METABOLITE PROFILES OF THE FERTILE AND NON-FERTILE SOILS FOR THE PLANTING OF *Mangifera indica L.*

Mohammad Zulfadhly Jan Jam[1,2], Nur Syaza Mohd Samsudin[1,2], Amir Anwar Raziq Amir Fuzil[1,2], Khawarizmi Mohd Aziz[2], Muhamad Azwat Abdullah[1], Mohd Salleh Rofiee[1,4], Teh Lay Kek[1,3], Mohd Zaki Salleh[1,3*]

[1] *Integrative Pharmacogenomic Institute, Universiti Teknologi MARA Selangor Branch, Puncak Alam Campus, 42300 Puncak Alam, Selangor, Malaysia.*

[2] *Faculty of Plantation and Agrotechnology, Universiti Teknologi MARA Melaka Branch, Jasin Campus, 77300 Merlimau, Melaka, Malaysia.*

[3] *Faculty of Pharmacy, Universiti Teknologi MARA Selangor Branch, Puncak Alam Campus, 42300 Puncak Alam, Selangor, Malaysia.*

[4] *Faculty of Health Sciences, Universiti Teknologi MARA Selangor Branch, Puncak Alam Campus, 42300 Puncak Alam, Selangor, Malaysia*

**Corresponding author: zakisalleh@uitm.edu.my*

Abstract

Mangifera indica L. is locally known as Harumanis. It is one of the popular cultivars of mango trees in Malaysia planted in the north of Peninsular Malaysia especially in Perlis because of the unique climate and soil condition. This study was carried out to differentiate the metabolites profiles of the fertile and non-fertile soil in *Mangifera indica L*. plantation. Soil from which the Harumanis was planted was sampled and the metabolites were profiled using LC/MS-QTOF. Soils were sampled from 2 different areas of the same farm, the fertile and non-fertile soil areas. The samples were dried, and the soil metabolites were extracted for metabolomics analysis using LC/MS-QTOF. A total of 307 metabolites were detected but only 13 metabolites were significantly differentiated between the fertile and non-fertile soil ($p < 0.05$, $\log_2 FC > 1.0$). The metabolites were characterised to different groups of compounds such as pesticide, alkaloid, amino acid, terpenoid and carbamate. Fertile soil may contain beneficial metabolites that influence the soil quality which affect the optimum growth of Harumanis.

Keyword: Soil metabolomics, *Mangifera indica L*., liquid-chromatography mass spectrometry, metabolites profile

Introduction

In Malaysia, there are different cultivars of mango such as Apple Mango (MA 194), Malgoa (MA 135, MA 171, MA 172, MA 200, MA 135, MA 200), Maha 65 (MA 165), Lebai Mohamad (MA 127) and Golek (MA 162) (Yusuf *et al*., 2018). However, one of the most popular cultivar mango is 'Harumanis' due to its flavor and soft texture. It has a high commercial demand with a rapidly growing market in Malaysia. Nevertheless, Harumanis grows well only in Perlis, Malaysia. The reasons that contribute to this observation include the types of climate and soil found in Perlis. Perlis is believed to have a unique fertile soil and temperature condition that allow Harumanis to grow as one of the best mangoes in the world (Uda *et al*., 2020).

Soil is a complex ecosystem for a variety of organisms which produce different types

of nutrients and organic materials. Soil is involved in many processes such as temperature regulation, carbon and nutrient cycling, water and temperature regulation, and decomposition which transform organic materials into different compounds (Hartmann *et al*., 2015). The organic metabolites such as amino acids, nucleobases, organic acids, fatty acids, carbohydrates secondary metabolites, and antibiotics have been detected in soil (Swenson and Northen, 2018). In a fertile soil, soil organisms efficiently turn nutrients and organic materials for plant yields, protect plants from diseases, and make the soil crumbly. Such a soil can easily be cultivated, absorbs rainwater well, and withstands capping/siltation and erosion (Berner *et al*., 2016). When it comes to maintaining soil fertility, the normal soil pH range between 5.0 - 6.5 plays an important factor for productivity followed by the content of phosphate and nitrogen content in soil (Block *et al*., 2015).

Soil metabolomics is a study of unbiased qualitative and relative quantitative analysis of all metabolites in a biological material. The untargeted soil metabolomics uses LC/MS or Gas Chromatography-Mass Spectrometry (GC/MS) to identify the compounds in the soil. LC/MS chromatography identifies the chemical compounds through the retention time, m/z values and fragmentation spectra. Metabolomics has been applied to understand the interaction between the micro-organisms with the environment which condition the soil and render it fertile or non-fertile via different metabolites that were produced (Rofiee *et al*., 2015). Soil metabolomics therefore has been used to detect biological responses of the soil microbes in different conditions (Song *et al*., 2020).

Thus far, there is a lack of study that investigate the metabolites profiles of the soil used to grow Harumanis in the farm. No information is available on how to improve the soil quality for the growth of Harumanis. Therefore, this study was carried out as an attempt to determine the different profiles of metabolites of the fertile and non-fertile soil planted with Harumanis tree. This study also aimed to evaluate the potential of metabolites that are suitable as indicators for soil quality representing fertile and non-fertile soils.

