UNIVERSITI PUTRA MALAYSIA

EFFICACY OF FUNGICIDE AGAINST MOULD (FUSARIUM SOLANI SACC.) AND STAIN (LASIODIPLODIA THEOBROMAE PAT.) FUNGI ON FOUR CLONES OF HEVEAWOOD

NORIDAH OSMAN

FH 2001 15

EFFICACY OF FUNGICIDE AGAINST MOULD (*FUSARIUM SOLANI* SACC.) AND STAIN (*LASIODIPLODIA THEOBROMAE* PAT.) FUNGI ON FOUR CLONES OF HEVEAWOOD

By

NORIDAH OSMAN

Thesis Submitted in Fulfilment of the Requirement for the Degree of Master of Science in Faculty of Forestry Universiti Putra Malaysia

March 2001

DEDICATION

Inspiration & Aspiration

IN LOVING MEMORY of MY LATE BELOVED FATHER
ARWAHYARHAM OSMAN B. HASHIM
THE FOUNDATION OF MY ACADEMIC CAREER,

MY LATE BELOVED GREAT GRANDMOTHER
ARWAHYARHAMAH SAENAH BT. TAHA,

AND

To my mother, RUSIAH@RASEAH JALIL; my grand mother,
JERIAH PERAL; my brother, IKWAN OSMAN;
my sister FARY AKMAL OSMAN; my youngest brother,
LOKMAN HAKIM OSMAN; my youngest sister,
TEH ZAWAHIR OSMAN, my antie, my uncle,
ROHIZA ATAN & READZUAN YUSOF

Abstract of thesis presented to the Senate of the Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

EFFICACY OF FUNGICIDE AGAINST *FUSARIUM SOLANI* SACC. AND *LASIODIPLODIA THEOBROMAE* PAT. FUNGI ON FOUR CLONES OF HEVEAWOOD

By

NORIDAH OSMAN

March 2001

Chairman : Zaidon Ashaari, Ph.D

Faculty : Forestry

New formulations of safe preservatives are being searched to treat heveawood to resolve discolouration problem so as to preserve and protect its original colour.

Sodium pentachlorophenate (NaPCP) has been widely used to control stain fungi on heveawood. However, this substance has been phased out due to its mamalian hazardous. This study was conducted to determine the efficacy of Evotek® 230 SE against wood staining fungi on four clones of heveawood.

A formulation of Evotek® 230 SE was tested at various concentrations to control wood staining fungi in four clones of heveawood. The solution concentrations used in this study were 0.1, 0.25 and 0.5 %. A concentration level of 0.5 and 1% for sodium pentachlorophenate was used as a standard.

Laboratory studies using freshly cut heveawood blocks, demonstrated the anti-fungal properties of Evotek® 230 SE. This formulation was effective for protection against stain fungi at a low level (0.1%) while sodium pentachlorophenate was effective only at 1.0%.

The efficacy of this formulation against *Fusarium solani* and *Lasiodiplodia theobromae* on different clones of heveawood was also observed. Evotek® 230 SE was effective to protect clones IAN 873 and RRIM 703 at low concentration (0.1%). However, RRIM 600 required higher concentration more than 0.25% to control the growth of the fungi.

Clone RRIM 703 was found to be the most resistant against the stain fungi. This is followed by BPM9 and RRIM 600. The infection of stain was observed at the end of the 1st week of incubation for clones IAN 873 and RRIM 703 and for the other two clones, the symptom showed up at the beginning of the 1st week.

The higher the starch content, the higher the rate of infection, the less resistant of heveawood to fungi. This was demonstrated by the characteristic of clone RRIM 600. Wood treatment analysis revealed that starch content in the wood was one of important element in determining the performance of preservative.

Among the four clones, RRIM 600 (0.92 %) contained the highest amount of starch when compared to BPM9 (0.73 %), IAN 873 (0.48 %) and RRIM 703 (0.39 %).

