Materials Science Forum
ISSN: 1662-9752, Vol. 1025, pp 110-115

Submitted: 2020-07-30
Revised: 2020-10-30
Accepted: 2020-10-30
Online: 2021-03-30

Study on Soil Properties Towards Formation of High-Quality Agarwood Resin in *Aquilaria Crassna*

Mohd Syafik Mohamad Hamdan[1,a*], Mohd Azrul Hisham Ismail[1,b], Siti Hajar Noor Shaarani[1,c] and Saiful Nizam Tajuddin[1,d]

[1]Bio Aromatic Research Centre of Excellence, Universiti Malaysia Pahang, Gambang Campus, Lebuhraya Tun Abdul Razak, 26300 Kuantan, Pahang Darul Makmur, Malaysia

[a*]msyafik@ump.edu.my, [b]mohd.azrulh88@gmail.com, [c]hajarnoor@ump.edu.my, [d]saifulnizam@ump.edu.my

Keywords: Agarwood, inoculant, soil, gas chromatography, sesquiterpenes

Abstract. Agarwood has been used for its fragrance and medicinal properties in Asian culture for centuries. In recent years, agarwood gains its popularity in the west due to its usage in perfume formulation. Unfortunately, the supply does not meet the augmentation market demand. This is mostly because of depleting nature agarwood reservoir and lack of induction technique that can produce agarwood consistently in the plantation. In this study, we look into a case where artificial inducing technique successfully produced high quality agarwood. To assure its quality, agarwood chip was analysed by Gas Chromatography for its chemical profiles. Discovered compounds were identified as sesquiterpene group which also had been characterized as major agarwood compound listed on previous studies. Few compounds that are detected such as δ-cadinene (0.20%), jinkoh-eremol (22.09%), epi-α-cadinol (4.74%), agarospirol (3.75%) and others. Soil condition that contributes into this agarwood formation; soil analysis on physical properties, chemical properties and nutrients content of the soil have been analyzed. Based on the findings, soil condition is an important factor to successfully induced high quality agarwood.

Introduction

Agarwood (known as gaharu in Malaysia, chen xiang in China, jinkoh in Japan, agar in India and oud in the Middle East) is a resinous wood that formed in *Aquilaria spp*. (Family: Thymalaeaceae) which is native in East Asia. *Aquilaria* trees have four different species that have been used for agarwood production, *A. malaccensis* can be found in Malaysia, Indonesia and India, *A. sinensis* in China, *A. crassna* in Thailand and Vietnam, and *A. filaria* in Philippines and Papua New Guinea [1]. Agarwood is a product of *Aquilaria* tree self-defence system. *Aquilaria* tree secrete resinous substance as a protective barrier to defend and suppress invading pathogen or destructive agent. Many of invading pathogen in wild or cultivated agarwood has been identified as fungi [2][3][4]. *Aquilaria* tree wood is white, less dense and odourless when it is healthy, as the tree get infected and resin secreted into the wood, it turns to brown, dense and has strong odour. Agarwood is rare to form by itself and it is estimated that less than 10% of *Aquilaria* population in forest will form agarwood [1].

Agarwood is prized for its aroma and medicinal value. It has very strong and complex but pleasant smell and there is few or no other natural source that have similar smell [5]. The aroma produces soul relaxing effect and gain special attention in many religions including Islam, Buddha, Hindu and Christian [6][7]. High market price of agarwood resin encourages some peoples to hunt the natural agarwood in the forest. This leads to the over exploitation of most agarwood producing trees whereby they have been cut indiscriminately as there is no way of knowing for sure the formation of agarwood in the tree. There are some efforts internationally and locally to preserve *Aquilaria spp*. in the nature, most agarwood producing *Aquilaria spp.* has been listed in Appendix II (potentially threatened species) by the Convention on International Trade in Endangered Species of Wild Fauna and Flora in 2005.