Materials and Methods

Collection and preparation of soil samples

The soils used in this study were collected from the Harumanis farm located at Kg. Tanah Pasir, Beseri, Perlis, Malaysia in April 2021. Two areas at the farm were first identified; one was the fertile land which had produced Harumanis with the desired texture of fruits such as soft and pulpy texture; while another was the non-fertile land which had produced Harumanis with fruits that are of less desirable quality such as less in weight, hard texture and sour in taste. Soil samples from three (3) different spots were collected randomly from fertile and non-fertile locations in the farm at a depth of 15 cm from the topsoil using the soil probe as suggested by Ackerson (2018). The soil samples were placed in clean plastic bags and labelled correctly. All the soil samples were sent to the laboratory for extraction of metabolites and further analysis.

Metabolite extraction, Sample drying and sieving

The soil samples (200 g) were weighed and placed in a 50 ml polypropylene conical tube and kept frozen at -80 degree celcius for 24 hours. The conical tubes were sealed with parafilm, and small holes were punched on the parafilm using a needle. The soil samples were placed in a vacuum concentrator (5301, Brinkmann, Eppendorf) to remove water content in the samples. The soil samples were weighed every 1 hour until the samples reached a constant weight (199 g). The dried soil samples were sieved using 2 mm mesh sieves and 2 grams of the

soil were transferred into 50 ml conical tubes and were labelled correctly (Swenson & Northen, 2018).

Extraction of polar metabolites

Eight (8) ml of LC/MS grade MeOH was added into each 50 ml conical tubes containing dried soil samples and were sonicated for 30 minutes. The tubes then were vortexed at 200 rpm for 1 hour with the angle at approximately 45° followed by centrifugation (Centrifuge 5804R, Eppendorf, USA) at 3,220 x g for 15 minutes at 4°C. The supernatant from each tube was collected and further filtered using a10 ml syringe with 0.22 µm filter disc. The filtrates were transferred into new 15 mL conical tubes. All extracted samples were dried using the vacuum concentrator (5301, Brinkmann, Eppendorf).

Sample preparation and Metabolite soil analysis

The dried extracted samples were reconstituted with 200 µL of mobile phase (ratio 1:1 of ddH_2O: acetonitrile) for each sample, vortexed for 1 min and centrifuged at 5,000 x g for 15 min. One hundred (100) µL of the supernatants were transferred into an insert and then 10 µL were injected into the LC/MS Q-TOF (Agilent Technologies 6520, Santa Clara, CA, USA). Each sample was injected four (4) times to obtain technical replicates for analysis.

Profiling of the Soil Metabolites using LC/MS Q-TOF

The extracted metabolites in the soil were profiled using LC/MS Q-TOF (Agilent Technologies 6520, Santa Clara, CA, USA) controlled by Agilent Mass Hunter Workstation Acquisition (B.02.01). The separation of the metabolites in the samples were performed using ZORBAX Eclipse Plus C18 column (1.8 microns) 2.1 mm x 100 mm (Agilent Technologies, SA, USA) maintained at 40°C in column oven. The system was operated with a flow rate of 0.25 mL/min with mobile phase A (0.1% formic acid in water) and mobile phase B (0.1% formic acid in acetonitrile) over a gradient of 18 minutes from 5% to 95% of mobile phase B. The mobile phase B was set to change from 95% to 5% over 12 minutes. The data were collected in positive electrospray ionization (ESI) mode. To ensure the mass accuracy, continuous internal calibration was performed during the analysis with reference mass of m/z 121.0529 ($C_6H_4N_4$) and, m/z 922.0098 ($C_{18}H_{18}O_6N_3P_3F_{24}$).

Data and Statistical Analysis

The raw mass spectrometry (MS) data was processed using the Agilent Mass Hunter Qualitative Analysis B.05.00 software (Agilent Technologies, Santa Clara, CA, USA) (Rofiee *et al*., 2015) to convert .d file into .CEF file. Data filtering, statistical analysis, principal component analysis and heat map analysis were done using Mass Profiler Professional (MPP) version B.12.01 (Agilent Technologies, Santa Clara, CA, USA) and MetaboAnalyst web-based tool. Heat map was used to visualize the data obtained in graphic form, while principal component analysis (PCA) was performed to represent different clusters of metabolites profiles between groups. The metabolites which were significantly different between groups ($p < 0.05$) were identified using ID Browser Identification linked to the METLIN database. Fold change analysis were performed to determine the metabolites that passed two-fold changes with $p < 0.05$.

Results

Plant Morphology

Soil fertility was determined based on the physical characteristics of the plant. The characteristics of Harumanis Mango (*Mangifera indica L.*) planted in fertile and non-fertile soil areas are shown in **Figure 1**.

