, occasionally with multiple proliferation. Fruits weight between 1.1 and 1.3 kg, cylindrical-shaped with dark purple peel ripening to an attractive orange-red. The flesh is deep golden-yellow and has a strong aroma and a sugar content between 17 °Brix on peat soils and 22 °Brix on mineral soils. It has a good storage life and are resistant to black-heart disorder or internal browning caused by low temperatures (Malezieux & Bartholomew, 2003).

Figure 2.6: Josaphine pineapple (MPIB, 2015b).

2.2.2.4 Moris

Moris pineapple (Figure 2.7) is quite common, all-purpose variety found everywhere, all year round and also known as ‘Mauritius’ or ‘Queen’. It has an elongated shape with small, prominent eyes that require a thick cut to be removed. When it is bitten into, it has a crisp crunch, unique flavour and sometimes sharp taste. This highly fibrous fruit is commonly used for jam making, cooked in curries or tossed in a rojak. Newer and sweeter varieties have made this less popular as an eating pineapple (Lee, 2010, June 20).

Figure 2.7: Moris pineapple (MPIB, 2015b).

2.2.2.5 Yan Kee

Yan Kee pineapple (Figure 2.8) is in the group of Queen pineapple. The Yan Kee reflects out for its rather odd shape. The long and tapering pineapple is planted in Klang and Sungai Pelek and is also known as the Selangor Sweet. The flesh is pale and translucent when entirely ripe. Its eyes are not deeply embedded and requires only shallow cuts in order to remove them. The Yan Kee has the highest juiciness level compared to other type and is the least fibrous of the pineapples and is very sweet. Its elegant, sweetish fragrance is reminiscent of melon. The season for Yan Kee is May-June and Oct-Nov (Lee, 2010, June 20).

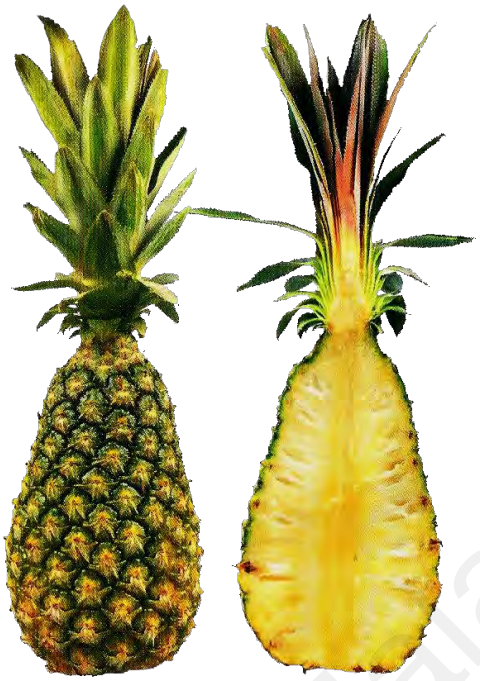

Figure 2.8: Yan Kee pineapple (MPIB, 2015b).

Table 2.1: Summary of pineapple cultivar widely cultivated in Malaysia (Medina & Garcia, 2005; MPIB, 2015b).

Cultivar	MD2	Sarawak	Josapine	Moris	Yan Kee
Group	Hybrid	Cayenne	Hybrid	Queen	Queen
Shape	Cylindrical	Tapering	Cylindrical	Tapering	Tapering
Weight range (kg)	1.3 – 2.5	1.5 – 2.5	1.0 – 1.3	0.8 – 2.0	1.0 – 2.1
Number of eyes	120 – 160	100 – 160	120 – 140	100 – 120	100 – 120
Peel colour	Bright green	Green-orange	Dark purple to orange-red	Dark green	Light green
Flesh colour	Golden yellow	Yellow	Golden yellow	Yellow	Clear white
Total soluble solid (°Brix)	12 – 19	14 – 17	16 – 17	12 – 14	11 – 14
Acidity (%)	0.05 – 0.3	0.3 – 1.2	0.5 – 1.2	0.4 – 0.7	0.6 – 0.8
Number of shoots	Two – three suckers, rarely have slips	Three-four suckers	Two – three suckers	Two or more suckers, robust slips	Robust slips
Another name	Golden ripe, Super sweet	Smooth Cayenne, Kew	-	Mauritius, Victoria, Sarikei	Selangor Sweet

2.2.3 Pineapple Cultivation

The pineapple plants growth cycle can be divided into three phases which are vegetative phase (involves the period from planting to floral differentiation), reproductive phase (flowering and fruiting) and propagative phase (begins at the productive phase, but continues after the fruits is harvested and also involves the development of slips and suckers until their harvest for planting) (Cunha, 2005). There are several factors that can influence the growth of pineapple plants such as weight of planting material, temperature, soil pH, irrigation, mineral nutrition, and field managements (drainage, weeds, pesticides, etc.). For commercial production, the common practices of pineapple cultivation usually use suckers as a planting material (Figure 2.9A) because of their size which shorten the crop cycle, will produce fruit in twelve to fourteen months after planting.

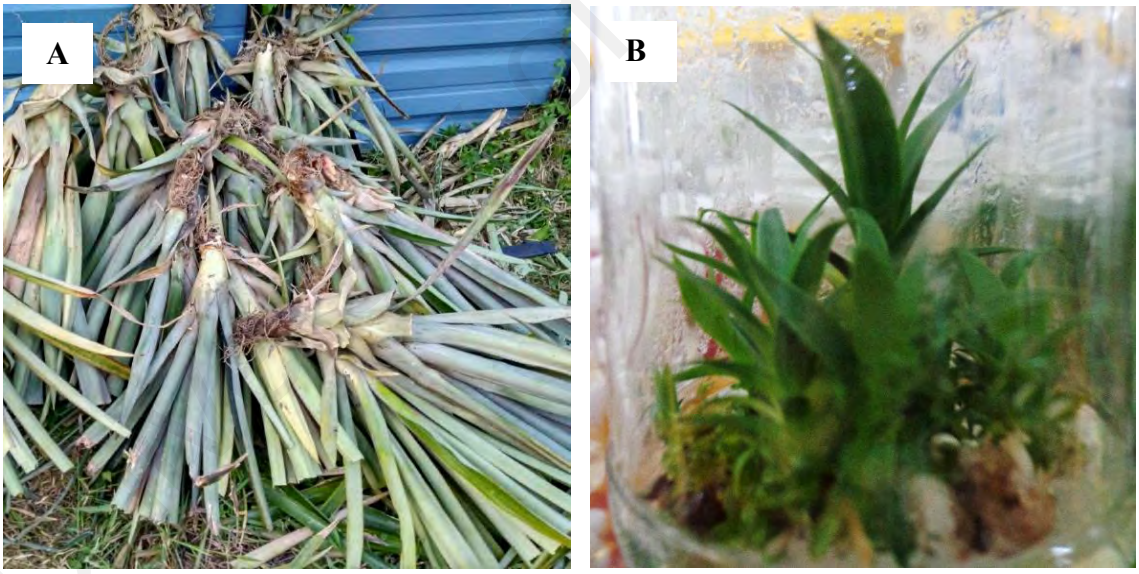

Figure 2.9: (A) Suckers and (B) tissue-cultured plantlets of MD2 pineapple plant.

However, for some of pineapple variety (e.g. MD2), the quantity of propagules was not enough and expensive to accommodate the demand for planting materials whereby 43000 propagules per hectare required for planting density of 30 x 60 x 90 cm. Single mother plant of MD2 pineapple only produced two-three propagules per cycle (18-20 months). Therefore, researchers have tried to improve the multiplication rates, whereby

this has led to the development of tissue culture technique of pineapple plants (Figure 2.9B). Some of the pineapple cultivars that have been successfully propagated through tissue culture include the MD2 (Danso et al., 2008; Hamid et al., 2013), Giant Kew (Amin et al., 2005), Smooth Cayenne (Usman et al., 2013), Josapine (Chan & Lam, 2002) and Maspine (Zuraida et al., 2011). Based on the research by Hamid et al., (2013) on MD2 pineapple plant, an average of five plantlets per explant was produced in just one month of cultures in the initiation media, which then further multiplicate in shoot multiplication media producing three-fold number of shoots. Thus, *in vitro* micropropagation approaches able to produce large number of propagules in the short of time and this might be a solution to the shortage of pineapple plantlets problem for commercial production. The usage of *in vitro* plantlets as planting material also can minimize the hectic earlier 'natural flowering' occurrence as well as reduce the pesticide used to plantlets prior to planting (Zuraida et al., 2011). However, this planting material is still not widely grown by farmers.

Furthermore, pineapple plant grows best and produces better fruits when grown at temperatures ranging from 22 to 32 °C (Souza & Reinhardt, 2007). At temperature above 32 °C the plant grows less well and if associated with solar radiation can burn the fruit during maturation phase (Malezieux & Bartholomew, 2003). Pineapple is well adapted to growing on acidic soils, a pH of 4.5 to 5.5 is ideal as iron in the soil become more readily available to the roots of plants (Lobo & Siddiq, 2017). Higher values can limit the availability of micronutrients (zinc, copper, iron and manganese) and favour the development of harmful microorganisms, like fungi of the genus Phytophthora, especially under wet conditions. However, the plant tolerates a high exchangeable aluminium and manganese content in the soil, a condition favoured on highly acidic soils (Souza & Reinhardt, 2007).

For water requirement, pineapple plants need less water than the vast majority of cultivated plants due to its morphological and physical characteristics (Souza & Reinhardt, 2007). Pineapple plants can withstand drought very well because of their stomata and use of the CAM pathway for photosynthesis (Lobo & Siddiq, 2017). It also has the capacity to store water in the hypoderm of the leaves, to collect water efficiently, including dew, in its through-shaped leaves also reduce transpiration by several mechanisms (Souza & Reinhardt, 2007). However, the greatest yield and the best fruit quality are obtained when the crop is well supplied with water (Souza & Reinhardt, 2007). A well distributed annual rainfall (839-1742 mm) and high relative humidity is important for optimal growth (Lobo & Siddiq, 2017). The pineapple's demand for water varies from 1.3 to 5.0 mm/day, depending on its state of development and on soil moisture (Souza & Reinhardt, 2007).

A good drainage is a basic requirement because they favour root development and reduce the risk of plant loss from fungal pathogens of genus Phytophthora (Souza & Reinhardt, 2007). Pineapple plant roots tend to be concentrated in the top 15 to 20 cm of soil (Souza & Reinhardt, 2007). However, the soil texture and density inhibit root growth and can prevent the roots from growing deeper (Py et al., 1987). Land that is flat or has a slope not exceeding 5% is preferred because it less susceptible to erosion which can be a serious problem because pineapple has a shallow root system (Souza & Reinhardt, 2007).

Weed competition is very harmful especially during first five to six months after planting (between planting and flower differentiation stage) due to slowly growth of pineapple plant and their superficial root system (Souza & Reinhardt, 2007). Therefore, the crop should be kept clean around this period, but during the fruit formation phase the presence of the weeds has practically no negative effect on the production (Souza & Reinhardt, 2007). The uncontrolled weeds will reduce the uptake of mineral nutrients,

water and sunlight where negatively effect to the growth, yield and quality of pineapple plants (Australia, Department of Health and Ageing, 2008; Eshetu et al., 2007). Some weeds commonly found in pineapple fields are *Digitaria sanguinalis*, *Dactyloctenium aegyptium*, *Paspalum conjugatum*, *Mimosa pudica* and *Echinochloa colona* (MPIB, 2015c). The weeds can be controlled by using herbicides such as diuron, ametryn, atrazine and bromacil or using soil surface mulching (MPIB, 2015c; Souza & Reinhardt, 2007). In addition, weeds are potential hosts for pests and viruses (Malezieux & Bartholomew, 2003).

There are a few insects pose a serious threat to pineapple cultivation, the Ant (*Solenopsis* sp. or *Araucomyrmex* sp.), the Mealybug (*Dysmicoccus brevipes*), the Pineapple Scale (*Diaspis bromeliae*) and the Red Mite (*Stigmacus floridanus*) (MPIB, 2015c). Mealybug colonies are tended by ants due to sugary secretion by them, the ants make a shelter of soil around the mealybugs and responsible to disperse the mealybug to other crops (Joy et al., 2013). Mealybugs first appear on roots, also found on the aerial parts of the plants, mainly in the leaf axils and on the developing fruit by feed on plant sap in the phloem of their host plants causing the plant to wilt (Joy et al., 2013). Mealybugs spread can be minimized by destroying the ant colonies by direct spraying their location using five percent malathion (MPIB, 2015c).

The floral differentiation of pineapple plants can be naturally induced or triggered artificially by using chemical substances such as 2-chloroetilfosfonic (ethephon), ethylene (C_2H_4) and calcium carbide (CaC_2) into the centre of the leaf rosette (Souza & Reinhardt, 2007). The addition of urea at concentration of two to three percent increases even more efficiency of the artificial induction (Cunha, 2005). The age or size of the plant is related to susceptibility of the pineapple plant to natural or artificial flowering where largest plants are more susceptible (Cunha, 2005). However, the successfulness of

inducing the pineapple flowering also related with daylength, temperature (26-28 °C), solar radiation (preferably during cloudy days or before 8:00 and after 17:00) and also hormones produced by the own plant (auxins) (Cunha, 2005; Souza & Reinhardt, 2007).

2.2.4 Postharvest Handling of Pineapple Fruits

During fruiting, it is crucial to cover the fruits to protect them from sun burn/damage (Malezieux & Bartholomew, 2003). As the fruit matures, the individual fruitlets slowly changes the colour from green to yellow which is a good indicator of fruit maturity (Malezieux & Bartholomew, 2003). The fruitlets mature progressively from the bottom part of the fruit to the top and become flatter (Joy & Rashida Rajuva, 2016). Once the fruit has been harvested, the amount of yellowing and change in surface colour should not be used as an indicator of fruit ripeness, since postharvest colour changes are not correlated with eating quality (Joy & Rashida Rajuva, 2016). The pineapple stops ripening when picked, only two components of quality that may changes after harvest which are external colour and texture of the flesh that soften over time (Joy & Rashida Rajuva, 2016). The ripening index as shown in Table 2.2.

Pineapple is a highly perishable fruit, therefore its important in determining the quality of the fruit and harvest times since pineapple cannot be store for long time (Australia, Department of Health and Ageing, 2008; Mirza et al., 2016). Prior to harvesting, sugar content should be assessed to ensure adequate sugar development since it not related with the fruit colour changes which more affected through agronomic and production factors (Joy & Rashida Rajuva, 2016). A minimum soluble solids content of ~12% and a maximum acidity of 1% will assure a baseline flavour that is acceptable to consumers (Lobo & Siddiq, 2017). The time of harvesting of pineapple depends on whether for domestic or international market. Other fruit quality indices include uniformity of size and shape; firmness; absence of decay; freedom from sunburn, cracks, bruising, internal

breakdown, brown spots, gummosis and insect damage and crown leaves should be green, medium length and erect (Australia, Department of Health and Ageing, 2008; Joy & Rashida Rajuva, 2016).

Table 2.2: The ripening index of pineapple fruits (MPIB, 2015c).

Ripeness Index	Fruit Colour	Descriptions
1	Dark green	Fruit is unripe and not suitable for fresh consumed. Firm flesh and high acidity.
2	Green with a little bit yellow	Fruit is almost matured and suitable for a long transportation journey through ships. Firm flesh with high acidity.
3	More green than yellow	Fruit is fully matured and suitable for a long journey through air shipping. Firm flesh, percentage of soluble solid increased while acidity decreased.
4	25% yellow	The fruit is almost ripe. Only suitable for the local market and fresh eaten. Firm and juicy flesh. High percentage of soluble solid.
5	50% yellow	The fruit is ripe. Only suitable for the local market and the best stage to be eaten freshly. Less firm and juicy flesh. High percentage of soluble solid.
6	75% yellow	The fruit is ripe. Only suitable for the local market. Soft flesh and juicier. Percentage of soluble solid decrease.
7	100% yellow	The fruit is overripe. Not suitable for the local market. Soft flesh with too much juice.

The pineapple is usually hand harvested by breaking or cutting the stalk a few centimetres below the fruit (Joy & Rashida Rajuva, 2016). Once harvested, fruit are prone to sunburn and therefore should be placed in shaded condition (Australia, Department of Health and Ageing, 2008). The main problems that affect pineapple fruit quality are

damage due to bruising during loading, transportation, unloading and conveying (Australia, Department of Health and Ageing, 2008). While stacking the fruits, the fruits are arranged in an upside down position where the crowns act as cushioning material to prevent the fruit injuries or bruises (Mirza et al., 2016). The transporting vehicle should allow for a good air circulation and protection of the produce from sunlight, in the absence of refrigeration, the fruit should be transported during the cooler part of the day (Joy & Rashida Rajuva, 2016).

Before the storage, the fruits should be soaked in water to reduce the temperature and wash with chlorinated water to remove the dirt, dust and live insect by thoroughly scrubbed on the fruit surface with a brush (Joy & Rashida Rajuva, 2016). As for fruit storage conditions, the fruits should be refrigerated at temperatures between 8 to 10 °C and 85 to 90% relative humidity, as soon as possible after harvested (Medina & Garcia, 2005; Mirza et al., 2016). Under these conditions, fruit with quarter-yellow ripening stage have a shelf life of approximately three to four weeks while at ambient temperature (30-32 °C) will result in a shortened shelf life as short as only a few days (Joy & Rashida Rajuva, 2016; Lobo & Siddiq, 2017; Medina & Garcia, 2005). However, the fruits are prone to get a chilling injury if stored below 7 °C (Medina & Garcia, 2005). Internal flesh browning, translucent or water-soaked flesh, incomplete colour development, wilting of crown and peel are typical symptoms of chilling injury (Lobo & Siddiq, 2017; Mirza et al., 2016).

2.2.5 Benefits of Pineapple

The pineapple was originally cultivated for consumed as a fresh fruit, however after development of processing industry, the fruits are now available in various forms especially canned fruit, juices, syrups and jams. Pineapple also is used as an ingredient in a variety of foods including pizzas, condiments, sweets, savouries, cakes, pastries, yogurt,

punches, ice creams etc. (Malezieux & Bartholomew, 2003). In the Philippines, high quality fibres were produced from pineapple leaves and were used to manufacture luxury clothing known as the 'pina cloth'. The fibre from stem and leaves also can be processed for making pulp in the paper industry (Lobo & Siddiq, 2017). As the innovative solution to the pineapple waste, the parts of the pineapple plant are used for silage and hay for livestock feed include the outer peel, the central core, and crown from canning industries (Joy & Rashida Rajuva, 2016).

Pineapple contains the proteolytic enzyme bromelain derive from the juice and stem of pineapples, which is used as a meat tenderising agent, baking processes, prevention of browning of pineapple juice and for medicinal purposes (Mirza et al., 2016). It has been reported to have valuable biological properties such as interfering with the growth of malignant cells, inhibiting plantlet aggregation, anti-inflammatory action after surgery, anti-thrombotic, fibrinolytic activities, reduction of swelling with physical injury and as a treating aid for digestive problem (Joy & Rashida Rajuva, 2016; Mirza et al., 2016). Moreover, the fruit is a good source of Cu, Mn, Ca, Zn, β-carotene, dietary fibre and contains significant amounts of vitamins A, BI, B6 and C (Australia, Department of Health and Ageing, 2008; Lobo & Siddiq, 2017).

2.3 Classification of Plant Nutrients

Plant acquire a several amounts of essential and non-essential nutrients in the growing medium for plant growth and reproduction (Barker & Pilbeam, 2007; Mitra, 2017). These elements are called essential since short supply of the nutrient affected the plant metabolism and growth, however excessive concentrations become toxic to the plant (Mitra, 2017). Their deficiency symptoms can be observed by raw eyes such as necrosis of leaf tips, but it also may present as symptoms of toxicity of another element (Mitra,

2017). However, this can be corrected by application of fertilizers. Plant nutrients are classified into two categories which are macronutrients and micronutrients.

2.3.1 Macronutrients

Macronutrients are applied in larger quantities to the plants which consists of structural elements (C, H, O), primary nutrients (N, P, K) and secondary nutrients (S, Ca, Mg) (Mitra, 2017). C, H and O are absorbed from the air or water by the process of photosynthesis, whereas other nutrients are typically obtained from the soil or nutrient solutions (Barker & Pilbeam, 2007). In the pineapple crop, the plant taken up the macronutrients in the following order; K > N > S > Ca > Mg > P (Pegoraro et al., 2014).

2.3.1.1 Nitrogen

Surface soils generally comprise 0.03-0.4% of total N, while 95% is in the organic form (Mitra, 2017). Plants taken up the N as nitrate (NO_3^-) or ammonium, (NH_4^+) ions but since NH_4^+ is toxic, it's not allowed to accumulate within the plants (Mitra, 2017). Urea and ammonium sulphate are the most common sources of N to the pineapple crop while other sources of N, such as potassium nitrate, ammonium nitrate as well as organic fertilizers can also be used (Souza & Reinhardt, 2007). In pineapple plants, nitrogen is required second after potassium especially during vegetative stage for the pineapple plant growth development. Based on the research, pineapple treated with 200 kg Nha^{-1} were 45% taller, increased in the D-leaf length, 26% higher in number of leaves and also resulted in the highest leaf area (242.36 cm^2) than untreated (control) plants (Omotoso & Akinrinde, 2013).

N is a component of amino acids, proteins, purine and pyrimidine rings of nucleic acids chlorophyll and enzymes. All of these compounds are involved in plant growth and metabolism; protein synthesis and chlorophyll synthesis (Barker & Pilbeam, 2007; Mitra,

2017). Symptoms of N deficiency appear as a result of the disruption of these processes. This appears first as yellowing of older leaves due to impaired photosynthesis and degradation of chloroplasts while growing leaves remain green (Mitra, 2017). Deficiency of N concentration in soils, poor in organic matter and under hot and sunny conditions show symptoms with a slow plant growth, small and narrow leaves, small and colourful fruit with a small crown and the absence of plantlets (Souza & Reinhardt, 2007).

In respect of fruit quality, study conducted have shown N deficiency decreased the fruit weight with crown of Jupi pineapple by 57.59%, the fruit length by 38.7%, and the fruit diameter by 22% (Ramos & da Rocha Pinho, 2014). However, the excess of N supplied on the plant during flowering also reduced the fruit weight. Average fruit weight per plant increase with N application levels up to 150 kg ha^{-1} but declined when supplied with 200 kg ha^{-1} (Omotoso & Akinrinde, 2013). N deficiency also reduced the peduncle diameter by 16.7% whereby the small diameter of peduncle may contribute to increase pineapple fruit drop and high percentage to get fruit sunburn (Ramos & da Rocha Pinho, 2014).

The percentage of acidity of fruit juice and total soluble solids (TSS) increased as N fertilizer rates decreased. The study has shown increased in titratable acidity in 85% and in the TSS in 11.2% with N deficiency (Ramos & da Rocha Pinho, 2014). Similar results were obtained by Omotoso & Akinrinde (2013), 40.1% reduction of fruit juice acidity relative to control as N fertilizer rates increased and the highest TSS was obtained in the plant crops that received 50 kg Nha^{-1}. They also reported that the significant decrease in TSS and Vitamin C with the highest N application level (200 kg Nha^{-1}) (Omotoso & Akinrinde, 2013). Ramos & da Rocha Pinho (2014) reported that an increased in the content of vitamin C by three-fold and reduction in pH by 18.7% in N deficiency compared to the fruit supplied with complete nutrient solution. Therefore, in order to

obtain a high yield and good quality of the fruits, nitrogen should not be applied excessively during flowering. However, during vegetative stage, pineapple plants need high N for vigorous growth.

2.3.1.2 Phosphorus

Total P in surface soils varies from 0.005 to 0.15%, 50% of it in organic form. P is primarily taken up by plants in the forms of phosphate ions such as HPO_4^{2-}, $H_2PO_4^-$ and PO_4^{3-} based on the pH of rhizosphere (Mitra, 2017). The main sources of P are fertilizers soluble in water such as single superphosphate, triple superphosphate, monoammonium phosphate and diammonium phosphate with 10-12% S (Souza & Reinhardt, 2007). Besides, magnesium thermophosphates (17% P_2O_5) also have been used as sources of P and Mg (nine % Mg). Adequate phosphorus in soil enhances the fundamental process of plants such as nitrogen fixation, photosynthesis, flowering, fruiting, seed production and also improves the development of both shoot and root systems (Orluchukwu & Adedokun, 2015).

There is a relatively small demand of P in pineapple plants. However, deficiency in P affect the plant growth such as causes delayed leaf development, reduction in number of leaves, more erect, long and narrow leaves, decreased photosynthetic capacity, stunted growth (reduced auxiliary shoot emergence and elongation), impaired flower development and roots with longer and less ramified hairs (Mitra, 2017; Souza & Reinhardt, 2007). Under condition of P deficiency, the roots are modified suitably to explore a larger volume of soil so as to absorb more P to meet the P-demand of plants (Mitra, 2017). Enhanced uptake of P by roots and translocation to shoots results in excess P accumulation in older leaves and may cause chlorosis and necrosis of leaf tips due to P-toxicity (Mitra, 2017).

A common visual deficiency symptom of phosphate in plants include dark bluish-green or purple shoot due to anthocyanin accumulation, which is more pronounced in the presence of excess N (Mitra, 2017; Souza & Reinhardt, 2007). Moreover, the old leaves have completely dry out at the tips, brown transverse striations and border of these leaves turn yellow from the tips (Souza & Reinhardt, 2007). This is because of enzymes in synthesis of anthocyanins reduced which protects nucleic acids from UV damage and chloroplast from photo-inhibitory damage (Mitra, 2017). Plant suffering from P deficiency also have small fruits and reddish in colour (Souza & Reinhardt, 2007).

2.3.1.3 Potassium

K content of soils is in the range of 0.5-2.5% (Mitra, 2017) and the largest amount accumulates in the plant (Souza & Reinhardt, 2007). However, the exchangeable K/Mg in the soil should be between 0.25 and 0.33 but not greater than 1.0. The concentration of K larger than Mg because of the antagonism between these nutrients in root uptake (Souza & Reinhardt, 2007). When given increasing amounts of K, a decrease in the concentrations of Ca and Mg was observed in the 'D' leaf of cultivar 'Perola', indicating competition for uptake by these nutrients (Souza & Reinhardt, 2007). K can be applied as potassium chloride (KCl), potassium sulfate (K_2SO_4), K and magnesium bisulfate (20% K_2O) and potassium nitrate (44% KNO_3).

K activates about 60 enzymes involved in various metabolic processes, such as photosynthesis, protein synthesis, oxidative metabolism and improves quality and stress tolerance of crops (Mitra, 2017). K also influences the crop productivity, although to a lesser extent than that of N (Souza & Reinhardt, 2007). The large demand of K concentration in pineapple plants often results in the symptoms of K deficiency, especially in soils with low K availability. The small yellow spots on leaves which increase in size, multiply and coalesce around the leaf borders, then dry out can be observe

visually on plant with K deficiency (Souza & Reinhardt, 2007). The plant also has more erect stature, a weak fruit peduncle, small fruit with low acidity and less aroma (Souza & Reinhardt, 2007).

2.3.1.4 Calcium

Ca is the fifth most abundant element and constitutes about 3.5% of the earth's crust. Most of the soils are moderate to rich in Ca^{2+} (0.7-1.5%), except strongly acidic tropical soils (0.1-0.3%) and calcareous soils in arid and semi-arid regions (1-30%) (Mitra, 2017). Plants have been classified according to their Ca^{2+} requirements: "Calcifuges", which grow in acid soils with low Ca^{2+} and capacity to tolerate toxic concentrations of Fe, Al and Mn (*Crassulaceae*, *Brassicaceae* and *Fabaceae*); 'Calcicoles', which grow in calcareous soils with high Ca^{2+} and tolerance to deficiency of P and Fe (*Apiales* and *Asterrales*) (Mitra, 2017).

Ca regulates various fundamental process such as cytoplasmic streaming, thigmotropism, gravitropism, cell division, cell elongation, cell elongation, cell differentiation, cell polarity, photomorphogenesis and plant defence and stress response (Mitra, 2017). Ca deficiency symptoms in the pineapple plants were described to have very small, short, narrow and breakable leaves and very short internode spacing but it occurs rarely, except in very poor soils (Souza & Reinhardt, 2007).

2.3.1.5 Sulphur

S is an essential plant nutrient and is considered as the fourth major nutrient after N, P and K. S supply is normally done by fertilizers that are at the same time sources of some of the primary macronutrients, like ammonium sulphate (23 to 24% S), potassium sulphate (17 to 18% S) and single superphosphate (10 to 12% S) (Souza & Reinhardt, 2007). However, application of S-fertilizers in optimum doses on clay minerals does not

have any residual effect since clay minerals do not bind sulphate and it is leached out of soil (Mitra, 2017).

S is a constituent of amino acid, cysteine and methionine, which are involved in maintaining protein structure and conformation, response to abiotic and biotic stress and plays an important ecological role in defence against herbivores and pathogens (Mitra, 2017). The deficiency symptoms of sulphur on plants were described as pale yellow to gold coloured foliage, pinkish leaf borders especially on older leaves, normal plant stature and very small fruit (Souza & Reinhardt, 2007). However, the occurrence of such symptoms was considered very rare in pineapples (Malezieux & Bartholomew, 2003).

2.3.1.6 Magnesium

Earth's crust contains about 1.93% of Mg. The Mg content of soil may vary from 0.1% coarse-textured humid soils to 4% in fine-textured soils from aid or semi-arid region (Mitra, 2017). Mg^{2+} ions unlike Ca^{2+} are more susceptible to leaching since they are not as strongly adsorbed to clay minerals or organic matter due to their large hydrated radius (Mitra, 2017). Therefore, Mg application is efficient using liquid (spray) form than applied in solid. For foliar sprays the concentration of magnesium sulphate in the solutions can vary between 0.5% and 2.5% (Souza & Reinhardt, 2007).

Mg^{2+} acts as cofactor of many enzymes, such as RNA polymerase, ATPases, protein kinases, phosphatases, carboxylases and glutathione synthetase whereby it was required for aggregation of ribosomes and is the central atom of chlorophyll molecule (Mitra, 2017). Plants with Mg deficiency have yellow old leaves, except were shaded by younger leaves when they stay green; yellow spots which turn brown in a controlled environment; drying of older leaves, which have not finished growing before the deficiency appears;

fruit without acidity, low in sugar content and without any flavour (Souza & Reinhardt, 2007).

2.3.2 Micronutrients

Micronutrients are essential elements to the plants at low concentrations but become toxic beyond a threshold concentration such as Zn, Fe and B (Mitra, 2017). Based on pineapple plants, the accumulation of micronutrients by the following decreasing order of uptake: Mn > Fe > Zn > B > Cu > Mo (Souza & Reinhardt, 2007).

2.3.2.1 Zinc

The Zn content of soil is within a range of 10-300 ppm (Mitra, 2017). Zn deficiency tends to be rare and is generally observed in low land soils, high pH due to excessive limestone application and calcareous soils (Mitra, 2017; Souza & Reinhardt, 2007). The deficiency symptoms in young plants are the centre of the leaf rosette is closed, rigid and cracked young leaves while in old plants, the basal leaves contain irregular veins, with the appearance of marble, and orange-yellow discoloration on the leaf borders and the tips are dry (Souza & Reinhardt, 2007).

2.3.2.2 Iron

Fe constitutes about 5% of earth's crust and most of the soils around the world are rich in iron (Mitra, 2017). Fe in soil is present in the form of an amorphous Fe $(OH)_3$ precipitate, which is the immediate source of Fe uptake by plants (Mitra, 2017). Fe can be applied as solutions of their salts, e.g. $FeSO_4.7H_2O$ which contain 20% Fe (Souza & Reinhardt, 2007). However, availability of Fe to plant roots depends on redox potential and pH of the soil (Mitra, 2017). Plant tissue concentration of one to five µM Fe is considered adequate and a concentration below one µM is likely to cause deficiency

(Mitra, 2017). Fe deficiency can occur under a range of conditions such as on soil with high pH, compacted soils, area of termite infestation and attack by pests which rapidly decline the root activity and also drought (Souza & Reinhardt, 2007).

The symptoms of Fe in plants results in development of chlorosis, starting in young leaves; the leaves are generally flaccid, wide and yellow with a green 'web' of conductor veins; dry old leaves and produced red fruit with a chlorotic crown (Souza & Reinhardt, 2007). When sprayed with large amounts of iron on the leaves, the green transverse lines can be observed (Souza & Reinhardt, 2007). A concentration above 10 µM may cause toxicity with reduction of growth parameters (Mitra, 2017). However, these limits may vary considerably among different plant species and their genotypes (Mitra, 2017).

2.3.2.3 Boron

B occurs in earth's crust in most of the igneous rocks at a concentration less than 10 µg g^{-1} and total B concentration in soils is mostly around seven to 80 µg g^{-1} whereby about 95% of soil B is not available to plants (Mitra, 2017). Availability of B decreases with an increase in pH above 6.3-6.5 and when due to drought (Mitra, 2017; Souza & Reinhardt, 2007). Furthermore, liming acid soils may cause temporary B deficiency since high Ca^{2+} concentration in alkaline soils or recently over limed soils negatively affects B availability (Mitra, 2017). Moreover, supplementation of higher doses of K-fertilizers may also affect B availability in the soils (Mitra, 2017). H_3BO_3 is the preferred form in which roots absorb B (Mitra, 2017).

B is essential for cell wall structure and functions (Mitra, 2017). B deficiency occurs at <20 µg g^{-1} B in mature leaf tissues results with a number of symptoms includes orange and yellow discoloration, which becomes brown on only one side of the leaf; minimum leaf growth to two thirds of its normal length and with dry tips; a tendency for the leaf to

curl; chlorosis of the young leaves with reddening of the dead borders at the apex and fruit with multiple crowns (Mitra, 2017; Souza & Reinhardt, 2007).

2.3.3 Beneficial Mineral Elements

2.3.3.1 Aluminium

Al is biotoxic, high level of Al in the soils affect the growth of crop roots and usually active in the acidic soil (Chen & Lin, 2010; Mota et al., 2016). Al toxicity occurs when the soil pH is below 5.5, low exchangeable bases and low organic matter content (Lima et al., 2014). Under those conditions, the insoluble Al may exist in soluble form in the soil (Chen & Lin, 2010; Lima et al., 2014; Mota et al., 2016). When the roots exposed to the cation, the cell begin to wrinkle due to disintegration of tissues of the epidermis and external portions of the cortex in the apices of the roots, in more severe cases, collapse (Lin & Chen, 2013). In studies by Mota et al. (2016) showed the root length and root dry matter of pineapple cv. 'Victoria' decreases with the increase in Al concentration in the nutrient solution (Mota et al., 2016). Similarly, pineapple cv. Tainung No.17 was chlorosis on young leaves, roots were short and coarse when grown in hydroponic solution (25-28 days) with 200 μM $AlCl_3$ and also dry weight decrease gradually with increasing the Al concentration (Lin, 2010).

Besides that, the long exposure and increasing concentration of Al inhibit the nutrients absorption by the root of plants such as Ca^{2+}, Mg^{2+}, K^+, NO_3^- and $H_2PO_4^-$ (Lin, 2010). According to Mota et al. (2016), the accumulation of N, P, K, Ca and Mg showed linear decrease in in the plant components (leaves, stems and roots) of pineapple cv. 'Victoria' with the increase of Al concentrations in the nutrient solution, which indicates the suppressive effect of Al on the absorption of the macronutrients (Mota et al., 2016). Similar results have been reported by Lin (2010) that the absorption of nutrients of Tainung No.17 mainly affected by Al was K, Ca and Mg at the treatment of 200 μM $AlCl_3$

and Fe, Mn, Cu at the treatment of 300 μM $AlCl_3$ (Lin, 2010). The decrease in the absorption of nutrients can be associated with the increase of injuries caused by Al in the root system and because the Al^{3+} competes with other cations such as Ca^{2+} and Mg^{2+} for binding sites in the apoplasm (Gupta et al., 2013; Mota et al., 2016). As a result, the inhibition of Ca^{2+} uptake by blocking Ca^{2+} channels in the plasma membrane and Mg^{2+} binding sites of transport proteins (Gupta et al., 2013).

However, some species has a mechanism that can tolerate high levels of exchangeable Al include the capacity to keep adequate nutrient content in the plant. The nutrient uptake by pineapple cv. Cayenne not affected with different concentration of Al concentration and Ca uptake increased as high Al concentration was supplied (Lin & Chen, 2011). Furthermore, the toxicity of Al reduces the content of chlorophyll, but it depends on the species, cultivar, time of exposure and Al concentration. Based on the experiments, chlorophyll index (Cl) of pineapple cv. 'Victoria' reduced as Al concentration increased using hydroponic cultivation. However, pineapple cv. 'IAC Fantastico' was not influenced by the presence of Al in the nutrient solution (Mota et al., 2016).

2.4 Factors of Nutrients Deficiency and Efficiency

The 'available nutrients' in the soils is the nutrient which is accessible to plant roots, then transported to the leaves and used according to their function in plant metabolism (Mengel et al., 2001; Roy et al., 2006). An optimal nutrient of crop requires sufficient available nutrients in the rootzone of the soil, rapid transport of nutrients in the soil solution towards the root surface, satisfactory root growth to access available nutrients, unimpeded nutrient uptake, and satisfactory mobility and activity of nutrients within the plant (Roy et al., 2006). The factors that can affect the availability of nutrients such as poor soil structure and fertility, lack of moisture, leaching of nutrient, competition with weeds, pests and diseases. The deficiency of nutrients in the soils contributed to chlorosis,

wilting, retarded growth and even death (necrosis) to the crops, in case of severe deficiency of certain nutrient (Roy et al., 2006). Therefore, the following factors should be taken seriously in the field managements to prevent nutrient deficiency or toxicity.

2.4.1 Soil Properties

The soil properties are critical edaphic factor affecting the response of plants to the existing and applied nutrients including physical (depth, soil texture and structure, water-holding capacity, soil organic matter (SOM) content), chemical (soil pH, cation exchange capacity (CEC) and both macro and micronutrients in sufficient and balanced amount), and biological (soil microbial biomass, beneficial soil microorganisms, and soil fauna) (Delgado & Gomez, 2016). The soil properties will determine the field management during planting for better yield of produce. The following section is a brief description of physical, chemical and biological properties of soil.

2.4.1.1 Soil Physical Properties

Soil physical properties determine the texture, structure, physical condition, tilth of the soil and generally the agronomical potential of soil. These are important as they influence the penetrability of roots, potential rooting volume, degree of aeration, living conditions for soil life, and nutrient mobility and uptake (Roy et al., 2006). Soil texture refers to the proportions of particles of different sizes in the soil such as clay (less than two μm), slit (two μm to 63 μm), sand (63 μm to two mm), gravel (two mm to two cm) and rocks (Mengel et al., 2001). These soil textures also determine other physical properties such as infiltration rate and some chemical properties such as cation exchange capacity (CEC) (Delgado & Gomez, 2016). For instance, when a soil contains a large proportion of sand, it is easier to cultivated, but since it is well aerated, excessive moisture drains away easily.

Therefore, the soil could not retain moisture which results in moisture stress to the plants. It also causes the plant nutrients to leach out very easily (Roy et al., 2006).

On the other hand, soil structure is the arrangement of individual particles of the fine soil fraction which bound together with organic matter in the soil into larger aggregates (Roy et al., 2006). These process also results in the arrangement of pores among these particles, and also the stability of this arrangement under external forces such as wetting and pressure (Delgado & Gomez, 2016). In contrast to texture, soil structure can be substantially modified by soil management such as tillage which can change soil structure rapidly (Delgado & Gomez, 2016). However, crumbly pieces formed by mechanical tillage are usually less stable (Roy et al., 2006). Structure modifies the effect of texture on regard to moisture and air relationships, availability of nutrients, action of microorganisms and root growth (Delgado & Gomez, 2016).