Many native *Aquilaria spp*. countries such as Malaysia have organized campaigns to raise awareness on Agarwood value and encourage people to cultivate the tree. Despite, the efforts seem futile as many trees is still illegally being cut and smuggled as well as the essential oil trading is hard to be monitored. Tree cultivation also gaining low acceptance in community as there is no available effective technique that ensure high quality agarwood formation. Many inoculants available in the markets may kill trees instead of successful induced agarwood or can only produce low quality agarwood, thus many small cultivators do not dare to take risk using the available products.

This study aims to identify relationship of soil condition contributing in inducing high quality agarwood. Therefore, we report the physical, chemical and nutrient analysis of the collected soil samples as it is the key to relate soil condition with successful induction of high quality agarwood.

Materials and Method

Plant Material and Growth Condition. Sample of *Aquilaria crassna* in the case study was grown in Chini, Pahang, Malaysia by a local villager next to his house. The tree was 4 years old when it was induced. The tree was induced by using commercial inoculant for 10 months before harvested and carved. Multiple holes were drilled 2 inches deep and inserted with 10 ml of the inoculant liquid and were sealed with bamboo sticks. The surrounded soil was layered with 15 cm of Empty Fruit Bunch (EFB) biofertilizer for every 6 months.

Grading. Agarwood chip was graded based on color appearance using agarwood grading scale (Figure 1) provided by Forestry Department Peninsular Malaysia (FDPM).

Gas Chromatography (GC) Analysis. The samples were exposed to SPME fiber (a 50/30 µm DVB/CAR/PDMS – divinylbenzene/carboxy/polydimethylsiloxane) at 40 °C for 30 min for volatile headspace adsorption**.** Chemical profile for wood chip sample was analysed using an Agilent Technologies 7890A Gas Chromatography system equipped with a flame ionization detector (FID). The column used was DB1-ms [30 m x 0.25 mm, 0.25 µm film thickness]. An Agilent Technologies 7683B Series injector was used in split less mode. The carrier gas was (He) at a flow rate of 1.2 mL/min in constant flow mode. The injection temperature was 250°C. The oven temperature profile was 60°C for one min then to 230°C at 3°C/min with a 5 min hold at 230°C for wood sample. Peak area and retention times were measured by electronic integration and qualitative composition was obtained by peak normalization of GC/FID data.

Soil Sampling. 500g soil samples were collected at 10 cm (O horizon) and 20 cm (A horizon) around the agarwood tree as well as the layered soil and stored in sealed sampling bags. Samples were dried in the oven at 37°C for 12 hours. The samples then were ground, homogenized, and sieved with 2-mm siever to remove stones and debris to achieve uniform samples [8].

Soil Analysis. The prepared samples were used to determine the physic-chemical characteristics of the soil including; organic matter (OM) content, pH, electrical conductivity, total Nitrogen, available Phosphorus, exchangeable basic cations (Ca, Mg and K) and cation exchange capacity (CEC). Most analysis were carried out in accordance with Malaysian Standard 678. The pH was measured using Eutech Instruments pH 700 pH meter. Conductivity was measured with Hanna Instrument EC 215 conductivity meter [9]. Determination of organic matter content measured with Thermolyne 30400 Muffle Furnace and LECO Trumac CNS Analyzer. Total Nitrogen, available Phosphorus and CEC were measured with Flow Injection Analysis (FIA) Foss A/S FIAstarTM 5000 Analyzer Module. Determination of soil exchangeable basic cation (K+, Ca2+ and Mg2+) was measured with Perkin Elmer Analyst 100 Flame Atomic Absorption Spectrometer (AAS).

Results and Discussion

Quality Based on Appearance. There are three types of agarwood resin gred (A, B and C). The harvested agarwood chip was identified as Grade A based on agarwood grading scale provided by FDPM [10]. Woodchips obtained from the inoculated tree fulfill the criterias for high grade agarwood resins. The colours are black and dark brown which match with Grade A in the scale shown in Figure 1. The criterias that have been considered during this agarwood grading scale development such as colour, usage and other supporting criteria such as shape, size, density and fragrance.