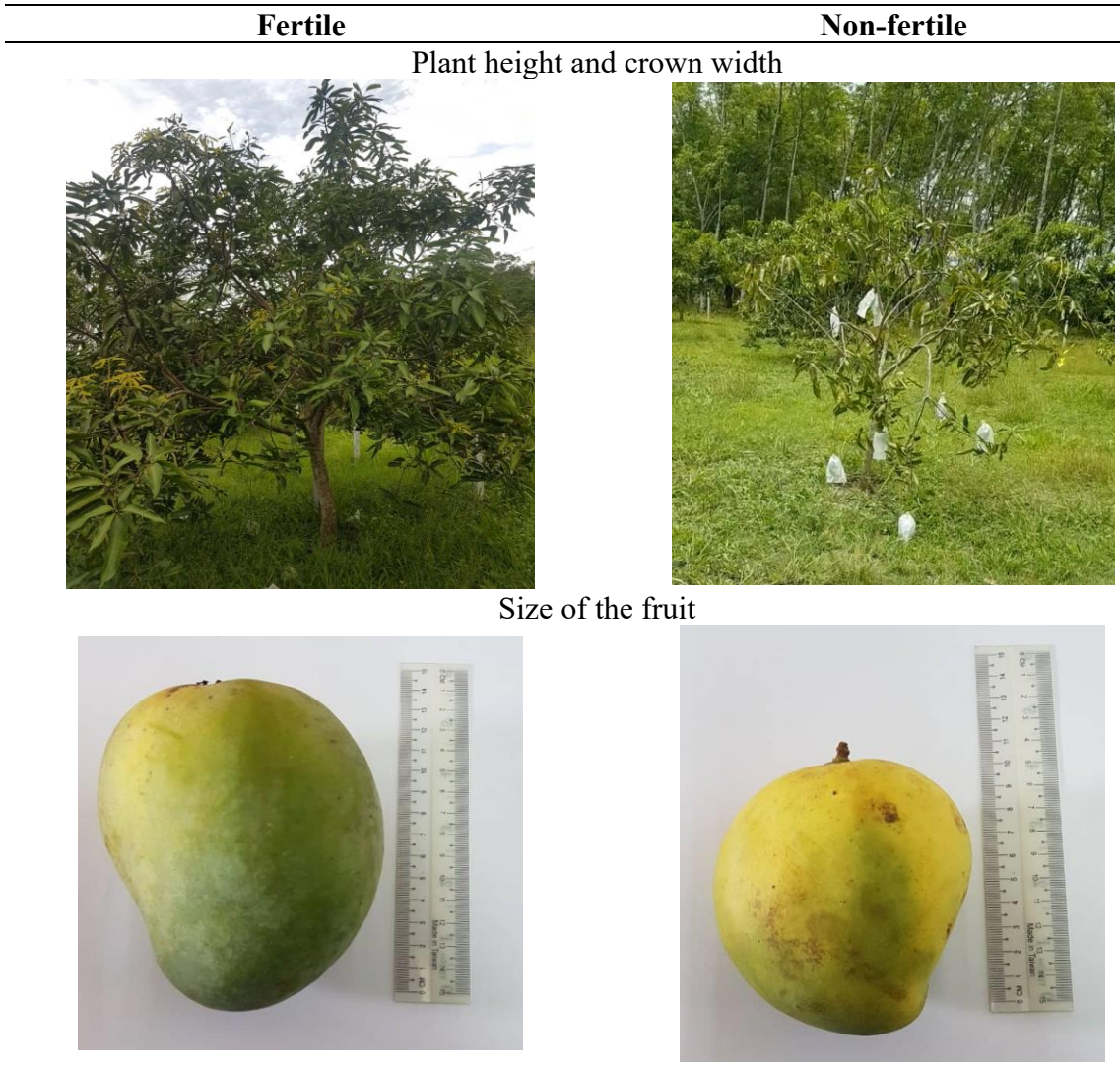

Figure 1 Comparison of plants and fruits of *Mangifera indica* L. planted in fertile and non-fertile soils.

The plant which grew in the fertile area has greater plant height and crown width, while the fruit was heavier and longer compared to the one grown in non-fertile soil (**Table 1**). Plant in the fertile area achieved greater height (3 meters) compared to the plant in non-fertile area (1.8 meters). The crown width of the plant in the fertile area was (2.5 meters) whilst the crown width of the plant in the non-fertile area was only 1 meter. In the fertile area, the maximum weight of Harumanis fruits was 650 g/pc, while the fruit in the non-fertile area was 400 g/pc. Harumanis fruits in the fertile area were longer, with a length of 16.3 cm compared to the fruits from the non-fertile area (11.4 cm).