As stated previously, soil physical properties are one of the factors that affect root elongation and enlargement, proliferation and water uptake which in turn affect plant nutrition. The requirements of plant roots in soils are deep rooting volume (ease of penetration and no restrictions on root growth); adequate available plant nutrients from soil reserves (external inputs or from N fixation); sufficient available water to support plants and soil life (also for nutrients transformations and for nutrient transport to the roots); facility for the drainage of excess water from rootzone to ensure the right air-water balance; and good soil aeration to meet the oxygen requirements of roots and for removal of surplus CO_2 (Roy et al., 2006).

Moreover, the SOM helps to maintain good aggregation, better soil aeration, protection of soil against erosion and increase water holding capacity and exchangeable K, Ca and Mg (Baligar et al., 2001; Roy et al., 2006). It also reduces P fixation, leaching of nutrients and decreases toxicities of Al and Mn. Furthermore, it influence particularly nutrient

mobilization from decomposed organic sources (N, P, S, Zn, etc); keeping the nutrients in available forms and protecting them against losses; gain a nutrient as a result of N fixation from the air; and promotion or retardation of growth through hormones (Roy et al., 2006). Best management practices such as addition of crops, green manures, compost, animal manure, use of cover crops, reduced tillage and avoiding burning of crop residues can significantly improve the level of SOM and contribute to the sustainability of the cropping system and higher nutrient use efficiency (Baligar et al., 2001).

2.4.1.2 Soil Chemical Properties

The soil pH is an important indicator of soil health whereby the soil reaction refers to its acidity or alkalinity. Soil reaction greatly influences the availability of several plant nutrients. For example, in the strongly acidic upland soils, phosphate is rendered less available but heavy metal nutrients (Cu, Fe, Mn and Zn) increases except for molybdenum (Mo), and Al become toxic below pH 4.5 (Roy et al., 2006). The rate of soil acidification often increases under leaching, intensive cropping and persistent use of acid-forming fertilizers which led to soil degradation (Roy et al., 2006). However, the application of calcium carbonate (lime) or similar soil amendment can decrease the soil acidity while unfavourable high pH values (alkali soils) can be decreased by amendment with materials such as gypsum ($CaSO_4 \cdot 2H_2O$), elemental S or iron pyrites (FeS_2) (Roy et al., 2006).

2.4.1.3 Soil Biological Properties

Soil biological properties are also interconnected with other soil physical and chemical properties such as aeration, SOM or pH as its affect the activity of many microorganisms in soils (Delgado & Gomez, 2016). The amount and type of organic matter also determine the biological activity in the soils since it is a carbon source for many organisms including soil microbiota (Delgado & Gomez, 2016). Moreover, the microbial activity mostly

controlled the rate of transformation of most nutrients into available forms (Roy et al., 2006). Microbial activity can be increased by soil management such as by improve the drainage, liming or organic amendments. Enhanced beneficial microbes such as rhizobia, diazotrophic bacteria and mycorrhizae in the rhizosphere can significantly affect the plant development by improved root growth by fixing atmosphere N2, suppressing pathogens, producing the growth regulator, enhancing root surface area to facilitate uptake of less mobile nutrients such as P and micronutrients, and mobilization and solubilization of unavailable organic/inorganic nutrients (Baligar et al., 2001; Delgado & Gomez, 2016). Infections of diseases and insects can reduce nitrogen uptake efficiency. Similarly, soil borne pathogens such as actinomycetes, bacteria, fungi, nematodes, and viruses present in the soil around roots lead to pathogenic stress and bring profound changes in the morphology and physiology of roots and shoots that reduces plant ability to absorb and use nutrient effectively (Baligar et al., 2001).

2.5 Sampling Technique for Soil and Plant Nutrient Analysis

There are several methods for soil sampling which are simple random sampling, systematic sampling, stratified sampling (Figure 2.10) and compositing (Pennock et al., 2006; Tan, 2005). Compositing is the mixing of sampling units to form a single sample which is then used for chemical analysis (Tan, 2005). The advantages of this method offers the increased in accuracy through the use of large numbers of sampling units per sample, by averaging the results of analysis from each of the sampling units (Tan, 2005). The soil sample can be obtained by using soil sampling tubes, augers, till spades or shovels depending on the depth and types of tool used. Sample can be dug from the soil surface (shallow sampling), or from deeper layers (deep sampling) (Tan, 2005). For disturbed shallow sampling, samples can be collected with an auger. For compositing, the collected units must be mixed thoroughly until homogenous mixture is attained.

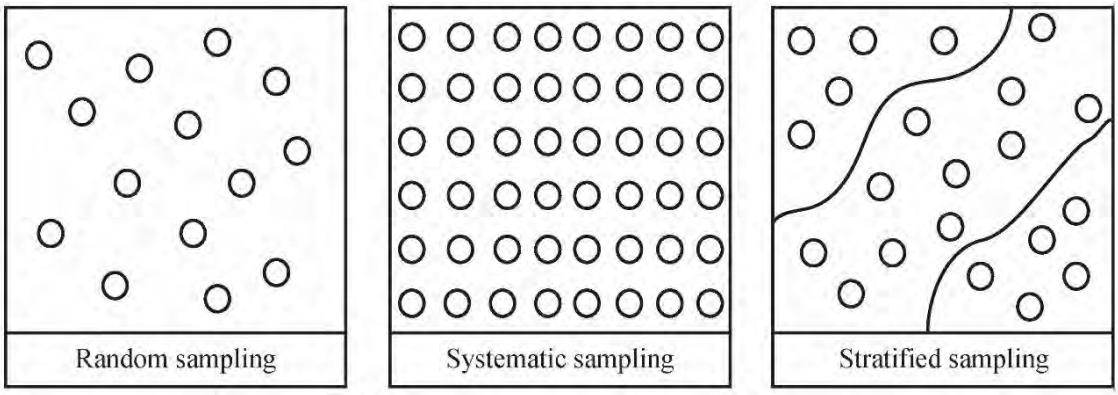

Figure 2.10: Illustration of random sampling, systematic sampling and stratified sampling plan for soil sampling.

Moreover, sample handling before analysis can affect soil test results. Therefore, prevention steps must be taken to avoid the occurrence of further chemical and biochemical reactions. Air drying is the most accepted procedure of sample preservation. Drying in the air may reduce the rate of possible reactions to continue in the disturbed soil sample. Microwave drying appears to change many nutrient test results compared to air-drying (Gelderman & Mallarino, 2012). The time needed to air dry soils may vary from two to 48 hours depending on soil, soil moisture, air temperature, air movement and humidity, pre-crushing, soils and the amount and thickness of the soil being dried (Gelderman & Mallarino, 2012). The temperature must not exceed 40 °C (Gelderman & Mallarino, 2012) because drying at elevated temperature may cause drastic changes on the physical, chemical, and biological characteristics of the soil sample (Tan, 2005). Early research showed that elevated drying can affect the results of K, N, P, S, Zn and perhaps other micronutrients (Gelderman & Mallarino, 2012; Tan, 2005).

According to Souza and Reinhardt (2007), the micronutrients that are most important for pineapples in many parts of the world, are Fe, Zn, Cu and B (Souza & Reinhardt, 2007). If micronutrient analyses are to be performed, all surfaces contacting the material should be made of stainless steel, plastic or wood (Gelderman & Mallarino, 2012). Additionally, a sample should not be allowed to stay moist for extended periods of time.

Crushing and grinding of soil sample are essential to produce uniform size of particles. Moreover, grinding is to reduce heterogeneity and to provide maximum surface area for physical and chemical reactions. Sieving is an essential part of homogenizing the sample after the grinding operation. The soil fraction more than two mm is usually discarded in soil chemical analysis (Tan, 2005).

In order to investigate the nutrient content in soil, plant samples should also be collected so that soil analysis can be interpreted and correlated with results of plant analysis. For pineapple plants, leaf sampling can be done at any point of the vegetative cycle, from the fourth month after planting until the induction of flowering, although within ± 15 days of floral induction has been adopted as the best time for leaf sampling (Souza & Reinhardt, 2007). It is believed that too young and very old leaves normally do not reflect the nutritional status of a plant and hence will provide wrong interpretations of analytical results. Contaminants, such as dust or soil, covering the plant samples should be removed by wiping the dust softly with a damp cloth (Tan, 2005). For analysis, the intermediate third of the non-chlorophyll portion (white) of the basal zone (Hawaiian technique) or the whole leaf (French technique) can be used (Teixeira et al., 2011).

2.6 Bioactive Compounds

Bioactive compounds in plants are compounds that produced as secondary metabolites which have pharmacological or toxicological effects in man and animals (Bernhoft, 2010). Secondary metabolites compounds such as flavonoids, alkaloids and tannins in plants appear to be randomly synthesised and some of them are found to hold important functions in the living plants (Bernhoft, 2010). The following section is a brief introduction of some of the main chemical groups in bioactive compounds.

2.6.1 Phenolic Compounds

Phenolic compounds constitute a large and ubiquitous group of phytochemicals. Phenolics are characterized as having at least one aromatic hydrocarbon ring attached with one or more hydroxyl groups where the simplest of the class are phenolic acids (C_6-C_1) such as gallic acid (Jagathan & Crozier, 2008). Phenolics are formed to protect plants from reactive oxygen species (ROS), photosynthetic stress and herbivory (Jagathan & Crozier, 2008). Moreover, phenolic compounds are closely associated with the sensory and nutritional quality of fresh and processed foods, also are good sources of natural antioxidants (Ho, 1992).

The most important single group of phenolics in food is flavonoids. Flavonoids are polycyclic structures consists of a central three-ring structure (Bernhoft, 2010; Jagathan & Crozier, 2008). Flavones, isoflavones, flavonoids, flavanols, flavanones, anthocyanins and proanthocyanidins are a part of flavonoids (Altemimi et al., 2017; Jagathan & Crozier, 2008). All compounds contain phenol groups involved in effect as general antioxidants and several structures reduce inflammation or carcinogenicity (Bernhoft, 2010).

Tannins, also known as proanthocyanidins are also another group of phenolic compounds. There are two types of tannins which are condensed tannins and hydrolysable tannins (Altemimi et al., 2017). Condensed tannins are large polymers of flavonoids and hydrolysable tannins are polymers composed of a monosaccharide core with several catechin derivatives attached. Tannins indiscriminately bind to proteins and larger tannins are used as astringents in cases of diarrhoea, skin bleeding and transudates (Bernhoft, 2010).

2.6.2 Alkaloids

Alkaloids are heterocyclic, nitrogen containing compounds and usually with potent activity and bitter taste (Bernhoft, 2010). The main groups of alkaloids are the benzylisoquinoline, tropane, terpenoid indole, nicotine, purine, pyrrolizidine, quinolizidine, steroidal glycoalkaloids, coniine and betalains alkaloids (Jagathan & Crozier, 2008; Zulak et al., 2007). Most of these alkaloids are found in herbal or medicinal plants with limited occurrence in fruit and vegetables. Moreover, alkaloids also protect plants against herbivores and pathogens (Jagathan & Crozier, 2008). Based on the previous research, the application of vermicompost helps in getting higher marketable alkaloid compound withaferin A and withanolide D in *Withania somnifera* Dunal compared to chemical fertilizer (Raja & Veerakumari, 2013).

2.6.3 Carotenoids

Carotenoids are group of pigments produced from eight isoprene molecules and contain 40 carbon atoms that are fat- or oil- soluble pigments found in green leaves and yellow, orange and red fruits (Jagathan & Crozier, 2008). During ripening of fruit, the chlorophyll in the shell degrades resulting in the yellowing of the fruit without any increase in carotenoid content except late in the senescence stage (Lobo & Siddiq, 2017; Py et al., 1987). Moreover, during yellowing of shell, the flesh also turns from white to yellow in concomitance with the accumulation of carotenoids (Lobo & Siddiq, 2017; Py et al., 1987).

Carotenoids can be divided into two classes; the oxygenated xanthophyll (contain carbon, hydrogen and oxygen) such as lutein, zeaxanthin and violaxanthin; and the hydrocarbon carotenes (purely hydrocarbon) such as β-carotenes, α-carotene and lycopene (Jagathan & Crozier, 2008). In human, four carotenoids which are β-carotenes, α-carotene, γ-carotenes and β-cryptoxanthin can be converted to vitamin A, also known

as retinol (Higuchi, 2014; Nieves, 2013). However, β-carotene is the most important precursors of vitamin A and is the most active as antioxidants among the carotenes (Jagathan & Crozier, 2008). The carotenes including α-, β- and γ- carotenes, along with lycopene and lutein which do not convert to vitamin A are suggested to provide protection against lung, breast, colorectal, prostate and uterine cancers (Ludwiczuk et al., 2017).

2.7 Overview of Antioxidants

Antioxidants are substance that protects other chemicals of the body from damaging oxidation reactions by reacting with free radicals (FR) and other reactive oxygen species (ROS) within the body, hence hindering the process of oxidation (Rodriguez-Roque et al., 2017). The term reactive oxygen species (ROS) refers to a group of molecules that includes not only free radicals but also non-radical species (without unpaired electrons). Free radicals (FR) are defined as any chemical species (atom, ion, or molecule) contain one or more unpaired electrons in their valency shell or outer orbit (Lobo et al., 2010; Phaniendra et al., 2015). For example, superoxide anion ($O_2^{\bullet -}$), hydroxyl radical ($^{\bullet}OH$), peroxyl ($ROO^{\bullet}$), hydroperoxyl ($HO_2^{\bullet}$) and alkoxyl ($RO^{\bullet}$) (Rodriguez-Roque et al., 2017).

The odd number of electron(s) makes its unstable, short lived and extremely reactive and cause damage to the cell by abstract electrons from other compounds or donate it to attain stability (Lobo et al., 2010; Phaniendra et al., 2015). ROS are generated in biological systems for a great variety of reasons such as free radicals help with cell growth, cell proliferation, and cell division; they regulate redox balance in the cell; they activate protein kinases that regulate gene function; and they regulate immune functions (Rodriguez-Roque et al., 2017). However, at higher concentration, the excess of ROS can damage the integrity of various biomolecules including lipids, proteins and DNA leading to increase in oxidative stress in various human diseases (Phaniendra et al., 2015). For

instance, diabetes mellitus, cardiovascular diseases, respiratory diseases, neurogenerative diseases, arthritis, cataracts as well as in aging process (Phaniendra et al., 2015).

Antioxidants from food prevents, reduces, retards, or inhibits the oxidation of easily oxidizable biomolecules such as proteins, lipids and carbohydrates, resulting in the delay of many chronic and degenerative disease (Rodriguez-Roque et al., 2017). This reaction is because antioxidant stable enough to donate an electron to free radical and neutralized it, as results reducing its capacity to damage (Lobo et al., 2010). However, as one antioxidant molecule can only react with a single free radical, antioxidant supply is not unlimited (Agnes & Anusuya, 2016). Therefore, there is a constant need to replenish antioxidants resources, whether synthetically (supplementation) or naturally such as in those fruits and vegetables. Vitamin C, vitamin E, carotenoids, phenolic compounds and chlorophylls are among the main food antioxidants (Rodriguez-Roque et al., 2017).

There are several different assays to estimate antioxidant capacities including 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Brand-Williams et al., 1995), 2,2-azinobis (3-ethyl-benzothiazoline-6-sulfonic acid) (ABTS) (Miller et al., 1993) and ferric reducing antioxidant power (FRAP) (Benzie & Strain, 1999). In recent years, these assays have been widely applied to analyse antioxidant capacity in pineapple fruits (Almeida et al., 2011; Kalaiselvi et al., 2012; Lu et al., 2014; Martínez et al., 2012; Yuris & Siow, 2014). In addition, previous research has found that with application of vermicompost can enhanced the total phenolics, total flavonoids and antioxidant activities of field grown cassava compared to inorganic fertilizer (NPK) (Omar et al., 2013). Considering of all of this evidence, it seems that pineapple is a good source for antioxidant and supplementation of vermicompost able to increase the bioactive compounds and antioxidant capacity in the fruits.

CHAPTER 3: METHODOLOGY

The section below describes the brief procedures of this research. To study the effect of vermicompost on MD2 pineapple plants (*in vivo* and *ex vitro*), a two-year field trial was conducted at the Glami Lemi Biotechnology Research Centre, University Malaya, Jelebu, Negeri Sembilan, Malaysia (3° 3' N latitude, 102° 3' E longitude) from January 2015 until December 2016. The fruits harvested from the plants were then used to determine their fruit quality. Along the planting period, soil and plant sample was collected and their nutrients content was determined. Also, the bioactivity potential of fruits was investigated and compared to fruits harvested from plants supplied with chemical fertilizer and control plants.

3.1 Cultivation of MD2 Pineapple Plants

The cultivation techniques and field management was based on the cultivation guidelines of pineapple provided by Malaysian Pineapple Industry Board (MPIB, 2015c). The experiment area selected was known as the warmest area in Malaysia with a mean monthly precipitation of 215 mm. The highest peak of monthly precipitation as recorded at nearest meteorological station was on April, October and November while the less precipitation (70 mm) in January to February (Puah et al., 2016). The monthly average temperature ranges from 23 °C (December – February) to 33 °C (March – April). The soil samples were collected randomly around cultivation field at a depth of 0-15 cm before the commencement of the experiment. The soil chemical properties of plantation sites were: pH 5.65, 57.90% electrical conductivity, 0.06% total N, 0.51% total C, 92.28 ppm available P, 0.21 cmol/kg exchangeable K, 0.85 cmol/kg exchangeable Ca, and 0.18 cmol/kg exchangeable Mg. The soil of experimental site was sandy loam. Prior to cultivation of plant, the experimental field and planting material was prepared as described in the following section.

3.1.1 Planting Material

Two hundreds of *in vivo* and *ex vitro* MD2 pineapple plants were purchased from Malaysian Pineapple Industry Board (MPIB), Negeri Sembilan and Plant Biotechnology Incubator Unit (PBIU) UM, respectively. *In vivo* plants were healthy grade 'A' suckers with 40 cm average height while *ex vitro* plantlets consisted of *in vitro* grown plantlets ready for acclimatization. All *in vivo* plants were soaked for 24 hours in water before planting to encourage rooting.

3.1.2 Field Preparation

In the beginning of fieldwork, the field was cleared with any weeds (Figure 3.1A). The field was ploughed and harrowed to obtain a good tilth suitable for planting the suckers and *ex vitro* plantlets of MD2 pineapple. A drains flow (1.5 x 1.2 x 1.2 m) was built around the plot to prevent standing water in the plot which may affect the plant growth. Six raised bed (15 to 20 cm, height) were made and was divided into four blocks each containing six beds. All beds were covered with plastic mulch with 0.03 mm of thickness (Figure 3.1B).

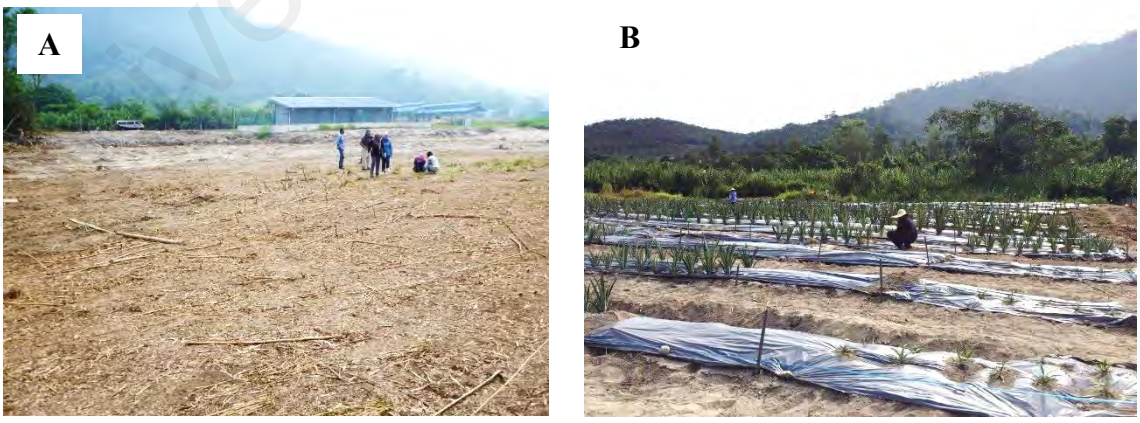

Figure 3.1: (A) The land was cleared from weeds and (B) pineapple plants were planted on the prepared beds covered with silver shines at Glami Lami Biotechnology Research Centre, Jelebu.

Figure 3.2 shows the illustration of field layout. Individual bed size was 3.0 m x 2.0 m. The beds and blocks were separated with a spacing of 1.0 m to ensure uninterrupted flow of irrigation for each individual plot. Moreover, the fence was built around the plot to prevent the attack from the wild boars.

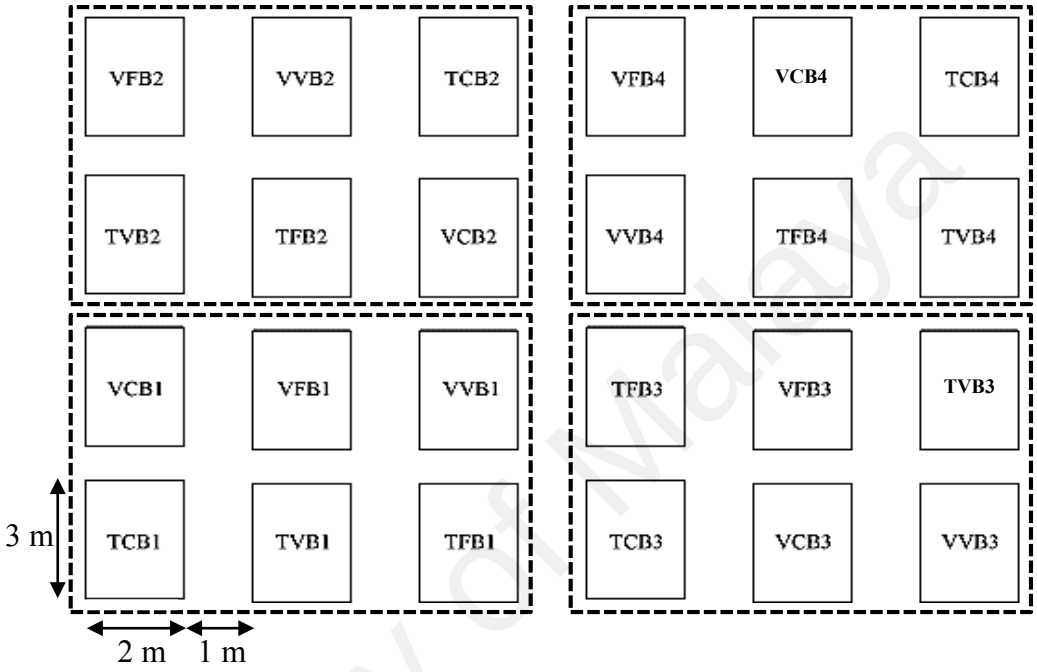

Figure 3.2: The illustration of field layout, individual bed size and arrangement of treatment plot. The treatment were assigned randomly to each plot based on factorial randomized complete block design. V: *in vivo*, T: *ex vitro*, C: control, F: treated with chemical fertilizer, V: supplied with vermicompost, B: block.

3.1.3 Planting Technique and Field Management

An average of 15 plants were planted in double rows for each plot with planting density 60 cm x 30 cm. A stake wood tied by a string was stretched on the bed to make a straight line for planting and as a marker to determine the distance between plants. A hole of 15 cm deep was made on the prepared bed by using *Tugal* wood for planting the *in vivo* MD2 pineapple plants. Then, soil surrounding the plant was pressed to keep the plant unmoved. Prior to planting of *ex vitro* plants, the shades were made in each plot. The shades were used until 12 months after planting. The *ex vitro* plants were planted in five to 10 cm deep hole.

The plants were irrigated as when necessary with sprinkler irrigation and the weeds were controlled periodically. The weeds on the plot were cleared by practice control culture method which is by using hand, pulling, cutting and digging. However, the weeds in between the plots, chemical method was applied. The herbicide (Diuron, 100 ml in 18 litre of water) was used when the growth of weeds uncontrolled caused of rainy season. In case of diseases especially caused by *Dysmicoccus brevipes*, insecticide (Malathion, 24 ml in 18 litre of water) was sprayed to the leaves to prevent the disease from spreading to other plants. Herbicide and insecticide were not applied after the flowering was induced.

3.1.4 Treatments application

The experimental design was arranged in factorial randomized complete block design (RCBD) with three treatments and four replicates consists of the growth of the pineapple plants supplemented with commercial vermicompost only and compared to those grown with commercial chemical fertilizer only, while the control plants were not supplied with any fertilizers or vermicompost products. The *in vivo* and *ex vitro* plants were analysed separately due to different in light capacity along the planting whereby *ex vitro* plants grown under the shades until 12 months after planting.

Commercial vermicompost (Earthworm^{TM}, Synergy Resources, Malaysia) was supplied twice at the recommended rate (10 t ha^{-1}), during transplanting and at seven months of planting for both *in vivo* and *ex vitro* plants. The vermicompost was incorporated into the soil by mixing with 10 cm of topsoil. The nutrient availability in vermicompost were 1.54% total N, 0.64% total P, 6.31% total K, 0.58% total Mg, 1.39% total Ca, 0.34% total S, 0.01% total Zn, 0% total B, 0.76% total Fe, and 1.04% total Al.

For treatments with chemical fertilizer, there were two types of fertilizer applied which were commercial chemical fertilizer (Nitrophoska® Green) and foliar sprays (micronutrients) based on the rate recommended by the Malaysian Pineapple Industry Board (MPIB, 2015c), with minor modifications. NPK (15:15:15) fertilizer granules was applied at the rate of 20 g per plant at one month, three months, seven months, and 14 months after planting (MAP). It was applied in between of two twin rows of pineapple plants or around each plant. A foliar fertilizer mix was sprayed two times at 1.5 months (640 g of hydrated lime, 42 g of copper sulphate, 42 g of zinc sulphate, 21 g of ferrous sulphate in 18 L of water) and 4.5 months (added 640 g of urea in 18 L of water) after planting. Fifty to 100 millilitres (50 - 100 ml) of fertilizer was sprayed onto the leaves of each plant.

3.2 Plant Growth Development

In order to study the effect of vermicompost on the MD2 pineapple productivity under field condition for both *in vivo* and *ex vitro* plants, firstly their morpho-physiology was investigated and compared to plants supplied with chemical fertilizers and control plants. The parameters determined in this study were plant height, number of leaves, length and width D-leaves, relative chlorophyll content and morphological feature of stomatal on D-leaf including stomatal density, stomatal size, stomatal length, stomatal width, pore length as well as pore aperture.

3.2.1 Plant Morphology

Twelve plants from each treatment were tagged randomly for data collection. On the vegetative parts, the data was collected every month on the number of leaves, plant height, length and width of D-leaf from two months after planting until fruit was harvested. Height of plants was measured with a meter ruler and the number of leaves was

determined by counting. The length of the D-leaf was measured with a meter ruler from three leaves per plant while the width of D-leaf was an average of three leaf per plant that measured in the middle part of the leaf. The D-leaf was identified by gathering all the leaves in the hands to form a vertical "bundle" in the centre of the plan where D-leaves are the longest ones.

3.2.2 Non-destructive Determination of Relative Chlorophyll Content

The relative chlorophyll content was measured with a chlorophyll meter (SPAD-502, Konica Minolta, Japan) every two months at three different places (tip, middle, basal) on one D-leaf per plants and the average value was calculated (Liu et al., 2012). The SPAD reading were taken before 09:00 or after 17:30 under the shades.

3.2.3 Observation of Pineapple D-leaf Surface by Field Emission Scanning Electron Microscopy (FE-SEM)

The D-leaf surface structure, stomatal density, stomatal size, stomatal length, width of stomatal, length of pore and stomatal aperture were determined after nine-months planting using Field Emission Scanning Electron Microscopy, FE-SEM (Quanta FEG 450, FEI, Australia).

3.2.3.1 Preparation of Sample

For preparation of sample, firstly, the D-leaf of nine-months-old samples from *in vivo* and *ex vitro* grown plants was collected freshly from plants. Then, it was washed with distilled water and blot dried with tissues. The leaf surface (abaxial and adaxial) was scrubbed lightly by using sharp scalpel to remove trichomes attached on the leaf surface where stomata can be found. Then, the leaf was cut into two parts taken from 30 cm from bottom of the leaf and away from the edge. Every part was cut into 0.3 cm x 0.3 cm each.

The same leaf was used to assure sampling consistency. The FE-SEM specimen stubs were tapped with black double-sided tape to prevent the samples from moving. The leaf was put on the stubs, one on abaxial side and the other one on adaxial side.

3.2.3.2 Measurement of Morphological Stomatal Features

The prepared stubs with samples were immediately put in the FE-SEM. It was done in a low vacuum mode because of excessive water content in the sample. The detector used was Large Field Low vacuum SED (LFD). To get optimal resolution images, the working distance was varied between images. The short working distances permitted optimal resolution. However, the observation must be made in a short time as possible to avoid the leaf from wrinkling due to the high pressure applied during the observation. Moreover, the microscope was operated at low accelerating voltage (5.00 kV) due to biological specimen was used.

The image of cross-section and transverse-section of the D-leaf was taken to show general structure of MD2 pineapple leaf and the differences of shield-shape trichomes structure on both sides. For morphological stomatal features, the measurements were made on four leaves (one leaf per plant) on both adaxial (upper) and abaxial (lower) leaf surface for each treatment. Stomatal were counted on a field of view of 0.295 mm (area of leaf) at a magnification of 500 times and stomatal density (d) was calculated as, d = number of stomata / areas of leaf. Stomatal size was calculated as the product of stomatal length and width of stomatal (μm^2). The stomata length, stomatal width, pore length and pore aperture were measured as described by Savvides et al. (2012) (Figure 3.3). All parameters were measured using imaging software, xT microscope control.

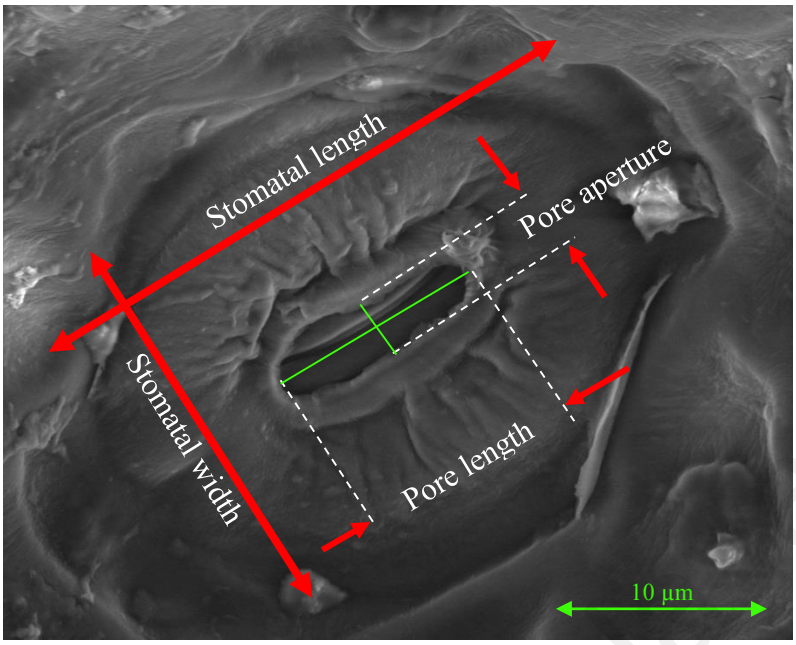

Figure 3.3: Illustration measurement of stomatal length, stomatal width, pore length and pore aperture.

3.3 Flowering Induction

Flowering was induced after nine months planting (*in vivo*) and 16 months after planting (*ex vitro*), based on the crop development stage. Fifty ml of Ethrel (2-chloroethyl phosphonic acid) solution (15 ml of Ethrel, 90 g of urea in 9 L of water) was sprayed at the centre of plants to induce the flowering (Figure 3.4A). The solution was sprayed either in the morning or late evening. After 30-45 days, the plants were checked for the successfulness of flowering induction by observed the formation of red bud (Figure 3.4B). If the red bud was not showed at the centre of plant, the second induction was made after 50 days of first induction. When the fruits were fully developed, the fruits were covered to avoid sunscorch. During hotter periods (>35 °C), sunburn is common to unshaded fruits. Sun scorched fruits show a bleached yellow-white skin which make it susceptible to attack by diseases.

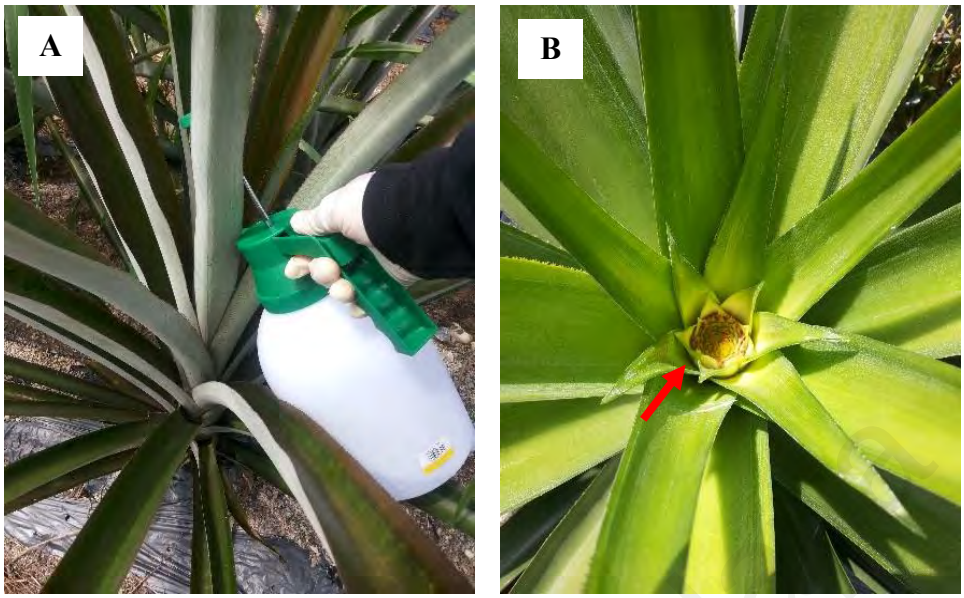

Figure 3.4: (A) The plants were induced with Ethrel by spraying at the centre of each plant. (B) The red arrow shows the formation of red bud which observed after 50 days of flowering induction.

3.4 Harvesting and Storage Condition of Fruits

The fruits were harvested when they were one-third ripe (Figure 3.5). Pineapple fruits was harvested by bending them over and the stalk was cut about 10 cm from the bottom of fruits. The fruits were harvested early in the morning or late in the evening to reduce the heat loads during pre-cooling of harvested fruits. After harvesting, the fruits were kept in the baskets, upside down on the crown to avoid injury and under the shades. Then, as soon as possible, the fruits were transported to the laboratory of the Plant Physiology, Institute of Graduate Studies, University of Malaya. Upon arrival, the fruits were unloaded by hand and washed with clean water to remove field heat, microbes or soil-contaminated fruit. The fruits sample free with diseases were kept at 5 to 7 °C until physical, chemical and sensory analysis were completed. The remaining flesh samples were freeze-dried using a freeze dryer (Labconco, USA) at -50 ˚C and stored at -80 °C until further analysis.

Figure 3.5: MD2 pineapple fruits were harvested when one-third ripe.

3.5 Analysis of Fruit Quality Attributes

A total of twelve randomly selected fruits at Index 5 of ripening stage (one-third ripe) were analysed for physical characteristics, physicochemical analysis, and sensory analysis for each treatment (*in vivo* and *ex vitro* MD2 pineapple). Figure 3.6 shows fruits of MD2 pineapple harvested from *in vivo* and *ex vitro* plants grown with different types of fertilizers in the field. To estimate the yield, the mean of fruit weight from each plot were calculated and multiplied by the plantation density in tonne per hectare.

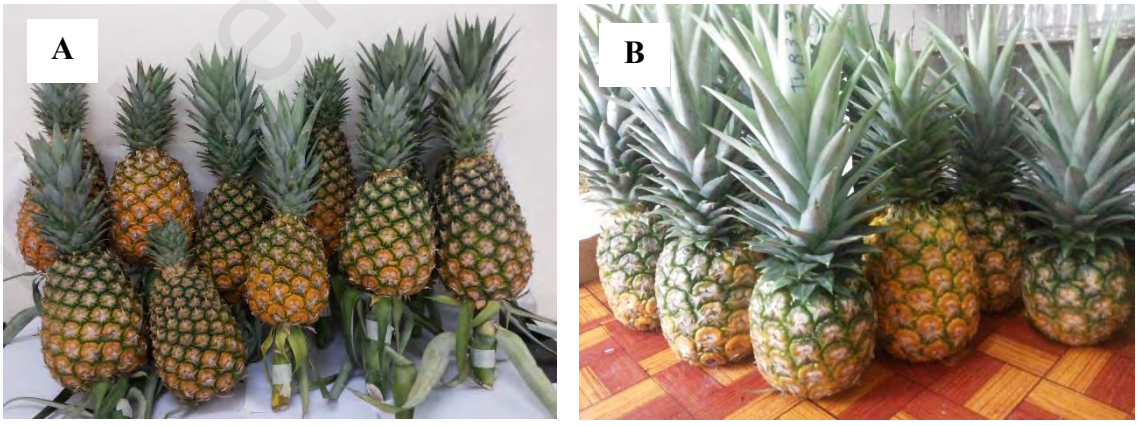

Figure 3.6: MD2 pineapple fruits harvested for analysis; (A) from *in vivo* plants, and (B) from *ex vitro* plants.

3.5.1 Measurement of Physical Characteristics of MD2 Pineapple Fruits

For physical properties of the fruits, fruit weight, fruit weight without crown, diameter of fruit, weight and length of crown, pulp firmness and core diameter were determined. The weight of fruits with crown, without crown and crown weight was determined using a digital balance (±1 g) (EK-6000*i*, A&D, Japan). Length of fruits was measured with a meter ruler and diameter of fruit was measured using digital callipers. The pineapple fruit was cut into two parts. At the bottom, middle and top of fruits, the pulp firmness was measured using fruit hardness tester (Nippon Optical Works, Japan) and defined as kilogram force (kg f) required to penetrate the tissue (Liew & Lau, 2012). Also, the core diameter was measured using a ruler at three different places, bottom, middle and top of fruits.

3.5.2 Determination of Physico-chemical Properties

The pulp was manually separated from the fruit and cut into small pieces to obtain homogeneous samples. The physicochemical properties of MD2 pineapple was determined by analysed the pH of fruit juice, total soluble solids (TSS), titratable acidity (TA), total solids and ascorbic acid content. Sugar-to-acid ratio was derived as the ratio of total soluble solids to titratable acidity.

3.5.2.1 Chemicals and Reagents

Sodium hydroxide (NaOH) was purchased from Macron Fine Chemicals, Sweden. 2, 6 – dichloroindophenol-indophenol sodium salt-dihydrate (DCPIP), phenolphthalein indicator solution (0.1%), and ascorbic acid were purchased from Systerm, Malaysia. Sodium bicarbonate ($NaHCO_3$), glacial acetic acid (CH_3COOH), and metaphosphoric acid $(HPO_3)_n$ were purchased from Friendemann Schmidt, USA. All chemicals and solvents were analytical grade.

3.5.2.2 pH Measurement

Thirty gram of pineapple pulp tissue and 90 ml of distilled water was added into a laboratory blender and blended for two minutes. The juice was filtered using filter paper. The filtrate was used to measure pH of juice at room temperature (25±2 °C) with a pH meter (Mettler Toledo, Switzerland) (Dadzie & Orchard, 1997). The mean of triplicates was taken as the sample pH. The pH meter was calibrate using pH 4 and pH 7 buffer solution prior to pH reading of samples.

