

**VARIATION OF NATURALLY AND
ARTIFICIALLY INDUCED AGARWOOD
RESIN CONTENT AND QUALITY OF *Gyrinops
walla* FOR COMMERCIAL EXTRACTION AND
ITS SEED GERMINATION**

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**Variation of Naturally and Artificially Induced
Agarwood Resin Content and Quality of *Gyrinops walla*
for Commercial Extraction and its Nursery
Establishment**

By

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DECLARATION

The work described in this thesis was carried out by me under the supervision of Dr. S.M.C.U.P. Subasinghe, Senior Lecturer, Department of Forestry and Environmental Science, University of Sri Jayewardenepura, Sri Lanka and Dr. D.S. Hettiarachchi, Research Scientist, Wescorp Group of Companies, 26, Coulson Way, Canning Vale, WA, Australia and a report on this has not been submitted in whole or in part to any university or any other institution for another degree / diploma


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This thesis is dedicated
to
my loving husband
Tharindu
and
my beloved parents
for their love, continued encouragement
and
tremendous support in every step that I make in my life...

TABLE OF CONTENTS

<i>List of figures</i>	<i>vi</i>
<i>List of tables</i>	<i>ix</i>
<i>Acknowledgement</i>	<i>xi</i>
<i>Abstract</i>	<i>xiii</i>
CHAPTER ONE: INTRODUCTION	1
CHAPTER TWO: LITERATURE REVIEW	6
2.1 General characteristics of agarwood.....	6
2.1.1 History of agarwood.....	7
2.2 Commercial uses of agarwood.....	8
2.2.1 Aroma production by burning.....	8
2.2.2 Traditional medicine.....	9
2.2.3 Perfume.....	10
2.2.4 Incense sticks.....	10
2.2.5 Food commodities.....	11
2.2.6 Other uses.....	12
2.3 Agarwood resin forming genera.....	13
2.3.1 <i>Aquilaria</i>	13
2.3.2 <i>Gonystylus</i>	19
2.3.3 <i>Aetoxylon</i>	20
2.3.4 <i>Gyrinops</i>	20
2.4 <i>Gyrinops walla</i>	21
2.4.1 Botanical description.....	21
2.4.2 Distribution.....	23
2.5 Chemistry of agarwood.....	25
2.5.1 Properties of essential oil.....	25
2.5.2 Physical properties.....	25
2.5.3 Chemical properties.....	25

2.6	Agarwood resin formation.....	26
2.7	Agarwood resin induction.....	28
2.7.1	Debarking.....	29
2.7.2	Drilling.....	30
2.7.3	Nailing.....	31
2.7.4	Aeration.....	32
2.7.5	Biological inoculation.....	33
2.7.6	Coppicing.....	34
2.7.7	Chemical methods.....	35
2.8	Agarwood resin extraction methods.....	36
2.8.1	Hydro-distillation.....	37
2.8.2	Steam distillation.....	40
2.8.3	Solvent extraction.....	41
2.8.4	Supercritical fluid carbon dioxide extraction.....	41
2.9	Resin content variation.....	42
2.10	Volatile components of agarwood resin.....	43
2.11	Agarwood oil analysis techniques.....	46
2.11.1	Electronic nose.....	46
2.11.2	Gas chromatography methods.....	47
2.11.3	Solid phase micro extraction (SPME).....	49
2.12	Grading of agarwood resins.....	50
2.13	Agarwood seed germination, nursery establishment and operations.....	54
2.13.1	Viability and germination of seeds.....	54
2.13.2	Seed storage.....	57
2.13.3	Nursery establishment.....	60
2.13.4	Nursery operations.....	61
2.13.5	Seed dormancy.....	66
2.13.6	Seed pre-treatment.....	69