Table 1 Characteristics of Harumanis Mango (*Mangifera indica L.*) from different soil area

Soil Area	Plant Height (m)	Crown Width (m)	Fruit Weight (g)	Fruit Length (cm)
Fertile	3.03 ± 0.15	2.53 ± 0.15	483.33 ± 152.75	16.33 ± 0.15
Non-Fertile	1.83 ± 0.15	1.07 ± 0.12	216.67 ± 76.38	11.37 ± 0.15

Metabolomics Analysis

Total Ion Chromatogram (TIC) for the fertile and non-fertile soil samples are presented in **Figure 2**. Analytical reproducibly within or between batches of MS data has a relative standard deviation of less than 20% (**Table 2**). This is in accordance to the requirement as previously published (Rofiee *et al*., 2015). A total of 307 metabolites were detected from the spectra. However, 13 metabolites were significantly different ($p < 0.05$) between the fertile and non-fertile soils. These metabolites were identified using METLIN database (http://metlin.scripts.edu) that is linked to Mass Profiler using Professional (MPP) version B.12.01 software (Agilent Technologies, Santa Clara, CA, USA). The data was analysed and presented in the form of Principal Components Analysis (PCA) (**Figure 3**) to observe similarities or differences of the clusters of the metabolites. The results showed that the metabolite profiles for the different soil samples were different. The PCA shows that PC1 and PC2 at 57% and 12.8% of the total variance, respectively. The PCA showed 2 clusters separated the fertile and non-fertile soils metabolites. However, there were overlapping metabolites profiles between the fertile and non-fertile soil samples. Fold change (FC) analysis (**Figure 4**) revealed the relative abundance of metabolites in each type of soil with $\log_2$ FC > 1.0. The abundance of 5-Butyl-2-ethyloxazole, songorine and dibucaine are higher in the fertile soil while diphenylcarbazide, dihydroretrofractamide B and cicutoxin were higher in the non-fertile soil.

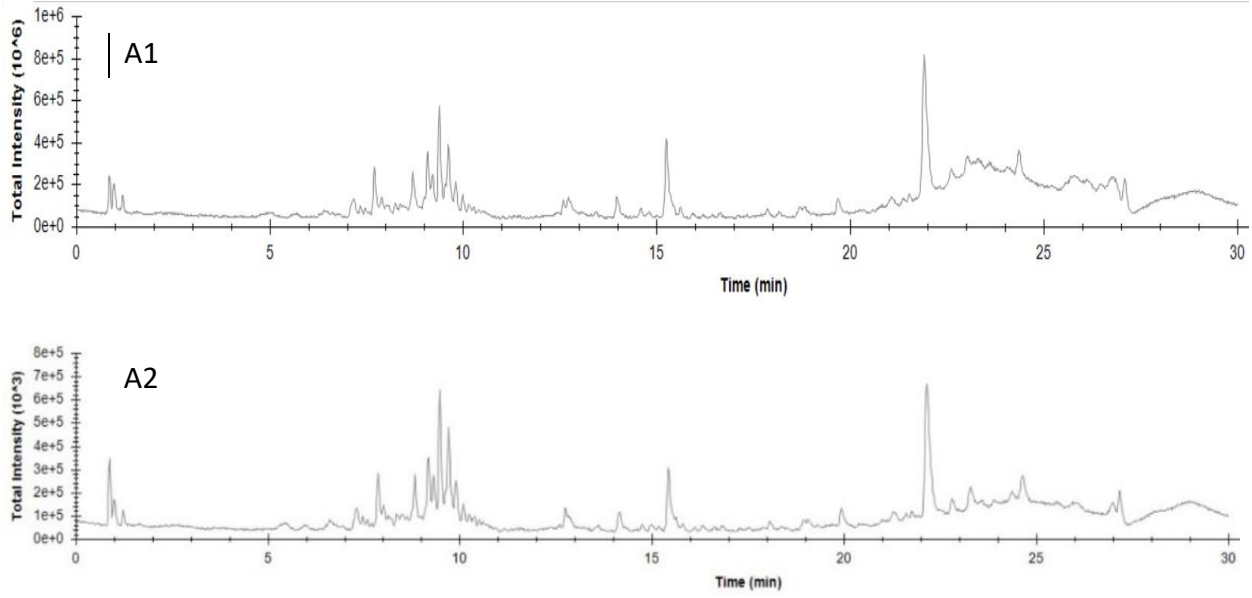

Figure 2 LC/MS Q-TOF TICs of soil samples taken from *Mangifera indica* plantation. Soil sample from fertile soil was labelled as A1 while non-fertile soil sample was labelled as A2.

Table 2 Relative standard deviation of soil metabolites

Compound	Mass diff (ppm)		
Reference Mass	Mean	SD	RSD (%)
922.01	5516.67	1079.66	19.5709
121.05	8746.97	505.92	5.7839