A scanning electron microscope study revealed that the present of intervessel pits, simple perforation plates, tyloses, crystal and starch have implications on penetration of preservatives.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk mendapatkan ijazah Master Sains

KEBERKESANAN RACUN KULAT TERHADAP *FUSARIUM SOLANI* SACC. AND *LASIODIPLODIA THEOBROMAE* PAT. PADA EMPAT KLON KAYU GETAH

Oleh

NORIDAH OSMAN

Mac 2001

Pengerusi: **Zaidon Ashaari, Ph.D.**

Fakulti: **Perhutanan**

Formulasi baru rawatan kayu getah dengan bahan kimia yang selamat masih dicari. Ia bertujuan mengatasi masalah kulat pewarna kayu getah sebagai mengekalkan dan mengawal warna asal kayu tersebut serta selamat buat pengguna.

Lazimnya, kayu getah akan direndam didalam bahan kimia selepas penebangan dilakukan. Bagi kilang-kilang kayu bahan kimia yang digunakan untuk mencegah serangan kulat pewarna ini adalah sodium pentachlorophenate (NaPCP). Meskipun, bahan kimia ini sangat berkesan mengawal masalah kulat pewarna tetapi, ia sangat beracun dan berbahaya kepada alam sekitar. Bahan kimia ini sekarang berada di dalam peringkat penyahgunaan.

Oleh itu, kajian ini bertujuan mencari bahan kimia baru dengan prestasi yang lebih baik bagi mengantikan kompoun NaPCP tadi. Formulasi bahan kimia baru

Evotek® 230 SE yang diiktirafkan lebih selamat telah diuji. Keberkesanannya diuji terhadap kulat pewarna kayu getah pada aras konsentrasi yang berbeza.

Di dalam kajian ini, pada aras konsentrasi 0.1%, 0.25% dan 0.5% bahan kimia Evotek® 230 SE diuji manakala aras konsentrasi 0.5% dan 1% bagi NaPCP digunakan sebagai kawalan. Kajian makmal ini bertujuan membentangkan unsur-unsur bahan kimia anti-kulat pewarna kayu getah.

Nyata, formulasi bahan kimia baru ini sangat berkesan mengatasi masalah kulat pewarna pada konsentrasi serendah 0.1% manakala kompon bahan kimia NaPCP berkesan pada konsentrasi lebih tinggi iaitu 1%. Dengan ini, bahan kimia baru Evotek® 230 SE adalah setanding dengan bahan kimia kawalan NaPCP. Ia berjaya menghalang kulat pewarna getah pada ketiga-tiga konsentrasi 0.1%, 0.25% dan 0.5%. Ini bermaksud, ia berupaya mengawal pertumbuhan kulat *Lasiodiplodia theobromae* dan *Fusarium solani.*

Kajian ini menunjukkan Evotek® 230 SE keberkesanan yang tinggi terhadap klon IAN873 dan RRIM703 walaupun pada konsentrasi yang rendah. Manakala, klon RRIM600 memerlukan konsentrasi yang tinggi pada 0.25% dan 0.5% untuk mencegah kulat pewarna.

Tahap perkembangan kulat juga berbeza di mana klon RRIM703 menunjukkan ketahanan yang lebih tinggi untuk dijangkiti kulat pewarna diikuti dengan klon

IAN873, klon BPM9 dan RRIM600. Klon RRIM600 amat mudah dijangkiti kulat pewarna *F. solani* dan *L. theobromae.* Jangkitan dapat dilihat pada awal pengkulturan dan klon yang lain pada penghujung minggu.

Semakin tinggi kandungan kanji semakin cepat serangan kulat dan semakin mudah kayu getah dijangkiti. Ini dibuktikan dengan klon RRIM600 yang mempunyai kandungan kanji yang tertinggi, Evotek® 230 SE gagal mencegah jangkitan kulat pewarna pada tahap konsentrasi yang rendah dan serangan awal kulat terhadap klon ini.

Kajian kandungan kanji dalam kayu getah menunjukkan bahawa klon RRIM600 mempunyai kandugan kanji tertinggi diikuti dengan klon BPM9, IAN873 dan RRIM703.

Penelitian mikroskop elektron mengambarkan bahawa kewujudan 'intervessel' pit/lubang, plat leliang, tilosis, hablur kristal dan kandungan kanji dalam kayu memberi kesan kepada penembusan bahan kimia. Unsur-unsur ini dapat dilihat pada klon-klon yang dikaji.

ACKNOWLEDGEMENTS

Firstly, Praise to Allah s.w.t for making the thesis possible.