3.5.2.3 Total Soluble Solids

The total soluble solids (TSS) were measured using a digital refractometer (PR-1, Atago, Japan). A 30 g of the pulp tissue in 90 ml of distilled water was blended with a laboratory blender for two minutes and filtered (Dadzie & Orchard, 1997). A single drop of the filtrate was placed on the prism of the refractometer at room temperature (25±2 °C). The recorded value was multiplied by three since the initial pulp sample was diluted three times with distilled water (Appiah et al., 2012). The refractometer was standardised with distilled water and the results were expressed in standard °Brix unit.

3.5.2.4 Titratable Acidity

Thirty grams (30 g) of the pulp was weighed and transferred into a blender plus 90 ml distilled water, blended for two minutes and then filtered. 25 ml of the filtrate was transferred into a 125 ml conical flask. Another 25 ml distilled water and four to five drops of phenolphthalein indicator was added. This solution was titrated against 0.1 N sodium hydroxide (NaOH) until there was a sharp colour change from light yellow to pink (Dadzie & Orchard, 1997). The titre volume of NaOH added was recorded and multiplied by the citric acid factor (0.07) to obtain the titratable acidity (Appiah et al., 2012). The results were expressed as g citric acid per kg of pineapple (g kg^{-1}).

3.5.2.5 Total Solids

Five gram of fruit pulp was weighed using electronic balance (±0.001 g) (FZ-2001-EX, A&D, Japan) and put in the oven (Memmert, Germany) at 60±5 °C until completely dried. The final weight was measured using an electronic balance (A&D, Japan). The percentage of total solids was calculated by following formula (AOAC, 2007; Bradley, 2010):

$$\text{Percentage of total solids (\%)} = \frac{\text{Initial weight (g)} - \text{Final weight (g)}}{\text{Initial weight (g)}} \times 100$$

3.5.2.6 Ascorbic Acid Content

Ascorbic acid was estimated by 2, 6 – Dichlorophenolindophenol visual titration method (AOAC, 2007; Pegg et al., 2010; Offia-Olua, 2015). Standard indophenol solution was prepared by dissolving 0.08 g of 2, 6 - dichloroindophenol in 50 ml distilled water, then 0.042 g of sodium bicarbonate ($NaHCO_3$) was added. The mixture was stirred to dissolve the solids. The solution was filtered, and the residue was washed with distilled water. After that, the solution was made up to 200 ml. For extraction solution, 15 g of metaphosphoric acid was mixed with 40 ml of 20% acetic acid solution and 200 ml of distilled water. Then, the solution was made up to 500 ml with distilled water.

The dye solution was then standardized against the ascorbic acid solution by titration. Standard solution of pure ascorbic acid was prepared by dissolving 0.05 g pure ascorbic acid in 100 ml of distilled water. 5 ml of the ascorbic acid standard solution was pipetted into a 250 ml conical flask. Then, 20 ml of distilled water and 10 ml of metaphosphoric/ acetic acid solution was added. Ascorbic acid solution was titrated until the first appearance of a faint pink colour persists for more than five seconds. The titration was repeated twice. The concentration was then expressed as mg ascorbic acid equivalent to

1 ml DCPIP. A blank measurement was performed using 5 ml distilled water instead of ascorbic acid. The blank was used to compare the colour.

For sample analysis, 5 g of fruit sample was weighed accurately into a 100 ml beaker. The sample was squeezed using a glass rod and then 15 ml of methaphosphoric/acetic acid solution was added. The mixture was stirred for a while and then only the solution was transferred into 250 ml conical flask. The residue in the beaker was washed with 30 ml of distilled water and then the washing was combined with the sample solution. The extracting sample solution was titrated immediately with DCPIP until the first appearance of pink colour that can persist for more than five seconds. The initial and the final readings of the burette was taken and used to calculate the average titre of dye used. The procedure was repeated twice for the each of sample. The calculation of ascorbic acid content as following:

$$\text{Ascorbic acid content (µg ascorbic acid /g FW of sample)} = X - B \times (F / E) \times (V/Y)$$

where, X = Average DCPIP for sample titration (ml)

B = Average DCPIP for sample blank titration (ml)

F = Titre of dye (mg ascorbic acid equivalent to 1 ml DCPIP)

E = Weight of sample (g)

V = Volume of initial sample solution (ml)

Y = Volume of sample aliquot titrated (ml)

3.5.3 Sensory Analysis

Organoleptic characteristics of the three pineapples flesh were carried out. Thirty untrained panellists (for *in vivo* fruits) and 20 untrained panellists (for *ex vitro* fruits) were randomly assigned among students and lecturers from University of Malaya. Assessors evaluated the small pieces of sliced pineapple fruit (1 cm x 1 cm) for colour, flavour,

odour, firmness and overall acceptability, then recorded their preferences in a survey's form (Appendix A). The samples were kept in aluminium foil to keep the moisture. The panellists were given mineral water to wash their mouth before every sample was tested.

Hedonic scales were used (Table 3.1), 1 to 3 hedonic scale was used for odour whereby flavour, colour, firmness and acceptability, 1 to 5 hedonic scale was used (Salomé et al., 2011) with slight modification. The preference towards the samples tested were analysed by using a 3-points Just-about-right (JAR) scales, by combining frequencies in scale 1 with scale 2 and assuming it as the lowest preferences, scale 3 as the middle preferences, and combining frequencies of scale 4 with scale 5, assuming it as the highest preferences by panellists. The data of frequencies is expressed in percentage of intensities of each attribute.

Table 3.1: A hedonic scale of flavour, odour, colour, firmness and acceptability for sensory evaluation of MD2 pineapple fresh pulp.

JAR	**Dislike**		**Like**	**Like very much**	
Hedonic scale	**1**	**2**	**3**	**4**	**5**
Flavour	Sour	Fairly sour	Sweet sour	Fairly sweet	Sweet
Odour	-	Off-odour	Slightly ripe-odour	Ripe-odour	-
Colour	Pale yellow	Slightly yellow	Bright yellow	Deep yellow	Brown
Firmness	Very firm	Firm	Fairly firm	Slightly firm	Soft
Acceptability	Dislike	Dislike slightly	Acceptable	Like slightly	Like very much

3.6 Soil Nutrient Analysis

Soil sampling was conducted on six months after planting and during the red bud stages. A stock sample was used to analyse the pH (2 mm sieved) and total elements content (P, K, Ca, Mg, S, Fe, Zn, B, Al) using Inductive Couple Plasma Optical Emission Spectrometer (725 ICP-OES, Varian, Australia) after sample extraction using aqua-regia method. Total N of the soil was analysed using Kjeldahl distillation method.

3.6.1 Preparation of Soil Sample

Soil samples were randomly collected using Dutch Auger at three different places of each treatment beds and bulk in one labelled clean plastic bag. The depth of soil that was taken was at 0 to 15 cm from the surface (Gopinath et al., 2008; Omotoso & Akinrinde, 2013). All soil samples were air dried in the glass house until completely dried. Then, soil samples were crushed using mortar and pestle and allowed the fraction to pass through a 2.0 mm laboratory sieve (Endecotts, England) for soil pH analysis. Then, the remaining stock samples (<2.0 mm) was sieved through 0.25 mm laboratory sieve (Endecotts, England) and was collected and stored in an airtight container as a stock sample for total element and total N analysis.

3.6.2 Measurement of pH in Soil

The pH of soil was measured in the supernatant suspension of a 1: 2.5 soil: distilled water (Shariff & Miller, 1989). Briefly, 10 g of soil was weighed into a plastic cup using electronic balance (±0.001 g) (FZ-2001-EX, A&D, Japan). Then, 25 ml of distilled water was added. The mixture was shaken for one hour with orbital shaker (ZP-200, Meditry Instrument, China) at 2.5 x 100 rpm and then the pH was determined with a pH electrode (PB-10, Sartorius, Germany). The pH reading was an average of triplicate. The pH meter was calibrated with pH 4, pH 7 and pH 10 buffer solution prior the pH reading of samples.

3.6.3 Determination of Total Nitrogen in Soil Sample

The total nitrogen in the soil was determined by Kjeldahl method (Bremmer & Mulvaney, 1982). Briefly, 0.50 g of soil (<0.25 mm) and 1 g of catalyst (sodium sulphate and selenium, 100:1) were added into digestion tube and were mineralized in 98% concentrated sulphuric acid on hotplate (Protech, Malaysia) for eight hours at 350 °C. After left overnight, 10 to 15 ml of reverse osmosis water were added and mixed using vortex mixer until all particles were dissolved. The sample then were transferred into distillation tube and 12 ml of 30% (v/v) sodium hydroxide was added. The sample were distilled for approximately four minutes using distillation unit (BŰCHI, Switzerland) and the distillate were collected in a conical flask contained 10 ml of 3% (v/v) boric acid and four to five drops of indicator (0.10 g of methyl red and 0.05 g of methylene blue in 100 ml of ethyl alcohol). The solution was titrated with 0.01 N of hydrochloric acid until colour changes from green to pink. Total nitrogen was calculated based on following formula:

$$\% \, \mathrm{N} = \frac{(\mathrm{a} - \mathrm{b}) \times \ 0.00014 \ \times \ \mathrm{c}}{\mathrm{s}} \times 100$$

where, 1 ml of 0.01 N HCl = 0.00014 g nitrogen

a = Amount of hydrochloric acid required for titration sample solution (ml)

b = Amount of hydrochloric acid required for titration blank (ml)

c = volume of sample solution used in distillation process (ml)

s = weight of air-dry sample (g)

3.6.4 Determination of Total Elements Content in Soil Sample

The content of other total elements (P, K, S, Ca, Mg, Zn, Fe, B, Al) were determined by aqua regia digestion method (Nieuwenhuize et al., 1991) with a few modification. A fine soil sample (<0.25 mm) of 0.5000 g was digested in 4ml of 3:1 (v/v) mixture of HCl

(35%) and HNO_3 (67%) on a hotplate (Protech, Malaysia) at 110 °C. After evaporation to near dryness, the sample was diluted with 10 ml of 1.2% (v/v) HNO_3 and were heated at 80 °C for 30 minutes. After left overnight, the sample were then filtered through Whatman no. 42 filters and again left overnight for solution to completely filtrate into 50 ml of volumetric flask (until filter paper completely dried). Then, the solution made up to 50 ml with reverse osmosis water. The filtrated solution was analysed by using Inductive Couple Plasma Optical Emission Spectrometer (725-ES ICP-OES, Varian, Australia).

3.7 Plant Nutrient Analysis

The nutritional status of the plant was analysed using the D-leaf as the sample which is considered as the best leaf that represents the nutritional state of the plant. Leaf sampling was conducted after six months planting and during the red bud stage and its nutrient content (N, P, K, S, Ca, Mg, Zn, Fe, B, Al) was determined. Total N was measured by using combustion method while other elements using dry ashing and digestion with nitric acid method.

3.7.1 Preparation of D-Leaf Sample

The nutrients in the plants were determined using the D-leaves. The D-leaf was identified by gathering all the leaves in the hands to form a vertical "bundle" in the centre of the plant where D-leaves are the longest ones. The samples were collected and washed with distilled water, then were blot dried before put into an oven (Memmert, Germany) at 70 to 75 °C until completely dried (Chen & Ma, 2001; Lin & Chen, 2011). The dried D-leaf samples were finely ground and kept in air-tight container until further analysis.

3.7.2 Determination of Total Nitrogen in D-leaf Sample

The total N for D-leaf samples were determined by dry combustion using a Nitrogen Determinator (FP-528, LECO, United Kingdom). Generally, 0.1±0.0005 g of finely ground leaf sample was weighed into tin foil cups (LECO, Switzerland). The tin foil was twisted to seal. Then, the recorded weight and code of sample was inserted into the machine. The sample was put into a machine and start button was pressed to analyse the sample. The samples were combusted at 950 °C in an oxygen rich atmosphere. The combustion gases were scrubbed of water and carbon dioxide and then was passed through a hot column. The resulting nitrogen gas was measured by thermal conductivity in a helium carrier. The Nitrogen Determinator was calibrate by using EDTA (LECO, USA) as a blank. The methods was based on the guideline describes in the equipment's manual (LECO, 2001).

3.7.3 Determination of Total Elements Content in D-leaf Sample

On the other hand, for total elements in D-leaves samples were determined by dry ashing method (Miller, 1998; White et al., 1985). A finely ground sample (0.5000 g, <0.25 mm) was placed in a porcelain crucible and heated in muffle furnace. The furnace temperature was slowly increased from room temperature to 550 °C for eight hours and left it to cool overnight. The grey ash residue was dissolved with a few drops of reverse osmosis water before 2 ml of hydrochloric acid (37% HCl) was added and heated slowly until dryness on sandbath. After 30 minutes, the residue was dissolved in 10 ml of 20% (v/v) HNO_3 and simmer on sandbath until 1/3 volume of mixture was obtained. Then, the solution was filtered through Whatman No.42 filters. The residue was washed with reverse osmosis water, filtrate and combined with the sample extract solution in the volumetric flask. The filtration process was left overnight for filter paper to completely dried and then diluted to 50 ml with reverse osmosis water. The filtrated solution was

analysed by using Inductive Couple Plasma Optical Emission Spectrometer (725-ES ICP-OES, Varian, Australia). A blank digest was carried out in the same way.

3.7.4 Standard of Total Element Analysis (ICP-OES)

In determination of the total elements in the soil and plant extract of MD2 pineapple plant (*in vivo* and *ex vitro*), the standard was used to calibrate the Inductive Couple Plasma Optical Emission Spectrometer (725-ES ICP-OES, Varian, Australia) before sample was analysed. Standard for K, Mg, Ca, Mg, Fe, B, Zn, and Al was obtained from Merck (Darmstadt, Germany). The multi-element standard was diluted in nitric acid with difference concentration (0 to 100 ppm) in the volumetric flask. In other hand, standard for sulphur and phosphorus was also purchased from Merck (Darmstadt, Germany) was diluted in distilled water (0 to 50 ppm). The blank was reverse osmosis water. The wavelength, range of concentration, linear equation and correlation coefficient of standard used to analyse soil samples at different period with ICP-OES was stated in the Appendix B.

3.8 Determination of Bioactive Compounds in Fruit Extract

On the other hand, after the investigation on the effect of nutrients content in the soil and plant to the growth and productivity of MD2 pineapple plants, the secondary metabolites and their bioactivity potential in the fruits was determined. This was important in plant defence mechanism in order to survive and inhibit molecular damage (Haripyaree et al., 2010). The brief procedure for qualitative and quantitative analysis was described in the next section.

3.8.1 Chemicals and Reagents

Methanol (CH_3OH), iron (III) chloride ($FeCl_3$), ascorbic acid, acetone (C_2H_4O), potassium persulfate ($K_2S_2O_8$) and sodium carbonate (Na_2CO_3) were purchased from Systerm, Malaysia. Folin-ciocalteau reagent, sodium acetate anyhydrous ($C_2H_3NaO_2$), hydrochloric acid (HCl) and gallic acid ($C_7H_6O_5$) were purchased from Merck, Germany. Lead (II) acetate ($(CH_3COO_2)_2Pb$), ferrous sulphate heptahydrate ($FeSO_4.7H_2O$), ferric chloride hexahydrate ($FeCl_3.6H_2O$), bismuth nitrate 5-hydrate ($Bi(NO_3)_3.5H_2O$), mercury (II) chloride ($HgCl_2$), potassium iodide (KI), iodine (I) and nitric acid (HNO_3) were obtained from R & M Chemical, UK whereas glacial acetic acid was purchased from Fisher Scientific, UK. 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and was purchased from Sigma, Germany; 2, 4, 6-tripyridyl-s-triazine (TPTZ) and 3-ethyl-benzothiazoline-6-sulfonic acid (ABTS) was purchased from Sigma-Aldrich, Switzerland. All chemicals and solvents used were analytical grade.

Dragendorff reagent was prepared by dissolved 8.0 g of bismuth nitrate in 12 ml of 30% (v/v) nitric acid. Then, 27.2 g of potassium iodide was dissolved in 50 ml of distilled water and the solution was added into the bismuth nitrate solution. The volume was adjusted to 100 ml distilled water. Mayer reagent was prepared by dissolved 1.36 g of mercury (II) chloride in 60 ml of distilled water (Solution A) and 5.0 g of potassium iodide was dissolved in 10 ml of distilled water (Solution B). The solution A and solution B were mixed, and the volume adjusted to 100 ml with distilled water. Wagner reagent was prepared by dissolved 2.0 g of potassium iodide in distilled water and 1.27 g of iodine was added into the solution. The solution was mixed thoroughly, and then the volume was adjusted to 100 ml with distilled water.

3.8.2 Sample Extraction

Five grams of freeze-dried samples were extracted with 150 ml of 99.8% methanol at room temperature for 48 hours under dark condition using orbital shaker (722-2T, Protech, Malaysia) at 100 rpm. The extracts were filtered through Whatman No. 2 filter paper and the filtrate was collected and stored at -20 °C. The residue was re-extracted and filtered. The extracts were pooled and centrifuged at 9000 rpm, 4 °C for 5 min. Then, the supernatant was concentrated to dryness using a Rotavapor® (R-3, Büchi Labortechnik AG, Switzerland) at 45 °C (Kalaiselvi et al., 2012). The concentration of the solvent-free extract was adjusted to 20 mg/mL using 99.8% methanol and stored at -20 °C in an air tight container until further analysis. As far as possible, all extraction procedures were performed under daylight protection.

3.8.3 Phytochemicals Screening

Chemical test was performed on the methanolic extract of pineapple pulp to identify bioactive secondary metabolites according to standard assay procedure as described by Solihah et al. (2012) with slight modifications.

3.8.3.1 Test for Phenols

2 ml of methanol extract was taken into waterbath (WNB-10, Memmert, Germany) at 45 to 50 °C. 2 ml of 3% $FeCl_3$ was added to the extract solution. Formation of green or blue colour indicated the presence of phenols.

3.8.3.2 Test for Tannins

1 ml of methanol extract was added to 1 ml of 3% of $FeCl_3$. A greenish black precipitate signified the presence of tannins.

3.8.3.3 Test for Flavonoids (I)

1 ml of methanol extract was added to 1 ml of 10% $(CH_3COO_2)_2Pb$ and gently shaken. Formation of muddy brownish precipitate indicated the presence of flavonoids.

3.8.3.4 Test for Flavonoids (II)

1 ml of methanol extract was added to 10% of $FeCl_3$. The mixture was shaken. Formation of woolly brownish precipitate indicated the presence of flavonoids.

3.8.3.5 Test for Alkaloids

1 ml of methanol extract was stirred with 5 ml of 1% HCl on a steam bath minutes and filtered. The filtrate was used to test for alkaloids I, alkaloids II and alkaloids III.

(a) ***Test for alkaloids I***

1 ml of Dragendorff reagent was added to 1 ml filtrate. The formation of cloudy orange indicated the presence of alkaloids.

(b) ***Test for alkaloids II***

1 ml of Mayer reagent was added to 1 ml of filtrate. Appearance of slight yellow colour indicated the presence of alkaloids.

(c) ***Test for alkaloids III***

1 ml of Wagner reagent was added to 1 ml of filtrate. The formation of turbid brown colour indicated the presence of alkaloids.

3.8.4 Antioxidant Assay of Fruit Extracts

The antioxidant capacity of the MD2 pineapple fruit extract was examined using three different assays (DPPH radical scavenging activity assay, ABTS radical scavenging activity assay and FRAP reducing power assay) and was correlated with phenolic content, chlorophyll α, chlorophyll b and total carotenoid.

3.8.4.1 Determination of Total Phenolic Contents

Total phenolic contents (TPC) of the fruit extracts were determined using the Folin–Ciocalteu (FC) method as described by Singleton *et al*. (Singleton et al., 1999). Briefly, 20 µl of sample (20 mg/ml) was mixed with 1.58 ml distilled water and 100 µl of 2 N Folic-Ciocalteu reagent. Then, 300 µl of 20% (w/v) Na_2CO_3 was added (30 s to 8 min) into the solution. The solution was incubated for two hours at room temperature, in the dark condition. The absorbance of the samples was read using a UV/Vis spectrophotometer (Lamda 25, Perkin Elmer, USA) at 765 nm. The blank and standard was prepared with the similar method. The standard solution of gallic acid (r^2= 0.99) was used to prepare the calibration curve (Appendix C). The TPC content of the samples was expressed as mg gallic acid equivalents (GAE)/ g of dried extract and was calculated based on the following formula:

$$\text{Total phenolic content (mg GAE / g dry extract)} = \frac{cV}{m}$$

where, c = concentration of gallic acid obtain from calibration curve (mg/ml)

V = volume of extract (ml)

m = weight of extract (g)

3.8.4.2 Determination of Total Carotenoids, Total Chlorophyll, Chlorophyll a and Chlorophyll b

Methanolic solutions of fruit extracts was analysed using a UV/Vis spectrophotometer (Lamda 25, Perkin Elmer, USA) at 470, 652.4 and 665.2 nm. The concentrations of total carotenoids, chlorophylls a and chlorophyll b were determined according to the formula by Lichtenthaler and Buschmann (Lichtenthaler & Buschmann, 2001) as follows:

$$C_{(x+c)}\ (mg/l) = \frac{1000\ A_{470} - (1.63\ Chl_a) - (104.96\ Chl_b)}{221}$$

$$C_a\ (mg/l) = 16.72\ A_{665.2} - 9.16\ A_{652.4}$$

$$C_b\ (mg/l) = 34.09\ A_{652.4} - 15.28\ A_{665.2}$$

3.8.4.3 DPPH Radical Scavenging Activity Assay

The DPPH assay was performed following Brand-Williams et al. (1995) with some modification. The positive control used was ascorbic acid. A 3 mM solution of DPPH radical solution in methanol was prepared, and 150 µl of this solution was mixed with 50 µl of methanolic extract of fruit (2 to 12 mg/ml), standard solution (0.01 to 1.00 mg/ml) and control (methanol) in different wells for triplicates. Then, the solution was allowed to stand for 30 minutes in the dark. After that, the absorbance was measured at 515 nm using the microplate spectrophotometer (Multiskan™ GO, Thermo Scientific, USA). Scavenging of free radicals by DPPH as percentage of radical scavenging activities (%RSA) was calculated as follows:

$$\text{DPPH radical scavenging activity (\%)} = \frac{A_o - A_1}{A_o} \text{ x } 10$$

where, A_o is the absorbance of the control, A_1 is the absorbance of samples.

The graph of DPPH radical scavenging activity percentage against concentration was plotted using a non-linear regression (third degree polynomial) as previously described by Samad et al. (2016) for methanolic fruit extract from *in vivo* plant treatment. For *ex vitro* plant treatment, linear regression was plotted between percentage of inhibition against the concentration (r^2= 0.99). The result was reported as concentration of sample required to reduce in 50% concentration of DPPH (IC_{50}) in mg/ml. The more potent antioxidant denotes by lower if IC_{50} value.

3.8.4.4 ABTS Radical Scavenging Activity Assay

For ABTS assay, the method described by Miller et al. (1993) was followed, but with few modifications. The stock solutions included 7.4 mM ABTS solution and 2.6 mM potassium persulfate solution. The working solution was prepared by mixing the two stock solutions in equal quantities and incubated for 12 to 16 hours at room temperature in the dark before use. The solution was then diluted by mixing with deionized water (18.2 $M\Omega cm^{-1}$) to obtain an absorbance of 0.70±0.02 units at 734 nm using the microplate spectrophotometer (Multiskan™ GO, Thermo Scientific, USA). Fresh ABTS solution was prepared for each assay. Fruit extracts (20 µl) at six different concentrations (2.0 to 12.0 mg/ml) were allowed to react with 200 µl of the ABTS solution for 10 min in a dark condition. Then the absorbance was taken at 734 nm using the spectrophotometer and the assay was performed in triplicates. The standard curve was plotted using non-linear regression (third degree polynomial) (*in vivo* plant) and linear regression (*ex vitro* plant) between percentage of inhibition against concentration (r^2= 0.99). The results were interpreted as 50% inhibition concentration (IC_{50}) values in mg/ml. The positive control used was ascorbic acid.

3.8.4.5 FRAP Reducing Power Assay

The FRAP assay was performed according to (Benzie & Strain, 1999) with some modification. The stock solutions included 300 mM acetate buffer (3.1 g C_2H_3NaOO and 16 ml C_2H_4O), pH 3.6, 10 mM TPTZ (2, 4, 6tripyridyl-s-triazine) solution in 40 mM HCl, and 20 mM $FeCl_3.6H_2O$ solution. The fresh working solution was prepared by mixing acetate buffer, TPTZ solution, $FeCl_3.6H_2O$ solution and distilled water in a ratio of 10:1:1:1.2 then warmed at 37 °C before using. Fruit extracts (10 µl) were allowed to react with 300 µl of the FRAP solution for 30 min in the dark condition. Readings of the coloured product (ferrous tripyridyltriazine complex) was then taken at 593 nm using microplate spectrophotometer (Multiskan™ GO, Thermo Scientific, USA). The standard curve was linear between 0.01 and 0.10 mg/ml of ferrous sulphate $FeSO_4.7H_2O$ (r^2= 0.99) (Appendix D). FRAP values were expressed in milligram of ferrous equivalent Fe (II) per gram of dried extract.

3.9 Determination and Quantification of Carotenoid Content

HPLC-MS analysis were performed on an HPLC system (1200 series, Agilent Technologies, USA), equipped with an autosampler, binary pump, injector, micro vacuum degasser, thermostatted column compartment and a UV-Vis diode array detection (DAD). HPLC analysis was performed according to the method described by Othman et al. (2015). All chemicals and solvents used were of analytical grade or higher suitable for HPLC and was filtered through a 0.22 µm PTFE syringe filter (Millipore). Acetone (C_2H_4O), ethyl acetate ($C_4H_8O_2$), potassium hydroxide (KOH) and sodium chloride (NaCl) were purchased from Systerm, Malaysia whereas methanol (CH_3OH), n-Hexane, and acetonitrile (CH_3CN) were obtained from Labscan, Thailand. 3,5-di-tert-4-butylhydroxytoluene (BHT) was purchased from Sigma, Switzerland. Water was prepared using a Mili-Q reagent water system.

3.9.1 Preparation of Sample

The pineapple fruit's pulp was cut and weighed to five grams. For each sample, the fresh pulp was pooled from three different fruits, mixed and immediately stored at -20 °C. Then, the fruit samples were freeze-dried for seven days using freeze dyer (Labconco, USA). The samples were ground into fine powder and stored at -80 °C until further analysis.

3.9.2 Extraction of carotenoids

For each sample, 1.0 g of freeze-dried fruit samples were rehydrated with 1.0 ml distilled water and soaked overnight at RT in 5 ml of acetone: methanol (7:3). Then, the mixture was vortexed and centrifuged at 13,500 g for two minutes (Thermo Scientific, Germany), where the supernatant was then collected and transferred into a foil covered 50 ml centrifuge tubes. The supernatant was centrifuged again at 13,500 g for five minutes to remove fine particulates. The extract was then stored at 4 °C in the dark, prior to analysis. For extraction of carotenoids, equal volume of hexane and distilled water (1:1) was added to the combined supernatants. The mixture was vortexed and centrifuged at 13500 g for one minute to collect upper hexane layer and the procedure was repeated until the hexane layer seemed colourless. The combined carotenoid layer (upper phase) was collected and dried completely under a gentle stream of oxygen-free nitrogen gas. Then, the vials were immediately capped and sealed with parafilm to prevent oxidation before being stored at -80 °C until subsequent analysis.

3.9.3 Saponification of carotenoid extract for HPLC analysis

A 100 µl of ethyl acetate was added into the carotenoid extract. Then 400 µl of acetonitrile and water (9:1) was added into the mixture. After that to give a final volume of 1 ml, 500 µl of 10% (w/v) methanolic potassium hydroxide solution was added. After

vortexing, the samples were incubated overnight in darkness at room temperature. The next day, 2 ml of hexane was added to extract the base carotenoids, followed by addition 2 ml of 10% NaCl until phase separation was achieved. The upper aqueous phase was removed and re-extracted again with 2 ml of hexane and the combined hexane extracts were washed three times with distilled water. The samples were then dried under a gentle stream of oxygen-free nitrogen and resuspended immediately in 250 µl ethyl acetate. Aliquots of 50 µl were used for spectrophotometric measurement of total carotenoid content to estimate any potential losses of carotenoids following saponification. The remaining sample (200 µl) was retained for analysis of individual carotenoid by HPLC.

3.9.4 Chromatographic Analysis

Sample (10 µl) was injected into a 5 µm, 4.6 × 250 mm ZORBAX SB-C18 end capped reverse phase column (Agilent Technologies, USA) at 20 °C. The mobile phase consisted of 9:1 (v/v) acetonitrile: water (eluent A) and 100% ethyl acetate (eluent B). The analysis was conducted using a gradient program: from 0 to 40% solvent B (0 to 20 min), from 40 to 60% solvent B (20 to 25 min), from 60 to 100% solvent B (25 to 25.1 min), 100% solvent B (25.1 to 35 min), 100% solvent B (35 to 35.1 min). Simultaneous monitoring was performed at 350 to 550 nm at a flow rate of 1.0 ml/min. The column was allowed to re-equilibrate in 100% solvent A for 10 min before the next sample injection. Peak height of less than 10 mAU was not detected. The fruit extracts were screened for 8 types of carotenoid (neoxanthin, violaxanthin, zeaxanthin, β-cryptoxanthin, α-carotene, β-carotene, lycopene, lutein), vitamin A (retinol) and vitamin E (α-tocopherol).

3.9.5 Identification and Quantification of Carotenoids, Vitamin A and E

The carotenoid compounds, vitamin A and E were identified in pineapple pulp by comparing their retention time with those respective standards. The quantification of the

mention compounds was calculated based on standard curves obtained from HPLC analysis, and the concentration of each carotenoid was expressed as µg per g dry weight samples (µg g^{-1} DW), while vitamin E was expressed as mg 100 g^{-1} DW. Vitamin A activity was expressed as µg of retinol equivalents (RE) 100 g^{-1} DW.

3.9.6 Preparation of the Standard Curve of Carotenoids, Vitamin A and E

In order to determined and quantified the carotenoids in the pulp MD2 pineapple plant (*in vivo* and *ex vitro*) fruit extract, the standards were prepared prior to analysis. The standard stock was diluted in an appropriate solvent (ethanol, hexane or acetone) with 0.01% (w/v) of BHT. The absorption maximum, retention time (Table 3.2) and spectral characteristics was observed as described by (Britton et al., 1995) to reveal the identity of individual carotenoid. The standard was injected into HPLC system and the linear regression equation and correlation coefficient (r^2) for each standard curve of α-carotene (CAS 7488-99-5), β-carotene (CAS 7235-40-7), neoxanthin (CAS 14660-91-4), violaxanthin (CAS 126-29-4), lutein (CAS 127-40-2), zeaxanthin (CAS 144-68-3), β-cryptoxanthin (CAS 472-70-8), lycopene (CAS 502-65-8), retinol (CAS 68-26-8) and α-tocopherol (CAS 59-02-9) was obtained and calculated using HPLC system software (Appendix E).

Table 3.2: Carotenoids, retinol and α-tocopherol retention time

Compound	Retention time (RT)
α-carotene	27.83
β-carotene	27.85
Neoxanthin	4.87
Violaxanthin	5.92
Lutein	9.96
Zeaxanthin	9.42
β-cryptoxanthin	21.56
Lycopene	26.61
Retinol	6.15
α-tocopherol	22.61

3.10 Cost Estimation

The cost of labour and fertilizer used throughout the study was also analysed. Total profit (per kg fruit and per plant per year) was then estimated by calculating the difference between the total value of the fruits produced (in Ringgit Malaysia; RM) and the total cost. In this study, the cost of labour was fixed at RM50/day per person, while the market price of the fruits was fixed at RM8 per kg (Times, 2017). The cost of the starting material (seedling), irrigation, and flower-inducing hormone were not included in the cost analysis.

3.11 Statistical Analysis

The statistical evaluation was analysed by using IBM SPSS version 24 (IBM Inc., Armonk, NY, USA). Normality test was performed at confidence level 95% and the data were considered normally distributed when $P > 0.05$. All data were presented as mean ± standard error of mean. General linear model (multivariate and repeated measures) was used to analyse the data taken by monthly (plant height, number of leaves, length and width of D-leaves, SPAD reading of D-leaves). For other parameters, the mean differences between the variables were determined by using one-way analysis of variance (ANOVA) and Duncan Multiple Range Test (DMRT). The differences were considered statistically significant when $P \leq 0.05$ and was indicated by different letters in the same row/column. Correlations among data were calculated using Pearson's correlation coefficient in bivariate correlations.

CHAPTER 4: RESULTS

4.1 Evaluation of Plant Growth Performance

In the present study, the growth performance of MD2 pineapple plants (*in vivo* and *ex vitro*) grown with different types of fertilizers was evaluated, where the plant height, number of leaves, length and width of D-leaf and chlorophyll content (estimated using SPAD meter) were recorded and measured monthly. Flowering induction was carried out when the plants have reached their optimum growth; after nine months of planting (MAP) for the *in vivo* plants and 15 MAP for the *ex vitro* plants. The outcome of these experiments was presented in the following sections.

4.1.1 Plant Height

The height of the MD2 pineapple plants were observed from two MAP until 13 MAP (*in vivo*) and 18 MAP (*ex vitro*). For *in vivo* plants, the plant height ranged from 41.2 cm to 106.5 cm. Data analysis revealed that MD2 pineapple plants supplemented with chemical fertilizer grew taller than plants supplemented with vermicompost and control plants, with mean plant height of 83.0 cm, 79.6 cm and 76.3 cm, respectively. However, the difference in height of plants supplemented with chemical fertilizer and vermicompost was not statistically significant (Table 4.1). The plant height of *ex vitro* plants ranged between 11 cm to 95 cm. Similar trend was observed, the height of both *in vivo* and *ex vitro* plants supplemented with vermicompost was comparable to that of plants supplemented with chemical fertilizer (Figure 4.1). Moreover, the height of plant treated with vermicompost was observed grew taller on 13 MAP to 15 MAP as well as on 18 MAP compared to plant treated with chemical fertilizer. The plants (both *in vivo* and *ex vitro*) treated with both types of fertilizers were also significantly taller than the control plants ($P \leq 0.05$).

Table 4.1: The mean of plant height grown with the effect supplementation of vermicompost and chemical fertilizer on *in vivo* and *ex vitro* MD2 pineapple plants in the field compared to control (unfertilized).

Treatment	*In vivo*	*Ex vitro*
Control	76.3 ± 1.3 [b]	47.1 ± 2.4 [b]
Chemical fertilizer	82.98 ± 1.3 [a]	57.3 ± 2.4 [a]
Vermicompost	79.6 ± 1.3 [ab]	57.0 ± 2.4 [a]

* Means ± standard error of mean value followed by different letters in column are significantly different using repeated measures ANOVA, Duncan's multiple range test at $P \leq 0.05$, n=12.

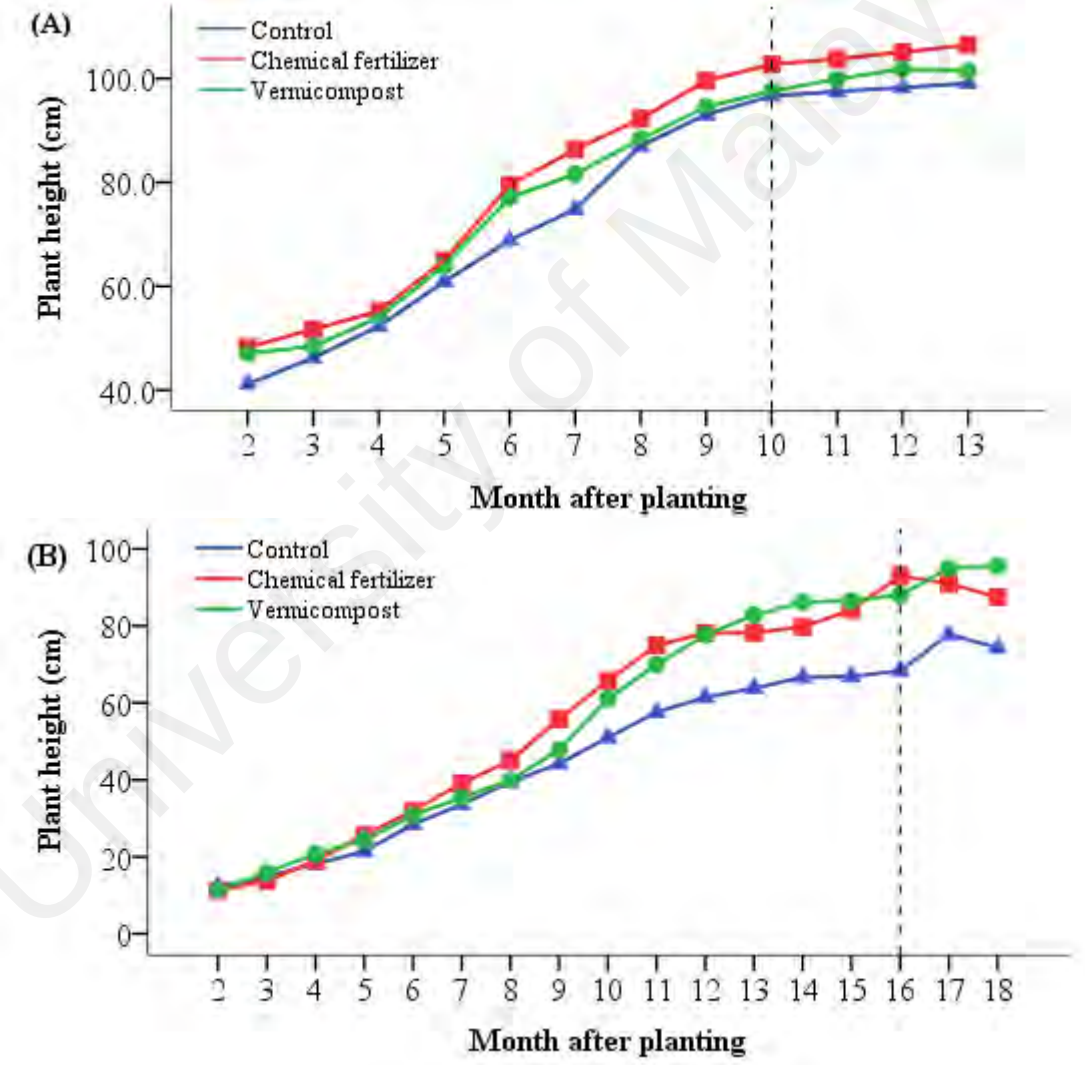

Figure 4.1: The mean of plant height by monthly with the effect of vermicompost and chemical fertilizer supplementation to the (A) *in vivo* and (B) *ex vitro* MD2 pineapple plants in the field compared to control. Each data point represents the mean of twelve replicates (n=12). The time for flowering induction was indicated by the dotted line.

4.1.2 Number of Leaves

In vivo grown plants supplemented with vermicompost were observed to produce comparable number of leaves with *in vivo* grown plants supplemented with chemical fertilizer (Table 4.2). For *ex vitro* plants, supplementation of chemical fertilizer produced the significantly highest number of leaves, compared to vermicompost and control ($P \leq 0.05$). However, there was no statistically difference between number of leaves of plant supplemented with vermicompost and untreated (control) plants for both type of plants (*in vivo* and *ex vitro*). After flowering (10 MAP), the number of leaves remained unchanged with time (Figure 4.2).