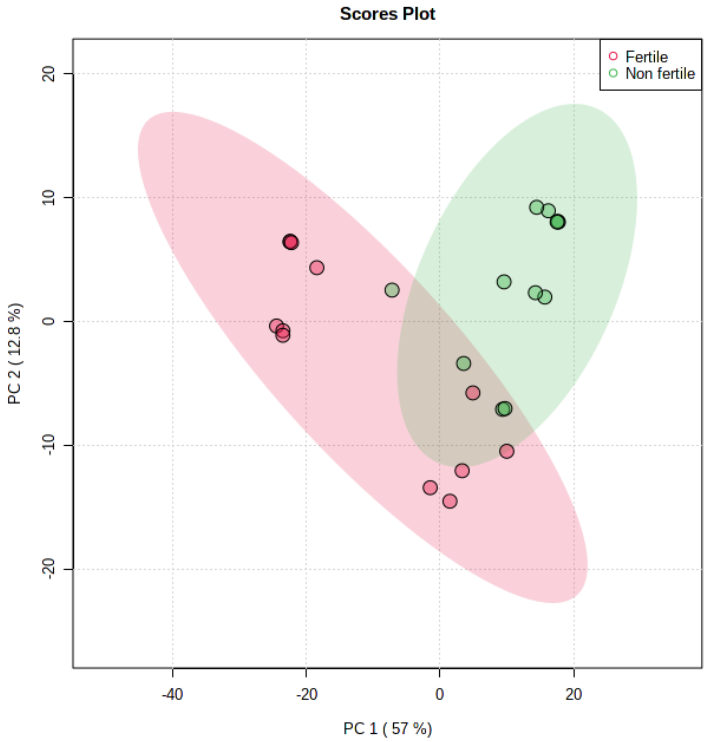

Figure 3 2D principal components score plot for all metabolites detected in the fertile and non-fertile soil.

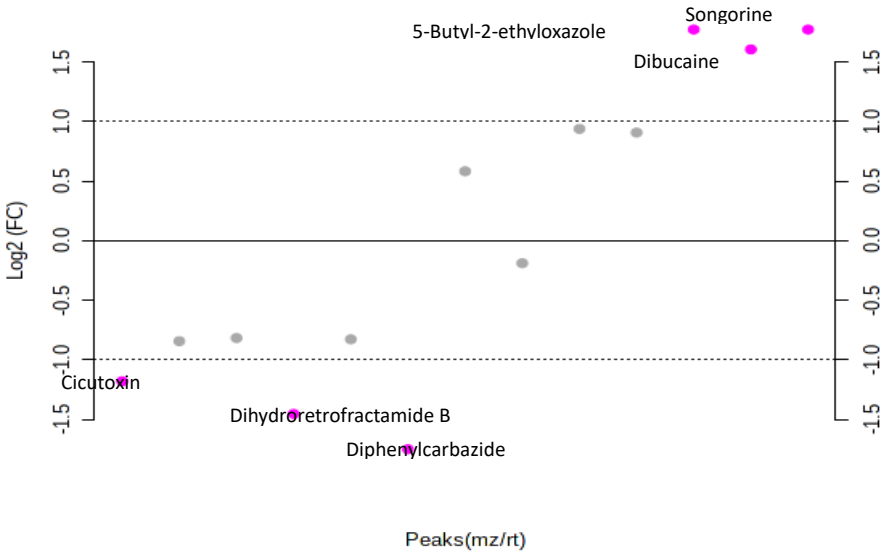

Figure 4 List of compounds with fold change analysis (log2 (FC) > 1.0)

Heatmap (**Figure 5**) was conducted using Euclidean distance and Ward's linkage. In comparison to the other replicates, blue and red hues represent lower or greater abundance of certain metabolites, with deeper hues suggesting more obvious differences. Most of the metabolites that were found in the fertile soil are hypoglycin, dibucaine, songorine, terbucarb, D-Fructofuranose 1, 5-Butyl-2-ethyloxa, Methyldopexamine, Leu lle Val, (E,E,E)-N-(2-Methylpropyl)hexadeca-2,6,8-trien-10-ynamide, dihydroretrofractamide B, diphenylcarbazide, cicutoxin and 4-O-Methylmelleolide. Fewer compounds were identified in the non-fertile soil such as dibucaine, songorine and 5-Butyl-2-ethyloxa. Diphenylcarbazide was not detected in the fertile soil based on heatmap analysis but relatively small amount of dihydroretrofracta was detected in the fertile soil.