CHAPTER THREE: MATERIALS AND METHODS.....	73
3.1 Identify agarwood resin content (w/w) and quality between <i>G. walla</i> trees and between different geographical regions.....	73
3.1.1 Sampling and data collection.....	73
3.1.2 Tree and geographical measurements.....	74
3.1.3 Determination of resin content.....	75
3.1.4 Determination of resin constituents.....	77
3.2 Identify relationships between resin content and constituents with tree parameters.....	78
3.3 Identify most suitable traditional agarwood resin inducement methods by comparing resin content and quality.....	78
3.3.1 Study area.....	78
3.3.2 Traditional inoculation methods used.....	78
3.3.3 Inoculation methods.....	80
3.3.4 Observations made and tissue sample collection.....	81
3.3.5 Determination of resin content and its constituents of agarwood resins formed due to traditional inoculation methods.....	82
3.4 Identify <i>Gyrinops walla</i> seed germination with different storage time.....	82
3.4.1 Seed collection and storage.....	82
3.4.2 Preparation of germination beds and seed sowing.....	83
3.4.3 Determination of germination percentage.....	84
3.4.4 Statistical Analysis.....	85
3.5 Identify <i>Gyrinops walla</i> seed germination with different treatments.....	85
3.5.1 Seed collection, storage and treatments.....	85
3.5.2 Statistical Analysis.....	86
3.6 Identify the optimum soaking method and hydro-distillation time for resin extraction.....	86
3.6.1 Sample collection and preparation.....	86
3.6.2 Determination of the optimum tissue mass: water ratio and soaking time	87
3.6.3 Determination of the optimum distillation time period.....	88
3.6.4 Determination of resin content and its constituents.....	89

CHAPTER FOUR: RESULTS.....	90
4.1 Identify agarwood resin content (w/w) and quality between <i>G. walla</i> trees and between different geographical regions.....	90
4.1.1 Variation of <i>Gyrinops walla</i> tree parameters.....	90
4.1.2 Variation of agarwood resin contents.....	91
4.1.3 Variations of tissue colours and extracted resin colours.....	92
4.1.4 Variation of resin constituents.....	93
4.2 Identify relationships between resin content and constituents with tree parameters.....	98
4.2.1 Correlation of resin content with tree parameters.....	98
4.2.2 Correlation of resin constituents with tree parameters and resin content..	98
4.3 Impacts of traditional agarwood resin induction methods.....	101
4.3.1 Variation of resin contents, tissue and resin colours due to different traditional methods.....	101
4.3.2 Variation of resin constituents due to different traditional methods.....	103
4.4 Identify <i>Gyrinops walla</i> seed germination with different storage time.....	109
4.4.1 Seed characteristics.....	109
4.4.2 Effect of storage conditions and storage period on seed germination percentage.....	109
4.5 Identify <i>Gyrinops walla</i> seed germination with different treatments.....	113
4.5.1 Seed characteristics.....	113
4.5.2 Effect of storage conditions and pre-sowing treatments.....	113
4.6 Identify the optimum soaking method and hydro-distillation time for resin extraction.....	118
4.6.1 Variation of colours of agarwood resins extracted by hydro-distillation	118
4.6.2 Variation of resin contents extracted with different soaking times and tissue mass: water ratios.....	118
4.6.3 Variation of hydro-distilled resin volume in different distillation time periods.....	119
4.6.4 Variation of resin constituents in different soaking distillation experiments.....	120

CHAPTER FIVE: DISCUSSION.....	128
5.1 Identify resin agarwood quantity and quality of <i>G. walla</i> growing in different locations.....	129
5.1.1 Variation of <i>Gyrinops walla</i> tree parameters.....	129
5.1.2 Variation of resin contents.....	129
5.1.3 Variations of tissue colours and extracted resin colours.....	130
5.1.4 Variation of resin constituents.....	132
5.2 Identify relationships between resin content and constituents with tree parameters.....	134
5.3 Identify most suitable traditional agarwood resin induction method.....	135
5.3.1 Variation of resin contents, tissue colours and resin colours between different traditional methods.....	135
5.3.2 Variation of resin constituents of agarwood resin formed due to different traditional methods.....	137
5.4 Identify <i>Gyrinops walla</i> seed germination with seed storage time.....	139
5.5 Identify <i>Gyrinops walla</i> seed germination with different treatments.....	140
5.6 Identify the optimum soaking method and hydro-distillation time for resin extraction.....	142
5.6.1 Variation of agarwood resin contents after different soaking times and tissue mass: water ratios.....	142
5.6.2 Variation of colours of agarwood resins extracted by hydro-distillation.....	143
5.6.3 Variation of agarwood resin volumes in different hydro-distillation time periods.....	144
5.6.4 Variation of resin constituents in hydro-distillation.....	144
CHAPTER SIX: CONCLUSIONS.....	146
CHAPTER SEVEN: RECOMMENDATIONS.....	149
REFERENCES.....	151
ANNEXES.....	172