Table 4.2: The mean of leaf number grown with the effect supplementation of vermicompost and chemical fertilizer on *in vivo* and *ex vitro* MD2 pineapple plants in the field compared to control (unfertilized).

Treatment	*In vivo*	*Ex vitro*
Control	42 ± 1 [b]	43 ± 1 [b]
Chemical fertilizer	47 ± 1 [a]	51 ± 1 [a]
Vermicompost	44 ± 1 [ab]	44 ± 1 [b]

* Means ± standard error of mean value followed by different letters in column are significantly different using repeated measures ANOVA, Duncan's multiple range test at $P \leq 0.05$, n=12.

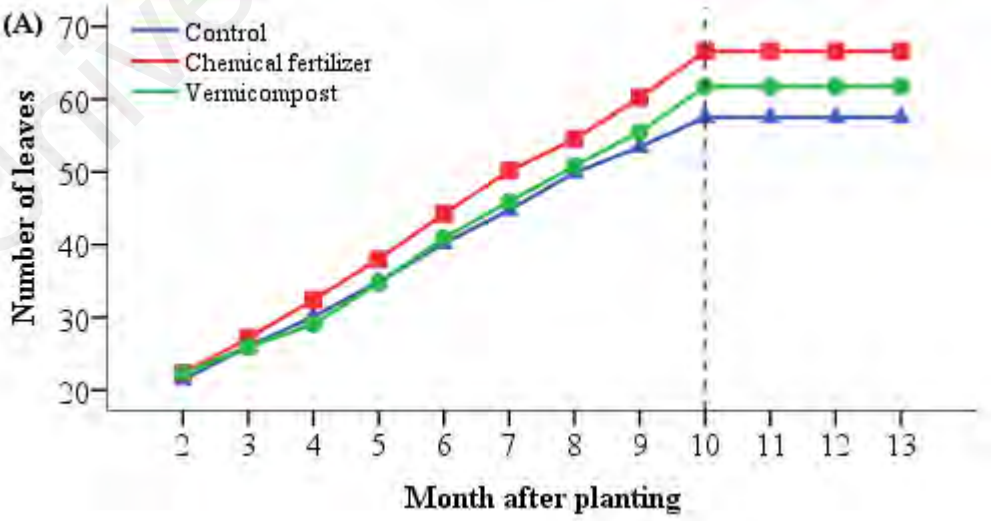

Figure 4.2: The mean of leaf number by monthly with the effect of vermicompost and chemical fertilizer supplementation to the (A) *in vivo* and (B) *ex vitro* MD2 pineapple plants in the field compared to control. Each data point represents the mean of twelve replicates (n=12). The time for flowering induction was indicated by the dotted line.

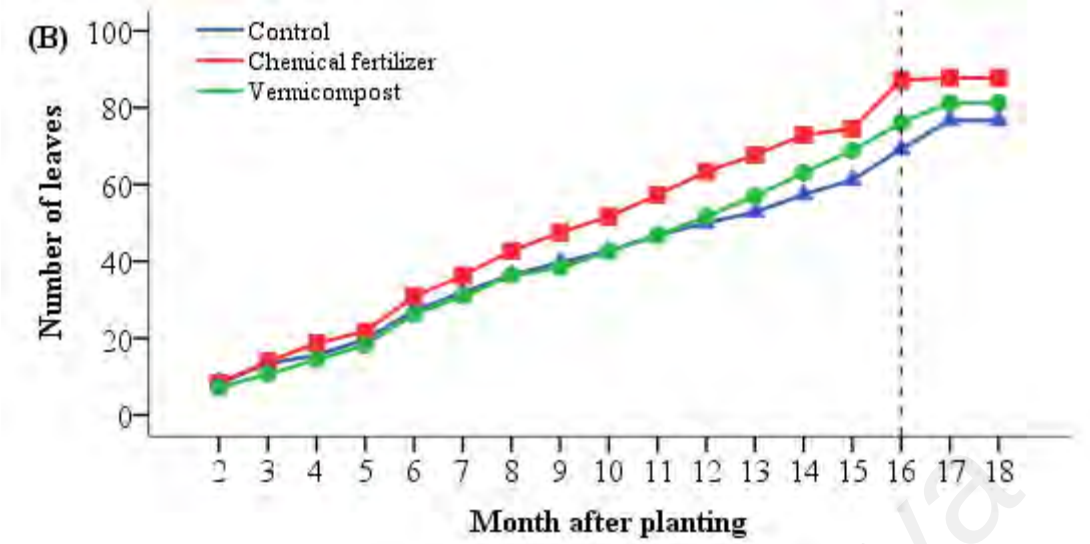

Figure 4.2, continued.

4.1.3 Length of D-leaf

Based on Table 4.3, it was shown that both *in vivo* and *ex vitro* plants supplemented with chemical fertilizer and vermicompost had D-leaves of similar length. However, data analysis revealed that the length of the D-leaf of the *ex vitro* plants treated with vermicompost showed a significantly marked increase compared to plants treated with chemical fertilizer after 13 MAP, possibly due to second supplementation of vermicompost to the soil (Figure 4.3B). On 18 MAP, the length of D-leaves was observed decrease for all treatments as the plants started to produce flowers.

Table 4.3: The mean of D-leaves length of *in vivo* and *ex vitro* MD2 pineapple plant grown with the effect supplementation of vermicompost and chemical fertilizer in the field compared to control (unfertilized).

Treatment	*In vivo*	*Ex vitro*
Control	67.5 ± 1.3 [a]	38.0 ± 1.9 [b]
Chemical fertilizer	70.2 ± 1.3 [a]	44.5 ± 1.9 [a]
Vermicompost	69.2 ± 1.3 [a]	46.0 ± 1.9 [a]

Means ± standard error of mean value followed by different letters in column are significantly different using repeated measures ANOVA, Duncan's multiple range test at $P \leq 0.05$, n=12.

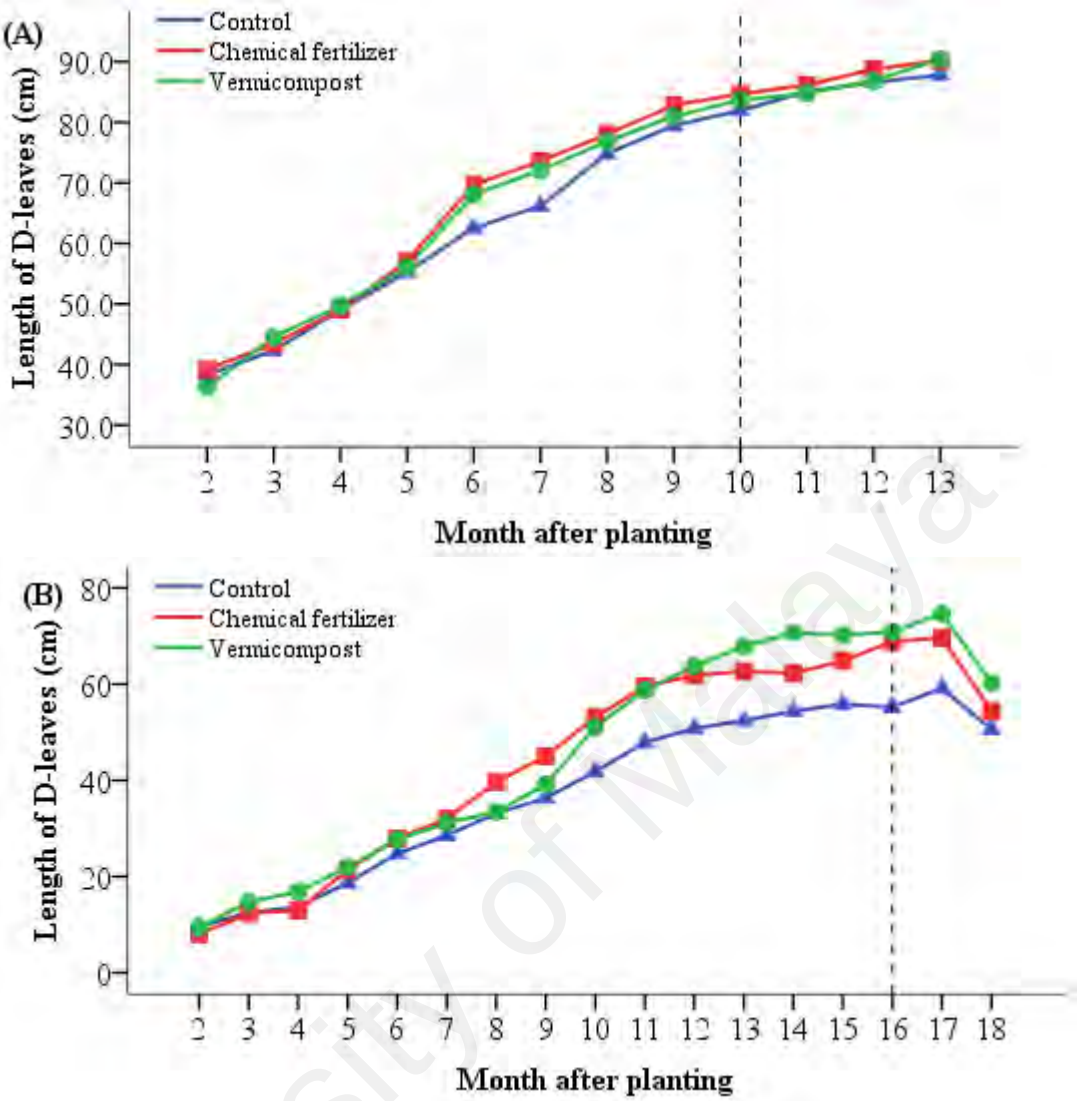

Figure 4.3: The mean of length of D-leaves by monthly with the effect of vermicompost and chemical fertilizer supplementation to the (A) *in vivo* and (B) *ex vitro* MD2 pineapple plants in the field compared to control. Each data point represents the mean of twelve replicates (n=12). The time for flowering induction was indicated by the dotted line.

4.1.4 Width of D-leaf

The width of D-leaves of both *in vivo* and *ex vitro* plants supplemented with vermicompost were comparable to that of chemical fertilizer, and both treatments produced wider leaves than control (Table 4.4). At six MAP, *in vivo* plants supplemented with chemical fertilizer showed significantly wider D-leaf size compared to control plants, with width of D-leaf of 6.5 cm and 4.6 cm respectively (Figure 4.4A). The width of the D-leaves nearly plateau after flowering was induced, resulting in the maximum width of 7.0 cm. Based on Figure 4.4B, after second supplementation of vermicompost

at eight MAP, *ex vitro* plants showed an increase in the width of D-leaf compared to plant treated with chemical fertilizer and control plants from 11 MAP until 15 MAP as they are still in their vegetative stage.

Table 4.4: The mean of D-leaves width of *in vivo* and *ex vitro* MD2 pineapple plant grown with the effect supplementation of vermicompost and chemical fertilizer in the field compared to control (unfertilized).

Treatment	***In vivo***	***Ex vitro***
Control	5.2 ± 0.1 [b]	3.8 ± 0.1 [b]
Chemical fertilizer	5.7 ± 0.1 [a]	4.3 ± 0.1 [a]
Vermicompost	5.4 ± 0.1 [ab]	4.2 ± 0.1 [a]

* Means ± standard error of mean value followed by different letters in column are significantly different using repeated measures ANOVA, Duncan's multiple range test at $p \leq 0.05$, n=12.

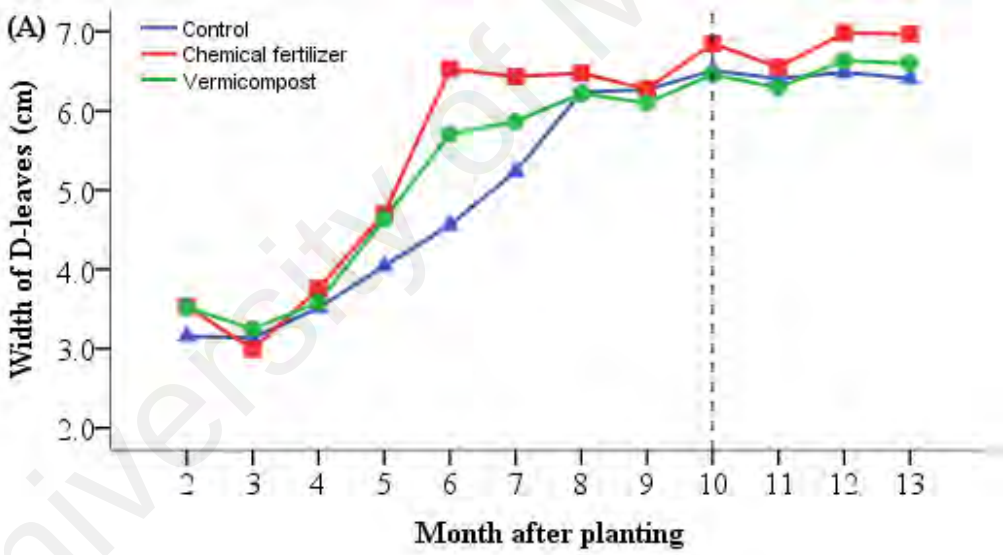

Figure 4.4: The mean of width of D-leaves by monthly with the effect of vermicompost and chemical fertilizer supplementation to the (A) *in vivo* and (B) *ex vitro* MD2 pineapple plants in the field compared to control. Each data point represents the mean of twelve replicates (n=12). The time for flowering induction was indicated by the dotted line.

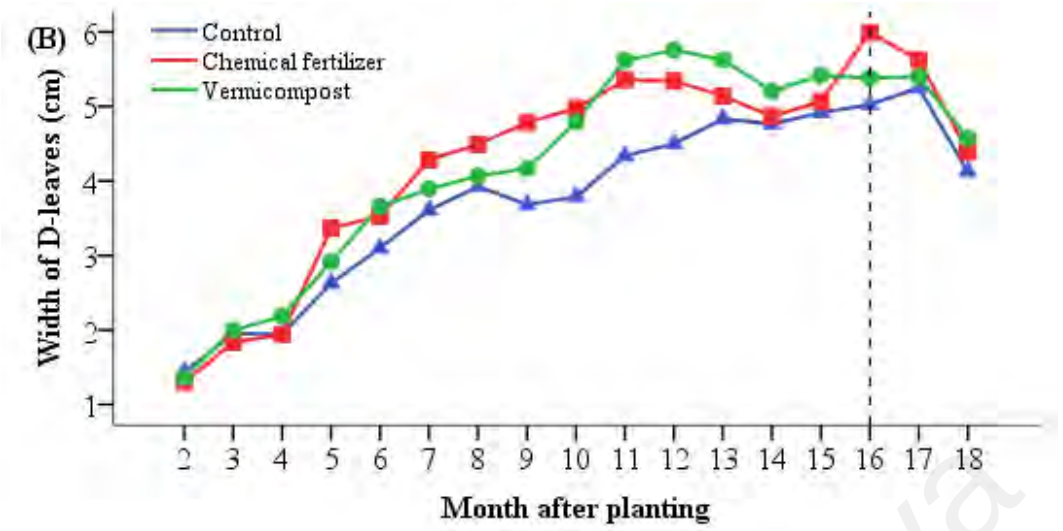

Figure 4.4, continued.

4.1.5 Relative Chlorophyll Content of D-leaves (SPAD)

In this study, the SPAD meter was used to estimate the chlorophyll and nitrogen content in the plants. For *in vivo* plants, the SPAD readings from two MAP until 12 MAP ranged between 56.19 to 81.73 SPAD (Figure 4.5). The SPAD reading of the leaves of *in vivo* grown plants drastically increased in plants supplemented with chemical fertilizer from six MAP, whereby the plants were already at their maturation stage. Data analysis also revealed that plants supplemented of chemical fertilizer significantly exhibited the highest chlorophyll content, $P \leq 0.05$ (Table 4.5). In contrast, plant supplemented with vermicompost exhibited the lowest chlorophyll content with 64.6 SPAD and 64.8 SPAD for *in vivo* and *ex vitro* plant, respectively. Nevertheless, the SPAD reading of pineapple leaves supplied with chemical fertilizer was observed to be greatly reduced on 12 MAP. Similar trend was observed on the chlorophyll content for *ex vitro* plants. However, the SPAD reading gradually increased after 12 MAP.

Table 4.5: The mean of relative chlorophyll content in D-leaves of *in vivo* and *ex vitro* MD2 pineapple plant grown with the effect supplementation of vermicompost and chemical fertilizer in the field compared to control (unfertilized).

Treatment	*In vivo*	*Ex vitro*
Control	65.1 ± 0.9 [b]	69.2 ± 1.0 [a]
Chemical fertilizer	71.7 ± 0.9 [a]	71.2 ± 1.0 [a]
Vermicompost	64.6 ± 0.9 [b]	64.8 ± 1.0 [b]

* Means ± standard error of mean value followed by different letters in column are significantly different using repeated measures ANOVA, Duncan's multiple range test at $P \leq 0.05$, n=12.

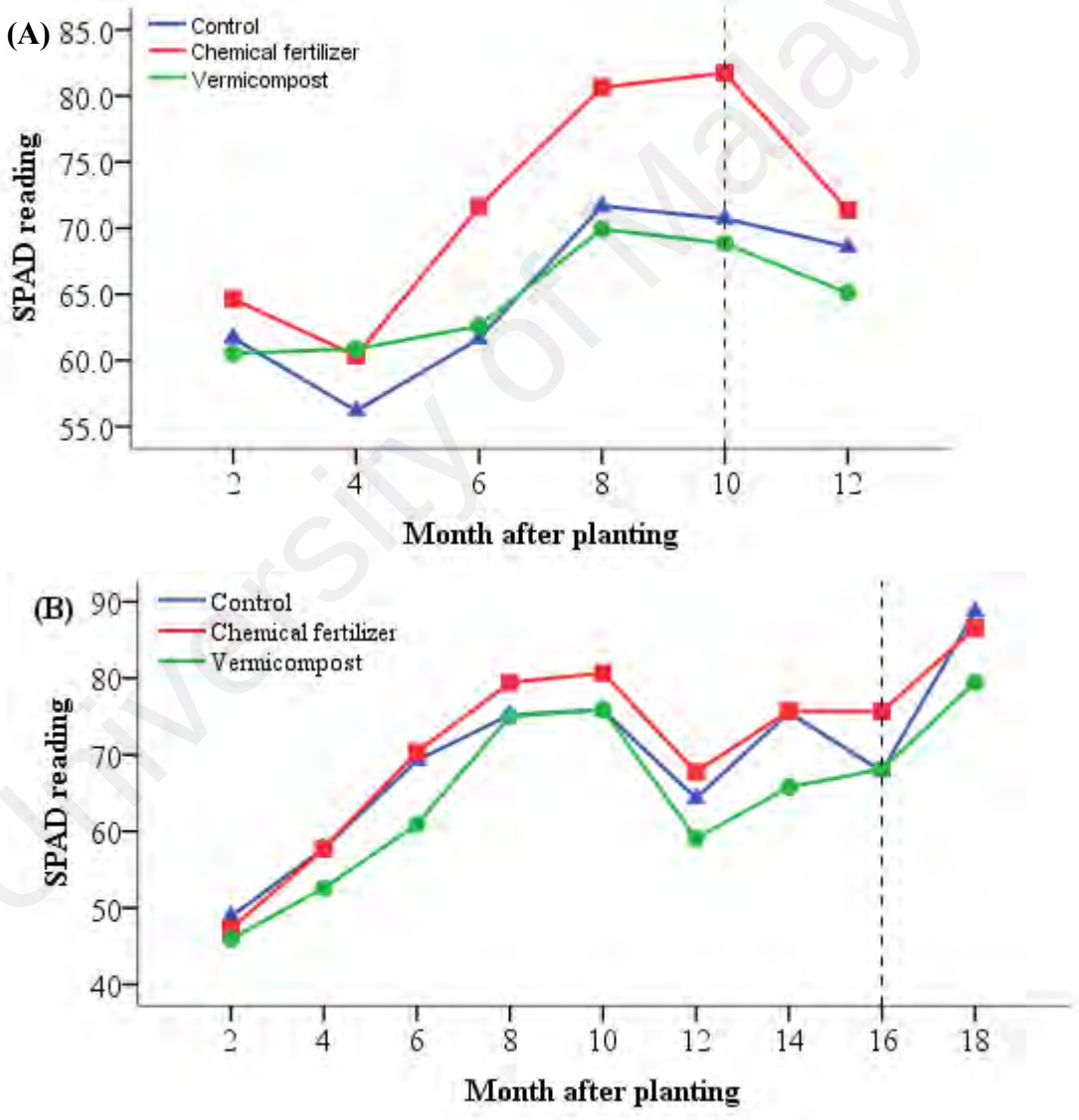

Figure 4.5: The mean of relative chlorophyll content every two months with the effect of vermicompost and chemical fertilizer supplementation to the (A) *in vivo* and (B) *ex vitro* MD2 pineapple plants in the field compared to control. Each data point represents the mean of twelve replicates (n=12). The time for flowering induction was indicated by the dotted line.

4.1.6 SEM Studies

A section across leaf (Figure 4.6A) shows the 'MD2' leaf structure consists of upper epidermis covered with thick and smooth cuticle; the water storage tissue, which nearly half of the leaf thickness, depending on the water status of the plant and the lower hypodermis with the stomata covered with dense flat and shield-shaped trichomes (Figure 4.6B).

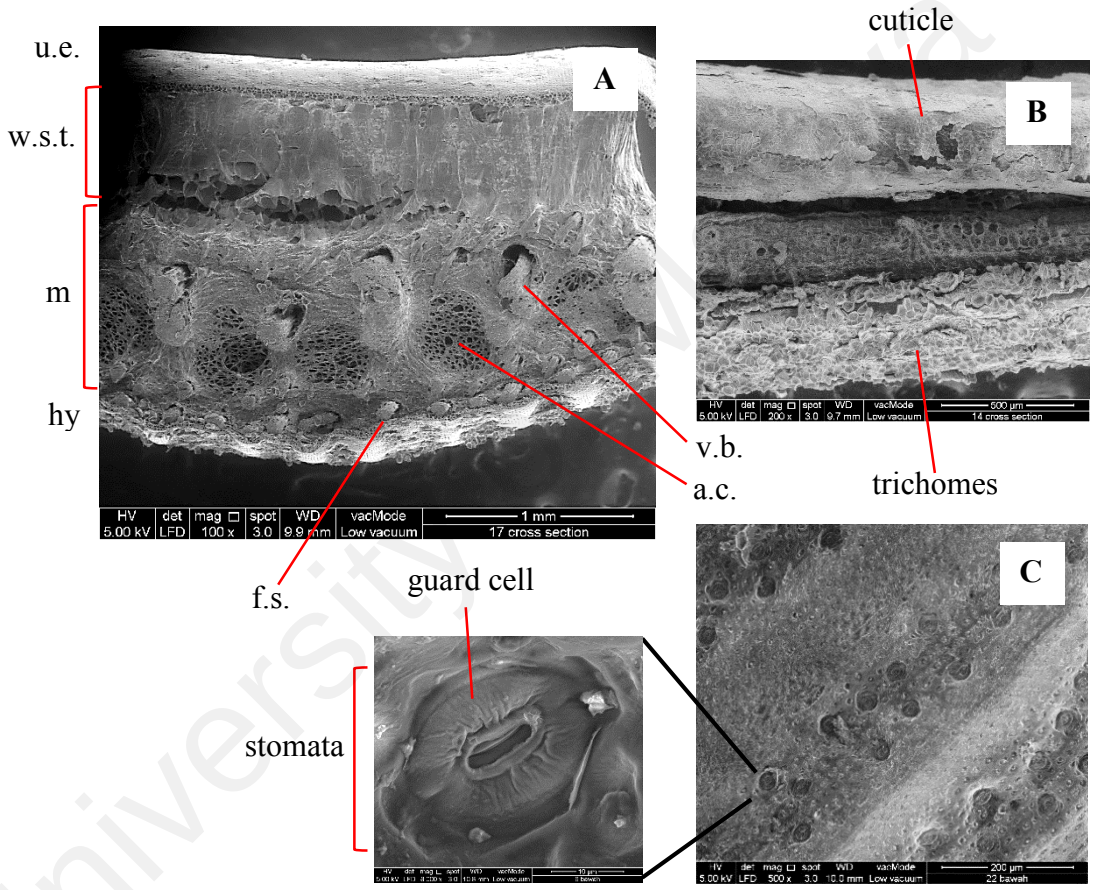

Figure 4.6: (A) The cross-transverse section of a MD2 leaf showing: u.e., upper epidermis; w.s.t., water storage tissue; m, mesophyll; hy, hypodermis; a.c., aerating canal; f.s., fiber strand; v.b., vascular bundle, (B) the longitudinal section of MD2 leaf showing differences of cuticle structure on abaxial surface and shield-shaped trichomes on adaxial surface, (C) the stomata structure and longitudinally arranged along the characteristic grooves of the abaxial leaf side (after removal of trichomes).

In order to measure the stomata, the cuticle and trichomes covered the surface of the leaf (adaxial and abaxial) were removed. Stomata are structures bounded together with a pair of guard cells on the epidermis of leaves. Based on the Figure 4.6C, the rows of stomata were observed on abaxial (lower) surface located in furrows that parallel to the longitudinal axis of leaves. However, after removal of cuticle on the upper epidermis of the D-leaf, no stomata were observed for both D-leaf of *in vivo* and *ex vitro* plants (Figure 4.7).

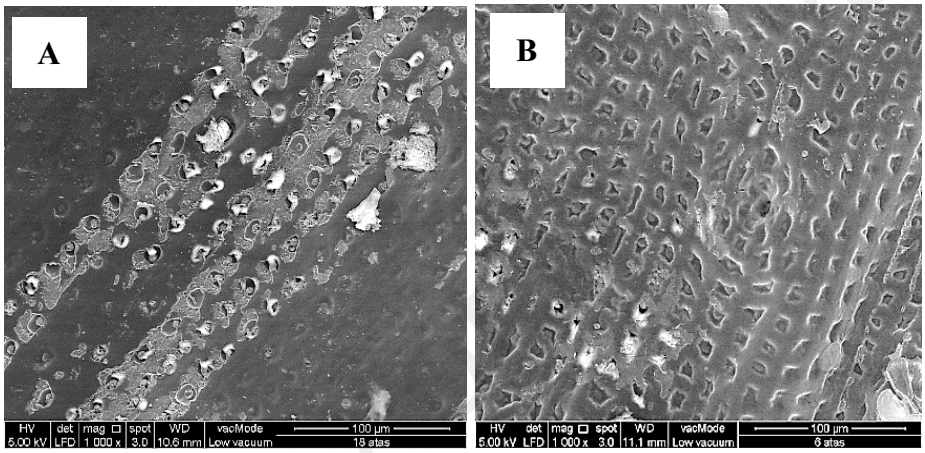

Figure 4.7: The adaxial surface (upper) of D-leaf after removal of shield-shaped trichomes on the surface (A) *in vivo* and (B) *ex vitro* of MD2 pineapple plants shows no stomata was observed.

4.1.6.1 *In vivo*

Based on Figure 4.8, stomata were discovered on the bottom (abaxial surface) of the *in vivo* pineapple plant's leaves after removal of shield-shaped trichomes. The rows of stomata were located in furrows that were parallel to the longitudinal axis of the leaves. The results showed that stomatal density of leaves of plant supplied with vermicompost (96.62 stomata per mm^2) was significantly higher than control plants (76.28 stomata per mm^2) at $P \leq 0.05$ (Table 4.6). However, there was no significant difference between plants treated with vermicompost and chemical fertilizer. Similar trend was observed on stomatal size whereby length of stomatal on D-leaves of plant treated with vermicompost significantly larger than chemical fertilizer and control but with similar width of stomatal.

Based on pore length of stomatal, plant supplemented with vermicompost showed widest pore length (9.64 µm), however there was no significance difference among treatments. Moreover, pore aperture of stomatal on D-leaf of plant treated with vermicompost (5.70 µm) also showed statistically significantly wider than pore aperture of stomatal from plant supplied with chemical fertilizer and control plants,4.60 µm and 4.35 µm respectively.

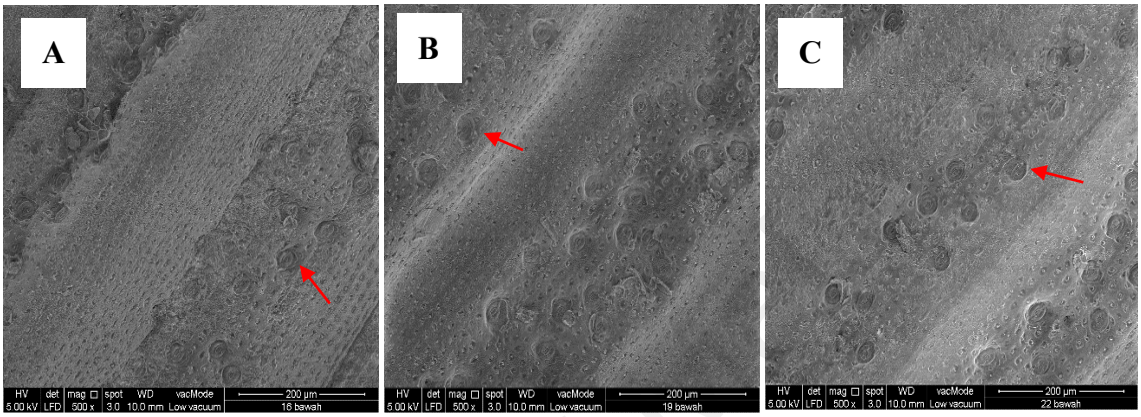

Figure 4.8: The abaxial surfaces (bottom) of *in vivo* D-leaf after removal of shield-shape trichomes on the surface (9 months after planting) as observed using FE-SEM at magnification of 500x under low vacuum; (A) control plants (B) plants supplemented with chemical fertilizer (C) plants supplemented with vermicompost. The arrow shows the stomata.

Table 4.6: The parameter of stomata in the lower epidermis of D-leaves from 9 months-old field grown *in vivo* MD2 pineapple plants supplemented with different types of fertilizers.

Treatment	Control	Chemical fertilizer	Vermicompost
Stomatal density (mm^2)	76.28 ± 7.64 ^{b}	85.60 ± 2.13 ^{ab}	96.62 ± 1.70 ^{a}
Stomatal size (μm^2)	566.78 ± 26.40 ^{b}	606.32 ± 19.77 ^{ab}	663.44 ± 12.45 ^{a}
Stomatal length (µm)	25.60 ± 0.38 ^{c}	26.41 ± 0.14 ^{b}	28.04 ± 0.11 ^{a}
Stomatal width (µm)	22.11 ± 0.72 ^{a}	22.96 ± 0.69 ^{a}	23.67 ± 0.52 ^{a}
Pore length (µm)	8.83 ± 0.13 ^{a}	9.10 ± 0.26 ^{a}	9.64 ± 0.61 ^{a}
Pore aperture (µm)	4.35 ±0.40 ^{b}	4.60 ± 0.18 ^{b}	5.79 ± 0.20 ^{a}

* Means ± standard error of mean followed by a different letter by row indicate significant differences by the Duncan Multiple Range Test (DMRT) at $P \leq 0.05$, n=4.

4.1.6.2 *Ex vitro*

For *ex vitro* plants, the stomatal distribution was similar to that of *in vivo* plants (Figure 4.9). In this study, stomatal density, stomatal size and some of stomatal features (stomatal length, stomatal width, pore length, pore aperture) were determined on D-leaves of pineapple plants supplied with different types of fertilizers on nine months after planting. The results showed that stomatal density of leaves of plant supplied with vermicompost (76.49 stomata per mm^2) was statistically significantly higher than control plants (56.78 stomata per mm^2) at $P \leq 0.05$ (Table 4.7). This research also found stomatal density was negatively correlated to stomatal size (r^2= -.570). However, other stomatal features were not significantly different among all treatments. Based on Pearson correlation coefficient, there was a strong, negative correlation between stomatal pore length and pore aperture, which statistically significant with r^2 = -.639 at $P \leq 0.05$.

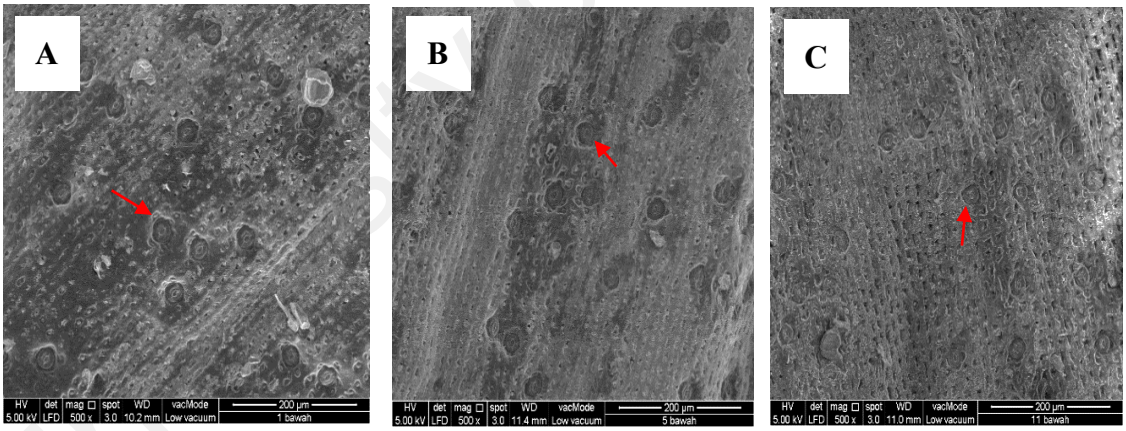

Figure 4.9: The abaxial surfaces (bottom) of *ex vitro* D-leaf after removal of shield-shape trichomes on the surface (9 months after planting) as observed using FE-SEM at magnification of 500x under low vacuum; (A) control plants (B) plants supplemented with chemical fertilizer (C) plants supplemented with vermicompost. The arrow shows the stomata.

Table 4.7: The parameter of stomata in the lower epidermis of D-leaves from 9 months-old field grown *ex vitro* MD2 pineapple plants supplemented with different types of fertilizers.

Treatment	Control	Chemical fertilizer	Vermicompost
Stomatal density (mm^2)	56.78 ± 1.62 [b]	73.45 ± 3.20 [a]	76.49 ± 0.72 [a]
Stomatal size (μm^2)	678.91 ± 19.45 [a]	658.54 ± 27.08 [a]	614.81 ± 6.02 [a]
Stomatal length (µm)	27.79 ± 0.25 [a]	27.48 ± 0.73 [a]	26.43 ± 0.62 [a]
Stomatal width (µm)	22.42 ± 0.48 [a]	23.94 ± 0.43 [a]	23.29 ± 0.46 [a]
Pore length (µm)	9.68 ± 0.01 [a]	9.58 ± 0.45 [a]	9.90 ± 0.21 [a]
Pore aperture (µm)	5.22 ± 0.11 [a]	5.03 ± 0.42 [a]	4.91 ± 0.36 [a]

* Means ± standard error of mean followed by a different letter by row indicate significant differences by the Duncan Multiple Range Test (DMRT) at $P \leq 0.05$, n=4.

4.2 Effect of Vermicompost on Fruit Quality Attributes

4.2.1 Physical Characteristics of Fruits

Figure 4.10 illustrates the different sizes of MD2 pineapple fruits harvested from *in vivo* plants treated with different types of fertilizer. Based on Table 4.8, *in vivo* pineapple plants supplied with chemical fertilizer was shown to produce the highest fruit yield, with 136.97 t ha^{-1} of pineapple fruits, while plants incorporated with vermicompost and control (unfertilized) plants produced 121.39 t ha^{-1} and 94.93 t ha^{-1} respectively. *In vivo* plants supplied with chemical fertilizer produced the largest fruits (2466 g), followed by plants supplied with vermicompost (2185 g) and control (1709 g). Based on the weight of the resulting fruits, the fruits can be graded to A grade (>1.7 kg), B grade (1.3 to 1.6 kg) and C grade (<1.3 kg) (Thalip et al., 2015). In this experiment, based on the fruit weight without crown, it was observed that both treatments with vermicompost and chemical fertilizer produced grade A fruits, while control plants produced only grade B fruits. However, the weight and length of the crown was the smallest when plants were supplied with vermicompost, while control plants produced the largest crowns. Moreover, the fruit's core size showed that fruits harvested from plants treated with chemical fertilizer

were significantly wider compared to control ($P \leq 0.05$). Similar trend was observed for pulp firmness where fruits produced from plants supplied with chemical fertilizer has less pulp firmness (0.671 kg f) compared to fruits produced from plant supplied with vermicompost (0.741 kg f) and control plants (0.771 kg f).

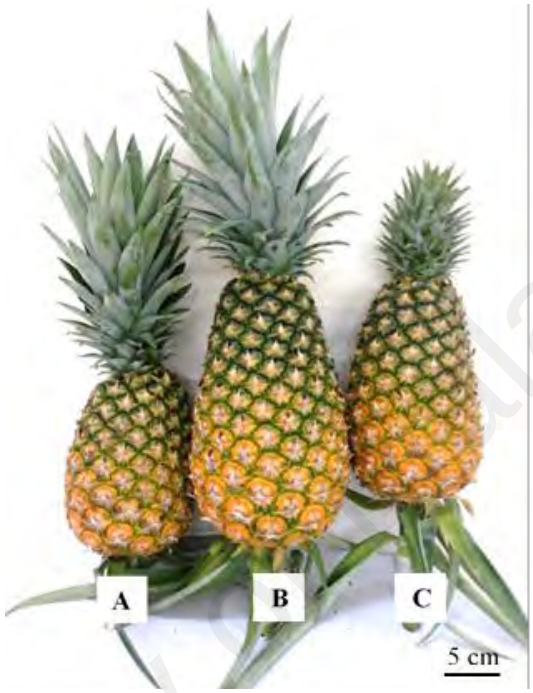

Figure 4.10: MD2 pineapple fruits harvested from *in vivo* plant; (A) control plants (B) plants supplemented with chemical fertilizer (C) plants supplemented with vermicompost.

Table 4.8: Morphological characteristics of fruits of *in vivo* grown MD2 pineapple with different types of fertilizers.

Treatment	Control	Chemical fertilizer	Vermicompost
Estimated yield (t ha^{-1})	94.93 ± 6.31 ^{c}	136.97 ± 0.89 ^{a}	121.39 ± 4.71 ^{b}
Fruit weight (g)	1709 ± 114 ^{c}	2466 ± 16 ^{a}	2185 ± 255 ^{b}
Fruit weight without crown (g)	1421 ± 103 ^{c}	2236 ± 23 ^{a}	1953 ± 68 ^{b}
Fruit diameter (cm)	10.9 ± 0.2 ^{b}	11.9 ± 0.3 ^{a}	11.6 ± 0.2 ^{a}
Fruit length (cm)	16.9 ± 0.0 ^{c}	24.5 ± 0.4 ^{a}	20.3 ± 0.8 ^{b}
Crown weight (g)	330 ± 10 ^{a}	247 ± 29 ^{b}	232 ± 86 ^{b}
Crown length (cm)	22.4 ± 0.8 ^{a}	18.5 ± 1.4 ^{b}	16.7 ± 1.4 ^{b}
Core diameter (cm)	2.2 ± 0.0 ^{b}	2.6 ± 0.3 ^{a}	2.4 ± 0.4 ^{ab}
Pulp firmness (kg f)	0.771 ± 0.004 ^{a}	0.671 ± 0.017 ^{b}	0.741 ± 0.007 ^{a}

* Mean ± standard error of mean within each row followed by a different letter indicates significant differences at $P \leq 0.05$ according to Duncan's multiple range test (DMRT), n=9.

Figure 4.11 shows the MD2 pineapple fruits harvested from the plants treated with different types of fertilizers. The physical analysis conducted on fruits harvested from *ex vitro* grown MD2 pineapple plants were shown in Table 4.9. The fruit yield was not statistically significantly different between plant supplied with vermicompost and plant treated with chemical fertilizer, but both significantly produced higher fruit yield compared to control plants ($P \leq 0.05$).