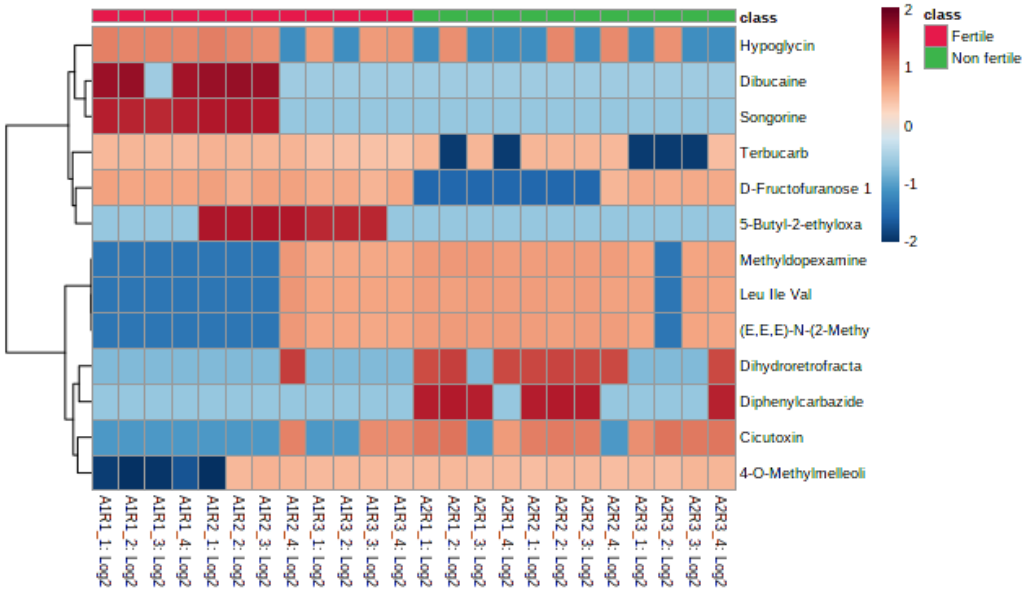

Figure 5 Heatmap showing expression profiles of each soil sample based on the most significant known metabolites identified by T-test. Metabolites are clustered by Euclidean distance and Ward's linkage

Discussion

The quality, health, and fertility of the soil are crucial in producing a healthy plant with an acceptable yield (Bending *et al.*, 2004). In this study, there were differences in the physical appearance of the *Mangifera indica L*. plants that grow at the areas with the fertile and non-fertile soils. Trees grown on the fertile soil were wider width crown and higher in height as well as bigger size of fruits compared to non-fertile soil. Based on the heatmap in **Figure 4**, several compounds such as alkaloid, amino acid, terpenoid, carbamate and metabolites from pesticide have been discovered in the soil. Li *et al.,* (2020) had reported that soil planted with different types of rice lines had prominent cluster of metabolites with slight differences in the replicated samples from the same group. However, Withers *et al.,* (2020) stated that there are similarities of metabolomic profiles among the different soil types.

A wide range of metabolites that are found in the soil originate from the microbial and plant sources with different abundance. The microorganisms in the soil play an important role in providing the unique soil metabolome. Different abundance of organic acid and sugar in the soils are related to the bacterial community (Hewavitharana *et al.*, 2019; Liu *et al.*, 2020). In addition, metabolites such as phenolic acid, flavonoids, alkaloids, and terpenoids have been reported to support the plants defence mechanism (Yang *et al.*, 2018). These plant metabolites

can be released to the soil via four pathways: volatilization and diffusion away from plant tissues, leaching of above-ground plant material, exudation from plant roots and also decomposition (Chomel *et al.*, 2016).

One of the metabolites found in the soil, D-fructofuranose is a saccharide detected mostly in the fertile soil which indicates that a higher sugar level is required compared to the non-fertile soil. Amino acids and saccharides can be considered as carbon sources which help in stimulating microbial activity. Amino acids also have significant roles in plant physiological processes including regulation of stress-responsive gene (Hewavitharana *et al.*, 2019; Zhao *et al.*, 2019). The metabolites that exist in both non-fertile soil and fertile soil in the present study include (E,E,E)-N-(2-Methylpropyl)hexadeca-2,6,8-trien-10-ynamide, methyldopexamine and amino acids such as hypoglycin and Leu-Ile-Val.

Another metabolite discovered in the soil was the 4-O-Methylmelleolide which found in honey mushroom *Armillaria mellea* (alkyl resorcinol ester derivative) and classified as terpenoids. It is stated that terpenoid is a secondary metabolite usually produced by plants, fungi and bacteria (Rivilla *et al.*, 2020). The metabolites were present in all of the non-fertile soil, with only a few in the fertile soil samples. Songorine an alkaloid metabolite isolated from the genus *Aconitum* also had been identified in the analysis. In the present study, it was observed that songorine and dibucaine were highly available in the fertile soil compared to the non-fertile soil. The function of alkaloid in plants is that they can improve the defence mechanism against stress, thus increasing the reproductive rates of the plant (Matsuura & Fett-Neto, 2015).

Some metabolites such as (E, E, E)-N-(2-Methylpropyl) hexadeca-2,6,8-trien-10-ynamide and terbucarb can be found in agricultural practices. Both of these metabolites are pesticide and herbicide, respectively. (E,E,E)-N-(2-Methylpropyl)hexadeca-2,6,8-trien-10-ynamide can be found in *Achillea ageratifolia,* while terbucarb is a carbamate compound which are usually used in agricultural practices (Zhou *et al*., 2011; Prashar & Shah, 2016). Pesticide can be harmful, toxic and contaminating to the soil and waterbodies such as lakes and rivers when not used accordingly (Aktar *et al.*, 2009).

Metabolite profiles of the soil can be used to indicate soil quality. The microorganisms in the soil were reported to remedy the heavy metal contaminated soil as well as reducing soil erosion and improve plant productivity by assisting in nutrient uptake from the soil (Prashar & Shah, 2016; Fierer, 2017). The usage of pesticide and the presence of plant exudates can affect the microorganisms' activities in the soil, thus impacting the abundance and the availability of metabolites released by the microorganisms (Swenson *et al.*, 2015; Prashar & Shah, 2016).