LIST OF FIGURES

Figure 2.1:	Illustrative map of global distribution of agarwood forming species....	15
Figure 2.2:	Capsule and seeds of five commonly cultivated <i>Aquilaria</i> species.....	18
Figure 2.3:	Botanical features of <i>Gyrinops walla</i>	23
Figure 2.4:	Distribution of <i>G. walla</i> in Sri Lanka.....	24
Figure 2.5:	Debarking of a tree stem.....	29
Figure 2.6:	Drilling the tree stem.....	30
Figure 2.7:	Nails inserting spirally in a tree trunk.....	31
Figure 2.8:	Plastic tubes inserted as aeration device to induce agarwood.....	32
Figure 2.9:	Application of the fungal inoculation technology on an agarwood producing tree at a community forest.....	34
Figure 2.10:	Hydro distillation process in extracting agarwood resin.....	38
Figure 2.11:	Boiler operated steam distillation unit.....	40
Figure 2.12:	Skeleton of hydrocarbons found in volatile agarwood resin.....	44
Figure 2.13:	Skeleton of oxygenated sesquiterpenes found in agarwood resin.....	44
Figure 2.14:	Different types of agarwood pieces and its imitation.....	50
Figure 2.15:	Broadcasting of rice seeds.....	63
Figure 2.16:	Hand dibbling of seeds.....	64
Figure 2.17:	Seed drill.....	65
Figure 2.18:	Key to seed dormancy types.....	68
Figure 3.1:	Extracting the dark colour tissues using chisel and hammer.....	74
Figure 3.2:	Size reduced, oven dried resinous tissues.....	75
Figure 3.3:	Apparatus of the solvent extraction method.....	76
Figure 3.4:	Filtering the extraction to the amber colour vial.....	76
Figure 3.5:	Extracted natural resin.....	76

Figure 3.6: DANI GC 1000 [®] Gas Chromatography Instrument with HT3000A [®] Auto Sampler.....	77
Figure 3.7: Study areas where sample collected.....	79
Figure 3.8: Traditional inoculation methods tested for <i>G. walla</i>	80
Figure 3.9: Inoculation pattern in a tree stem.....	81
Figure 3.10: Sampling technique.....	81
Figure 3.11: <i>G. walla</i> seeds separate for each treatment.....	82
Figure 3.12: Measuring seed diameter by vernier caliper.....	83
Figure 3.13: Seeds sown in the sand bed.....	84
Figure 3.14: Watering to the germination beds.....	84
Figure 3.15: Mixing of pulverized tissues to obtain an even sample.....	87
Figure 3.16: Grounded tissue particles were soaked in water.....	88
Figure 3.17: Hydro distillation apparatus.....	89
Figure 4.1: Distribution of average resin content % (\pm SE).....	91
Figure 4.2: Gas chromatographic fingerprint of <i>G. walla</i> extract from Maliboda, MB-04 sample.....	94
Figure 4.3: Gas chromatographic fingerprint of <i>G. walla</i> extract from Neboda, NB-10 sample.....	94
Figure 4.4: Composition of major sesquiterpenes, fatty acids and 2-(2-phenylethyl) chromones (+ SE).....	97
Figure 4.5: Average resin content variation at the inoculation point, at 10 cm above and 10 cm below (+ SE).....	103
Figure 4.6: Gas chromatographic fingerprint of agarwood resins inoculated with NaCl.....	105
Figure 4.7: Gas chromatographic fingerprint of agarwood resins inoculated with iron nails.....	105
Figure 4.8: Variations of major resin constituents present in agarwood resins after traditional inoculations (+ SE).....	108
Figure 4.9: Effect of <i>G. walla</i> seeds storage conditions and storage period on seed germination percentage after 16 weeks.....	110