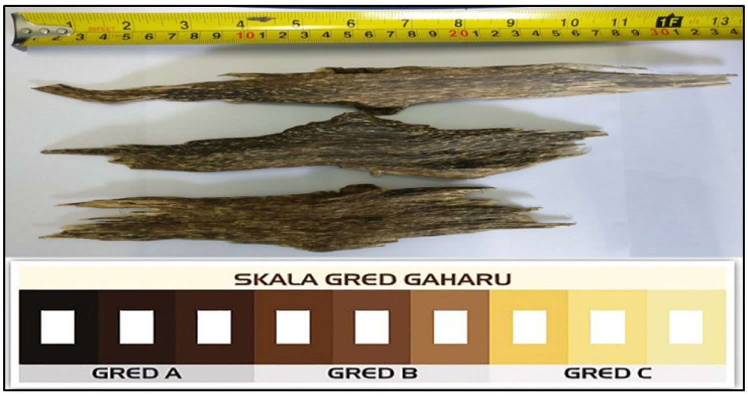

Figure 1. Comparison of agarwood chips with grading scale from FDPM grading manual 2015.

Quality Based on Chemical Profiles. Chromatographic analysis shows that the agarwood chips samples have high density of agarwood resins. GC-FID have been used to analyse the presence of compounds in the woodchip samples. It shows that the resins obtained from the inoculation process is high quality agarwood resins. The identified chemical compounds are listed in the Table 1. The highest peak was identified as jinkoh-eremol with abundance of 22.09%. Jinkoh-eremol is a sesquiterpene hydrocarbon and known as a major compound in agarwood based on previous study [11]. Other presence sesquiterpene compounds that are identified as major agarwood compounds are caryophyllene oxide, guaiol, agarospirol and eudesmol [12]. Thus, the presence of major agarwood compounds in the woodchip and high concentration of jinkoh-eremol supporting the wood chip is high quality. As reviewed by [7], tertiary valencene such as jinkoh-eremol and kusunol have been used as the marker compounds to identify agarwood quality in the *Aquilaria* species.

Table 1. Chemical composition of volatile *A. crassna* inoculated wood based on GC-FID analysis

Compounds	DB1	Relative peak area (%)
Sesquiterpene hydrocarbons		
δ-cadinene	1523	0.20
Oxygenated sesquiterpenes		
nor-ketoagarofuran	1558	1.42
caryophyllene oxide	1580	0.43
guaiol	1608	0.69
1,5-epoxy-no-ketoguaiene	1614	0.37
Agarospirol	1622	3.75
epi-α-cadinol	1639	4.74
jinkoh-eremol	1642	22.09
kusunol	1650	2.73
bulnesol	1657	1.20
dehydrojinkoh-eremol	1671	5.12
epi-α-bisabolol	1679	0.76
α-bisabolol	1685	1.29
selina-3,11-dien-9-ol	1718	3.11
9,11-eremophiladien-8-one	1741	1.36
guaia-1(10),11-diene-9-one	1752	0.83
Selina-4,11-dien-14-al	1763	2.22
guaia-1(10),11-dien-15-ol	1768	0.28
selina-3,11-dien-14-oic acid	1775	0.19
dihydrokaranone	1795	3.00
guaia-1(10),11-dien-15-al	1802	1.09
karanone	1814	2.25
oxo-agarospirol	1822	2.77
eudesmol	1862	6.66
Sesquiterpene hydrocarbon (%)		**0.20**
Oxygenated sesquiterpenes (%)		**68.35**
Total area (%)		**68.55**
Number of compounds		**24**

ND-Non Detected; Id KI – Identified Kovat Index ; Ref. KI – Reference Kovat Index [22].

Soil Analysis. Soil sample that have been obtained from plot area of inoculated trees have been analyzed and determined its electrical conductivity, organic matter, cation exchange capacity (CEC), available phosphorus, pH, total nitrogen and available nutrients content. Results for the analysis shown in Table 2.