I am indebted to the Department of Science Education, Ministry of Education for the funding under PASCA and the Faculty of Forestry, UPM for permitting me to continue my studies beyond my B.Sc..

It also pleases me to convey my sincere gratitude to my Supervising Committee, Chairman Dr. Zaidon Ashaari, for the guidance, support, understanding and supervision.

My deepest appreciation is also due to my other committee members Dr. Nathan Ganapathi and Assoc. Prof. Mohd. Zin Jusoh for the constructive criticism, patience and full understanding as well as for the invaluable advice and encouragement throughout my masters programme.

My special thanks is extended to Dr. Mannoor Narayanan Balakrishnan Nair who gave me the opportunity to visit the Wood Science Laboratory in Kerala, India. I also wish to thank him for his constructive criticism in editing my thesis.

My dearest thanks to Mr. Ed Sutherland and Assoc. Prof. Gloria A. Manaarpac for their comments and kind encouragement throughout the completion of my thesis.

I would like to acknowledge Mr. Hee Lau Kong (AgrEVO company) and Mr. Ayeru Singaram (Malaysian Timber Industry Board) for providing the chemical Evotek® 230 SE and Sodium pentachlorophenate used in this study.

I also wish to extend my thanks to Dr. Ramli b. Othman of the Rubber Research Institute of Malaysia (RRIM), Mr. Wan Razak b. Wan Jusoh and Mr. Sulaiman Mohd. Nazir of Unit Tanaman Perladangan, UPM for the heveawood sample used in the study, Dr. Rahim Hj. Sudin and Mr. Jalali Hj. Salleh of Forest Research Institute of Malaysia (FRIM) for their helps in starch study.

Appreciation is also extended to the staff of the Faculty of Forestry, especially to Ms. Halimah bt. Hussien, who assisted through out my study in the pathological work. My thanks also due to Mr. Rahmat Ismail, Mr. Baharom Zainal and Mr. Abd. Jalal Aman for assisting in processing the heveawood, Ms. Aidah Marsidi and Mr. Harmaen Ahmad Saffian for their kind assistance rendered.

I wish to thank Assoc. Prof. Dr. Kamis Awang for his encouragement and Dr. V. Prakash, Dr. Wong Ee Ding and Pn. Khamziah Abd. Kudus their help during my study. I must cite my friends, Astinah Watis Jersery, John Keen Chubo, Goh Kun Hwa for the useful interaction during the difficult times in my study, I thank you. And last but not least, to the many others who have contributes in one way or another to the completion of this study, I extend my sincere gratitude for their kind contributions.

TABLE OF CONTENTS

		Page
DEDICATION		ii
ABSTRACT		iii
ABSTRAK		vi
ACKNOWLEDGEMENTS		ix
APPROVAL SHEETS		xi
DECLARATION FORM		xiii
LIST OF TABLES		xvi
LIST OF FIGURES		xvii
LIST OF ABBREVIATIONS		xx
GLOSSARY		xxii
CHAPTER		
1	INTRODUCTION	1
	General Background	1
	Objective of This Study	6
2	LITERATURE REVIEW	7
	Wood Staining Fungi	7
	Factors Favouring Growth of Fungi	7
	Characteristics of Sap-Stain Fungi	11
	Wood Preservation	21
	Preservative Treatment Process	23
	Preservative Chemicals	24
	Sodium pentachlorophenate (NaPCP)	27
	Evotek® 230 SE	28
	Alternative Preservatives for Wood Staining Fungi	30
	Heveawood	32
	Clone	34
	Characteristics	36
	Durability	38
	Preservation	42
3	MATERIALS AND METHODS	46
	Preparations of Samples	46
	Growth of Heveawood Staining Fungi	48
	Fungi Used in This Study	48
	Identification of Fungi Using the Slide Culture	