Figure 4.10: Seed germination by the direct sowing method.....	111
Figure 4.11: Seed storage vs. germination test trial.....	111
Figure 4.12: Effect of storage temperature and storage period on seed cumulative germination percentage of <i>G. walla</i> seeds.....	112
Figure 4.13: Effect of seed treatments after two weeks storage of <i>G. walla</i> seeds.....	114
Figure 4.14: Effect of seed treatments with six weeks storage.....	115
Figure 4.15: Control of the seed treatment vs. germination test trial.....	116
Figure 4.16: Seeds germinated in 0.1% citric acid treatment after six months storage.....	116
Figure 4.17: Effect of pre-sowing treatment, storage temperature and storage period on seed germination percentage of <i>G. walla</i> seeds.....	117
Figure 4.18: Distribution of average resin content percentages (w/w) between soaking time.....	119
Figure 4.19: Gas chromatographic fingerprints of a <i>G. walla</i> hydro-distilled resin sample soaked for seven days at 1:15 tissue:water ratio.....	125
Figure 4.20: Gas chromatographic fingerprints of a <i>G. walla</i> hydro-distilled resin sample soaked for nine days at 1:20 tissue: water ratio.....	126
Figure 4.21: Composition of major resin constituents identified in hydro-distilled resin samples of <i>G. walla</i> tissues under different soaking-distillation methods (+SE).....	127

LIST OF TABLES

Table 2.1:	Accepted names, distribution, and conservation status of <i>Aquilaria</i> species.....	16
Table 2.2:	Vegetative and reproductive characteristics of cultivated <i>Aquilaria</i> species.....	17
Table 2.3:	Commonly used agarwood grades found in the Malaysian market.....	52
Table 3.1:	Location description of the samples collected.....	73
Table 3.2:	Parameters assessed and instruments used for the sampled trees.....	74
Table 3.3:	Optimum soaking and distillation plan.....	88
Table 4.1:	Summary of the mean values of tree parameters (\pm SE).....	90
Table 4.2:	Variations of tissue colour and resin colour with Munsell colour codes.....	92
Table 4.3:	Variation of resin constituents identified in <i>G. walla</i> trees of Selected populations.....	95
Table 4.4:	Summary of the correlation test of resin contents with tree parameters at 95% probability level.....	98
Table 4.5:	Correlations of resin content and constituents.....	100
Table 4.6:	Variations of resin content, resinous tissue colour and extracted resin colour with Munsell colour codes.....	101
Table 4.7:	Summary of GLM ANOVA test of resin content with inoculation treatment, sample collection point and their interaction.....	102
Table 4.8:	Variation of resin constituents in agarwood resins formed due to different traditional methods.....	106
Table 4.9:	Effect of storage temperature, storage periods and their interaction on seed germination in <i>G. walla</i>	109
Table 4.10:	Effect of storage temperature, storage duration, sowing treatments and their interaction on seed germination in <i>G. walla</i>	113
Table 4.11:	Variations of hydro-distilled resin colours with Munsell colour codes.....	118

Table 4.12:	Hydro-distilled resin volume variation in each treatment in different distillation time periods.....	120
Table 4.13:	Composition variation of constituents identified in <i>G. walla</i> hydro-distilled resin samples.....	123
Table 4.14:	Effect of resin constituents, soaking time periods, tissue:water ratios and their interaction on hydro-distillation treatments in <i>G. walla</i>	122

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WATUTHANTIRIGE NILUKA HASINI DE ALWIS

ABSTRACT

Gyrinops walla, a member of the family Thymelaeaceae produces a valuable resin called agarwood which is used for religious, cultural and medicinal purposes in many countries. Agarwood is formed as a result of a self-defense mechanism towards a stress caused by several factors which can be physical, chemical and biological. The ability of agarwood production in *G. walla* was discovered in 2012 and adequate information is still lacking on plantation establishment, agarwood inducement and resin extraction for this species. Therefore the present study was conducted to identify: the variation of the contents and qualities of the agarwood resins formed due to natural causes in *G. walla*; most effective low cost traditional agarwood resin inducement methods; the process of nursery establishment based on the seed storage and pre-sowing treatments; and the best agarwood resin extraction process by further developing hydro-distillation process.