One of the limitations of this study is that most of the metabolites cannot be identified using the existing database for soil. Fernie *et al*., (2004) also encountered the problem of having unknown analytes measured during the metabolite profiling of soil. Several databases need to be used in order to gather the information regarding the metabolites present in the soil. The metabolites database on roots exudation and microbial products are more accessible for the identification of metabolites obtained from soil (Pétriacq *et al.*, 2017).

Many aspects need to be considered when the quality and fertility of soil are investigated such as pH of soil, availability of nutrients, organic matter and moisture. Bioformulation which helps to improve soil fertility has been suggested as an option. Bioformulation is a chemical containing microorganism and their metabolites that can improve plant development by making nutrients available for absorption and activating the plants defence mechanisms. Specific metabolite as formulation composition may cause concerns due to the required amount of metabolite concentration needed and purification (Arora & Mishra, 2016). Thus, beneficial microorganism can be used in developing bioformulation. Besides that, the usage of excessive chemical pesticide in the farm needs to be reduced and replaced with biopesticide.

Metabolite profiling for the fertile and non-fertile soils provide information on the soil quality required for optimum plant growth and yield. This information is useful for the farmers to strategize their cultivation. Besides, it also helps in discussing the possibility of factors that affect plant performance when planting in specific areas. The relationship of physical, chemical and biological properties of the soil needs to be considered to create suitable environment that encourages the thrive of diverse soil microflora that benefit the growth of the plant.

Conclusion

In conclusion, soil metabolites can be one of the factors affecting the soil quality and fertility in the plantation area. Microorganisms in the soil affect plant growth in the plantation area due to their metabolites, which are either beneficial compounds or non-beneficial compounds for plant development. Besides, the plant also produces its metabolites which are released to the soil, contributing to the metabolite profiling of the soil. Hence, the Harumanis mango tree can be improved by managing the metabolites in terms of growth and quality after checking the composition of metabolites.

Ethics Statement

The research does not require research ethics approval.

Authors Contribution

Concept – Mohd Zaki Salleh; Design – Teh Lay Kek; Supervision – Mohd Salleh Rofiee and Khawarizmi Mohd Aziz; Materials – Amir Anwar Raziq Amir Fuzi; Data Collection and/or Processing – Nur Syaza Mohd Samsudin and Mohammad Zulfadhly Jan Jam; Literature Search – Muhamad Azwat Abdullah; Writing Manuscript - Mohammad Zulfadhly Jan Jam; Nur Syaza Mohd Samsudin and Amir Anwar Raziq Amir Fuzil; Critical Review – Teh Lay Kek and Mohd Zaki Salleh

Acknowledgement

The research was done at Universiti Teknologi MARA (UiTM). The authors would like to thank the Integrative Pharmacogenomics Institute (iPROMISE), UiTM Puncak Alam Campus for supporting the study**.**

Conflict of interests

The authors declare that they have no conflict of interests.

References

Ackerson, J. P. 2018. Soil sampling guidelines; Agronomy: AY-368-W. *Purdue University*, 6. Retrieved 7 June 2021 from https://www.extension.purdue.edu/extmedia/AY/AY-368-w.pdf

Aktar, W., Sengupta, D. and Chowdhury, A. 2009. Impact of pesticides use in agriculture: Their benefits and hazards. *Interdisciplinary Toxicology* 2(1): 1–12.

Arora, N. K., and Mishra, J. 2016. Prospecting the roles of metabolites and additives in future

bioformulations for sustainable agriculture. *Applied Soil Ecology* 107(June): 405–407.

Berner, Alfred and et al. (2016) The Basics of Soil Fertility. Basic guide. Research Institute of Organic Agriculture (FiBL) and The Organic Research Centre, Elm Farm, CH-5070 Frick and UK.

Bending, Gary D., Turner, M. K., Rayns, F., Marx, M. C., & Wood, M. 2004. Microbial and biochemical soil quality indicators and their potential for differentiating areas under contrasting agricultural management regimes. *Soil Biology and Biochemistry* 36(11): 1785–1792.

Block, B. C. D., & Bengal, W. 2015. Soil Fertility and Its ' Impact on Agricultural Productivity : A Study in Sapar Soil Fertility and Its ' Impact on Agricultural Productivity : A Study in Sapar Mouza , Burdwan-I C . D . Block , West Bengal Kshudiram Chakraborty. International Journal of Humanities and Social Sciences (IJHSSS) 2(November): 196–206.

Chomel, M., Guittonny-Larchevêque, M., Fernandez, C., Gallet, C., DesRochers, A., Paré, D., … Baldy, V. 2016. Plant secondary metabolites: a key driver of litter decomposition and soil nutrient cycling. *Journal of Ecology* 104(6): 1527–1541.

Fernie, A. R., Trethewey, R. N., Krotzky, A. J., & Willmitzer, L. 2004. Metabolite profiling: From diagnostics to systems biology. *Nature Reviews Molecular Cell Biology* 5(9): 763–769.

Fierer, N. 2017. Embracing the unknown: Disentangling the complexities of the soil microbiome. *Nature Reviews Microbiology* 15(10): 579–590.