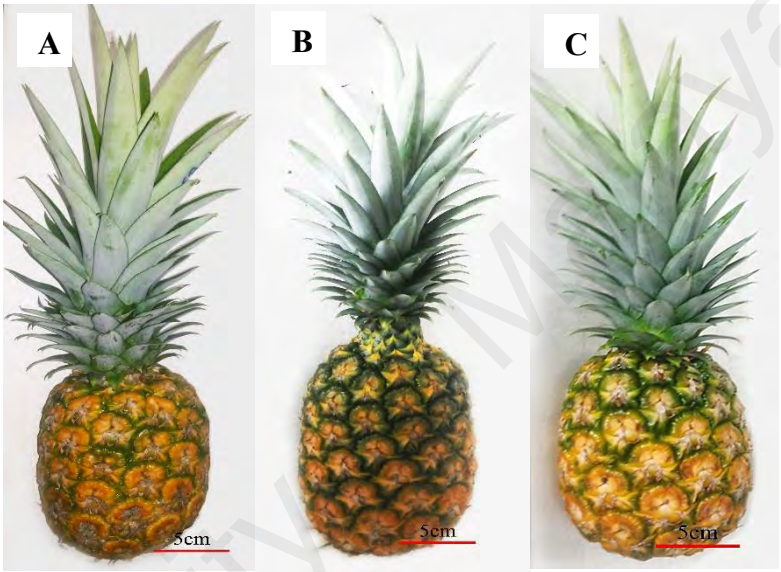

Figure 4.11: MD2 pineapple fruits harvested from *ex vitro* plant; (A) control plants, (B) plants supplemented with chemical fertilizer and (C) plants supplied with vermicompost.

The fruit's weight ranged from 1248 g to 1734 g. Plant supplied with chemical fertilizer produced largest fruits, followed by vermicompost and control plants but vice versa for the crown weight. Based on the weight of resulting fruits, the fruits can be graded to A grade (>1.7 kg), B grade (1.3 to 1.6 kg) and C grade (<1.3 kg) (Thalip et al., 2015). Plant supplied with chemical fertilizer produced grade A fruits (1734 g), grade B fruits for vermicompost treatment (1540 g), while control plants produced grade C fruits (1248 g). However, based on diameter and length of fruits, there was no significant difference between fruit produced by plant supplied with chemical fertilizer and vermicompost. Overall, pineapple plants treated with chemical fertilizer was observed to produce highest yield of fruit, largest fruit with smaller crown and core size, followed by plants supplied

with vermicompost and control. The fruits harvested from unfertilized plants showed similar trends with fruits from *in vivo* plants, whereby the fruits were smaller in size with large crowns. The fruit's core size and pulp firmness showed no difference for all treatments. In general, the fruits of *ex vitro* plants were smaller in size and have larger crown compared to fruits from *in vivo* plants.

Table 4.9: Morphological characteristics of fruits of *ex vitro* grown MD2 pineapple supplemented with different types of fertilizers.

Treatment	Control	Chemical fertilizer	Vermicompost
Estimated yield (t ha^{-1})	64.74 ± 3.58 ^{b}	90.46 ± 4.62 ^{a}	85.55 ± 4.26 ^{a}
Fruit weight (g)	1248 ± 51 ^{c}	1734 ± 63 ^{a}	1540 ± 77 ^{b}
Fruit weight without crown (g)	865 ± 62 ^{c}	1436 ± 68 ^{a}	1195 ± 78 ^{b}
Fruit diameter (cm)	10.5 ± 23 ^{b}	11.9 ± 0.3 ^{a}	12.0 ± 0.3 ^{a}
Fruit length (cm)	12.3 ± 0.6 ^{b}	15.0 ± 0.6 ^{a}	14.5 ± 0.7 ^{a}
Crown weight (g)	398 ± 23 ^{a}	288 ± 22 ^{b}	337 ± 5 ^{ab}
Crown length (cm)	27.6 ± 1.0 ^{a}	22.7 ± 1.2 ^{b}	27.8 ± 0.8 ^{a}
Core diameter (cm)	1.8 ± 0.1 ^{a}	1.7 ±0.1 ^{a}	1.8 ± 0.1 ^{a}
Pulp firmness (kg f)	0.72 ± 0.02 ^{a}	0.69 ± 0.03 ^{a}	0.68 ± 0.02 ^{a}

* Mean ± standard error of mean within each row followed by a different letter indicates significant differences at $P \leq 0.05$ according to Duncan's multiple range test (DMRT), n=12.

4.2.2 Physicochemical Properties

The pH of fruit juice, TSS (total soluble solids), TA (titratable acidity), sugar-to-acid ratio, total solid and ascorbic acid content of fruits of *in vivo* grown MD2 pineapple can be observed in Table 4.10. In comparison to the control (11.13 °Brix), the TSS of fruit juice from fruits harvested from plants treated with chemical fertilizer was lower (9.93 °Brix), followed by vermicompost (9.32 °Brix). TA of the fruit juices ranged from 0.40 g kg^{-1} to 0.43 g kg^{-1} and was found to be not significantly different among all treatments. Based on data analysis, fruit juice from fruit harvested from pineapple plants treated with vermicompost contained lowest sugar-to-acid ratio in the fruits, among all treatments.

Higher ascorbic acid content (17.334 µg AA/g fresh weight (FW) fruit) was observed in fruits produced from plants supplied with chemical fertilizer, followed by vermicompost (3.588 µg AA/g FW fruit) and unfertilized (control; 3.468 µg AA/g FW fruit) plants.

Table 4.10: Physicochemical properties of fruits of *in vivo* grown MD2 pineapple produced from application of different types of fertilizers.

Treatment	Control	Chemical fertilizer	Vermicompost
pH	4.23 ± 0.05 [a]	4.21 ± 0.04 [a]	4.18 ± 0.01 [a]
Total soluble solid (°Brix)	11.13 ± 0.31 [a]	9.93 ± 0.48 [ab]	9.32 ± 0.56 [b]
Titratable acidity (g kg^{-1})	0.40 ± 0.04 [a]	0.43 ± 0.04 [a]	0.43 ± 0.02 [a]
Sugar:acid ratio	27.83	23.09	21.67
Total solid (% wt/wt)	14.933 ± 0.766 [a]	14.096 ± 0.515 [a]	14.225 ± 0.776 [a]
Ascorbic acid (µg AA/g FW fruit)	3.468 ± 0.766 [b]	17.334 ± 2.196 [a]	3.588 ± 0.928 [b]

* Mean ± standard error of mean within each row followed by a different letter indicates significant differences at $P \leq 0.05$ according to Duncan's multiple range test (DMRT), n=12. AA, ascorbic acid; FW, fresh weight.

Based on Table 4.11, the pH of the fruit juice from *ex vitro* pineapple plants supplied with vermicompost and chemical fertilizer was found to be more acidic compared to fruit from unfertilized (control) plants. Results of this study showed that the fruit acidity was significantly influenced by the fertilization. In increasing order, the lowest acidity of fruit recorded was produced from unfertilized (control) plant > chemical fertilizer > vermicompost, ranged from 0.300 g kg^{-1} to 0.386 g kg^{-1}. Similar trend was observed on ratios between soluble solids and acid. There was a negative significant correlation between sugar to acid ratio and titratable acidity (r^2= -0.758, $P \leq 0.01$).

However, there was no significant difference on total soluble solid among treatments. The percentage of total solid higher in fruits harvested from plants supplemented with vermicompost (20.841%) was significantly higher compared to fruits harvested from plants treated with chemical fertilizer and unfertilized (control) plants, with total solids

of 17.804% and 18.044% respectively. In addition, the ascorbic acid content in the fruits harvested from plants supplied with vermicompost was the highest (44.577 µg AA/g FW fruit) followed by fruits from unfertilized (control) plants (37.477 µg AA/g FW fruit) and plants treated with chemical fertilizer (7.896 µg AA/g FW fruit). In terms of chemical characteristics, fruits from plant supplied with vermicompost produced competitive results with chemical fertilizer, but significantly contained higher total solids and ascorbic acid content.

Table 4.11: The physicochemical analysis of fruits of *ex vitro* grown MD2 pineapple supplemented with different types of fertilizers.

Treatment	Control	Chemical fertilizer	Vermicompost
pH	4.86 ± 0.15 [a]	4.48 ± 0.07 [b]	4.42 ± 0.04 [b]
Total soluble solid (°Brix)	12.6 ± 0.3 [a]	12.1 ± 0.2 [a]	12.6 ± 0.4 [a]
Titratable acidity (g kg^{-1})	0.30 ± 0.03 [b]	0.32 ± 0.03 [ab]	0.39 ± 0.03 [a]
Sugar:acid ratio	42.00	37.81	32.31
Total solid (% wt/wt)	18.044 ± 0.530 [b]	17.804 ± 1.012 [b]	20.841 ± 1.023 [a]
Ascorbic acid (µg AA/g FW fruit)	37.477 ± 1.452 [a]	7.896 ± 1.404 [b]	44.577 ± 7.467 [a]

* Mean ± standard error of mean within each row followed by a different letter indicates significant differences at $P \leq 0.05$ according to Duncan's multiple range test (DMRT), n=12. AA, ascorbic acid; FW, fresh weight.

4.2.3 Sensory Analysis

Figure 4.12 shows the percentage of panellist's preferences toward fresh MD2 pineapple fruits produced from *in vivo* grown plants supplied with chemical fertilizer, vermicompost and control plants. Based on the fruit's colour, the fruit colour of plants supplemented with chemical fertilizer was liked by all. The overall acceptability results showed all panellists (100%) preferred fruits derived from plants supplied with chemical fertilizer and control plants. Besides, based on all sensory attributes, fruits from plants

treated with chemical fertilizer showed the highest percentage of preferences. However, data analysis revealed that there was no significant difference among all treatments.

Based on the sensory analysis of fruits harvested from *ex vitro* plants (Figure 4.13), in terms of overall acceptability, 85% of the panellists liked the fruits from unfertilized plants the most, followed by vermicompost (70%) and chemical fertilizer (60%). Based on all sensory attributes, the highest percentage of panellists preferred fruits from control plants more than other treatments. Overall, in terms of panellists' preference on sensory qualities, data analysis revealed that there was no significant difference between fruits produced from plants supplied with vermicompost and chemical fertilizer.

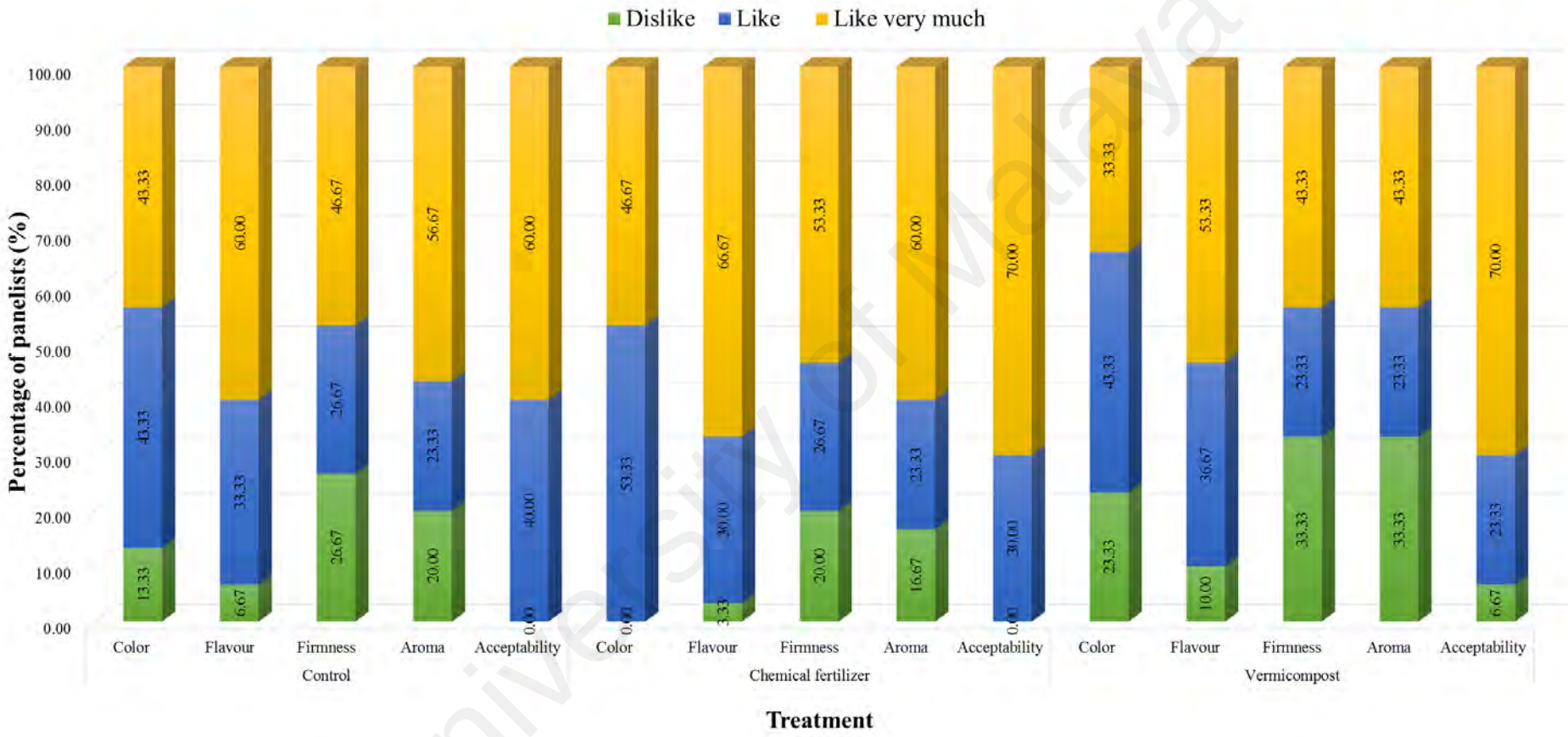

Figure 4.12: Comparison between the sensory attributes of fruits produced from *in vivo* grown MD2 pineapple plants supplied with different types of fertilizers, depicted in terms of percentage and based on the JAR method.

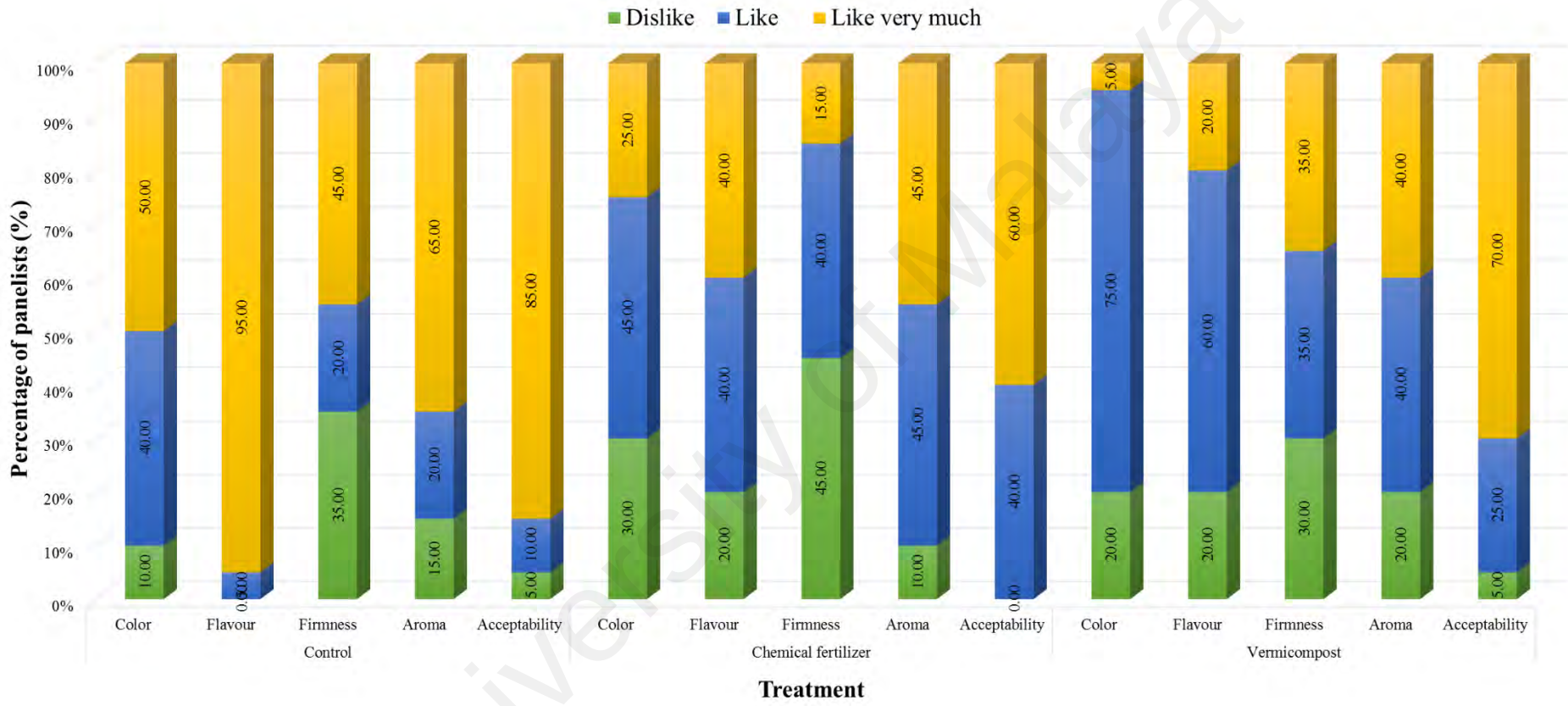

Figure 4.13: Comparison between the sensory attributes of fruits produced from *ex vitro* grown MD2 pineapple plants supplied with different types of fertilizers, depicted in terms of percentage and based on the JAR method.

4.3 Effect of Vermicompost on Nutrients Content in the Soil and Plant

4.3.1 Soil pH after 6 Months of Planting and during the Red Bud Stage

Based on Table 4.12, at six months after planting (S1), when the *in vivo* pineapple plants were at the vegetative stage, all treatments showed soil pH were maintained in the range of recommended pH which is 4.5 to 6.5. However, at red bud stage or during emergence of inflorescence (S2), soils supplemented with chemical fertilizer showed lower than range of soil pH for pineapple plants (pH 4.30). In contrast, a significant increase in soil pH (5.77) was obtained after second application of vermicompost, although it was still in the range of recommended pH for pineapple plants.

Table 4.12: Soil pH at six months of planting (S1) and during red bud stage (S2) used for growing the *in vivo* MD2 pineapple plants in the field.

Time of sampling	Control	Chemical fertilizer	Vermicompost
S1	5.44 ± 0.21^{a}	4.79 ± 0.09^{b}	4.99 ± 0.17^{ab}
S2	5.45 ± 0.15^{a}	4.30 ± 0.11^{b}	5.77 ± 0.13^{a}

* Mean ± standard error of mean within each row followed by a different letter indicates significant differences at $P \leq 0.05$ according to Duncan's multiple range test (DMRT), n=4.

The soil pH from *ex vitro* plants was more acidic during the red bud stages compared to after six months of planting for all treatments (Table 4.13). The soil pH of unfertilized plants drastically decreased from pH 5.21 to 3.66 where it was the lowest pH among all treatments. However, soils supplemented with vermicompost still showed soil pH within the recommended pH range for pineapple plants. The soil supplied with vermicompost leads to significant increase in the pH of soil compared to soil treated with chemical fertilizer and unfertilized (control) soils for both samplings.

Table 4.13: shows pH of soil at six months of planting (S1) and during red bud stage (S2) used for growing the *ex vitro* MD2 pineapple plants in the field.

Time of sampling	Control	Chemical fertilizer	Vermicompost
S1	5.21 ± 0.10 [ab]	4.95 ± 0.04 [b]	5.80 ± 0.30 [a]
S2	3.66 ± 0.08 [b]	3.68 ± 0.05 [b]	4.90 ± 0.29 [a]

* Mean ± standard error of mean within each row followed by a different letter indicates significant differences at $P \leq 0.05$ according to Duncan's multiple range test (DMRT), n=4.

4.3.2 Nutrients Concentration in the Soil

Data on the soil nutrients content (N, P, K, Mg, S, Ca, Fe, Zn, B, and Al) taken on six months after planting (S1) and red bud stages (S2) at the plots grown with MD2 pineapple plants (*in vivo*) are presented in Table 4.14. Data analysis indicated that there was no significant difference among all nutrients content determined in the soils after being supplied with chemical fertilizer and vermicompost on S1 and S2, except for calcium (Ca). Total nitrogen (N) in the soils ranged from 0.03% to 0.15% and was higher in soils supplemented with vermicompost compared to soils supplied with chemical fertilizer, with N content of 0.15% and 0.09% respectively. The N concentration increased from S1 to S2 when soils were supplied with vermicompost twice along the cycle. However, no increase in N concentration was observed when chemical fertilizer was supplied periodically. The K concentration also decreased in the soils at S2. In contrast, the nutrients content in soils supplemented with vermicompost showed increment of all nutrients measured, except for K content.

The nutrients content in soils grown with *ex vitro* MD2 pineapple plants was presented in Table 4.15 for sample taken on six months after planting (S1) and during the red bud stages (S2). On six months after planting (S1), the P and Mg content in the soil supplemented with vermicompost was significantly higher compared to unfertilized (control) soils. Moreover, the K content in the soil showed a significant difference between soil supplied with chemical fertilizer (0.07%) and unfertilized soil (0.06%) at *P*

≤0.05. There was no difference for almost of nutrients content analysed in soils for all treatments.

During the red bud stage, soils supplied with vermicompost showed the highest nutrients content with N (0.18%), P (0.04%), Mg (0.06%), S (0.02%), Ca (0.09%), Fe (0.69%), Zn (41.40 mg kg^{-1}) and B (1.90 mg kg^{-1}) compared to soil supplemented with chemical fertilizer and unfertilized soil. The difference in macronutrients (N, Mg, S and Ca) content was found to be statistically significant at $P \leq 0.05$. The N content was observed to increase two-fold higher than during 6 months after planting (S1). Similar trend was observed for P content in soil treated with chemical fertilizer and vermicompost. However, the K, Zn and B contents were decreased in all treatments and only soil supplied with vermicompost showed an increased in Ca content and decreased in Al content.

Table 4.14: Concentrations of soil nutrients six months after planting (S1) and during red bud stage (S2) grown with *in vivo* MD2 pineapple plants.

Total elements	Control		Chemical fertilizer		Vermicompost	
	S1	S2	S1	S2	S1	S2
N (%)	0.03 ± 0.02 ^{c}	0.07 ± 0.02 ^{bc}	0.09 ± 0.02 ^{abc}	0.09 ± 0.02 ^{abc}	0.10 ± 0.02 ^{ab}	0.15 ± 0.01 ^{a}
P (%)	0.02 ± 0.00 ^{a}	0.02 ± 0.00 ^{a}	0.04 ± 0.02 ^{a}	0.04 ± 0.01 ^{a}	0.03 ± 0.01 ^{a}	0.04 ± 0.00 ^{a}
K (%)	0.08 ± 0.02 ^{ab}	0.05 ± 0.00 ^{b}	0.10 ± 0.02 ^{a}	0.07 ± 0.01 ^{ab}	0.09 ± 0.01 ^{ab}	0.07 ± 0.01 ^{ab}
Mg (%)	0.04 ± 0.00 ^{a}	0.04 ± 0.00 ^{a}	0.05 ± 0.01 ^{a}	0.05 ± 0.01 ^{a}	0.05 ± 0.01 ^{a}	0.06 ± 0.01 ^{a}
S (%)	0.01 ± 0.00 ^{a}	0.01 ± 0.00 ^{a}	0.02 ± 0.01 ^{a}	0.02 ± 0.00 ^{a}	0.02 ± 0.01 ^{a}	0.02 ± 0.00 ^{a}
Ca (%)	0.06 ± 0.01 ^{ab}	0.06 ± 0.01 ^{ab}	0.09 ± 0.02 ^{a}	0.04 ± 0.01 ^{b}	0.08 ± 0.01 ^{a}	0.08 ± 0.01 ^{ab}
Fe (%)	0.64 ± 0.05 ^{a}	0.68 ± 0.04 ^{a}	0.71 ± 0.06 ^{a}	0.80 ± 0.13 ^{a}	0.73 ± 0.09 ^{a}	0.80 ± 0.10 ^{a}
Zn (mg kg^{-1})	34.76 ± 3.12 ^{a}	35.81 ± 8.49 ^{a}	38.26 ± 1.99 ^{a}	33.20 ± 5.00 ^{a}	40.05 ± 4.31 ^{a}	37.63 ± 2.94 ^{a}
B (mg kg^{-1})	2.81 ± 0.86 ^{a}	2.22 ± 0.73 ^{a}	3.02 ± 0.75 ^{a}	2.17 ± 0.70 ^{a}	2.10 ± 1.16 ^{a}	2.56 ± 1.61 ^{a}
Al (%)	2.74 ± 2.09 ^{a}	2.85 ± 0.29 ^{a}	3.46 ± 0.53 ^{a}	4.13 ± 0.88 ^{a}	3.11 ± 0.36 ^{a}	3.48 ± 0.70 ^{a}

* Mean ± standard error of mean within each row followed by a different letter indicates significant differences at $P \leq 0.05$ according to Duncan's multiple range test (DMRT), n=4.

Table 4.15: Concentrations of soil nutrients on six months after planting (S1) and during red bud stage (S2) grown with *ex vitro* MD2 pineapple plants.

Total elements	Control		Chemical fertilizer		Vermicompost	
	S1	S2	S1	S2	S1	S2
N (%)	0.06 ± 0.02 ^{c}	0.10 ± 0.02 ^{bc}	0.06 ± 0.01 ^{c}	0.13 ± 0.02 ^{b}	0.06 ± 0.01 ^{c}	0.18 ± 0.02 ^{a}
P (%)	0.02 ± 0.00 ^{b}	0.02 ± 0.00 ^{b}	0.02 ± 0.00 ^{b}	0.03 ± 0.01 ^{ab}	0.03 ± 0.01 ^{ab}	0.04 ± 0.00 ^{a}
K (%)	0.06 ± 0.00 ^{bc}	0.04 ± 0.00 ^{c}	0.07 ± 0.00 ^{a}	0.06 ± 0.01 ^{bc}	0.07 ± 0.01 ^{ab}	0.05 ± 0.01 ^{c}
Mg (%)	0.04 ± 0.00 ^{c}	0.03 ± 0.00 ^{c}	0.04 ± 0.00 ^{bc}	0.04 ± 0.01 ^{c}	0.05 ± 0.01 ^{ab}	0.06 ± 0.00 ^{a}
S (%)	0.01 ± 0.00 ^{b}	0.01 ± 0.00 ^{b}	0.01 ± 0.00 ^{b}	0.01 ± 0.00 ^{b}	0.02 ± 0.00 ^{b}	0.02 ± 0.00 ^{a}
Ca (%)	0.05 ± 0.01 ^{b}	0.03 ± 0.01 ^{b}	0.05 ± 0.00 ^{b}	0.04 ± 0.01 ^{b}	0.07 ± 0.02 ^{ab}	0.09 ± 0.01 ^{a}
Fe (%)	0.61 ± 0.04 ^{a}	0.59 ± 0.03 ^{a}	0.65 ± 0.07 ^{a}	0.66 ± 0.08 ^{a}	0.67 ± 0.07 ^{a}	0.69 ± 0.01 ^{a}
Zn (mg kg^{-1})	34.32 ± 2.98 ^{a}	28.38 ± 3.13 ^{a}	33.49 ± 2.14 ^{a}	27.62 ± 2.25 ^{a}	47.31 ± 17.33 ^{a}	41.40 ± 8.08 ^{a}
B (mg kg^{-1})	4.39 ± 1.16 ^{a}	0.74 ± 0.10 ^{c}	2.90 ± 0.73 ^{ab}	1.74 ± 0.58 ^{bc}	2.52 ± 0.20 ^{abc}	1.90 ± 0.19 ^{bc}
Al (%)	2.41 ± 0.35 ^{a}	2.44 ± 0.47 ^{a}	2.80 ± 0.47 ^{a}	3.29 ± 0.60 ^{a}	2.94 ± 0.71 ^{a}	2.81 ± 0.70 ^{a}

***** Mean ± standard error of mean within each row followed by a different letter indicates significant differences at $P \leq 0.05$ according to Duncan's multiple range test (DMRT), n=4.

4.3.3 Nutrients Concentration in the D-leaf

Table 4.16 shows the macronutrients content in the D-leaf of MD2 pineapple plants (*in vivo*). The total N means ranged from 0.69% to 1.44% in the leaf. At the red bud stages (S2), all of the treatments showed a reduction of N content in the leaf samples, however, these values were still adequate for the growth of pineapple plants (N >0.66%) (Ramos et al., 2009). Based on Pearson's correlation analysis, a strong significant correlation was observed between N content in the soil and in the plants, when supplied with chemical fertilizer ($P \leq 0.05$, $r^2 = -0.970$), indicating sufficient N uptake from the soils. Although higher N content was observed in the soil supplied with vermicompost, the correlation between the N content in the soil and plants was weak ($r^2 = 0.236$). In this study, Ca concentrations in the soil supplied with chemical fertilizer at S2 showed the lowest concentration compared to other treatments, with only 0.04% Ca. The low availability of Ca in the soils has also affected the Ca uptake by the pineapple plants. The *in vivo* grown plants also showed a deficiency of S content at S2 for all treatments (ranged from 0.04% to 0.05%), whereby these values were below the recommended range for crop growth: 0.056% (Ramos et al., 2009).

The Fe, Zn, B and Al content in *in vivo* grown MD2 pineapple leaves are detailed in Table 4.10. The Fe levels in the leaves ranged from 46.93 mg kg^{-1} to 73.32 mg kg^{-1} which was below the recommended range (100-200 mg kg^{-1}) for crop growth (Malezieux & Bartholomew, 2003). The Zn content was twofold above the level considered ideal for the growth of pineapple plants, with Zn content ranging from 21.00 mg kg^{-1} to 29.76 mg kg^{-1} at S2. In addition, the application of vermicompost onto the soils also increased the B content in *in vivo* MD2 pineapple D-leaves at S1 (11.36 mg kg^{-1}). At S2, the plants treated with vermicompost has low B content, although it was still above the level considered insufficient to support plant growth. This is in direct contrast to the plants

supplemented with chemical fertilizer and control (unfertilized) plants, where the B content during S2 was found too low, and thus was not sufficient for plant growth.

In this study, the accumulation of macronutrient by plant showed a similar pattern for all treatment at six months after planting by the following decreasing order of uptake: K >N > Ca > Mg > P > S. Based on Table 4.11, the N content in the D-leaf of *ex vitro* MD2 pineapple at S1 ranged between 0.68% to 0.77%, whereby these values were lower than the ideal concentration of N during vegetative stage (4 months after planting) reported by Malavolta (1.5% to 1.7%). Similar trend was observed for P, K and Ca concentrations. However, there was no significant difference between the N, P, K and Ca content in the D-leaf of *ex vitro* plants supplemented with vermicompost compared to plants supplied with chemical fertilizer and unfertilized plants. According to Malavolta, the Mg content in *ex vitro* plants supplied with vermicompost (0.20%) showed an ideal concentration (0.18% to 0.20%) for pineapple growth (Souza & Reinhardt, 2007). Moreover, the D-leaf of *ex vitro* plants supplemented with vermicompost had significantly higher S content than plants supplemented with chemical fertilizer. Although vermicompost was supplied six months before sampling, nutrients uptake by plant showed a competitive result with plant supplied with chemical fertilizer.

The N content in the D-leaf of *ex vitro* plants (for all treatments) was considered as inadequate for pineapple plants at floral induction as reported by Ramos et al. (2009). Similar trend was observed on the K, Ca and S concentration, although it was still considered as adequate to support plant growth. Other than that, the level of P content in the D-leaf was observed to be higher in plant supplied with vermicompost compared to other treatments. The Mg concentration in the *ex vitro* plants supplied with vermicompost and the unfertilized plants (during S2) was close to the ideal Mg content for plants during flowering. In contrast, the Mg level in *ex vitro* plants supplied with chemical fertilizer

was observed to be the lowest. There was a statistically significant positive correlation between soil pH with P content (r^2= 5.88, $P \leq 0.05$) and Mg content (r^2= 0.778, $P \leq 0.01$) in the D-leaves at the red bud stage.

In terms of micronutrients content, at both S1 and S2, the Fe content (for all treatments) in the *ex vitro* plants were observed to be lower than the ideal concentration required for pineapple plants (Table 4.12). Nevertheless, *ex vitro* plants supplemented with vermicompost at S1 showed higher Zn concentration (46.99 mg kg^{-1}) than required range recommended by Malavolta (17 to 39 mg kg^{-1}). However, during S2, the unfertilized *ex vitro* plants contained more Zn than plants supplied with chemical fertilizer and vermicompost, however, their range of Zn concentration was still considered to be adequate for plants during flowering. In contrast, the B concentration in the *ex vitro* plants (for all treatments) was lower than ideal concentration during flowering and was near to the B-deficiency level for the plants. Overall, the *ex vitro* plants supplemented with vermicompost showed higher content of micronutrients during S1. However, the micronutrients content recorded in the *ex vitro* plants supplied with vermicompost during S2 was comparable to that of *ex vitro* plants supplied with chemical fertilizer.

Table 4.16: Concentration of macronutrients (%) in the D-leaf of *in vivo* MD2 pineapple plants six months after planting (S1) and during red bud stage (S2).

Time of sampling	Treatment	N	P	K	Ca	Mg	S
		%					
S1	Control	1.20 ± 0.14 ^{ab}	0.16 ± 0.02 ^{b}	1.97 ± 0.25 ^{b}	0.36 ± 0.05 ^{a}	0.24 ± 0.01 ^{ab}	0.07 ± 0.01 ^{bc}
	Chemical fertilizer	1.44 ± 0.10 ^{a}	0.19 ± 0.04 ^{ab}	3.29 ± 0.08 ^{a}	0.27 ± 0.04 ^{b}	0.18 ± 0.01 ^{c}	0.08 ± 0.00 ^{b}
	Vermicompost	1.16 ± 0.07 ^{b}	0.26 ± 0.04 ^{a}	3.43 ± 0.47 ^{a}	0.20 ± 0.02 ^{bc}	0.25 ± 0.03 ^{a}	0.10 ± 0.01 ^{a}
S2	Control	0.69 ± 0.05 ^{c}	0.15 ± 0.01 ^{b}	1.87 ± 0.25 ^{b}	0.26 ± 0.03 ^{bc}	0.19 ± 0.01 ^{bc}	0.04 ± 0.00 ^{d}
	Chemical fertilizer	1.04 ± 0.03 ^{b}	0.16 ± 0.02 ^{b}	2.30 ± 0.03 ^{b}	0.16 ± 0.02 ^{c}	0.11 ± 0.02 ^{d}	0.05 ± 0.00 ^{cd}
	Vermicompost	0.75 ± 0.05 ^{c}	0.17 ± 0.02 ^{b}	2.14 ± 0.25 ^{b}	0.22 ± 0.02 ^{bc}	0.18 ± 0.02 ^{c}	0.05 ± 0.00 ^{d}
Malavolta [(1)]		1.5-1.7	0.23-0.25	3.9-5.7	0.5-0.7	0.18-0.20	-
Dalldorf & Langenegger [(2)]		1.5-1.7	±0.10	2.2-3.0	0.8-1.2	±0.30	-
Ramos et al. [(3)]		1.48/0.66	0.14/0.07	2.3/1.16	0.44/0.13	0.23/0.09	0.15/0.06

* Means ± standard error followed by a different letters by column indicate significant differences by the Duncan Multiple Range Test (DMRT) at $P \leq 0.05$, n=4. [(1)]Ideal concentration at 4 month (whole leaf), source: (Souza & Reinhardt, 2007) [(2)]Ideal concentrations at the inflorescence emergence (whole leaf), source: (Malezieux & Bartholomew, 2003; Souza & Reinhardt, 2007). [(3)]Ideal concentrations / deficiency concentration at floral induction (Ramos et al., 2009).

Table 4.17: Concentration of micronutrients in the D-leaf of *in vivo* MD2 pineapple plants six months after planting (S1) and during red bud stage (S2).

Time of sampling	Treatment	Fe	Zn	B	Al
		mg kg^{-1}			
S1	Control	61.93 ± 6.18 [a]	39.82 ± 5.50 [a]	5.58 ± 0.89 [bc]	57.61 ± 11.43 [a]
	Chemical fertilizer	64.84 ± 16.54 [a]	44.14 ± 5.04 [a]	5.68 ± 0.55 [bc]	44.47 ± 9.96 [a]
	Vermicompost	73.32 ± 20.35 [a]	41.63 ± 9.83 [a]	11.36 ± 1.82 [a]	48.41 ± 11.99 [a]
S2	Control	65.26 ± 14.97 [a]	29.76 ± 3.30 [ab]	4.33 ± 0.47 [bc]	19.43 ± 1.03 [b]
	Chemical fertilizer	46.93 ± 9.49 [a]	21.00 ± 1.63 [b]	2.98 ± 0.15 [c]	10.59 ± 1.29 [b]
	Vermicompost	68.69 ± 17.60 [a]	22.13 ± 1.51 [b]	6.33 ± 0.83 [b]	11.73 ± 3.19 [b]
Malavolta [(1)]		600-1000	17-39	-	-
Dalldorf & Langenegger [(2)]		100-200	±10	30	-
Ramos et al. (2009) [(3)]		-	-	20/5.6	-

* Means ± standard error followed by a different letters by column indicate significant differences by the Duncan Multiple Range Test (DMRT) at $P \leq 0.05$, n=4. [(1)]Ideal concentration at 4 month (whole leaf), source: (Souza & Reinhardt, 2007) [(2)]Ideal concentrations at the inflorescence emergence (whole leaf), source: (Malezieux & Bartholomew, 2003; Souza & Reinhardt, 2007). [(3)]Ideal concentrations / deficiency concentration at floral induction (Ramos et al., 2009).

Table 4.18: Concentration on macronutrients in the D-leaf of *ex vitro* MD2 pineapple plants six months after planting (S1) and during red bud stage (S2).

Time of sampling	Treatment	N	P	K	Ca	Mg	S
		%					
S1	Control	0.68 ± 0.05 ^{a}	0.21 ± 0.04 ^{bc}	2.33 ± 0.23 ^{a}	0.29 ± 0.03 ^{ab}	0.22 ± 0.01 ^{b}	0.07 ± 0.01 ^{a}
	Chemical fertilizer	0.75 ± 0.10 ^{a}	0.13 ± 0.01 ^{c}	1.80 ± 0.10 ^{bc}	0.26 ± 0.02 ^{ab}	0.15 ± 0.01 ^{cd}	0.05 ± 0.00 ^{b}
	Vermicompost	0.77 ± 0.05 ^{a}	0.15 ± 0.01 ^{bc}	2.17 ± 0.11 ^{ab}	0.29 ± 0.03 ^{ab}	0.20 ± 0.03 ^{bc}	0.06 ± 0.00 ^{a}
S2	Control	0.55 ± 0.07 ^{ab}	0.17 ± 0.01 ^{bc}	1.53 ± 0.19 ^{c}	0.30 ± 0.03 ^{a}	0.24 ± 0.01 ^{b}	0.06 ± 0.01 ^{a}
	Chemical fertilizer	0.66 ± 0.08 ^{a}	0.23 ± 0.04 ^{ab}	1.75 ± 0.04 ^{bc}	0.21 ± 0.02 ^{b}	0.13 ± 0.02 ^{d}	0.07 ± 0.00 ^{a}
	Vermicompost	0.42 ± 0.08 ^{b}	0.30 ± 0.03 ^{a}	1.55 ± 0.14 ^{c}	0.26 ± 0.02 ^{ab}	0.37 ± 0.04 ^{a}	0.07 ± 0.00 ^{a}
Malavolta [(1)]		1.5-1.7	0.23-0.25	3.9-5.7	5.0-7.0	0.18-0.20	-
Dalldorf & Langenegger [(2)]		1.5-1.7	±0.10	2.2-3.0	8.0-1.2	±0.3	-
Ramos et al. [(3)]		1.48/0.66	0.14/0.07	2.3/1.16	0.44/0.13	0.23/0.09	0.15/0.06

* Means ± standard error followed by a different letters by column indicate significant differences by the Duncan Multiple Range Test (DMRT) at $P \leq 0.05$, n=4. [(1)]Ideal concentration at 4 month (whole leaf), source: (Souza & Reinhardt, 2007) [(2)]Ideal concentrations at the inflorescence emergence (whole leaf), source: (Malezieux & Bartholomew, 2003; Souza & Reinhardt, 2007). [(3)]Ideal concentrations / deficiency concentration at floral induction (Ramos et al., 2009).