150 *G. walla* trees growing naturally in nine different geographical locations of wet zone, Sri Lanka: viz., Horawala, Kalatuwawa, Karandana, Maliboda, Neboda, Yagirala, Mirigama, Kalawana and Suriyakanda, were selected for the present study. *G. walla* trees growing in the selected areas were carefully observed for the stem damages and wounds. Collected agarwood resinous tissues of the damaged areas in non-destructive manner were size reduced and the resins extraction was done using solvent extraction method. Resin constituent analysis was done using GC-FID method. Pearson correlation was done to identify the relationships between naturally produced resin content and constituents with tree diameter and total height. Seven types of

traditional inoculation methods were tested with healthy non-infected *G. walla* trees growing in Kalawana and Mathugama Divisional Secretariat Divisions to identify the most effective inoculation method. Agarwood formed tissues were collected after 12 months of inoculation and resinous tissues were extracted by solvent extraction and constituent analysis was done by GC-FID method. Fresh, healthy *G. walla* seeds were collected from Mathugama Divisional Secretariat Division to identify the potential of storage. Seeds were stored in three temperature levels viz., room temperature, 8° C and (-)10° C and six storage durations up to six weeks. Stored seeds were sown at weekly intervals and direct sowing was done without storing as the control. Six types of pre-treatments with controls were also tested in the present study. Five methods were used for this purpose for *G. walla* seeds stored at three temperature levels, viz., room temperature, 8° C and (-)10° C for two storage durations, viz., two and six weeks. In order to identify optimum conditions to extract agarwood by hydro-distillation, agarwood formed tissues were separated from *G. walla* trees of Mirigama Divisional Secretariat Division. These tissues were size reduced to 5-10 mm and mixed evenly to obtain a uniform sample. Two tissue mass to water ratios (1:15 and 1:20) with six soaking days (3, 5, 7, 9, 11, 13) were tested for this experiment. Based on a pre-test, hydro-distillation was continued to 72 hours, while taking resin volume measurements at 24 hour interval. Resin constituents were identified using GC-FID method.

Average resin content of the naturally formed agarwood was varied from 2.19% to 4.92%, however it was not significantly different between selected nine locations, although higher resin content variations were found within and between populations. 21 different constituents were identified in naturally formed agarwood resins of *G. walla* which belonged to seven classes, viz., vetispirane, selinene, cardinane, guaiene, eremophilane, 2-(2-phenyl)-chromone and fatty acids. It was not possible to build mathematical relationships between resin content and resin constituents with tree parameters as resin content and most constituents were not significantly correlated with tree diameter and height. A significant difference ($F=5.50$; $p=0.000$) was found of

resin contents between different tested traditional inoculation methods. Among these methods, the highest average resin content ($4.38\% \pm 0.41$) was recorded from the trees inoculated with sodium chloride. In the traditionally induced agarwood resins of *G. walla*, 28 different constituents of nine classes were identified. Trees inoculated with sodium chloride showed the most important resin constituents, viz., phenyl butanone, agarofuran, agarospirol, guaienes, eremophilanes, valencane and phenyl chromone derivatives.

Most *G. walla* seeds started their germination within one to two weeks after sowing. Direct sowing method showed the highest germination percentage (73.3%), while seeds stored at 8° C for two and four weeks showed the second highest germination percentage (26.7%). The results confirmed that *G. walla* seeds are sensitive to the desiccation and therefore cannot be stored for a long period. Results of the pre-sowing treatment showed the highest germination (46.7%) was recorded from the control, viz., direct sowing without storage and pre-treatment which was followed by 40.0% of germination after treating with 0.05% gibberellic acid after two weeks of storage at room temperature.

Optimum conditions to extract agarwood by hydro-distillation were soaking tissues for nine days in 1:20 tissue to water ratio, distilled for 72 hours continuously, which gave the highest average resin content of 0.057%. 21 different constituents with six classes of sesquiterpenes, fatty acid and 2-(2-phenylethyl)-chromone derivatives were found in agarwood resins of *G. walla* extracted by hydro-distillation. Agarospirol and agarofuran were present in all agarwood resins of hydro-distilled samples.

According to the findings, the Maliboda population had the highest average resin content, where Suriyakanda population had the highest agarospirol content. The seeds of *G. walla* were found to be recalcitrant.