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

WATUTHANTIRIGE NILUKA HASINI DE ALWIS

M.Phil **2017**

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

By

Watuthantirige Niluka Hasini de Alwis

Thesis submitted to the University of Sri Jayewardenepura for the award of the Degree of Master of Philosophy in Forestry on 30th June 2017

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 anther degree / diploma

.....

W.N.H. de Alwis

Date: 28. 11. 2018.

CERTIFICATE OF APPROVAL

We certify that the above statement made by the candidate is true and this thesis is suitable for submission to the University for the purpose of evaluation.

Signature : .....

Internal Supervisor

Prof. S.M.C.U.P. Subasinghe

Head,

Department of Forestry and Environmental Science,

University of Sri Jayewardenepura,

Sri Lanka

Date : 28/11/18

Signature : .....

External Supervisor

Dr. D.S. Hettiarachchi

Research Scientist,

Wescorp Group of Companies,

26, Coulson Way,

Canning Vale, WA,

Australia

Date : 22nd November 2018

We certify that all the corrections, additions and amendments have been done in accordance with the comments and suggestions made by the members of the viva-voce examination.

Signature : .....

Internal Supervisor

Prof. S.M.C.U.P. Subasinghe

Head,

Department of Forestry and Environmental Science,

University of Sri Jayewardenepura,

Sri Lanka

Date : 28/11/18

Signature : .....

External Supervisor

Dr. D.S. Hettiarachchi

Research Scientist,

Wescorp Group of Companies,

26, Coulson Way,

Canning Vale, WA,

Australia

Date : 22nd November 2018

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

LIST OF FIGURES

LIST OF TABLES

ACKNOWLEDGEMENT

Firstly, I would like to express my sincere gratitude to my Supervisor Prof. S.M.C.U.P. Subasinghe, Head, Department of Forestry and Environmental Science, University of Sri Jayewardenepura for the continuous support and advice to complete this study. I also deeply appreciate the valuable guidance and knowledge given by my External Supervisor, Dr. D.S. Hettiarachchi, Research Scientist, Wescorp Group of Companies, Australia to complete the study.

This study would not have been possible without the financial support of the National Research Council of Sri Lanka and Sadaharitha Plantations Limited under the Public-Private-Partnership Program (PPP 12-57). My sincere thanks also goes to Mr. Sathis Nawarathne, Chairman of Sadaharitha Plantations Limited, for providing me an opportunity to conduct this study as a Research & Development Executive, and for providing nursery facilities for seed experiments.

I gratefully acknowledge Dr. Prashanthi Gunawardene, and Prof. Nilanthi Bandara, Former Heads of the Department of Forestry & Environmental Science for allowing me to use the laboratory facilities in the Department. Thanks are also extended to Dr. Asitha Cooray, Coordinator of the Central Instrumental Facility of Faculty of Applied Sciences, University of Sri Jayewardenepura for providing access to the GC-MS facilities.

My thanks are also extended to Mr. Chathura Karunathunga, Manager of the Agarwood Project, Mr. Janath Erandaka, Forestry Manager, Mr. Kanchana Waduge, Assistant Manager

of the Agarwood Nursery, Sadaharitha Plantations for supporting my study in numerous ways by providing transport, labour facilities and *G. walla* seed samples.

I also take this opportunity to express my gratitude to Mr. I.D. Wijesinghe, Mr. S. Weththasinghe, Senior Technical Officers of Department of Forestry and Environmental Science, University of Sri Jayewardenepura, Mr. G.D. Nishantha, Caretaker of Yagirala Forest Reserve and all other non-academic staff members of the Department of Forestry and Environmental Science for their support rendered in many ways to complete this research project in successful manner. I am also thankful to Mr. K.S. Wijenayake, Senior Technical Officer, Department of Zoology and Mrs. R.M. Munasinghe, Technical Officer of Central Instrumental Facility for their support.

Special thanks are also extended to Ms. H.I.D. Hitihamu, Ms. Upekha Mandakini, Ms. K.P. Millaniayage, Mr. C.I. Deshapriya, Ms. S.A.Y. Medhavi, Ms. A.A.P. Dias, Ms. S. Vithanage and Ms. A.N.G.C.K. Vidurangi and all my friends supported me in numerous ways to complete this study.

Last but not least I would like to thank my parents, my husband and my family for their continuous encouragement and support given throughout to complete this research successfully.

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

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^{o} C and (-)10^{o} 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^{o} C and (-)10^{o} 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%±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^{o} 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.