Table 4.19: Concentration of micronutrients in the D-leaf of *ex vitro* MD2 pineapple plants six months after planting (S1) and during red bud stage (S2).

Time of sampling	Treatment	Fe	Zn	B	Al
		mg kg^{-1}			
S1	Control	127.37 ± 38.55 ^{a}	43.41 ± 5.65 ^{ab}	9.48 ± 0.72 ^{a}	67.14 ± 28.59 ^{a}
	Chemical fertilizer	45.50 ± 9.46 ^{b}	32.16 ± 2.50 ^{b}	4.96 ± 0.23 ^{b}	32.70 ± 11.38 ^{a}
	Vermicompost	69.97 ± 22.86 ^{ab}	46.99 ± 6.47 ^{a}	8.00 ± 0.89 ^{a}	37.52 ± 30.71 ^{a}
S2	Control	40.65 ± 5.14 ^{b}	32.18 ± 1.87 ^{b}	8.43 ± 0.45 ^{a}	53.28 ± 12.55 ^{a}
	Chemical fertilizer	45.93 ± 9.58 ^{b}	13.95 ± 1.39 ^{c}	7.24 ± 1.00 ^{a}	39.90 ± 22.33 ^{a}
	Vermicompost	41.77 ± 9.73 ^{b}	14.14 ± 2.41 ^{c}	9.49 ± 0.69 ^{a}	22.61 ± 3.83 ^{a}
Malavolta [(1)]		600-1000	17-39	-	-
Dalldorf & Langenegger [(2)]		100-200	±10	30	-
Ramos et al. (2009) [(3)]		-	-	20/5.6	-

* Means ± standard error followed by a different letters by column indicate significant differences by the Duncan Multiple Range Test (DMRT) at $P \leq 0.05$, n=4. [(1)]Ideal concentration at 4 month (whole leaf), source: (Souza & Reinhardt, 2007) [(2)]Ideal concentrations at the inflorescence emergence (whole leaf), source: (Malezieux & Bartholomew, 2003; Souza & Reinhardt, 2007). [(3)]Ideal concentrations / deficiency concentration at floral induction (Ramos et al., 2009).

4.3.4 Correlations between Fruit Quality Attributes with Nutrients Content in the D-leaf

The correlation between the nutrients content with the fruit quality attributes was also analysed, to determine the relationship between fruit quality with nutrient availability in the plants. Based on the Table 4.20, data analysis revealed that the total nitrogen (N) in the D-leaf of i*n vivo* plants showed significantly positive correlation with fruit weight (r^2= 0.816, $P \leq 0.01$), crown weight (r^2=0.588, $P \leq 0.05$) and fruit diameter (r^2= 0.877, $P \leq 0.01$). Moreover, a negative strong correlation also was obtained between N content and TSS of fruit juice with coefficient of r^2= -0.507, indicating that an increase in N content strongly reduces the TSS of fruit juice. In contrast, the K content in the D-leaf of *in vivo* showed significant negative correlation with pH and TSS (r^2= -0.641 and -0.667, respectively at $P \leq 0.05$), indicating that the increase in K content would lead to the decrease of pH of fruit juice and total soluble solids. Moreover, a strong positive correlation was observed between K content in D-leaf of *in vivo* and TA (r^2= 0.712, $P \leq 0.05$), which implied that TA will increase with increasing K. Other than that, the analysis also revealed that the increase of N content showed a strong correlation with ascorbic acid content (r^2= 0.904, $P \leq 0.01$).

For *ex vitro* plants (Table 4.21), the produced fruits showed different effect with the fertilizer compared to *in vivo* plants where the K content in the leaf showed a strong significant correlation with crown length (r^2= -0.759, $P \leq 0.01$), pH of fruit juice (r^2= -0.802, $P \leq 0.01$) and TA (r^2= 0.644, $P \leq 0.05$) indicating that the increment in the accumulation of K content in the leaf would lead to decrease in crown length and increase the acidity of fruit. However, increasing with N content in the leaf would decrease TSS (r^2= -0.795, $P \leq 0.01$), TA (r^2= -0.777, $P \leq 0.01$), total solid (r^2= -0.850, $P \leq 0.01$) and ascorbic acid content (r^2= -0.699, $P \leq 0.05$) in the fruit whereby decrease the quality of fruit.

Table 4.20: Pearson's correlation coefficient between fruit attributes and nutrients content in the D-leaf of *in vivo* MD2 pineapple during red bud stage (S2).

Parameters	Physical									Physico-chemical					Nutrients content in the D-leaf (S2)								
	Estimated yield	Fruit weight	Fruit weight w/o crown	Crown weight	Fruit diameter	Fruit length	Crown length	Core size	Pulp firmness	pH	TSS	TA	Total solid	Ascorbic acid	N	P	K	Mg	S	Ca	Fe	Zn	B
Fruit weight	1.000**	1																					
Fruit weight without crown	.993**	.993**	1																				
Crown weight	.807**	.807**	.805**	1																			
Fruit diameter	.835**	.835**	.870**	.513	1																		
Fruit length	-.529	-.530	-.617*	-.419	-.699*	1																	
Crown length	-.522	-.522	-.601*	-.396	-.618*	.911**	1																
Core size	.605*	.605*	.594*	.671*	.398	-.302	-.200	1															
Pulp firmness	-.664*	-.664*	-.624*	-.611*	-.514	.107	-.043	-.410	1														
pH	-.147	-.147	-.209	.069	-.335	.386	.437	.209	-.326	1													
TSS	-.454	-.454	-.470	-0.152	-.661*	.285	.401	.126	.175	.269	1												
TA	.651*	.651*	.665*	0.507	.737**	-.353	-.438	.098	-.361	-.697**	-.481**	1											
Total solid	.448	.448	.374	.388	.231	.216	.405	.572	-.614*	.128	.376*	-.141	1										
Ascorbic acid	.748**	.748**	.753**	.546	.838**	-.375	-.245	.620*	-.675*	-.005	-.433	.110	.474	1									
N	.816**	.816**	.823**	.588*	.887**	-.420	-.283	.453	-.631*	-.237	-.507	.333	.501	.904**	1								
P	-.029	-.028	.017	-.112	.169	-.406	-.234	-.114	.231	-.594*	-.049	.370	-.125	-.077	.138	1							
K	.472	.472	.519	.303	.549	-.453	-.542	-.174	-.076	-.641*	-.667*	.712*	-.350	.299	.504	.226	1						
Mg	-.410	-.410	-.464	-.291	-.728**	.541	.387	-.272	.355	.056	.528	.046	-.068	-.689*	-.608*	.106	-.278	1					
S	.580*	.580*	.552	.670*	.258	-.124	-.140	.769**	-.566	.277	-.003	.045	.396	.453	.255	-.515	-.082	-.219	1				
Ca	-.464	-.465	-.537	-.456	-.692*	.684*	.520	-.124	.364	.192	.422	-.150	.058	-.549	-.623*	-.071	-.561	.846**	-.104	1			
Fe	-.046	-.047	-.083	-.220	-.268	.151	.012	-.013	.038	-.043	.173	.107	-0.158	-.277	-.384	.007	-.078	.608*	.217	.616*	1		
Zn	-.639*	-.639*	-.691*	-.460	-.732**	.786**	.784**	-.106	.161	.512	.562	-.459	.186	-.397	-.576	-.523	-.769**	.334	.070	.567	.095	1	
B	-.108	-.108	-.105	-.099	-.289	-.109	-.162	-.269	.253	-.275	.381	.181	-.129	-.591*	-.363	.464	-.060	.680*	-.285	.382	.428	-.128	1
Al	-.504	-.504	-.565	-.373	-.655*	.739**	.600*	-.339	.240	.586*	.284	-.462	-.035	-.443	-.525	-.664*	-.315	.318	-.090	.393	.024	.649*	-.238

** Correlation is significant at the 0.01 level (2-tailed).

* Correlation is significant at the 0.05 level (2-tailed).

Table 4.21: Pearson's correlation coefficient between fruit attributes and nutrients content in the D-leaf of *ex vitro* MD2 pineapple during red bud stage (S2).

Parameters	Physical									Physico-chemical					Nutrients content in the D-leaf (S2)								
	Estimated yield	Fruit weight	Fruit weight w/o crown	Crown weight	Fruit diameter	Fruit length	Crown length	Core size	Pulp firmness	pH	TSS	TA	Total solid	Ascorbic acid	N	P	K	Mg	S	Ca	Fe	Zn	B
Fruit weight	.979**	1																					
Fruit weight without crown	.875**	.868**	1																				
Crown weight	.898**	.842**	.864**	1																			
Fruit diameter	.877**	.827**	.753**	.909**	1																		
Fruit length	-.454	-.399	-.528	-.624*	-.626*	1																	
Crown length	-.295	-.265	-.360	-.367	-.484	.696*	1																
Core size	-.050	-.095	-.039	.214	.106	-.309	-.185	1															
Pulp firmness	-.108	-.125	-.352	-.380	-.321	.411	.266	-.215	1														
pH	.173	.142	-.044	-.048	-.061	.370	.523	-.419	.435	1													
TSS	-.462	-.518	-.240	-.224	-.195	.026	-.365	.100	-.203	-.566	1												
TA	-.097	-.109	.153	.179	.116	-.210	-.428	.391	-.646*	-.837**	.682*	1											
Total solid	-.093	-.178	.109	.146	.106	-.084	-.354	.024	-.442	-.533	.798**	.814**	1										
Ascorbic acid	-.393	-.454	-.360	-.247	-.300	.553	.518	.103	-.086	-.077	.426	.388	.512	1									
N	.112	.213	.004	-.134	-.106	-.002	.240	-.341	.223	.536	-.795**	-.777**	-.850**	-.669*	1								
P	.287	.363	.407	.151	-.087	.224	.393	-.035	.112	-.012	-.393	.014	-.166	.088	.111	1							
K	-.033	-.006	.046	.051	.212	-.328	-.759**	.281	-.287	-.802**	.462	.644*	.443	-.181	-.366	-.249	1						
Mg	-.071	-.141	-.001	.126	-.184	.325	.551	.214	-.015	.067	.136	.211	.340	.689*	-.446	.410	-.424	1					
S	.092	.109	.135	.054	.129	-.072	-.306	.020	-.158	-.601*	.135	.534	.454	.127	-.315	.268	.681*	-.058	1				
Ca	-.157	-.156	-.244	-.155	-.265	.565	.804**	-.052	.046	.710**	-.341	-.449	-.354	.371	.265	.051	-.769**	.441	-.624*	1			
Fe	-.147	-.184	.032	-.266	-.293	.054	-.212	-.015	.502	-.014	.226	-.092	.022	-.160	-.079	.167	.121	-.105	.089	-.328	1		
Zn	-.606*	-.611*	-.761**	-.611*	-.546	.465	.553	.125	.348	.475	-.101	-.506	-.489	.221	.259	-.370	-.475	.024	-.643*	.667*	-.080	1	
B	-.049	.010	-.090	-.121	-.272	.624*	.819**	-.015	.158	.222	-.408	-.160	-.221	.455	.133	.669*	-.395	.599*	.139	.552	-.182	.148	1
Al	-.257	-.306	-.351	-.388	-.246	-.091	.047	-.040	.385	.286	-.123	-.426	-.387	-.103	.214	-.251	-.303	-.377	-.326	-.019	.300	.480	-.373

** Correlation is significant at the 0.01 level (2-tailed).

* Correlation is significant at the 0.05 level (2-tailed).

4.4 Bioactive Compounds in the Methanolic Fruit Extract of MD2 Pineapple

4.4.1 Phytochemicals Constituents

The phytochemical constituents in the methanolic extracts of the pineapple fruits (*in vivo*) was shown in Table 4.22. The results of qualitative analysis on each fruit pulp extract only showed the presence of flavonoids and tannins. Phenols and alkaloids were absent in all samples. However, for fruit extract of MD2 pineapple produced from *ex vitro* plants, only flavonoids were detected in all treatments (Table 4.23).

Table 4.22: Phytochemical analysis of methanolic extract of *in vivo* MD2 pineapple fruits produced from plants supplied with different types of fertilizers in the field.

Chemical constituents	Control	Chemical fertilizer	Vermicompost
Phenol	-	-	-
Flavonoids I	+	+	+
Flavonoids II	+	+	+
Tannins	+	+	+
Alkaloids I	-	-	-
Alkaloids II	-	-	-
Alkaloids III	-	-	-

- absent; + present

Table 4.23: Phytochemical analysis of methanolic extract of *ex vitro* MD2 pineapple fruits produced from plants supplied with different types of fertilizers in the field.

Chemical constituents	Control	Chemical fertilizer	Vermicompost
Phenol	-	-	-
Flavonoids I	-	-	-
Flavonoids II	+	+	+
Tannins	-	-	-
Alkaloids I	-	-	-
Alkaloids II	-	-	-
Alkaloids III	-	-	-

- absent; + present

4.4.2 Chlorophyll a, Chlorophyll b, Total Carotenoids, Total Chlorophyll, Total Phenolic Contents, and β-carotene Content.

Table 4.24 showed the results of pigment content of methanolic fruit extracts from *in vivo* MD2 pineapple grown with different types of fertilizers. Based on the results, both chlorophyll a and b content were found to be higher in fruits extract from plants treated with chemical fertilizer followed by vermicompost and control plants. However, fruit extract from control treatment contained highest total carotenoid with 66.341 µg/g and the lowest value was from the methanolic fruit extract of plant treated with vermicompost (30.657 µg/g). Similar trend was observed on the total pigments.

Table 4.24: Pigments and total carotenoid contents of methanolic fruit extracts of MD2 pineapple fruit produced from *in vivo* plants field-grown with different types of fertilizers.

Treatment	Control	Chemical fertilizer	Vermicompost
C_a (µg/g)	1.055 ± 0.078 ^{c}	2.438 ± 0.038 ^{a}	1.866 ± 0.000 ^{b}
C_b (µg/g)	3.194 ± 0.096 ^{c}	7.203 ± 0.073 ^{a}	5.496 ± 0.000 ^{b}
$C_a + C_b$ (µg/g)	4.249 ± 0.041 ^{c}	9.640 ± 0.040 ^{a}	7.362 ± 0.000 ^{b}
$C_{(x+c)}$ (µg/g)	66.341 ± 0.116 ^{a}	44.276 ± 0.084 ^{b}	30.657 ± 0.013 ^{c}
C_a / C_b ratio	0.332 0.035 ^{a}	0.339 ± 0.009 ^{a}	0.340 ± 0.000 ^{a}
$(C_a + C_b)/C_{(x+c)}$ ratio	0.005 ± 0.001 ^{a}	0.008 ± 0.000 ^{b}	0.011 ± 0.000 ^{c}
Total (µg/g)	70.590 ± 0.115 ^{a}	53.916 ± 0.066 ^{b}	38.019 ± 0.013 ^{c}

* Means ± standard error of mean value followed by different letters in rows are significantly different using ANOVA, Duncan's test, $P \leq 0.05$, n=3. C_a chlorophyll a, C_b chlorophyll b, $C_a + C_b$ total chlorophyll a and b, $C_{(x+c)}$ total carotenoid (xanthophyll and carotene).

Based on the Table 4.25, the total phenolics (TPC) of fruits varied from 5.983 mg GAE/g dry extract to 8.895 mg GAE/g dry extract, where fruits produced from *in vivo* plants supplied with chemical fertilizer showed higher TPC followed by control and vermicompost. Besides that, the extract also was subjected to HPLC analysis to detect the presence and quantify various carotenoids, ie. neoxanthin, violaxanthin, zeaxanthin, lutein, β-cryptoxanthin, lycopene, α-carotene, β-carotene, vitamin A (retinol) and E (α-tocopherol). However, only β- carotene was detected in the extracts from all samples

(Appendix F). The pulp extract of fruits grown with chemical fertilizer significantly showed higher amount of β-carotene (44.2 µg/ g DW) compared to vermicompost, (28.4 µg/ g DW) and control (2.3 µg/ g DW) at $P \leq 0.05$.

Table 4.25: The concentration of total phenolic content (TPC) and β-carotene in methanolic fruit extracts of MD2 pineapple fruit produced from *in vivo* plants field-grown with different types of fertilizers.

Treatment	Total phenolics (mg GAE/g dE)	β-carotene (µg/g DW)
Control	5.983 ± 0.001 [b]	2.3 ± 0.2 [c]
Chemical fertilizer	8.895 ± 0.002 [a]	44.2 ± 0.7 [a]
Vermicompost	4.859 ± 0.001 [c]	28.4 ± 2.4 [b]

* Mean ± standard error of mean within each row followed by a different letter indicate significant differences at $P \leq 0.05$, (n=3). GAE, gallic acid equivalents; dE, dry extract; DW, dry weight.

In contrast, the fruit extracts from *ex vitro* MD2 pineapple supplied with vermicompost contained significantly higher chlorophyll a (0.977 µg/g), chlorophyll b (3.094 µg/g) and total chlorophyll content (4.071 µg/g) compared to other treatments ($P \leq 0.05$) (Table 4.26). However, there was no significant difference on ratio of chlorophyll a and chlorophyll b among treatments.

Table 4.26: Pigments and total carotenoid contents of methanolic fruit extracts of MD2 pineapple fruit produced from *ex vitro* plants field-grown with different types of fertilizers.

Treatment	Control	Chemical fertilizer	Vermicompost
C_a (µg/g)	0.525 ± 0.014 [b]	0.627 ± 0.026 [b]	0.977 ± 0.086 [a]
C_b (µg/g)	2.349 ± 0.077 [b]	2.128 ± 0.098 [b]	3.094 ± 0.136 [a]
$C_a + C_b$ (µg/g)	2.874 ± 0.074 [b]	2.754 ± 0.072 [b]	4.071 ± 0.70 [a]
$C_{(x+c)}$ (µg/g)	2.834 ± 0.030 [b]	3.080 ± 0.060 [a]	2.890 ± 0.054 [b]
C_a / C_b ratio	0.224 ± 0.011 [a]	0.297 ± 0.026 [a]	0.319 ± 0.043 [a]
$C_a + C_b$ / $C_{(x+c)}$ ratio	1.015 ± 0.032 [b]	0.896 ± 0.041 [b]	1.410 ± 0.049 [a]
Total (µg/g)	5.708 ± 0.065 [b]	5.834 ± 0.012 [b]	6.961 ± 0.031 [a]

* Means ± standard error of mean value followed by different letters in rows are significantly different using ANOVA, Duncan's test, $P \leq 0.05$, n=3. C_a chlorophyll a, C_b chlorophyll b, $C_a + C_b$ total chlorophyll a and b, $C_{(x+c)}$ total carotenoid (xanthophyll and carotene).

On the other hand, plant supplied with chemical fertilizer produced fruits with significantly higher amount of total carotenoid, 3.080 µg/g at $P \leq 0.05$. This is line with the findings obtained in this study, the pineapple produced with vermicompost contained significantly higher total chlorophyll, thus yielding lower carotenoid content in the latter. Similar trend was found on *in vivo* MD2 pineapple fruit. Moreover, similar to the results observed in *in vivo* plants, fruit extract from plant supplied with vermicompost contained the lowest total phenolics, 6.055 mg gallic acid (GAE) per g dry extract, followed by chemical fertilizer (6.083 mg GAE/g dry extract) and control plants (8.212 mg GAE/g dry extract) (Table 4.27). Interestingly, the fruit extract from *ex vitro* plants supplied with chemical fertilizer was observed to contain α-carotene (26.9 µg/g DW) and β-carotene (9.0 µg/g DW), but both were absent in the fruit extracts from *ex vitro* plants supplied with vermicompost and control (unfertilized) plants (Appendix F).

Table 4.27: The concentration of total phenolic content (TPC), α-carotene and β-carotene in methanolic fruit extracts of MD2 pineapple fruit produced from *ex vitro* plants field-grown with different types of fertilizers.

Treatment	Total phenolics (mg GAE/g dE)	α-carotene (µg/g DW)	β-carotene (µg/g DW)
Control	8.212 ± 0.567 [a]	ND	ND
Chemical fertilizer	6.083 ± 0.273 [b]	26.9 ± 2.5	9.0 ± 1.6
Vermicompost	6.055± 0.141 [b]	ND	ND

* Mean ± standard error of mean within each row followed by a different letter indicate significant differences at $P \leq 0.05$, (n=3). GAE, gallic acid equivalents; dE, dry extract; DW, dry weight.

4.4.3 Correlations between Bioactive Compounds with Nutrient Content in Plants.

The correlation between the nutrients content in *in vivo* and *ex vitro* plants during red bud stages with the bioactive compounds in the fruits were analysed. Based on Table 4.28, total N in the D-leaf of *in vivo* plants showed significant positive correlation with chlorophyll b, total chlorophyll, total carotenoid, total pigment and β-carotene contents;

with r^2 of 0.699, 0.690, -.0737, -.0700 and 0.670 respectively at $P \leq 0.05$. This indicated that the increase of total N in D-leaf of *in vivo* pineapple plants will increase the chlorophyll b, total chlorophyll, and β-carotene content but decrease the total carotenoid and total pigment in the fruits. Besides that, the K content in the D-leaf of *in vivo* plants showed strong positive correlation with chlorophyll a, chlorophyll b, total chlorophyll and β-carotene in the methanolic fruit extract, with r^2 of 0.761, 0.778, 0.776 and 0.720 respectively ($P \leq 0.05$). On the other hand, the K content in the D-leaf of *ex vitro* plants (Table 4.29) showed significant positive correlation with total carotenoid (r^2= 0.880, $P \leq 0.01$).

Table 4.28: Pearson's correlation coefficient between bioactive compounds and nutrients content in the D-leaf of *in vivo* MD2 pineapple plants during red bud stage (S2).

Parameters	Chl a	Chl b	Total Chlorophyll	Total carotenoid	Total pigments	Total phenolics	β-carotene	N	P	K	Mg	S	Ca	Fe	Zn	B
Chlorophyll a	1															
Chlorophyll b	.984**	1														
Total chlorophyll	.991**	.999**	1													
Total carotenoid	-.682*	-.677*	-.680*	1												
Total pigments	-.589	-.583	-.586	.993**	1											
Total phenolics	.620	.634	.632	.137	.256	1										
β-carotene	.984**	.990**	.992**	-.713*	-.624	.587	1									
N	.656	.699*	.690*	-.737*	-.700*	.158	.670*	1								
P	.548	.589	.581	-.203	-.128	.567	.576	.138	1							
K	.761*	.778*	.776*	-.463	-.383	.561	.720*	.504	.226	1						
Mg	-.485	-.575	-.554	.349	.294	-.371	-.538	-.608*	.106	-.278	1					
S	-.188	-.209	-.204	-.394	-.469	-.692*	-.195	.255	-.515	-.082	-.219	1				
Ca	-.588	-.707*	-.678*	.455	.390	-.434	-.665	-.623*	-.071	-.561	.846**	-.104	1			
Fe	-.537	-.656	-.627	.427	.368	-.392	-.611	-.384	.007	-.078	.608*	.217	.616*	1		
Zn	-.922**	-.924**	-.926**	.653	.568	-.566	-.925**	-.576	-.523	-.769**	.334	.070	.567	.095	1	
B	-.181	-.200	-.196	-.024	-.060	-.281	-.114	-.363	.464	-.060	.680*	-.285	.382	.428	-.128	1
Al	-.726*	-.763*	-.756*	.791*	.749*	-.179	-.797*	-.525	-.664*	-.315	.318	-.090	.393	.024	.649*	-.238

** Correlation is significant at the 0.01 level (2-tailed).

* Correlation is significant at the 0.05 level (2-tailed).

Table 4.29: Pearson's correlation coefficient between bioactive compounds and nutrients content in the D-leaf of *ex vitro* MD2 pineapple plants during red bud stage (S2).

Parameters	Chl a	Chl b	Total Chlorophyll	Total carotenoid	Total pigments	Total phenolics	N	P	K	Mg	S	Ca	Fe	Zn	B
Chlorophyll a	1														
Chlorophyll b	.696*	1													
Total Chlorophyll	.852**	.969**	1												
Total carotenoid	.078	-.568	-.387	1											
Total pigments	.924**	.905**	.979**	-.189	1										
Total phenolics	-.610	-.217	-.368	-.560	-.517	1									
N	-.238	.035	-.056	-.168	-.098	.019	1								
P	.233	.141	.183	.024	.200	-.452	.111	1							
K	.237	-.347	-.172	.880**	.013	-.633	-.366	-.249	1						
Mg	.180	.396	.351	-.390	.286	.165	-.446	.410	-.424	1					
S	.094	-.370	-.238	.831**	-.068	-.648	-.315	.268	.681*	-.058	1				
Ca	-.252	.104	-.011	-.572	-.139	.700*	.265	.051	-.769**	.441	-.624*	1			
Fe	.199	-.268	-.127	.328	-.062	-.156	-.079	.167	.121	-.105	.089	-.328	1		
Zn	-.678*	-.463	-.571	-.283	-.671*	.856**	.259	-.370	-.475	.024	-.643*	.667*	-.079	1	
B	-.097	-.119	-.120	.011	-.125	.086	.133	.669*	-.395	.599*	.139	.552	-.182	.148	1
Al	-.568	-.344	-.446	-.244	-.529	.464	.214	-.251	-.303	-.377	-.326	-.019	.300	.480	-.373

** Correlation is significant at the 0.01 level (2-tailed).

* Correlation is significant at the 0.05 level (2-tailed).

4.5 Antioxidant Activity of Fruit Extracts

The fruit pulp extract was also examined for radical scavenging and antioxidant activities using three different assay methods; DPPH, ABTS and FRAP. The IC_{50} value for DPPH and ABTS assays, as well as the FRAP values of the methanolic extracts of MD2 pineapple fruits produced from *in vivo* plants are depicted in Table 4.30. For DPPH assay, the fruits extract from *in vivo* pineapple plants supplied with vermicompost and chemical fertilizer showed stronger scavenging activity against DPPH radicals than the control plants. Similar observation was recorded for ABTS assay, where the scavenging activity of the extracts against ABTS radicals in decreasing order is; chemical fertilizer > vermicompost > control.

Table 4.30: Antioxidant capacities determined by DPPH, ABTS and FRAP assays in methanolic extracts of MD2 pineapple fruits produced from *in vivo* plants grown with different types of fertilizers.

Antioxidant assay	Ascorbic acid (standard)	Control	Chemical fertilizer	Vermicompost
DPPH, IC_{50} (mg/ml)	0.034 ± 0.002^{c}	5.133 ± 0.101^{a}	2.909 ± 0.050^{b}	3.239 ± 0.213^{b}
ABTS, IC_{50} (mg/ml)	0.057 ± 0.004^{d}	8.393 ± 0.100^{a}	5.777 ± 0.130^{c}	7.290 ± 0.188^{b}
FRAP (mg FE/g dE)	36.198 ± 4.398^{a}	0.368 ± 0.005^{b}	0.402 ± 0.017^{b}	0.276 ± 0.020^{b}

* Mean ± standard error of mean within each row followed by a different letter indicate significant differences at $P \leq 0.05$, (n=3). FE, ferric equivalent; dE, dry extract.

On the other hand, the FRAP assay estimates the antioxidant power, which is the reducing ability of the substances involved in the transfer of electron in the reaction. Based on Table 4.30, the FRAP reducing power of fruit extract of *in vivo* plants supplied with vermicompost is lower than fruit extracts from plants supplied with chemical fertilizer and control (unfertilized) plants with 0.276 mg of $FeSO_4$ equivalent/ g of dried

extract. All of treatments exhibited lower antioxidant capacity compared to the ascorbic acid (standard) for all assays tested.

Furthermore, the antioxidant capacity in fruit pulp of *ex vitro* plant was presented in Table 4.31. Contrasting results were observed compared to that of *in vivo* plants, where the highest antioxidant potential against DPPH radicals were recorded in fruit extracts produced from unfertilized *ex vitro* plants (control) but still lower than standard (ascorbic acid). The scavenging activity of the extracts against DPPH radicals in decreasing order is; control > vermicompost > chemical fertilizer. On the other hand, the scavenging activity against $ABTS^{\bullet+}$ radicals exhibited by the fruit extracts of *ex vitro* plants supplied with different types of fertilizers showed no significant difference ($P \leq 0.05$). Moreover, the FRAP reducing power exhibited by the fruits extracts of *ex vitro* plants supplied with vermicompost was observed to be comparable to that of chemical fertilizer, but both showed lower FRAP values compared to control (unfertilized) plants. Similar trend to *in vivo* MD2 pineapple fruit extract, all treatments showed lower antioxidant capacities than ascorbic acid (standard).

Table 4.31: Antioxidant capacities determined by DPPH, ABTS and FRAP assays in methanolic extracts of MD2 pineapple fruits produced from *ex vitro* plants grown with different types of fertilizers.

Antioxidant assay	Ascorbic acid (standard)	Control	Chemical fertilizer	Vermicompost
DPPH, IC_{50} (mg/ml)	0.050 ± 0.001 ^{d}	6.022 ± 0.036 ^{c}	8.660 ± 0.102 ^{a}	8.250 ± 0.035 ^{b}
ABTS, IC_{50} (mg/ml)	0.065 ± 0.002 ^{c}	7.361 ± 1.775 ^{a}	10.502 ± 1.791 ^{ab}	12.559 ± 0.126 ^{a}
FRAP (mg FE/g dE)	29.074 ± 4.800 ^{a}	0.301 ± 0.030 ^{b}	0.181 ± 0.014 ^{b}	0.220 ± 0.021 ^{b}

***** Mean ± standard error of mean within each row followed by a different letter indicate significant differences at $P \leq 0.05$, (n=3). FE, ferric equivalent; dE, dry extract.

4.5.1 Correlations between Antioxidant Potential with Bioactive Compounds Composition.

Pearson's correlation analysis was conducted to determine the relationship between the antioxidant potential exhibited by the fruits extracts with its bioactive compound's composition. As observed in Table 4.32, the antioxidant potential exhibited by the extracts was directly related to the chlorophyll a and chlorophyll b contents of the extracts. Analysis of results revealed strong negative correlations between antioxidant activity of the extracts against DPPH radicals with the total carotenoid, chlorophyll a, chlorophyll b and β-carotene with r^2 values of -0.674, -0.814, -0.839 and -0.715 respectively ($P \leq 0.01$). These indicated that the IC_{50} of the extract against DPPH would significantly decrease with increasing concentration of total carotenoid, chlorophyll a, chlorophyll b and β-carotene. Moreover, ABTS assay also negatively correlated with total phenolics (r^2 = -0.493), chlorophyll a (r^2 = -0.499), chlorophyll b (r^2 = -0.527) and β-carotene (r^2 = -0.637) (significant at $P \leq 0.05$ level). In contrast, FRAP assay showed positive correlation with total phenolics, total carotenoid, chlorophyll a and chlorophyll b.

Table 4.32: Pearson's correlation coefficient between antioxidant potential with bioactive compounds composition.

Parameters	Total phenolics	Total carotenoid	Chlorophyll a	Chlorophyll b	β-carotene	DPPH	ABTS
Total phenolics	1						
Total Carotenoid	-.017	1					
Chlorophyll a	.174	.537*	1				
Chlorophyll b	.259	.500*	.981**	1			
β-carotene	.573	-.013	.921**	.936**	1		
DPPH	-.216	-.674**	-.814**	-.839**	-.715**	1	
ABTS	-.493*	-.437	-.499*	-.527*	-.637*	.747**	1
FRAP	.539*	.750**	.557*	.577*	.384	-.746**	-.690**

** Correlation is significant at the 0.01 level (2-tailed).
* Correlation is significant at the 0.05 level (2-tailed).

4.6 Cost Analysis of Vermicompost Usage versus Chemical Fertilizer

A cost analysis was also conducted to determine the effectiveness of using vermicompost as a replacement of chemical fertilizer in the field. Based on the results depicted in Table 4.33, the cost of organic supplement per plant per year (vermicompost) was 1.86 times higher than the cost of chemical fertilizer. However, due to the vermicompost's characteristics as a slow release fertilizer, the labour cost (per plant per year) for field maintenance for plants supplemented with vermicompost was 2.5 times lower (RM3.13) than that needed for plants supplemented with chemical fertilizer (RM7.81). In this study, the profit obtained from fruits produced by plants supplemented with vermicompost was slightly higher than that obtained using chemical fertilizer. However, the profit is estimated to significantly increase if the planting density of the seedlings is increased and if the field size is bigger.

Table 4.33: Cost analysis of MD2 pineapple fruits grown with vermicompost and chemical fertilizer.

Cost Analysis		**Chemical Fertilizer**	**Vermicompost**
Fertilizer	Cost per kg fruit	RM0.98	RM2.06
	Cost per plant per year	RM2.42	RM4.50
Labour	Cost per kg fruit	RM3.17	RM1.43
	Cost per plant per year	RM7.81	RM3.13
Total cost (labour + fertilizer)	Cost per kg fruit	RM4.15	RM3.49
	Cost per plant per year	RM10.23	RM7.63
Market price of fruits		RM 8.00 per kg	
Profit	Profit per kg fruit	RM3.85	RM4.51
	Profit per plant per year	RM9.50	RM9.86

Note: Labour cost = RM50/day per person. Excludes costs of starting material (seedling), irrigation and flower inducing hormone.

CHAPTER 5: DISCUSSION

5.1 Effect of Vermicompost on Morpho-physiology of MD2 Pineapple Plants in the Field

Based on the current study, two times application of vermicompost with the amount of 10 tan ha^{-1} to the soils grown with *in vivo* and *ex vitro* MD2 pineapple plants is comparable to the plants supplied with chemical fertilizer in terms of their growth performance in the field. Generally, there was no significant difference between *in vivo* plants supplemented with vermicompost and chemical fertilizer on plant height, number of leaves, length and width of D-leaves. However, *in vivo* plant supplemented with vermicompost was not significant with control plants in terms of growth performance in the field. In addition, after six months after planting, *in vivo* plants supplemented with chemical fertilizer showed a significant wider of D-leaf compared to other treatments (Figure 4.4A). This is might be due to low in N content in the D-leaf, where *in vivo* plant treated with chemical fertilizer contained higher total N content (1.44%) compared to plant treated with vermicompost (1.16%) and unfertilized plant (1.20%). Deficiency of N concentrations symptoms includes slow plant growth and narrower leaves (Souza & Reinhardt, 2007). Moreover, the last supplementation of vermicompost was on prior to planting whereby explained the lower in N content. Similar trend was observed for *ex vitro* plant supplemented with vermicompost whereby contained low N (0.42%) in the D-leaf on red bud stage (S2, 17 MAP), thus affect the growing of new leaves. For future research, the vermicompost application is suggested to apply again after five months of planting interval.

Furthermore, data analysis revealed that the length of the D-leaf of the *ex vitro* plants treated with vermicompost showed a significantly marked increase compared to plants treated with chemical fertilizer after 13 MAP, possibly due to second supplementation of

vermicompost to the soil (Figure 4.3B). Similar result was obtained by previous research on Queen varieties of pineapple plant whereby the influence of vermicompost (20 tons ha^{-1} $year^{-1}$) was clearly greater on second year of planting (Chaudhuri et al., 2016). The effect of second application of vermicompost also can be observed on width of the D-leaf of *ex vitro* plants where the width of D-leaves drastically increased after eight MAP (Figure 4.4B). It also might be due to weather condition whereby width of D-leaves drastically increased during raining season on April (three MAP, 15 MAP), October (9 MAP) and November (10 MAP). Between February and April 2016, the Super El Nino 2015/2016 condition has taken place and caused drought nationwide which was among the strongest since previously reported on 1997/1998 (MMD, 2016). As a result, the width of D-leaves decreased during the event.

Increasing in light intensity due to removal of shades on 12 MAP also could possibly a reason to a dropped in width of D-leaves. Plants grown in warmer and dried climates tend to have smaller leaves to reduce water loss through transpiration while in more wetter environment with low light intensity, larger leaves are more prevalent because the attendant water cost is less critical (Chitwood et al., 2012; Peppe et al., 2011). In these extreme conditions (13-15 MAP), the plants treated with vermicompost showed highest width of the D-leaves compared to plant supplied with chemical fertilizer and unfertilized *ex vitro* plant (control). The properties of vermicompost which known to have high water holding capacity further helps with the water economy of pineapple plants. Based on previous study, the water holding capacity was reported increased on the vermicompost added soils grown with black gram compared to control (untreated), 52.21% and 49.26% respectively (Tharmaraj et al., 2010).

A non-destructive and rapid method to estimate the chlorophyll and nitrogen status in the leaves can be measured by using chlorophyll meter (SPAD-502). The SPAD meter values shows the relative greenness existing in the crop canopy and values lower than 40 indicated impairment in photosynthesis process (Netto et al., 2005). In this study, data analysis revealed that plants supplemented with vermicompost exhibited the lowest chlorophyll content compared to chemical fertilizer and control for both type of plants (Table 4.5). This result is in agreement with those obtained by El-Hassan et al. (2017), the chlorophyll reading of green bean plants supplied with vermicompost decreased compared to mineral fertilizers, 29.30 SPAD and 33.27 SPAD respectively. Alaboz et al. (2017) also reported pepper (*Capsicum annuum*) plant treated with 0.75 w/w vermicompost (60.7 SPAD) contain lower chlorophyll content compared to unfertilized plants (64.9 SPAD) under field capacity with 80% soil moisture level. Moreover, the chlorophyll content of *ex vitro* plants was observed to drastically reduced on 12 MAP, the lowest precipitation obtained along the year. In the event of water shortage, the chlorophyll content was decreased (Ghahfarokhi et al., 2015) due to less of water content in the leaves which reduced the rate of chlorophyll synthesis in the leaves (Lawlor, 2002).

Majic et al. (2008) reported that SPAD values and the leaf N concentration show a highly significant correlation (r values ranged from 0.51** to 0.81**) which presents major physiological growth stages in potato crop. Similar observation was found in this study, whereby data analysis revealed that there was a strong significant correlation between the SPAD values (10 MAP) and N levels in the leaves of *in vivo* plants (r^2= 0.877, $P \leq 0.01$) at S2 (Appendix G). Similar trend was observed on *ex vitro* plants. There was a significant different between SPAD values (18 MAP) and N content in the leaves at S2 (r^2= 0.639, $P \leq 0.05$) (Appendix H). Based on Figure 4.5, the SPAD values of D-leaves from *in vivo* plants supplied with chemical fertilizer showed a steady increase from four MAP until the emergence of flowering (10 MAP). This might be because at the

vegetative stage, chemical fertilizer and foliar sprays were periodically supplied to the soils and plants.