Hartmann, M., Frey, B., Mayer, J., Mäder, P., & Widmer, F. 2015. Distinct soil microbial diversity under long-term organic and conventional farming. *ISME Journal* 9(5): 1177–1194.

Hewavitharana, S. S., Klarer, E., Reed, A. J., Leisso, R., Poirier, B., Honaas, L., … Mazzola, M. 2019. Temporal Dynamics of the Soil Metabolome and Microbiome During Simulated Anaerobic Soil Disinfestation. *Frontiers in Microbiology* 10(October): 1–17.

Li, Y., Xu, L., Letuma, P., & Lin, W. 2020. Metabolite profiling of rhizosphere soil of different allelopathic potential rice accessions. *BMC Plant Biology* 20(1): 1–21.

Liu, K., Ding, X., & Wang, J. 2020. Soil metabolome correlates with bacterial diversity and co-occurrence patterns in root-associated soils on the Tibetan Plateau. *Science of the Total Environment* 735:139572.

Matsuura, H.N.; Fett-Neto, A.G. 2015. Plant alkaloids: Main features, toxicity, and mechanisms of action. In Plant Toxins; Gopalakrishnakone, P., Carlini, C.R., Ligabue-Braun, R., Eds.; Springer: Dordrecht, The Netherlandd, pp. 1–15

Prashar, P., & Shah, S. 2016. Impact of Fertilizers and Pesticides on Soil Microflora in Agriculture. *Sustainable Agriculture Reviews*, pp.331–361.

Pétriacq, P., Williams, A., Cotton, A., McFarlane, A. E., Rolfe, S. A., & Ton, J. 2017. Metabolite

profiling of non-sterile rhizosphere soil. *Plant Journal* 92(1): 147–162.

Martin-Rivilla, H., Gutierrez-Mañero, F. J., Gradillas, A., P. Navarro, M. O., Andrade, G., & Lucas, J. A. 2020. Identifying the Compounds of the Metabolic Elicitors of Pseudomonas fluorescens N 21.4 Responsible for Their Ability to Induce Plant Resistance. *Plants* 9(8): 1020.

Rofiee, M. S., Yusof, M. I. M., Abdul Hisam, E. E., Bannur, Z., Zakaria, Z. A., Somchit, M. N., … Salleh, M. Z. 2015. Isolating the metabolic pathways involved in the hepatoprotective effect of Muntingia calabura against CCl4-induced liver injury using LC/MS Q-TOF. *Journal of Ethnopharmacology* 166: 109–118.

Song, Y., Li, X., Yao, S., Yang, X., & Jiang, X. 2020. Correlations between soil metabolomics and bacterial community structures in the pepper rhizosphere under plastic greenhouse cultivation. *Science of the Total Environment* 728: 138439.

Swenson, T. L., Jenkins, S., Bowen, B. P., & Northen, T. R. 2015. Untargeted soil metabolomics methods for analysis of extractable organic matter. *Soil Biology and Biochemistry* 80: 189–198.

Swenson, T. L., & Northen, T. R. 2018. Untargeted Soil Metabolomics Using Liquid Chromatography–Mass Spectrometry and Gas Chromatography–Mass Spectrometry. *Methods in Molecular Biology*, pp.97–109.

Uda, M. N. A., Gopinath, S. C. B., Hashim, U., Hakimi, A., Afnan Uda, M. N., Anuar, A., … Parmin, N. A. 2020. Harumanis Mango: Perspectives in Disease Management and Advancement using Interdigitated Electrodes (IDE) Nano-Biosensor. *IOP Conference Series: Materials Science and Engineering* 864(1): 012180

Withers, E., Hill, P. W., Chadwick, D. R., & Jones, D. L. 2020. Use of untargeted metabolomics for assessing soil quality and microbial function. *Soil Biology and Biochemistry* 143: 107758.

Yang, L., Wen, K. S., Ruan, X., Zhao, Y. X., Wei, F., & Wang, Q. 2018. Response of plant secondary metabolites to environmental factors. *Molecules* 23(4): 1–26.

Yusuf, A., Rahman, A. M. A., Zakaria, Z., Wahab, Z., & Kumar, S. V. 2018. Assessment of variability pattern of flesh color in 'harumanis' mango (Mangifera indica l.) from diverse perlis geographical origin. *Food Research* 2(6): 564–571.

Zhao, L., Zhang, H., White, J. C., Chen, X., Li, H., Qu, X., & Ji, R. 2019. Metabolomics reveals that engineered nanomaterial exposure in soil alters both soil rhizosphere metabolite profiles and maize metabolic pathways. *Environmental Science: Nano* 6(6): 1716–1727.

Zhou, J., Xie, G. and Yan, X. 2011. Encyclopedia of Traditional Chinese Medicines - Molecular Structures, Pharmacological Activities, Natural Sources and Applications. Berlin: Springer-Verlag, p.608.

Zivy, M., Wienkoop, S., Renaut, J., Pinheiro, C., Goulas, E., & Carpentier, S. 2015. The quest for tolerant varieties: the importance of integrating “omics” techniques to phenotyping. *Frontiers in Plant Science* 6*:* 448.