Table 2. Soil Analysis Parameters

Sample	Electrical Conductivity (mmho cm^{-1})	Organic Matter (%)	CEC (meq/ 100g)	Available P (mg kg^{-1})	pH	Total N (%)	Mg^{2+} Saturation (%)	K^{+} Saturation (%)	Ca^{2+} Saturation (%)
O Horizon	0.773	1.62	1.62	5	5.95	0.11	5	3	11
A Horizon	0.452	1.31	1.31	4	6.67	0.09	10	7	24
EFB	0.761	6.38	6.38	31	6.40	0.24	25	3	34

Physical properties. Soil contained low amounts of organic matter (OM) content at 1.62% (O horizon) and 1.31% (A horizon). The OM content is decrease with depth. The low OM is due to the new layered soil during the house construction. Organic matter will help in better holding water and nutrients in the soil. According to [13], soil organic matter play major role as it is required to reflect the quality and quantity of soil controlling primary productivity and also being part of biogeochemical cycles of major nutrient elements. As the soil was new layer to the area, the mineral content and organic matter will not be the same as other area. Besides, low OM also might due to other factor such as leaching. Based on [14], OM classification in both soils are categorized low to medium class (<10%). The low value of organic matter indicates the soil is subjected to the leaching.

Chemical properties. The pH values are 5.95 and 6.67 for 10 cm and 20 cm soil respectively. 10 cm and 20 cm soil pH values are categorized moderately acidic and normal according to Burt et. al [15]. 10 cm soil is low in carbonate but satisfactory for most plant while 20 cm soil is in ideal range for most crops. The low pH is one of unique features of Malaysia's soil [16]. On the other hand, the soil samples have moderately acidic and neutral pH which might be due to the frequent application of the EFB fertilizer which has neutral pH itself. For 10 cm soil, pH value showed lower than 20 cm value could be due to leaching. The value of electrical conductivity was 0.773 mmho cm^{-1} for 10 cm soil and 0.452 mmho cm^{-1} for 20 cm soil. Based on [9], 10 cm and 20 cm soil electrical conductivity are classified as high and medium respectively which satisfactory for most plant growth. 10 cm soil high conductivity value attributed to frequent fertilization applied on the land plot. The Cation Exchange Capacity (CEC) value for 10 cm and 20 cm soils are 1.62 meq/100g and 1.31 meq/100g respectively. The value decreases with depth which may attribute to higher OM content in the 10 cm soil and categorized as low [14].

Available nutrients. The amount of available phosphorus in 10 cm and 20 cm are 5 and 4 mg kg^{-1} respectively. According to [17], the 10 cm value is scaled as moderate which suitable for plant growth and 20 cm value is scaled as low but satisfactory for most plant. The value of total nitrogen was 0.11% in 10 cm and 0.09% in 20 cm. The values are categorized as normal for mineral soils and suitable for plant growth [18]. The value of calcium ion saturation in 10 cm and 20 cm are 11% and 24% respectively. Both values are very low compare to the ideal value which are 70-90% and 40% for highly weathered soil [29]. The low values attributed to the low value of calcium ion saturation from the applied EFB fertilizer. The value of magnesium ion saturation in 10 cm and 20 cm is 5% and 10% respectively. 10 cm value is categorized as low and 20 cm value is ideal based on the guideline. The value of potassium ion saturation in 10 cm and 20 cm are 3% and 7% respectively. Based on the guide 10 cm value is classified as low and 20 cm is ideal for plant growth. All ion saturation appeared higher with soil depth due to the nutrient leaching.

Conclusion

The land plot soil has low organic matter and was leached. It has neutral pH, moderate electrical conductivity and very low CEC. The available nutrient in the soil is satisfactory. Available phosphorus is moderate and total nitrogen is normal. Calcium ion saturation is low, magnesium ion saturation is ideal and potassium ion saturation is ideal. Analysis of data collected suggested the land plot soil is fertile and satisfactory for plant growth. Very low CEC indicated occurrence of nutrients leaching but analysed data show satisfactory level of nutrients in soil. This occurrence is attributed to frequent application of fertilizer by the land plot owner. This study might suggest the importance of fertile soil to form high quality agarwood. Fertile soil allow tree to grow healthily and fight invading pathogen by producing protective resin during inoculation period.

Acknowledgement

This study was funded by University-Industry Collaboration (UIC) Grant (UIC160903), Universiti Malaysia Pahang. The authors fully acknowledged Universiti Malaysia Pahang for the approved fund which makes this important research viable and effective.