Stomatal density and stomatal characteristics (e.g. stomatal size, stomatal length) are indicators of acclimation and adaptation to environments changes such as light intensity (Custodio et al., 2016; Pompelli et al., 2010), temperature (Tian et al., 2016), water status (Drake et al., 2013), leaf nutrients and soil nutrients content (Tian et al., 2016). In this study, stomatal density, stomatal size and some of stomatal features were determined on D-leaves of pineapple plants supplied with different types of fertilizers on nine months after planting. Generally, stomatal density of the pineapple leaves is low, about 80 stomata mm^{-2} (d'Eeckenbrugge & Leal, 2003). However, in this study, the stomatal density of *in vivo* plants supplied with chemical fertilizer (85 stomata per mm^2) and vermicompost (96.62 stomata per mm^2) were found higher than previous study. Moreover, the results showed that stomatal density of leaves for both *in vivo* and *ex vitro* plant supplied with vermicompost was statistically significantly higher than control plants at $P \leq 0.05$. The higher stomatal density on D-leaves of plant supplied with vermicompost could be due to high water holding capacity which reduced the water-stress to the plants, thus under these favourable conditions, pore length of stomatal also showed wider than other treatments.

However, *ex vitro* plants showed lower stomatal density compared to *in vivo* plants. The differences in stomatal density between *ex vitro* and *in vivo* plants might be due to different in light intensity whereby *ex vitro* plants grown under shade and *in vivo* plants grown under direct sunlight. Plants cultivated under high light intensity contained high stomatal densities as an adaptation to optimize long-term gas exchange, increase transpiration efficiency, reduce leaf temperature with better photosynthetic activity (Custodio et al., 2016). This research found stomatal density was negatively correlated to

stomatal size (r^2 = -0.570). Similar findings were found on previous studies (Pompelli et al., 2010; Tian et al., 2016). Under favourable conditions, high stomatal densities with the speed of small stomata that can open and close more rapidly allowing better regulation of gas exchange and transpiration for plant photosynthesis (Tian et al., 2016). Therefore, the plant with faster stomatal response help to reduced water loss in high evaporative demand and fluctuating light (Drake et al., 2013).

5.2 Effect of Vermicompost on Fruit Attributes

5.2.1 Physical Characteristics

The results of this study indicated that physically the fruits produced from *in vivo* plants grown with chemical fertilizer were larger in size, followed by vermicompost and control, but vermicompost had the smallest crown. Similar trend was observed on *ex vitro* plants, but plant supplied with vermicompost had average crown size. However, the fruits from *ex vitro* plants smaller than *in vivo* plants with ranged from 1248 g to 1734 g but heavier than commercialized MD2 pineapple fruit reported by previous study, 1132.8 g (Lu et al., 2014). This might be due to smaller plant size at forcing of flowering. Plant size at forcing influences the number of florets (eyes) per fruit developed where the larger the stump, the greater the number of florets possible (Paull & Chen, 2003). It could also be due to low N content. The total N content in the D-leaves of *ex vitro* plants was considered deficient for plant growth during red bud stages where plant supplemented with vermicompost contained the lowest N content among treatment. Based on the previous study, the fruit weight of 'Smooth Cayenne' pineapple tends to increase with N fertilizer application (Paull & Chen, 2003). Similar results were reported in a previous study on Jupi pineapple, where N deficiency had resulted in a 57.59% reduction of fruit weight (with crown); fruit length reduced by 38.7%, and the fruit diameter reduced by 22% (Ramos & da Rocha Pinho, 2014).

An important indicator of fruit freshness is their firmness especially after the storage. In this study, fruit produced from *in vivo* plants supplied with vermicompost were significantly firmer than fruits grown on chemical fertilizer. These results are in agreement with those of previous studies, where strawberries harvested from plants receiving vermicompost (seven tan ha^{-1}) were significantly firmer than those harvested from plants receiving inorganic fertilizer only (Singh et al., 2008). A possible explanation for this might be because of the excess of N which can contribute to a reduction in fruit pulp firmness (Py et al., 1987). The amount of N in the D-leaves of *in vivo* plants treated with chemical fertilizer was higher than in plants treated with vermicompost and unfertilized plants during red bud stages. Based on the Pearson's correlation coefficient (Table 4.20), total N showed negative significant correlation with pulp firmness (r^2= -0.631, $P \leq 0.05$) indicating that the increase in N could reduce the pulp firmness. Moreover, several report have shown that Ca also responsible for firmness of fruits such as apple (Beavers et al., 1994), peach (Gupta et al., 2011) and blueberry (Ochmian, 2012). Ca ions have a role in linking adjacent acidic pectin polymers in cell walls, whereby there are major changes in the pectin-rich middle lamella region of cells during ripening (Tucker, 1993). Reduced Ca levels in the cell walls would result in the structural failure to the tissues. However, in this study, no correlation was found between Ca content in the D-leaves and pulp firmness for both fruits produced from *in vivo* and *ex vitro* plants.

5.2.2 Physico-chemical Properties

The sugar-to-acid ratio is usually used as a measure of consumer taste preference. To obtain high quality of pineapple fruit, a sugar-to-acid ratio range from 20 to 40 were recommended (Lu et al., 2014; Soler, 1992). In this study, the sugar-to-acid ratio fell within the recommended range (21.67 to 42.00). The range of TSS of fruits produced from *in vivo* plants were from 9.32 °Brix to 11.13 °Brix and for *ex vitro* plants ranged

from 12.1 °Brix to 12.6 °Brix. The highest level in TSS content was obtained from fruits harvested from control (unfertilized) plants and *ex vitro* plants supplemented with vermicompost which might be due to the low N content in the D-leaves compared to chemical fertilizer. The total N in D-leaves of *in vivo* plants showed a strong negative correlation with TSS of fruit juice with a coefficient of r^2= -0.507, indicating that the low in N content resulted in the increase of the TSS of fruit juice (Table 4.20). Similarly, significant correlation was observed on the fruits produced from *ex vitro* plants (r^2 = -0.795, $P \leq 0.01$) (Table 4.21). The low N content in the D-leaves reduced the size of fruits but increased the TSS content. These results are in accordance with that reported by Darnaudery et al. (2016), whereby the highest level of TSS was observed in 'Queen Victoria' pineapple fruit, and was found to be caused by the decrease in N content in the D-leaves.

The pH of juice was decreased when titratable acidity (TA) increased. There was a significant correlation on TA and pH of juice of both fruits harvested from *in vivo* and *ex vitro* with value of r^2 = -0.697 and r^2 = - 0.837 at $P \leq 0.01$, respectively (Table 4.20). The pH values ranged from 4.18 to 4.23 for fruit juice from *in vivo* plants, which in accordance with the results obtained in literature which ranged from 3.58 to 4.24 from 26 pineapple genotypes grown in China (Lu et al., 2014), however the range pH of fruit juices from *ex vitro* plants slightly higher (4.42 to 4.86). Previous studies also have reported that fruit acidity tended to increase with increased in K content (Razzaque & Hanafi, 2001). The results of this study also showed a significant increase in acidity when K content in the D-leaves increased for both *in vivo* plants (r^2 = 0.712) and *ex vitro* plants (r^2= 0.644) at $P \leq 0.05$. In terms of fruit acidity, the application of vermicompost at 10 t ha^{-1} had been reported to result in lower fruit acidity in 'Chandler' strawberry compared to addition of inorganic nutrients (Singh et al., 2008). In contrast, the fruits harvested from *ex vitro* plants supplemented with vermicompost had higher fruit acidity compared to other

treatments. Moreover, pineapple fruits harvested from *in vivo* plants supplemented with vermicompost in this study were observed to have comparable fruit acidity levels with fruits harvested from plants supplied with chemical fertilizer and control plants.

The nutritional value of fruits is characterized by antioxidant content such as ascorbic acid (vitamin C). In this experiment, fruits harvested from *in vivo* plants supplied with chemical fertilizer showed high values of ascorbic acid but fruits from *ex vitro* plants had lowest ascorbic acid. The inconsistency may be due to exposure to air and light whereby vitamin can be easily destroyed (Njoku et al., 2011). However, the results from *ex vitro* plants supplied with vermicompost in agreement with previous studies where both ascorbic acid and titratable acidity showed high value (Darnaudery et al., 2016). The range of ascorbic content obtained in this investigation are far below compared to those of other studies (Darnaudery et al., 2016; Lu et al., 2014; Wardy et al., 2009). In terms of chemical characteristics, fruits from *in vivo* plant supplied with vermicompost produced competitive results with chemical fertilizer, but for *ex vitro* plants resulting fruits significantly contained higher total solids and ascorbic acid content.

5.2.3 Sensory Analysis

Figure 4.12 and figure 4.13 represent the sensory analysis on attributes of colour, flavour, aroma, firmness and overall acceptance conducted on fresh cut of MD2 pineapple harvested from plants supplemented with chemical fertilizer, vermicompost and unfertilized plants*, in vivo* and *ex vitro* plants respectively. For *in vivo* plants, all panellists preferred fruits derived from plants supplied with chemical fertilizer and control plants which contain higher TSS. What is surprising is that highest overall acceptability for *ex vitro* plants was fruits harvested from control plants whereby no panellists dislike the flavour of these fruits. This result may be explained by the fact that it contains the highest

sugar-to-acid ratio compared to other treatments, therefore sweeter and preferred by panellists.

5.3 Effect of Vermicompost on Soil and Plant Nutrients Content

5.3.1 Soil pH

Soil pH has direct impact on availability of nutrients in the soils for plant growth. The current study found that the soil pH showed significant variation when different types of fertilizers were applied. The soil pH was found to increase when vermicompost was applied twice in one season into the soils grown with *in vivo* plants. Similar results have been previously reported (Gopinath et al., 2008), where a significant increase of soil pH was observed after two consecutive applications of vermicompost (two years of transition) onto wheat plants grown on mildly acidic soils, compared to the application of NPK fertilizers. The pH of vermicompost-treated soils (at S1) fell below the control at the beginning due to the formation of organic acids through the degradation of organic compounds, which are easily mineralized later under aerobic conditions. This produces ammonium, which can subsequently increase the soil pH (Beck-Friis et al., 2003) and also reduce the potential of Al and Mn toxicity that can injured root tips (Nada et al., 2011). On the other hands, the soil supplied with vermicompost grown with *ex vitro* plants leads to significant increase in the pH of soil compared to soil treated with chemical fertilizer and unfertilized (control) soils for both samplings. Based on previous studies, the acidity of soil decreased with the increased level of vermicompost application (Chaudhuri et al., 2016, Zaman et al., 2015) supports similar findings in our present experiment.

In contrast, this study also found that the continuous application of chemical fertilizer decreased the soil pH over time for both soils grown with *in vivo* and *ex vitro* MD2 pineapple plants. These results were in agreement with the findings from other studies

that showed that the supplementation of NPK fertilizer also decreased the soil pH (Brar et al., 2015; Han et al., 2016; Milosevic & Milosevic, 2009). This might be due to the usage of chemical fertilizer that contained 9% $NH4^+$ and 6% NO_3^- as the source of nitrogen to the soils. The acidifying effect of nitrification and leaching of NO_3^- decreased the soil pH due to increasing the H^+ accumulation in the soils, caused by their release from NH_4^+ (Bolan & Hedley, 2003).

In addition, these will also adversely affect the pH of poorly buffered soils, when a high rate of N is applied to sandy soils that are low in Ca. These were in agreement with the results obtained in this study, where the Ca content in the soils supplied with chemical fertilizer grown with *in vivo* plants was found to be reduced by 56% during S2 (red bud stage), which in turn had resulted in a low soil pH (Table 4.14). This also justifies the increase of the pH of the soil added with vermicompost, as it contained twice the amount of Ca compared to soils supplied with chemical fertilizer (Table 4.14). Similar trend was observed for *ex vitro* plants. There was a strong positive correlation between soil pH and Ca level in the soil supplemented with vermicompost grown with *ex vitro* plants at both sampling time with value of r^2= 0.936 (S1) (Appendix I) and r^2= 0.921 (S2) (Appendix J). These results are in accordance with the earlier reported findings Angelova et al. (2013), which showed that amendment with 10 g kg^{-1} of vermicompost resulted in an increase in soil pH, with a high correlation coefficient between exchangeable Ca and soil pH (r = 0.90).

5.3.2 Nutrients Content in the Soil and D-leaf of MD2 Pineapple Plant

Based on Table 4.14, there was no significant difference between almost all nutrients content analysed in soils supplied with chemical fertilizer, vermicompost and control plot grown with *in vivo* pineapple plants after six months of planting except N content. Most of total elements higher in value when soil was supplemented with chemical fertilizer,

followed by vermicompost and control. On the other hand, during red bud stage, soils incorporated with vermicompost either higher or on par with soils treated with chemical fertilizer and statistically significantly higher than unfertilized (control) soils. Similar results was reported by Zaman et al. (2015), total N, available P, exchangeable K, Ca, Mg, available S, Zn and B was significantly increased with 10 t ha^{-1} vermicompost application compared to unfertilized soils. This also might be because of second supplementation of vermicompost before flowering was induced. Vermicompost is a 'slow-release fertilizer' (Kashem et al., 2015), this properties allowed nutrient to accumulate in the soils before its mineralized and then release the essential nutrients needed by plants overtime. As a result, significantly higher of N level was obtained in the soils supplemented with vermicompost. Similar trend was observed on soil nutrients content grown with *ex vitro* plants (Table 4.15). Moreover, only soil supplied with vermicompost showed an increase in Ca content and decrease in Al content in the soils. This could be explained the higher in soil acidity compared to soil supplied with chemical fertilizer and unfertilized soil as described in section 5.3.1.

Pineapple plants were observed to contain statistically different amount of nutrients taken up from the soils. For both S1 and S2, total nitrogen uptake for *in vivo* plants supplied with vermicompost was significantly lower than chemical fertilizer even though the N content in the soils was slightly higher than soils supplied with chemical fertilizer (Table 4.16). Similar trend was observed during red bud stage for *ex vitro* plants (Table 4.17). This probably due to the properties of vermicompost which is a 'slow-release fertilizer' while the chemical fertilizers contain N in the form that immediately available for plant uptake (Rajiv et al., 2010). The N content important in the vegetative stage for rapid growth, but the excess or deficiency of N during flowering will affect the fruit quality (Souza & Reinhardt, 2007).

The N content in the D-leaf of *ex vitro* plants (for all treatments) was decrease during S2 and was considered as inadequate for pineapple plants at floral induction as reported by Ramos et al. (2009). However, the low N uptake by plant during flowering showed a positive result to the chemical properties of fruit produced. According to Ramos and da Rocha Pinho (2014), the deficiency of N increased in the TSS of Jupi pineapple by 11.2%, TA by 85% and vitamin C content by three-fold compared to the fruit supplied with complete nutrient solution. Similar pattern were obtained by Omotoso and Akinrinde (2013), 40.1% reduction of fruit juice acidity relative to control as N fertilizer rates increased and the highest TSS was obtained in the plant crops that received lowest N fertilizer, 50 kg Nha^{-1}. In this study, the Pearson's correlation coefficient analysis revealed that the N content in the D-leaves of *ex vitro* plants showed a significantly negative correlation with TSS (r^2 = -0.795, $P \leq 0.01$), TA (r^2 = -0.777, $P \leq 0.01$), total solids (r^2 = -0.850, $P \leq 0.01$) and ascorbic acid content (r^2 = -0.669, $P \leq 0.05$) in the resulting fruits as previously describes in section 5.2.2.

Moreover, *in vivo* plants supplemented with vermicompost were lower in Ca content in the D-leaves, while control plants have the highest at S1. The reduced in the Ca concentration in the D-leaves might be because of competition with K for nutrient uptake. Similar trend was observed during red bud stages where high content of K reduced the Ca content in the D-leaves. There was a significant negative correlation between K and Ca content in the D-leaves of *in vivo* plants with coefficient of r^2= -0.682 at $P \leq 0.05$ (S1) (Appendix K). Similar results were obtained in D-leaf of cultivar 'Smooth Cayenne' when given high amount of K, the nutrient uptake for Ca decreased (Teixeira et al., 2011). In addition, unlike Ca^{2+}, Mg^{2+} ions are more susceptible to leaching, since they are not as strongly adsorbed to clay minerals or organic matter due to their large hydrated radius (Mitra, 2017).

At S2, the S, Fe, and B content in all of the samples were considered inadequate for plant growth, except the B concentration in the D-leaves of *in vivo* plants supplied with vermicompost (Ramos et al., 2009; Souza & Reinhardt, 2007). In general, soils that are well provided with organic matter and with pH less than 7 will not usually face B deficiency (Basso & Suzuki, 2001; Maeda et al., 2011). However, the supplementation of higher doses of K-fertilizers may also affect B availability in the soils (Mitra, 2017). In this study, a positive significant correlation was found between the K and B content in the soils grown with *in vivo* plants during S2 with a coefficient of r^2= 0.594 at $P \leq 0.05$ (Appendix L). Moreover, the application of S fertilizers onto clay minerals at optimum doses does not have any residual effect, since clay minerals do not bind sulfate and thus, it is leached out of the soil (Mitra, 2017).

The D-leaves of *ex vitro* plants supplemented with vermicompost contained higher macro and micronutrients compared to plants supplemented with chemical fertilizer on six MAP. Although vermicompost was supplied six months before sampling, nutrients uptake by plant showed a competitive result with plant supplied with chemical fertilizer. This probably due to the properties of vermicompost which is a 'slow-release fertilizer', allows the plants to absorb these nutrients over time (Kashem et al., 2015). Based on previous study, the nutrient content of different plant components such as roots, shoots and the fruits also improved as vermicompost was supplied to the soils (Theunissen et al., 2010).

As the soil pH drops below 5, Al is solubilized into toxic forms (Gupta et al., 2013). Excess of Al^{3+} in the soil enters roots, then inhibit the root growth which limits water and interferes with the uptake, transport and utilization of most of mineral elements. Under Al stress, the deficiency of some essential nutrients including P, K, Ca, Mg and Fe can be easily detected its symptoms in plant (Gupta et al., 2013). According to Mota et al. (2016),

increment in Al concentration reduced the accumulation of K and Mg in the roots, K in the stem and N, P and K in the pineapple cv. 'Vitoria' plant. Similarly, in this experiment, Al concentration negatively correlated with N, P, and K content in the D-leaves of *in vivo* plants during S2 (Table 4.20, Appendix L), means with the increases accumulation of Al concentration could reduce the N, P and K content in the D-leaves. However, there was no effect on P and Mg content in the D-leaves of *in vivo* plants supplemented with vermicompost which showed higher than recommended range on both samplings. In contrast, based on the analysis of both samplings (S1 and S2), *ex vitro* plants showed lower than recommended range in N, P, K, Ca and Fe content in the D-leaves for all treatments, but there was no significant correlation with Al content in the D-leaves (Appendix J). *Ex vitro* plant supplied with vermicompost showed the lowest accumulation of Al during S2, possibly related to the mechanism of defence to Al toxicity such as P elements can help to retard the entry of Al in the apoplast by formation of insoluble compounds such as $Al_4(PO_4)_3$ (Mota et al., 2016).

5.4 Bioactive Compounds in the Methanolic Fruit Extract of MD2 Pineapple

5.4.1 Phytochemical Screening

Phytochemicals are bioactive non-nutrient plant compounds found in fruits and other plant foods which naturally occurring substances that could act as antioxidant and anti-inflammatory, then reduced the risk of major chronic diseases (Crosby et al., 2008). They also could produce to provide protection against abiotic stresses such as UV-B irradiation, heat stress, low water potential or mineral deficiency (Jagathan & Crozier, 2008). In this study, methanolic extract of freeze dried MD2 pineapple harvested from both *in vivo* and *ex vitro* plants were screened for presence of secondary metabolites such as phenol, flavonoids, alkaloids and tannins.

Flavonoids are acclaimed for their antioxidant and antimicrobial activity. Flavonoids test was positive for both fruit extracts of *in vivo* and *ex vitro* plants for all treatments. Similar results were obtained in previous studies (Gunwantrao et al., 2016). Tannins were only found in fruits extracts from *in vivo* plants. Tannins are acclaimed for their free-radical scavenging activities, antiviral, antimicrobial, anti-inflammatory properties and also used in medicine as astringent (Agnes & Anusuya, 2016). The detection of tannins in fruits indicates their potential health benefits.

Alkaloid are a diverse group of secondary metabolites that protect plants against herbivores and pathogen which mostly found in herbal or medicinal plants with limited occurrence in fruits and vegetables (Jagathan & Crozier, 2008). Phenol and alkaloid were not detected in all samples tested. However, the findings of the current study do not support the previous studies. The differences of the results might be due to different extraction process or different solvent was used to extract the fruits. Phenol and alkaloids were detected in pineapple fruits extracted with ethanol and water (Agnes & Anusuya, 2016; Gunwantrao et al., 2016).

5.4.2 Detection and Quantification of Bioactive Compounds in the Fruit Extracts

The current study found that pineapple fruits (*in vivo*) grown with chemical fertilizer and vermicompost had twice the amounts of total chlorophylls, 20 and 10 times the amount of β-carotene than control fruits, respectively. However, application of chemical fertilizer significantly yielded fruits with higher amounts of phenolics, chlorophylls, carotenoid and β-carotene compared to fruits grown with vermicompost. In contrast, the fruits harvested from *ex vitro* pineapple plants supplied with vermicompost significantly contained more chlorophyll than other treatments ($P \leq 0.05$). It is somewhat surprising that no β-carotene was detected in fruits extract grown with vermicompost and control fruits. This is line with total carotenoid in methanolic fruits extract of *ex vitro* plant grown

with chemical fertilizer which contained significantly higher than other treatments. These observations were largely due to the difference in nutrient composition of the fertilizers, which affects soil nutrient availability and nutrient uptake in the pineapple crops, to be used for growth and during fruiting. Moreover, carotenoid contents are also influenced by several pre- and post-harvesting factors such as ripening time, production practice, growing locations as well as climatic conditions, including light and temperature (Saini et al., 2015).

Application of chemical fertilizer significantly increased the total N content in the D leaves of A. *comosus* var. MD2 plants might be due to nitrate accumulation in the plant leaves. Similar observation was reported by Wang and Li (2004), the utilization of nitrate N fertilizer to the plants will lead to accumulation of nitrates in the vegetables. However, the accumulation was more apparent when nitrate N fertilizer is used, compared to usage of ammonium N (Wang & Li, 2004). Nitrogen played a very important role for growth, reproduction and maintenance of photosynthetic capacity of plants (Crous et al., 2010; Mou et al., 2012). Chlorophyll concentrations is positively correlated with plant N status (Crosby et al., 2008). This is in line with the findings of the present study, where chlorophyll b content in the methanolic fruit extract was positively correlated with total N in the D-leaves of *in vivo* pineapple plants with r^2 value of 0.699 at $P \leq 0.05$ (Table 4.28). However, for *ex vitro* plants, the chlorophyll content in the fruit extract showed a weak negative correlation to total N.

K is essential for fruit production as it affects sugar concentration, regulates pH and fruit acidity and is involved in synthesis of phenolic compounds (Brunetto et al., 2015). In the current study, the methanolic fruit extract from *in vivo* plants supplied with chemical fertilizer showed high TPC content, in line with high K content in the D-leaves whereby strong correlation was obtained with r^2 value is 0.561. Besides that, it was also

observed that an increase in K content will significantly increase the β-carotene (r^2= 0.720), chlorophyll a (r^2= 0.761), chlorophyll b (r^2=0.778) and total chlorophyll (r^2= 0.776) contents in the pineapple fruits (*in vivo*) at $P \leq 0.05$ (Table 4.28). In previous studies conducted on tomato plants, it was found that K fertilization can affect carotenoid biosynthesis, specifically lycopene (Afzal et al., 2015; Almeselmani et al., 2009; Serio et al., 2007; Taber et al., 2008). It has been reported that the relationship between chlorophyll and carotenoid contents in plants are highly influenced by K status of the plant (Trudel & Ozbun, 1970, 1971). However, the ratio of chlorophyll to carotenoid content changes during ripening where chlorophyll content will decrease as carotenoids increase as fruit ripens (Trudel & Ozbun, 1970). This is in line with the findings obtained in the study for *in vivo* plants, where the pineapple fruits produced with chemical fertilizer had higher total chlorophyll content than fruits produced with vermicompost, thus yielding lower carotenoid content in the latter.

Phenolics are formed to protect plants from reactive oxygen species (ROS), photosynthetic stress and herbivory (Jagathan & Crozier, 2008). They also could produce to provide protection against abiotic stresses such as UV-B irradiation, heat stress, low water potential or mineral deficiency (Jagathan & Crozier, 2008). In this study (Table 4.27), methanolic fruit extract obtained from *ex vitro* plant supplied with vermicompost significantly contained the lowest total phenolics, 6.055 mg gallic acid (GAE) per g dry extract, followed by chemical fertilizer (6.083 mg GAE/g dry extract) and control plants (8.212 mg GAE/g dry extract). Similar result was found on previous study whereby total phenolic content in methanolic extracts of C. *nutans* leaves was significantly higher in control plant (unfertilized) compared to plant supplied with chemical fertilizer and plant amended with vermicompost (Yusof et al., 2018). Moreover, it could possibly due to Al stress whereby control plants contained highest Al accumulation in the plant (Table 4.19).

According to Meriño-Gergichevich et al. (2010), Al-toxicity triggers an increase in ROS which may increase or inhibit antioxidant activities to scavenge ROS.

5.4.3 Antioxidants Capacities of Fruit Extracts

Fruits contain many compounds that show antioxidant functionalities. The role of antioxidants is their interaction with oxidative free radicals. Several methods have been developed to estimate the total antioxidant activity of different plant materials. Usually, these methods measure the ability of antioxidants to scavenge specific radicals, to inhibit lipid peroxidation or to chelate metal ions (Martínez et al., 2012). For more complete picture of the antioxidant capacity of pineapple fruit extracts, more than one method should be used (Almeida et al., 2011; Martínez et al., 2012). In the present of study, the free radical scavenging activity of the pineapple fruit extracts was tested through DPPH, ABTS and FRAP assays which are the most widely used.

Based on the DPPH assay, fruits from *in vivo* plants supplemented with chemical fertilizer and vermicompost showed similar IC_{50} values, with significantly stronger DPPH radicals scavenging activities than control plants (Table 4.30). In contrast, for *ex vitro* plants, the free radical scavenging potential is stronger in fruit extracts of control plants (Table 4.31). It has been found that total carotenoid, chlorophyll a, and chlorophyll b and β-carotene showed a strong negative correlation with DPPH radicals (Table 4.32). This study supports evidence from previous observations where TPC present in the pineapple extracts are not the main contributor to the radical scavenging activity of the extracts (Yuris & Siow, 2014). However, there were studies showed correlation between DPPH assay and TPC in pineapple fruit extracts (Almeida et al., 2011; Alothman et al., 2009; Haripyaree et al., 2010; Lu et al., 2014; Oliveira et al., 2009).

The ABTS assay is based on the ability of antioxidants to scavenge the long-life radical cation ABTS and usually used for testing the preliminary radical scavenging activity of a compound or plant extract. The percentage of inhibition pattern of the ABTS radicals was similar to DPPH, whereby fruits from *in vivo* plants treated with chemical fertilizer showed the least IC_{50} values, followed by vermicompost and control (Table 4.30). A lower value of IC_{50} indicates a higher antioxidant activity. However, there was no significant different for *ex vitro* plants. Moreover, ABTS assay showed significant negative correlation with TPC, chlorophyll a, chlorophyll b and β-carotene at $P \leq 0.05$.

The FRAP assay estimates the antioxidant power, which is the reducing ability of the substances involved in the transfer of electron in the reaction (Marikkar et al., 2016). According to Table 4.30, fruit extracts from *in vivo* plants supplemented with chemical fertilizer and vermicompost treatments not significantly difference in their reducing power ($P \leq 0.05$). The lowest reducing power among all treatments was exhibited by fruit extracts from vermicompost treatment, with 0.276 mg of $FeSO_4$ equivalent/ g of dried extract. Similarly, fruit extracts from *ex vitro* plants supplemented with vermicompost comparable to chemical fertilizer but their reducing power lower compared to control plants. A strong significant correlation was observed between FRAP and TPC with r^2= 0.539 at $P \leq 0.05$. In contrast, previous research found a weak correlation when corrected TPC of fruit extracts of Josephine, Morris and Sarawak pineapples was plotted against FRAP (r^2= 0.158) (Yuris & Siow, 2014). There was also a significant correlation with total carotenoid, chlorophyll a, chlorophyll b and total chlorophyll (Table 4.32). Overall, all treatments exhibited weak antioxidant capacities compared to ascorbic acid (positive control) for DPPH, ABTS and FRAP assays.

5.5 Cost Analysis

In this study, a rough cost analysis was conducted to determine the effectiveness of using vermicompost to replace chemical fertilizer in the field. It was observed that while the cost of vermicompost (per plant per year) was higher, the labour cost for field maintenance was significantly lower than that needed for plants supplemented with chemical fertilizer. This was due to its characteristic as a slow release fertilizer, which therefore does not require frequent incorporation into the soil. Based on these two criteria, the estimated profit obtained from fruits produced with vermicompost was observed to be slightly higher than that obtained using chemical fertilizer. However, the profit is estimated to significantly rise if the planting density of the seedlings and field size are increased. Also, other parameters of field maintenance such as the cost of irrigation and pest control could also play a major role in determining total profit (especially in big plantations). The cost of irrigation and pest control in vermicompost-treated areas are estimated to be significantly lower (data not shown) due to the excellent water-holding capacity of the vermicompost and its pest-repelling benefits (Adhikary, 2012; Arancon & Edward, 2005). Various studies have reported that vermicompost increases the 'biological resistance' in plants (due to Actinomycetes) and protect them against pest and diseases either by repelling or by suppressing them (Sinha, 2009).

CHAPTER 6: CONCLUSION

6.1 Conclusion

The preceding results showed that the utilization of vermicompost at the rate 10 t ha^{-1} with two-split application throughout the planting period produced comparable results (in terms of the growth of *in vivo* and *ex vitro* MD2 pineapple plants) with that obtained using conventional practices through regular supplementation with chemical fertilizer but lower SPAD reading. In comparison to the control, the application of vermicompost produced fruits that were significantly larger in size with smaller crowns for both types of plants. Moreover, *ex vitro* plant supplemented with vermicompost produced fruits contained higher TSS (12.6 °Brix), TA (0.39 g kg^{-1}), total solids (20.841% wt/wt), ascorbic acid content (44.577 µg AA/g FW fruit), total chlorophyll (4.071 µg/g) and stronger DPPH radicals scavenging activities (8.250 mg/ml) compared to chemical fertilizer.

The results of soil analysis showed that the application of vermicompost had significantly increased the soil pH and was able to retain the nutrients content in the soils. Moreover, the soil contains readily-available nutrients, which increase the plant nutrient uptake. It also maintains the soil pH and has ability to hold water in the soil, as indicated by the increase in the stomatal density with 96.62 stomata per mm^2 and 76.49 stomata per mm^2 for *in vivo* and *ex vitro* plants, respectively. However, some of the nutrient uptake by the plants was lower than the concentration required for pineapple growth, similar to when chemical fertilizer was used. Thus, it could be deduced that both types of fertilizers (chemical fertilizer and vermicompost) could not supply the ideal concentration of nutrients required by pineapple plants, when they are used as the sole nutrient provider for plants grown on sandy soil. Therefore, further research needs to be carried out to identify the best ratio of the combination of vermicompost and chemical fertilizer.

With respect to biochemical properties, this study has shown that the application of vermicompost to pineapple plants produced fruits of good quality with high content of bioactive compounds, although slightly lower than in fruits produced with chemical fertilizer. Similar trends were observed in terms of antioxidant potential of the fruit extracts. These also imply that vermicompost cannot completely replace chemical fertilizer for production of fruits with high content of phytoconstituents but could be used as additional supplement to reduce environmental pollution and ensure agricultural sustainability.

On the other hands, the most obvious finding emerged from this study was that the application of vermicompost to pineapple plants, both *in vivo* and *ex vitro* contained the lowest total phenolic content, compared to in fruits produced with chemical fertilizer and control. Phenolic compounds tend to increase in plant tissues infested by piercing-sucking insects as biochemical defence against herbivorous arthropods such as mealy bugs (Golan et al., 2017). This may also be an evidence of vermicompost potential to protect the pineapple plants against pest's attack.

6.2 Further Study

The present study lays the groundwork for future research on implementation of vermicompost in pineapple cultivation. Further research needs to be carried out in order to validate the best rate of application of vermicompost on MD2 pineapple plants, also the time for its supplementation to the soils. The low uptake of N from vermicompost might be due to majority of nutrients contain in vermicompost from organic origin whereby not readily available for plants. Thus, they need to be mineralized in order to become available for plants by the action of microorganisms (Eckhardt et al., 2016). Therefore, it is essential to know the efficiency index of the N from vermicompost, in order to establish agronomic recommendations concerning the correct dosages and period

of adhibition. In spite of its limitations, the study certainly adds to our understanding of the possibility of usage of vermicompost in pineapple plantation.

REFERENCES

Adhikary, S. (2012). Vermicompost, the story of organic gold: A review. *Agriculture Sciences, 3*(7), 905-917.

Afzal, I., Hussain, B., Basra, S. M. A., Ullah, S. H., Shakeel, Q., & Kamran, M. (2015). Foliar application of potassium improves fruit quality and yield of tomato plants. *Acta Scientiarum Polonorum Hortorum Cultus, 14*(1), 3-13.

Agarwal, S., Sinha, R. K., & Sharma, J. (2010). Vermiculture for sustainable hoticulture agronomic impact studies of earthworms, cow dung compost and vermicompost vis-a-vis chemical fertilizers on growth and yield of lady's finger (*Abelmoscus esculentus*). *International Journal Global Environmental Issues, 10*(3/4), 366-377.

Agnes, J. X., & Anusuya, A. (2016). Phytochemical screening and *in vitro* antioxidant activity of *Ananas comosus*. *International Journal of Research in Pharmacology & Pharmacotherapeutics, 5*(2), 162-169.

Aksakal, E. L., Sari, S., & Angin, I. (2015). Effects of vermicompost application on soil aggregation and certain physical properties. *Land Degradation & Developement*, 1-13.

Alaboz, P., Isildar, A. A., Mujdeci, M., & Senol, H. (2017). Effects of different vermicompost and soil moisture levels on pepper (*Capsicum annuum*) grown and some soil properties. *Yuzuncu Yil University Journal of Agricultural Sciences, 27*(1), 30-36.

Alidadi, H., Saffari, A. R., Ketabi, D., Peiravi, R., & Hosseinzadeh, A. (2014). Comparison of vermicompost and cow manure efficiency on the growth and yield of tomato plant. *Health Scope, 3*(4), 1-5.

Almeida, M. M. B., de Sousa, P. H. M., Arriaga, Â. M. C., do Prado, G. M., de Carvalho Magalhães, C. E., Maia, G. A. & de Lemos, T. L. G. (2011). Bioactive compounds and antioxidant activity of fresh exotic fruits from northeastern Brazil. *Food Research International, 44*(7), 2155-2159.

Almeselmani, M., Pant, R. C., & Singh, B. (2009). Potassium level and physiological response and fruit quality in hydroponically grown tomato. *International Journal of Vegetable Science, 16*(1), 85-99.

Alothman, M., Bhat, R., & Karim, A. A. (2009). Antioxidant capacity and phenolic content of selected tropical fruits from Malaysia, extracted with different solvents. *Food Chemistry, 115*(3), 785-788.

Altemimi, A., Lakhssassi, N., Baharlouei, A., Watson, D. G., & Lightfoot, D. A. (2017). Phytochemicals: Extraction, isolation, and identification of bioactive compounds from plant extracts. *Plants, 6*(42), 1-23.

Amin, M. N., Rahman, M. M., Rahman, K. W., Ahmed, R., Hossain, M. S., & Ahmed, M. B. (2005). Large scale plant regeneration *in vitro* from leaf derived callus cultures of pineapple [*Ananas comosus* (L.) Merr. cv. Giant Kew]. *International Journal of Botany, 1*(2), 128-132.

Aminifard, M. H., & Bayat, H. (2016). Effect of vermicompost on fruit yield and quality of bell pepper. *International Journal of Horticultural Science and Technology, 3*(2), 221-229.

Amouei, A. I., Yousefi, Z., & Khosravi, T. (2017). Comparison of vermicompost characteristics produced from sewage sludge of wood and paper industry and household solid wastes. *Journal of Environmental Health Science & Engineering, 15*(5), 1-6.

Angelova, V. R., Akova, V. I., Artinova, N. S., & Ivanov, K. I. (2013). The effect of organic amendments on soil chemical characteristics. *Bulgarian Journal of Agricultural Science, 19*(5), 958-971.

AOAC. (2007). *Official methods of analysis of AOAC International* (18th ed. rev. 2). Gaithersburg, MD: AOAC International.

Appiah, F., Kumah, P., & Oppong, D. (2012). Predicting the consumer acceptability of dried MD2 and *Smooth cayenne* pineapple pulps from chemical composition. *Journal of Food Research, 1*(2), 210-216.

Arancon, N. Q., & Edward, C. A. (2005). *Effect of vermicompost on plant growth.* Columbus, OH: Soil Ecology Laboratory, The Ohio State University.

Atiyeh, R. M., Subler, S., Edwards, C. A., Bachman, G., Metzger, J. D., & Shuster, W. (2000). Effects of vermicomposts and composts on plant growth in horticultural container media and soil. *Pedobiologia, 44*, 579-590.

Ayyobi, H., Olfati, J.-A., & Peyvast, G.-A. (2014). The effects of cow manure vermicompost and municipal solid waste compost on peppermint (*Mentha piperita* L.) in torbat-e-jam and rasht regions of Iran. *International Journal of Recycling of Organic Waste in Agriculture, 3*(4), 147-153.

Azarmi, R., Ziveh, P. S., & Satari, M. R. (2008). Effect of vermicompost on growth, yield and nutrition status of tomato (*Lycopersicum esculentum*). *Pakistan Journal of Biological Sciences, 11*(14), 1797-1802.

Baldotto, L. E. B., Baldotto, M. A., Canellas, L. P., Bressan-Smith, R., & Olivares, F. L. (2010). Growth promotion of pineapple 'Victoria' by humic acid and *Burkholderia* spp. during acclimatization. *Revista Brasileira de Ciencia do Solo, 34*, 1593-1600.

Baligar, V. C., Fageria, N. K., & He, Z. L. (2001). Nutrient use efficiency in plants. *Communications in Soil Science and Plant Analysis, 32*(7-8), 921-950.

Banful, B., Adjei, P. Y., & Idun, I. A. (2011). The influence of two pre and post-planting fungicides on the growth and fruiting of MD2 pineapple (*Ananas comosus* L. (Merr). *Agriculture and Biology Journal of North America, 2*(6), 876-879.

Barker, A. V., & Pilbeam, D. J. (2007). *Handbook of plant nutrition*. Boca Raton, FL: Taylor & Francis.

Basso, C., & Suzuki, A. (2001). Solos e nutrição. In EPAGRI (Ed.), *Nashi: A pêra-japonesa* (pp. 139-160). Florianópolis, BR: Epagri/JICA.

Beavers, W. B., Sams, C. E., Conway, W. S., & Brown, G. A. (1994). Calcium source affects calcium content, firmness, and degree of injury of apples during storage. *HortScience, 29*(12), 1520-1523.

Beck-Friis, B., Smårs, S., Jönsson, H., Eklind, Y., & Kirchmann, H. (2003). Composting of source-separated household organics at different oxygen levels: Gaining an understanding of the emission dynamics. *Compost Science & Utilization, 11*(1), 41-50.

Benzie, F. F., & Strain, J. J. (1999). Ferric reducing/ antioxidant power assay: Direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. *Methods in Enzymology, 299*, 15-23.

Bernhoft, A. (2010). A brief review on bioactive compounds in plants. *Proceedings of the The Norwegian Academy of Science and Letters*, 11-17.

Bolan, N. S., & Hedley, M. J. (2003). Role of carbon, nitrogen, and sulfur cycles in soil acidification. In Z. Rengel (Ed.), *Handbook of soil acidity* (pp. 29-56). New York, NY: Marcel Dekker.

Bradley, J. R. L. (2010). Moisture and total solids analysis. In S. S. Nielsen (Ed.), *Food analysis* (4th ed., pp. 190-191). Berlin, DE: Springer Science+Business Media.

Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *Lebensmittel Wissenschaft und Technologia, 28*, 25-30.

Brar, B. S., Singh, J., Singh, G., & Kaur, G. (2015). Effects of long term application of inorganic and organic fertilizers on soil organic carbon and physical properties in maize-wheat rotation. *Agronomy, 5*, 220-238.

Bremmer, J. M., & Mulvaney, C. S. (1982). Nutrient total. In A. L. Page, R. H. Miller & D. R.. Keeney (Eds.), *Methods of soil analysis* (pp. 595-624). Madison, Wisconsin: American Society of Agronomy.

Britton, G., Liaaen-Jensenm, S., & Pfander, H. (1995). *Carotenoids* (Vol. 1). Basel, CH: Birkhauser Verlag.

Brunetto, G., Melo, G. W. B. D., Toseli, M., Quartieri, M., & Tangliavini, M. (2015). The role of mineral nutrition on yield and fruit quality in grapvine, pear and apple *Revista Brasileira de Fruticultura, 37*(4), 1089-1104.

Chan, Y. K., & Lam, P. F. (2002). Field performance of tissue-cultured 'Josapine' pineapple. *Journal of Tropical Agriculture and Food Science, 30*(1), 1-6.

Chaudhuri, P. S., Paul, T. K., Dey, A., Datta, M., & Dey, S. K. (2016). Effects of rubber leaf litter vermicompost on earthworm population and yield of pineapple (*Ananas comosus*) in West Tripura, India. *International Journal of Recycling of Organic Waste in Agriculture, 5*, 93-103.

Chen, J.-H., & Lin, Y.-H. (2010). Effect of aluminum on variations in the proteins in pineapple roots. *Soil Science and Plant Nutrition, 56*, 438-444.

Chen, M., & Ma, L. Q. (2001). Comparison of three aqua regia digestion methods for twenty Florida soils. *Soil Science Society of America Journal, 65*(2), 491-499.

Chitwood, D. H., Headland, L. R., Filiault, D. L., Kumar, R., Go´mez, J. M. J. n., Schrager, A. V., . . . Maloof, J. N. (2012). Native environment modulates leaf size and response to simulated foliar shade across wild tomato species. *PloS ONE, 7*(1), e29570.

Crosby, K., Jifon, J., & Leskovar, D. (2008). Agronomy and the nutritional quality of vegetables. In F. A. Tomas-Barberan & M. I. Gil (Eds.), *Improving the health-promoting properties of fruit and vegetable products* (pp. 392-407). Cambridge, England: Woodhead Publishing Limited.

Crous, K. Y., Reich, P. B., Hunter, M. D., & Ellsworth, D. S. (2010). Maintenance of leaf N controls the photosynthetic CO_2 response of grassland species exposed to 9 years of free-air CO_2 enrichment. *Global Change Biology, 16*(7), 2076-2088.

Cunha, G. A. P. D. (2005). Applied aspects of pineapple flowering. *Bragantia, 64*(4), 499-516.

Custodio, R. A., Neto, S. E. A., Junior, P. C. P. F., Neto, R. d. C. A., & Silva, I. F. (2016). Morpho-anatomy of leaves and yield of pineapple plant in intercropping with cassava. *Bioscience Journal, 32*(4), 839-848.

d'Eeckenbrugge, G. C., & Leal, F. (2003). Morphology, anatomy and taxonomy. In D. P. Bartholomew, R. E. Paull & K. G. Rohrbach (Eds.), *The pineapple: Botany, production and uses* (pp. 13-32). Wallingford, UK: CAB International.

d'Eeckenbrugge, G. C., Sanewski, G. M., Smith, M. K., Duval, M.-F., & Leal, F. (2011). Ananas In C. Kole (Ed.), *Wild crop relatives: Genomic and breeding resources, tropical and subtropical fruits* (pp. 21-22). Verlag Berlin Heidelberg, NY: Springer.

Dadzie, B. K., & Orchard, J. E. (1997). *Routine post-harvest screening of banana/ plantain hybrids: Criteria and methods*. Rome, IT: International Plant Genetic Resources Institute.

Danso, K. E., Ayeh, K. O., Oduro, V., Amiteye, S., & Amoatey, H. M. (2008). Effect of 6-benzylaminopurine and a-naphthalene acetic acid on *in vitro* production of MD2 pineapple planting materials. *World Applied Sciences Journal, 3*(4), 614-619.

Darnaudery, M., Fournier, P., & Lechaudel, M. (2016). Low-input pineapple crops with high quality fruit: Promising impacts of locally integrated and organic fertilisation compared to chemical fertilisers. *Experimental Agriculture, 54*(2), 286-302.

Delgado, A., & Gomez, J. A. (2016). The soil: Physical, chemical and biological properties. In F. J. Villalobos & E. Fereres (Eds.), *Principles of agronomy for sustainable* (pp. 15-26). Cham, CH: Springer.

Department of Health and Ageing (2008). *The biology of Ananas comosus var. comosus (pineapple)*. Australia: Office of the Gene Technology Regulator.

Domínguez, J., Edwards, C. A., & Webster, M. (2000). Vermicomposting of sewage sludge: Effect of bulking materials on the growth and reproduction of the earthworm *Eisenia andrei*. *Pedobiologia, 44*, 24-32.

Drake, P. L., Froend, R. H., & Franks, P. J. (2013). Smaller, faster stomata: Scaling of stomatal size, rate of response, and stomatal conductance. *Journal of Experimental Botany, 64*(2), 495-505.

Eckhardt, D. P., Redin, M., Jacques, R. J. S., Lorensini, F., Santos, M. L., Weiler, D. A., & Antoniolli, Z. I. (2016). Mineralization and efficiency index of nitrogen in cattle manure fertilizers on the soil. *Ciência Rural, Santa Maria, 46*(3), 472-477.

El-Hassan, S. A., Elwanis, M. A., & El-Shinawy, M. Z. (2017). Application of compost and vermicompost as substitutes for mineral fertilizers to produce green beans. *Egyptian Journal of Horticulture, 44*(2), 155-163.

Eshetu, T., Tefera, W., & Kebede, T. (2007). Effect of weed management on pineapple growth and yield. *Ethiopian Journal of Weed Management, 1*(1), 29-40.

FAO. (2017). Food outlook: Biannual report on global food markets. In S. Altendorf (Ed.), *Special feature: Global prospects for major tropical fruits* (Vol. November 2017, pp. 69-81). Rome, IT: Food and Agriculture Organization of the United Nations.

Fernandez, G., & Pomilio, A. B. (2003). Optimized growth conditions and determination of the catalytic type of the peptidase complex from a novel callus culture of pineapple (*Ananas comosus*). *Molecular Medicinal Chemistry, 1*, 39-49.

Gelderman, R. H., & Mallarino, A. P. (2012). Soil sample preparation. In J. R. Brown (Ed.), *Recommended chemical soil test procedures for the North Central Region*. Columbia, MO: Missouri Agricultural Experiment Station SB 1001.

Ghahfarokhi, M. G., Mansurifar, S., Taqhizadeh-Mehrjardi, R., Saeidi, M., Jamshidi, A. M., & Ghasemi, E. (2015). Effects of drought stress and rewatering on antioxidant systems and relative water content in different growth stages of maize (*Zea mays* L.) hybrids. *Archives of Agronomy and Soil Science, 61*(4), 493-506.

Gilmartin, A. J., & Brown, G. K. (1987). Bromeliales, related monocots, and resolution of relationships among Bromeliaceae subfamilies. *Systematic Botany, 12*, 493-500.

Golan, K., Sempruch, C., Go´rska-Drabik, E., Czerniewicz, P., Łagowska, B., Kot, I., . . . Leszczyn´ski, B. (2017). Accumulation of amino acids and phenolic compounds in biochemical plant responses to feeding of two different herbivorous arthropod pests. *Arthropod-Plant Interactions, 11*, 675-682.

Gopinath, K. A., Saha, S., Mina, B. L., Pande, H., Kundu, S., & Gupta, H. S. (2008). Influence of organic amendments on growth, yield and quality of wheat and on soil properties during transition to organic production. *Nutrient Cycling in Agroecosystems, 82*(1), 51-60.

Gunwantrao, B. B., Bhausaheb, S. K., Ramrao, B. S., & Subhash, K. S. (2016). Antimicrobial activity and phytochemical analysis of orange (*Citrus aurantium* L.) and pineapple (*Ananas comosus* (L.) Merr.) peel extract. *Annals of Phytomedicine, 5*(2), 156-160.

Gupta, N., Gaurav, S. S., & Kumar, A. (2013). Molecular basis of aluminum toxicity in plants: A review. *American Journal of Plant Sciences, 4*, 21-37.

Gupta, N., Jawandha, S. K., & Gill, P. S. (2011). Effect of calcium on cold storage and post-storage quality of peach. *Journal of Food Science and Technology, 48*(2), 225-229.

Hamid, N. S., Bukhori, M. F. M., & Jalil, M. (2013). Direct and indirect plant regenerations of pineapple *var*. MD2 (*Ananas comosus* L.). *Malaysian Applied Biology, 42*(1), 61-66.

Han, S. H., An, J. Y., Hwang, J., Kim, S. B., & Park, B. B. (2016). The effects of organic manure and chemical fertilizer on the growth and nutrient concentrations of yellow poplar (*Liriodendron tulipifera* Lin.) in a nursery system. *Forest Science and Technology, 12*(3), 137-143.

Haripyaree, A., Guneshwor, K., & Damayanti, M. (2010). Evaluation of antioxidant properties of phenolic extracted from *Ananas comosus* L. *Notulae Scientia Biologicae, 2*(2), 68-71.

Higuchi, M. (2014). Antioxidant properties of wheat bran against oxidative stress. In R. R. Watson, V. R. Preedy & S. Zibadi (Eds.), *Wheat and rice in disease prevention and health* (pp. 181-199). San Diego, CA: Academic Press.

Ho, C.-T. (1992). Phenolic compounds in food. *Proceedings of the Phenolic Compounds in Food and Their Effects on Health I*, 2-7.

Jagathan, I. B., & Crozier, A. (2008). Overview of health-promoting compounds in fruit and vegetables. In F. A. Tomas-Barberan & M. I. Gil (Eds.), *Improving the health-promoting properties of fruit and vegetable products* (pp. 3-10). Cambridge, England: Woodhead Publishing Limited.

Joy, P. P., Anjana, R., & Soumya, K. K. (2013). *Pests of pineapple and their management*. Pineapple Research Station, IN: Kerala Agricultural University.

Joy, P. P., & Rashida Rajuva, T. A. (2016). *Harvesting and post-harvest handling of pineapple*. Pineapple Research Station, IN: Kerala Agricultural University.

Kalaiselvi, M., Ravikumar, G., Gomathi, D., & Uma, C. (2012). *In vitro* free radical scavenging activity of *Ananas comosus* (L.) Merill peel. *International Journal of Pharmacy and Pharmaceutical Sciences, 4*(2), 604-609.

Kashem, M. A., Sarker, A., Hossain, I., & Islam, M. S. (2015). Comparison of the effect of vermicompost and inorganic fertilizers on vegetative growth and fruit production of tomato (*Solanum lycopersicum* L.). *Open Journal of Soil Science, 5*, 53-58.

Khaliq, S. J. A., Al-Busaidi, A., Ahmed, M., Al-Wardy, M., Agrama, H., & Choudri, B. S. (2017). The effect of municipal sewage sludge on the quality of soil and crops. *International Journal of Recycling of Organic Waste in Agriculture, 6*(4), 289-299.

Kmeťová, M., & Kováčik, P. (2014). The impact of vermicompost application on the yield parameters of maize (*Zea mays* L.) observed in selected phenological growth stages (BBCH-SCALE). *Acta Fytotechnica et Zootechnica, 17*(4), 100-108.

Kumar, D. S., Kumar, P. S., Kumar, V. U., & Anbuganapathi, G. (2014). Influence of biofertilizer mixed flower waste vermicompost on the growth, yield and quality of groundnut (*Arachis hypogea*). *World Applied Science Journal, 31*(10), 1715-1721.

Lamma, O. A., & Moftah, M. A. (2016). Effect of vermicompost on antioxidant levels in *Andrographis paniculata*. *International Journal of Applied and Pure Science and Agriculture, 2*(3), 160-164.

Lawlor, D. W. (2002). Limitation to photosynthesis in water-stressed leaves: stomata vs. metabolism and the role of ATP. *Annals of Botany, 89*(7), 871-885.

Lazar, M., Mitre, V., Tripon, F. A., & Badiu, D. (2014). The potential use of vermicompost in orchards. *Buletin of University of Agricultural Sciences and Veterinary Medicine Horticulture Cluj-Napoca. Horticulture, 71*(1), 56-58.

Lazcano, C., Arnold, J., Tato, A., Zaller, J. G., & Dominguez, J. (2009). Compost and vermicompost as nursery pot components: effects on tomato plant growth and morphology. *Spanish Journal of Agricultural Research, 7*(4), 944-951.

LECO (2001). *Nitrogen analyzer FP-528 instruction manual*. St. Joseph, MI, USA: LECO Corporation.

Lee, K. Y. (2010, June 20). Pineapple passion, *The Star Malaysia,* p. M7.

Li, Y., Liu, Q., Liu, F., Zhu, P., Zhang, L., Zhou, X., . . . Cheng, Y. (2016). Effects of different ratios of sewage sludge and cattle manure on growth and propagation of *Eisenia fetida*. *PLoS ONE, 11*(6), 1-13.

Lichtenthaler, H. K., & Buschmann, C. (2001). Chlorophylls and carotenoids: Measurement and characterization by uv-vis spectroscopy. In R. E. Wrolstad, T. E. Acree, H. An, E. A. Decker, M. H. Penner, D. S. Reid, . . . P. Sporns (Eds.), *Current protocol in food analytical chemistry*. New York, NY: John Wiley & Sons.

Liew, C. Y., & Lau, C. Y. (2012). Determination of quality parameters in Cavendish banana during ripening by NIR spectroscopy. *International Food Research Journal, 19*(2), 751-758.

Lima, R. L. S., Severino, L. S., Ferreira, G. B., Azevedo, C. A. V. de, Sofiatti, V., & Arriel, N. H. C. (2014). Soil exchangeable aluminum influencing the growth and leaf tissue macronutrients content of castor plants. *Resvista Caatinga, 27*(4), 10-15.

Lin, Y.-H. (2010). Effects of aluminum on root growth and absorption of nutrients by two pineapple cultivars [*Ananas comosus* (L.) Merr.]. *African Journal of Biotechnology, 9*(26), 4034-4041.

Lin, Y.-H., & Chen, J.-H. (2011). Effects of aluminum on nutrient uptake in different parts of four pineapple cultivars. *Journal of Agricultural Research, 6*(6), 1438-1446.

Lin, Y. H., & Chen, J. H. (2013). Aluminium resistance and cell-wall characteristics of pineapple root apices. *Journal Plant Nutrition Soil Science, 176*, 795-800.

Liu, Z. A., Yang, J. P., & Yang, Z. C. (2012). Using a chlorophyll meter to estimate tea leaf chlorophyll and nitrogen contents. *Journal of Soil Science and Plant Nutrition, 12*(2), 339-348.

Lobo, M. G., & Siddiq, M. (2017). Overview of pineapple production, postharvest physiology, processing and nutrition. In M. G. Lobo & R. E. Paull (Eds.), *Handbook of pineapple technology: Production, postharvest science, processing and nutrition* (pp. 1-15). Hoboken, New Jersey: John Wiley & Sons.

Lobo, V., Patil, A., Phatak, A., & Chandra, N. (2010). Free radicals, antioxidants and functional foods: Impact on human health. *Pharmacognosy Reviews, 4*(8), 118-126.

Lu, X.-H., Sun, D.-Q., Wu, Q.-S., Liu, S.-H., & Sun, G.-M. (2014). Physico-chemical properties, antioxidant activity and mineral contents of pineapple genotypes grown in China. *Molecules, 19*, 8518-8532.

Ludibeth, S.-M., Marina, I.-E., & Vicenta, E. M. (2012). Vermicomposting of sewage sludge: Earthworm population and agronomic advantages. *Compost Science & Utilization, 20*(1), 11-17.

Ludwiczuk, A., Skalicka-Woźniak, K., & Georgiev, M. I. (2017). Terpenoids. In S. Badal & R. Delgoda (Eds.), *Pharmacognosy* (pp. 233-266). Boston, USA: Academic Press.

Maeda, A. S., Buzetti, S., Boliani, A. C., Benett, C. G. S., Filho, M. C. M. T., & Andreotti, M. (2011). Foliar fertilization on pineapple quality and yield. *Pesquisa Agropecuária Tropical, 41*(2), 248-253.

Majic, A., Poljak, M., Sabljo, A., Knezovic, Z., & Horvat, T. (2008). Effeciency of use chlorophyll meter and cardy-ion meter in potato nitrogen nutrition supply. *Cereal Research Communications, 36*, 1431-1434.

Majlessi, M., Eslami, A., Saleh, H. N., Mirshafieean, S., & Babaii, S. (2012). Vermicomposting of food waste: Assesing the stability and maturity. *Iranian Journal of Environmental Health Science & Engineering, 9*(25), 1-6.

Malezieux, E., & Bartholomew, D. P. (2003). Plant nutrition. In D. P. Bartholomew, R. E. Paull & K. G. Rohrbach (Eds.), *The pineapple: Botany, production, and uses* (pp. 143-165). Wallingford, UK: CAB International.

Marikkar, J., Tan, S., Salleh, A., Azrina, A., & Shukri, M. (2016). Evaluation of banana (*Musa* sp.) flowers of selected varieties for their antioxidative and anti-hyperglycemic potentials. *International Food Research Journal, 23*(5), 1988-1995.

Martínez, R., Torres, P., Meneses, M. A., Figueroa, J. G., Pérez-Álvarez, J. A., & Viuda-Martos, M. (2012). Chemical, technological and *in vitro* antioxidant properties of mango, guava, pineapple and passion fruit dietary fibre concentrate. *Food Chemistry, 135*(3), 1520-1526.

Medina, J. D. L. C., & Garcia, H. S. (2005). *Pineapple: Post-harvest operations*. In D. Mejia (Ed.), *Post-harvest compedium*. Instituto Tecnologico de Veracruz: Food and Agriculture Organization of the United Nations.

Mengel, K., Kirkby, E. A., Kosegarten, H., & Appel, T. (2001). *Principles of plant nutrition* (5th ed.). Dordrecht, NL: Springer Science+Business Media.

Meriño-Gergichevich, C., Alberdi, M., Ivanov, A. G., & Reyes-Díaz, M. (2010). Al^{3+}-Ca^{2+} interaction in plants growing in acid soils: Al-phytotoxicity response to calcerous amendments. *Journal of Soil Science and Plant Nutrition, 10*(3), 217-243.

Miller, N. J., Diplock, A. T., Rice-Evans, C., Davies, M. J., Gopinathan, V., & Milner, A. (1993). A novel method for measuring antioxidant capacity and its application to monitoring the antioxidant status in premature neonates. *Clinical Science, 84*(4), 407-422.

Miller, R. O. (1998). High-temperature oxidation: Dry ashing. In Y. P. Karla (Ed.), *Handbook of references methods for plant analysis* (pp. 53-56). Boca Raton, FL: CRC Press.

Milosevic, T., & Milosevic, N. (2009). The effect of zeolite, organic and inorganic fertilizers on soil chemical properties, growth and biomass yield of apple trees. *Plant, Soil and Environment, 55*(12), 528-535.

Mirza, A. A., Senthilkumar, S., & Singh, S. K. (2016). A review on trends and approaches in post-harvest handling of pineapple. *Journal of Chemical and Pharmaceutical Sciences, 9*(4), 2885-2889.

Mitra, G. (2017). Essential plant nutrients and recent concepts about their uptake. In M. Naeem, A. A. Ansari & S. S. Gill (Eds.), *Essential plant nutrients: Uptake, use efficiency, and management* (pp. 3-35). Cham, CH: Springer.

MMD. (2016). *Annual report*. Kuala Lumpur, MY: Malaysian Meteorological Department.

Mota, M. F. C., Pegoraro, R. F., Batista, P. S. C., Pinto, V. O., Maia, V. M., & Silva, D. F. (2016). Macronutrients accumulation and growth of pineapple cultivars submitted to aluminium stress. *Revista Brasileira de Engenharia Agrícola e Ambiental, 20*(11), 978-983.

Mou, Z.-m., Yan, N., Li, S.-y., & Hu, H. (2012). Nitrogen requirements for vegetative growth, flowering, seed production, and ramet growth of *Paphiopedilum armeniacum* (orchid). *HortScience, 47*(5), 585-588.

MPIB. (2015a). *Laporan tahunan status industri* (pp. 25-35). Johor, MY: Malaysian Pineapple Industry Board.

MPIB. (2015b, March 16). *Kultivar nanas*. Retrieved from http://www.mpib.gov.my/kultivar/

MPIB. (2015c). *Pengurusan tanaman nenas pelbagai kultivar*. Johor, MY: Malaysian Pineapple Industry Board.

Nada, W. M., van Rensburg, L., Claassens, S., & Blumenstein, O. (2011). Effect of vermicompost on soil and plant properties of coal spoil in the lusatian region (Eastern Germany). *Communications in Soil Science and Plant Analysis, 42*(16), 1945-1957.

Netto, A. T., Campostrini, E., Oliveira, J. G. A., & Bressan-Smith, R. E. (2005). Photosynthetic pigments, nitrogen, chlorophyll a fluorescence and SPAD-502 readings in coffee leaves. *Scientia Horticulturae, 104*, 199-209.

Nieuwenhuize, J., Poley-Vos, C. H., Akker., A. H. v. d., & Delft, W. v. (1991). Comparison of microwave and conventional extraction techniques for the deteermination of metals in soils, sediment and sludge samples by atomic spectrometry. *Analyst, 116*, 347-351.

Nieves, J. W. (2013). Alternative therapy through nutrients and nutraceuticals. In R. Marcus, D. Feldman, D. W. Dempster, M. Luckey & J. A. Cauley (Eds.), *Osteoporosis* (4th ed., pp. 1739-1749). San Diego, US: Academic Press.

Njoku, P. C., Ayuk, A. A., & Okoye, C. V. (2011). Temperature effects on vitamin C content in citrus fruits. *Pakistan Journal of Nutrition, 10*(12), 1168-1169.

Ochmian, I. (2012). The impact of foliar application of calcium fertilizers on the quality of highbush blueberry fruits belonging to the '*Duke*' cultivar. *Notulae Botanica Horti Agrobotanici, 40*(2), 163-169.

Offia-Olua, B. I. E., O. A. (2015). Production and evaluation of the physico-chemical and sensory qualities of mixed fruit leather and cakes produced from apple (*Musa pumila*), banana (*Musa sapientum*), pineapple (*Ananas comosus*). Nigerian Food Journal. *Nigerian Food Journal, 33*(2015), 22-28.

Oliveira, A. C., Valentim, I. B., Silva, C. A., Bechara, E. J. H., Barros, M. P., Mano, C. M., & Goulart, M. O. F. (2009). Total phenolic content and free radical scavenging activities of methanolic extract powders of tropical fruit residues. *Food Chemistry, 115*(2009), 469-475.

Omar, N. F., Hassan, S. A., & Yusoff, U. K. (2013). Comparative evaluation of organic and inorganic fertilizers on total phenolic, total flavonoid antioxidant activity and cyanogenic glycosides in cassava (*Manihot esculenta*). *African Journal of Biotechnology, 12*(18), 2414-2421.

Omotoso, S. O., & Akinrinde, E. A. (2013). Effect of nitrogen fertilizer on some growth, yield and fruit quality parameters in pineapple (*Ananas comosus* L. *Merr.*) plant at Ado-Ekiti Southwestern, Nigeria. *International Research Journal of Agricultural Science and Soil Science, 3*(1), 11-16.

Oo, A. N., Iwai, C. B., & Saenjan, P. (2013). Soil properties and maize growth in saline and nonsaline soils cassava - Industrial waste compost and vermicompost with or without earthworms. *Land Degradation & Development*, 1-12.

Orluchukwu, J. A., & Adedokun, O. M. (2015). Response of growth and yield of pineapple (*Ananas comosus*) on spent mushroom substrates and inorganic fertilizer in south-south, Nigeria. *International Journal of Plant & Soil Science, 8*(6), 1-5.

Othman, R., Zaifuddin, M. F., & Hassan, M. N. (2015). Potential sources for lipid soluble food colorants from selected Malaysian traditional vegetables. *Malaysian Journal of Analytical Sciences, 19*(1), 268-254.

Owureka-Asare, M., Agyei-Amponsah, J., Agbemavor, S., Apatey, J., Sarfo, A., Okyere, A., . . . Dodobi, M. (2015). Effect of organic fertilizers on physical and chemical quality of sugar loaf pineapple (*Ananas comosus* L) grown in two ecological sites in Ghana. *African Journal of Food, Agriculture, Nutrition and Development, 15*(2), 9982-9995.

Pathma, J., & Sakthivel, N. (2012). Microbial diversity of vermicompost bacteria that exhibit useful agricultural traits and waste management potential. *SpringerPlus, 1*(26), 1-19.

Paull, R. E., & Chen, C.-C. (2003). Postharvest physiology, handling and storage of pineapple. In D. P. Bartholomew, R. E. Paull & K. G. Rohrbach (Eds.), *The pineapple: Botany, production and uses* (pp. 253-274). USA: CAB International.

Pegoraro, R. F., de Souza, B. A. M., Maia, V. M., da Silva, D. F., Medeiros, A. C., & Sampaio, R. A. (2014). Macronutrient uptake, accumulation and export by the irrigated 'Vitória' pineapple plant. *Revista Brasileira de Ciência do Solo, 38*(3), 896-904.

Pegg, R. B., Landen, J. W. O., & Eitenmiller, R. R. (2010). Vitamin analysis. In S. S. Nielsen (Ed.), *Food analysis* (4th ed., pp. 190-191). Berlin, Germany: Springer Science+Business Media.

Pennock, D., Yates, T., & Braidek, J. (2006). Soil sampling designs. In M. R. Carter & E. G. Gregorich (Eds.), *Soil sampling and methods of analysis* (2nd ed.). Bacon Raton, FL: Taylor & Francis Group.

Peppe, D. J., Royer, D. L., Cariglino, B. r., Oliver, S. Y., Newman, S., Leight, E., . . . Wright, I. J. (2011). Sensitivity of leaf size and shape to climate: global patterns and paleoclimatic applications. *New Phytologist, 190*, 724-739.

Phaniendra, A., Jestadi, D. B., & Periyasamy, L. (2015). Free radicals: Properties, sources, targets, and their implication in various diseases. *Indian Journal of Clinical Biochemistry, 30*(1), 11-26.

Pompelli, M., Martins, S., Celin, E., Ventrella, M., & DaMatta, F. (2010). What is the influence of ordinary epidermal cells and stomata on the leaf plasticity of coffee plants grown under full-sun and shady conditions?. *Brazilian Journal of Biology, 70*(4), 1083-1088.

Puah, Y. J., Huang, Y. F., Chua, K. C., & Lee, T. S. (2016). River catchment rainfall series analysis using Holt-Winters method. *Journal of Earth System Science, 125*(2), 269-283.

Py, C., Lacoueilhe, J. J., & Teison, C. (1987). *The pineapple: Cultivation and uses*. Paris, France: G. P. Maisonneuve et Larose.

Raja, G., & Veerakumari, L. (2013). Influence of vermicompost on the yield and alkaloid content of *Withania somnifera*. Dunal. *International Journal of Advanced Biological Research, 3*(2), 223-226.

Rajiv, K. S., Sunita, A., Krunal, C., & Dalsukh, V. (2010). The wonders of earthworms & its vermicompost in farm production: Charles Darwin's 'friends of farmers', with potential to replace destructive chemical fertilizers from agriculture. *Agricultural Sciences, 1*(2), 76-94.

Ramos, M. J. M., Monnerat, P. H., Pinho, L. G. d. R., & Pinto, J. A. d. A. (2009). Growth and flowering of 'Imperial' pineapple plants under macronutrient and boron deficiency. *Acta Horticulturae,* 822, 139-146.

Ramos, M. J. M., & da Rocha Pinho, L. G. (2014). Physical and quality characteristics of Jupi pineapple fruits on macronutrient and boron deficiency. *Natural Resources, 5*, 359-366.

Ray, M. (2016). Vermicompost: A boon to crop production. *International Journal of Plant Protection, 9*(2), 619-627.

Razzaque, A. H. M., & Hanafi, M. M. (2001). Effect of potassium on growth, yield and quality of pineapple in tropical peat. *Fruits, 56*, 45-49.

Rodriguez-Roque, M. J., Soliva-Fortuny, R., & Martin-Belloso, O. (2017). Methods for determining the antioxidant capacity of food constituents. In E. M. Yahia (Ed.), *Fruit and vegetable phytochemicals: Chemistry and human health* (2nd ed., Vol. I & II, pp. 803). Hoboken, NJ: John Wiley & Sons Ltd.

Roy, R. N., Finck, A., Blair, G. J., & Tandon, H. L. S. (2006). *Plant nutrition for food security: A guide for integrated nutrient management* (Vol. 16). Rome, IT: Food and Agriculture Organization of the United Nations.

Saini, R. K., Nile, S. H., & Park, S. W. (2015). Carotenoids from fruits and vegetables: Chemistry, analysis, occurence, bioavailability, and biological activities. *Food Research International, 76*, 735-750.

Salomé, Y. E. S., Laurent, K. K., Irénée, B. J. P., Patrice, K., & Hilaire, K. T. (2011). Comparison of pineapple fruit characteristics of plants propagated in three different ways: By suckers, micropropagation and somatic embryogenesis. *Journal of Nutrition and Food Sciences, 1*(4), 1-8.

Samad, M. A., Hashim, S. H., Simarani, K., & Yaacob, J. S. (2016). Antibacterial properties and effects of fruit chilling and extract storage on antioxidant activity, total phenolic and anthocyanin content of four date palm (*Phoenix dactylifera*) cultivars. *Molecules, 21*(4), 419.

Savvides, A., Fanourakis, D., & leperen, W. v. (2012). Co-ordiantion of hydraulic and stomata conductances across light qualities in cucumber leaves. *Journal of Experimental Botany, 63*(3), 1135-1143.

Serio, F., Leo, L., Parente, A., & Santamaria, P. (2007). Potassium nutrition increases the lycopene content of tomato fruit. *Journal of Horticultural Science and Biotechnology, 82*(6), 941-945.

National Resources Conservation Service (2018, January 15). Classification for kingdom plantae down to species *Ananas comosus (L.)* Merr. Retrieved from https://plants.usda.gov/java/ClassificationServlet?source=display&classid=ANCO30

Shamsudin, R., Daud, W. R. W., Takrif, M. o., & Hassan, O. (2009). Physico-mechanical properties of the Josapine pineapple fruits. *Pertanika Journal of Science and Technology, 17*(1), 117-123.

Shariff, A. H. M., & Miller, H. G. (1989). Site fertility and its influence on the stocking of dipterocarp species in the tropical rain forest of peninsular Malaysia. *Journal of Tropical Forest Science, 4*(3), 189-201.

Singh, R., Sharma, R. R., Kumar, S., Gupta, R. K., & Patil, R. T. (2008). Vermicompost substitution influences growth, physiological disorders, fruit yield and quality of strawberry (*Fragaria* x *ananassa* Duch.). *Bioresource Technology, 99*(17), 8507-8511.

Singleton, R. M., Orthofer, V. L., & Lamuela-Raventos, R. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods in Enzymology, 299*, 152-178.

Sinha, R. K. (2009). Earthworms: The miracle of nature (Charles Darwin's 'unheralded soldiers of mankind & farmer's friends'). *Environmentalist, 29*, 339-340.

Soler, A. (1992). *Pineapple*. Paris, France: CIRAD-IRFA.

Solihah, M., Rosli, W., & Nurhanan, A. (2012). Phytochemical screening and total phenolic content of Malaysin *Zea mays* hair extracts. *International Food Research Journal, 19*(4), 1533-1538.

Song, X., Liu, M., Wu, D., Griffiths, B. S., Jiao, J., Li, H., & Hu, F. (2015). Interaction matters: Synergy between vermicompost and PGPR agents improves soil quality, crop quality and crop yield in the field. *Applied Soil Ecology, 89*, 25-34.

Souza, L. F. d. S., & Reinhardt, D. H. (2007). Pineapple. In L. A. Crisóstomo & A. Naumov (Eds.), *Fertilizing for high yield and quality: Tropical fruit of Brazil* (IPI Bulletin No.18 ed., pp. 179-201). Horgen, CH: International Potash Institute.

Suhane, R. K. (2007). Vermicompost. *Rajendra Agriculture University*, 88.

Suneetha, C., Siddique Ahamed Khan, M., Muralinath, E., Jaffar, S., Ibrahim, M., & Guruprasad, M. (2011). Effect of vermicompost on the antioxidant levels of *Coleus aromaticus*. *International Journal of Science Innovations and Discoveries, 1*(3), 422-427.

Taber, H., Perkins-Veazie, P., Li, S., White, W., Rodermel, S., & Xu, Y. (2008). Enhancement of tomato fruit lycopene by potassium is cultivar dependent. *HortScience, 43*(1), 159-165.

Tan, K. H. (2005). Principles of soil sampling. In Kim H. T. (Ed.), *Soil sampling, preparation, and analysis* (2nd ed., pp. 1-24). Boca Raton, FL: Taylor & Francis.

Teixeira, L. A. J., Quaggio, J. A., Cantarella, H., & Mellis, E. V. (2011). Potassium fertilization for pineapple: Effects on soil chemical properties and plant nutrition. *Revista Brasileira de Fruticultura, 33*(2), 627-636.

Thalip, A. A., P.S., T., & Ng, C. (2015). The MD2 'Super Sweet' pineapple (*Ananas comosus*). *UTAR Agriculture Science Journal, 1*(4), 14-17.

Tharmaraj, K., Ganesh, P., Kolanjinathan, K., Kumar, R. S., & Anandan, A. (2010). Influence of vermicompost and vermiwash on physico chemical properties of black gram cultivated soil. *International Journal of Recent Scientific Research, 3*, 77-83.

Theunissen, J., Nhakidemi, P., & Laublsher, C. P. (2010). Potential of vermicompost produced from plant waste on the growth and nutrient status in vegetable production. *International Journal of Physical Sciences, 5*(13), 1964-1973.

Tian, M., Yu, G., He, N., & Hou, J. (2016). Leaf morphological and anatomical traits from tropical to temperate coniferous forests: Mechanisms and influencing factors. *Scientific Reports, 6*, 19703.

Times, T. S. (2018, August 19). Malaysian pineapple in demand in China. *The Straits Times*. Retrieved from https://www.straitstimes.com/asia/se-asia/malaysian-pineapple-in-demand-in-china.

Trudel, M. J., & Ozbun, J. L. (1970). Relationship between chlorophyll and carotenoids of ripening tomato fruit as influenced by potassium nutrition. *Journal of Experimental Botany, 21*(4), 881-886.

Trudel, M. J., & Ozbun, J. L. (1971). Influence of potassium on carotenoid content of tomato fruit. *Journal of the American Society for Horticultural Science, 96*(6), 763-765.

Tucker, G. A. (1993). Introduction. In G. B. Seymour, J. E. Taylor & G. A. Tucker (Eds.), *Biochemistry of fruit ripening* (pp. 454). London, England: Chapman & Hall.

Usman, I. S., Abdulmalik, M. M., Sani, L. A., & Muhammad, A. N. (2013). Development of an efficient protocol for micropropagation of pineapple (*Ananas comosus* L. var. smooth cayenne). *African Journal of Agricultural Research, 8*(18), 2053-2056.

Wang, Z., & Li, S. (2004). Effects of nitrogen and phosphorus fertilization on plant growth and nitrate accumulation in vegetables. *Journal of Plant Nutrition, 27*(3), 536-556.

Wardy, W., Saalia, F. K., Steiner-Asiedu, M., Budu, A. S., & Sefa-Dedeh, S. (2009). A comparison of some physical, chemical and sensory attributes of three pineapple (*Ananas comosus*) varieties grown in Ghana. *African Journal of Food Science, 3*(1), 22-25.

White, R. T., JR, & Douthit, G. E. (1985). Use of microwave oven and nitric acid-hydrogen peroxide digestion to prepare botanical materials for elemental analysis by inductive couple plasma emission spectroscopy. *Journal Association of Official Analytical Chemists, 68*(4), 766-769.

Yang, L., Li, F., & Chu, H. (2014). Effects of food waste compost on soil microbial populations, tomato yield, and tomato quality. *Communications in Soil Science and Plant Analysis, 45*(8), 1049-1058.

Yousefi, A. A., & Sadeghi, M. (2014). Effect of vermicompost and urea chemical fertilizers on yield and yield components of wheat (*Triticum aestivum*) in the field condition. *International Journal of Agriculture and Crop Sciences, 7*(12), 1227-1230.

Yuris, A., & Siow, L.-F. (2014). A comparative study of the antioxidant properties of three pineapple (*Ananas comosus* L.) varieties. *Journal of Food Studies, 3*(1), 40-56.

Yusof, Z., Ramasamy, S., Mahmood, N. Z., & Yaacob, J. S. (2018). Vermicompost supplementation improves the stability of bioactive anthocyanin and phenolic compounds in *Clinacanthus nutans* Lindau. *Molecules, 23*(1345), 1-13.

Zaman, M. M., Chowdhury, M. A. H., Islam, M. R., & Uddin, M. R. (2015). Effects of vermicompost on growth and leaf biomass yield of stevia and post harvest fertility status of soil. *Journal of the Bangladesh Agricultural University, 12*(2), 169-174.

Zucco, M. A., Walters, S. A., Chong, S.-K., Klubek, B. P., & Masabni, J. G. (2015). Effect of soil type and vermicompost applications on tomato growth. *International Journal of Recycling of Organic Waste in Agriculture, 4*(2), 135-141.

Zulak, K. G., Liscombe, D. K., Ashihara, H., & Facchini, P. J. (2007). Alkaloids. In A.Crozier, M.N. Clifford & H. Ashihara (Eds.), *Plant secondary metabolites* (pp. 102-136). Oxford, England: Blackwell Publishing Ltd.

Zuraida, A. R., Shahnadz, A. H. N., Harteeni, A., S., R., Radziah, C. M. Z. C., & S., S. (2011). A novel approach for rapid micropropagation of maspine pineapple (*Ananas comosus* L.) shoots using liquid shake culture system. *African Journal of Biotechnology, 10*(19), 3859-3866.

LIST OF PUBLICATIONS AND PAPERS PRESENTED

Publication

Mahmud, M., Abdullah, R., & Yaacob, J. (2018). Effect of vermicompost amendment on nutritional status of sandy loam soil, growth performance, and yield of pineapple (*Ananas comous* var. MD2) under field conditions. *Agronomy*, *8*(9), 183.

Paper Presented

Growth performance, morphology characteristics and physicochemical properties of pineapple variety MD2 treated with vermicompost as supplement in the field. 4th International Conference Sustainable Agriculture, Food and Energy (SAFE 2016), University of Ruhuna, Sri Lanka, 20 October 2016 -22 October 2016. Oral presentation.