References

[1] Akter, S., Islam, M. T., Zulkefeli, M., Khan, S., Agarwood Production - A Multidisciplinary Field to be Explored in Bangladesh, International Journal of Pharmaceutical and Life Sciences. 2(1) (2013) 22-32.

[2] Mohamed, Rozi., Jong, Phai., Abd Kudus, Kamziah., Fungal inoculation induces agarwood in young *Aquilaria malaccensis* trees in the nursery, Journal of Forestry Research. 25 (2014) 201-204.

[3] Mohamed R., Jong PL., Nurul Irdayu I., Succession patterns of fungi associated to wound-induced agarwood in wild *Aquilaria malaccensis* revealed from quantitative PCR assay, World J Microbiol Biotechnol. 30 (2014) 2427-2436.

[4] Tan, J.-J., X.-X. Gao, W.-M. Zhang, W. Lei, and L.-H., Molecular identification of endophytic fungi from *Aquilaria sinensis* and artificial agarwood induced by pinholes-infusion technique, African J. Biotechnol. 12 (2013) 3115–3131.

[5] Sen, S., Dehingia, M., Talukdar, N. et al., Chemometric analysis reveals links in the formation of fragrant bio-molecules during agarwood *(Aquilaria malaccensis)* and fungal interactions, Sci Rep. 7 (2017) 44406.

[6] Lim, T. and A. Noorainie, Wood for the Trees: a review of the agarwood (gaharu) trade in Malaysia, TRAFFIC Southeast Asia. 2010.

[7] Naef, R., The volatile and semi-volatile constituents of agarwood, the infected heartwood of Aquilaria species: A review, Flavour and Fragrance Journal. 26 (2011) 73–87.

[8] Mehta, R., P. Kumar, S. Goyal., Chemo Toxicity Investigation and Physio Chemical Analysis of Composite Samples of Chemical Waste Effluent with Soil, International Journal of Information Research and Review, 3 (2016) 2115–2117.

[9] Radojevic, M., V. N. Bashkin, Practical Environmental - Analysis, 2nd ed. Cambridge, UK: The Royal Socety of Chemistry. 2006.

[10] Forestry Department Peninsular Malaysia, Manual in Grading of Agarwood, Alamedia Sdn. Bhd., Selangor, Malaysia. 2015.

[11] Tajuddin, S. N., M. M. Yusoff, Chemical composition of volatile oils of *Aquilaria malaccensis* (Thymelaeaceae) from Malaysia, Natural Product Communications. 5 (2010) 1965–1968.

[12] Liu, Y., J. Wei, Z. Gao, Z. Zhang, J. Lyu., A Review of Quality Assessment and Grading for Agarwood. Chinese Herb. Med. 9 (2017) 22–30.

[13] Burke, I. C., C. M. Yonker, W. J. Parton, C. V. Cole, K. Flach, D. S. Schimel., Texture, Climate, and Cultivation Effects on Soil Organic Matter Content in U. S. Grassland Soil, Soil Science Society of America Journal. 53 (1989) 800–805.

[14] Folland, C. J., M. S. K. Acres, B.D., R.P. Bower, P.A. Borrough, The soils of Sabah. Classification and Description, Land Resource Study No. 20, Land Resource Division, Volume 1. Ministry of Overseas Development, England. 1975.

[15] Burt, R., Kellogg Soil Survey Laboratory Methods Manual. Soil Survey Investigations Report No. 42, Version 5.0. U.S. Department of Agriculture, Natural Resources Conservation Service, Lincoln, Nebraska. 2014.

[16] Gasim, M. B., B. S. Ismail, S.-I. Mir, S. Abd. Rahim, M. E. Toriman, The Physico-chemical Properties of Four Soil Series in Tasik Chini, Pahang, Malaysia, Asian J. Earth Sci. 4 (2011) 75-84.

[17] The Potash Development Association, Soil analysis: Key to Nutrient Management Planning, Potash Development Association, United Kingdom. 2011.

[18] Jones, J. B., Laboratory Guide for Conducting Soil Tests and Plant Analysis. CRC Press LLC, New York, US. 2001.