	Technique	51
	Preparation of Culture Media	53
	Preparation of Ioculum	53
	Preservative Treatment of Heveawood Against Staining Fungi	54
	Treatment of Wood Blocks For Testing	54
	Preparation of Preservative Solution	56
	Treating Wood Blocks	57
	Inoculation and Incubation Period	59
	Evaluation of Staining	59
	Statistical Analysis	61
	Determination of Starch Content	61
	Microscopic Structure	65
4	RESULTS AND DISCUSSIONS	68
	Preservative Treatment of Heveawood	68
	Efficacy of Evotek® 230 SE Against Wood Staining Fungi of *Fusarium solani* and *Lasiodiplodia theobromae*	68
	Efficacy of Sodium pentachlorophenate (NaPCP) Product Against Sapstain Fungus of *Lasiodiplodia theobromae*	78
	Effect of Solution Concentration on Development of Stain Fungi	82
	Effect of Hevea Clones on the Growth of Stain Fungi	85
	The Growth and Development of Staining Fungi	87
	Fusarium Solani	93
	Lasiodiplodia Theobromae	94
	Starch Content in Heveawood	97
	Microscopic Structure of Different Clones of Heveawood in Relation to Preservative and Fungi	101
5	CONCLUSIONS AND RECOMMENDATIONS	114
	REFERENCES	117
	APPENDICES	138
	BIODATA OF THE AUTHOR	148

LIST OF TABLES

Table		Pages
2.1	Active ingredients of anti-sapstain preservatives evaluated in Malaysia	26
2.2	Some common biodeteriorating organisms of fungi on partially seasoned and seasoned heveawood	40
3.1	Number of heveawood samples for each treatment combinations	49
3.2	The preservatives and the concentrations used for treating solution	57
3.3	Rating scale of wood staining fungi on heveawood	60
4.1	Mean stain ratings and PEC of untreated and treated Evotek® 230 SE Heveawood blocks (clone IAN 873)	71
4.2	Mean stain ratings and PEC of untreated and treated Evotek® 230 SE Heveawood blocks (clone RRIM 703)	73
4.3	Mean stain ratings and PEC of untreated and treated Evotek® 230 SE heveawood blocks (clone BPM 9)	75
4.4	Mean stain ratings and PEC of untreated and treated Evotek® 230 SE heveawood blocks (clone RRIM 600)	77
4.5	Mean stain ratings and PEC of heveawood blocks infected By *Lasiodiplodia theobromae*	79
4.6	Summarised Results of Analysis of Variance on Rating Score	81
4.7	Mean of starch content of different clone heveawood	98

LIST OF FIGURES

Figures		Pages
2.1	*Fusarium solani* var. solani (Toussoun and Nelson, 1961)	16
2.2	*Fusarium solani*, asci and ascospores; conidio and conidiophores (Booth, 1971)	17
2.3	*Lasiodiplodia theobromae* from Gerbera (Natthaporn, 1987)	19
2.4	Conidia of *Lasiodiplodia theobromae* (Natthaporn, 1987) (400 X)	20
2.5	Step needed in protecting heveawood against sapstain attacks (Mohd. Dahlan *et al*., 1994)	43
3.1	Heveawood log at the RRIM trial plot	47
3.2	*Lasiodiplodia theobromae* cultures on heveawood	50
3.3	*Fusarium solani* cultures on heveawood	50
3.4	A schematic diagram of the apparatus used to identify fungi using the slide culture technique (top view)	52
3.5	Slide culture apparatus	52
3.6	Schematic diagram showing the inoculation of the treated wood blocks in a humidity chamber. A.Top view. B. Side view	55
3.7	Schematic diagram of a arrangement of test material in a 600-ml beaker for dip-process	58
3.8	Schematic flow chart of the procedure to determine starch content in heveawood	64
3.9	Calibration curve for starch analysis obtained from spectophotometric measurements	65
3.10	Schematic flow of the sample preparation of Scanning Electron Microscopy (SEM)	67
4.1	Wood block of clone RRIM 703. A. Untreated block. B. Block treated with Evotek® 230 SE.	69

0.5% Evotek® 230 SE

4.2 Wood block of clone IAN 873. A. Block treated with 0.5% Evotek® 230 SE B. Untreated block 69

4.3 Hevea with *F. solani.* White mycelium can be seen in the early stages after 2 weeks of incubation 88

4.4 *L. theobromae* on the control wood block after four weeks of incubation 88

4.5 Perithecium of Fusarium, Asci of *Fusarium solani* split open showing, asci and ascopore (x100) 89

4.6 Fruiting body with macroconidia of *Fusarium solani* (X 100) 89

4.7 Macroconidia of *F. solani* (x400) 90

4.8 Fruiting body and conidiospores of *L. theobromae* (X 100) 91

4.9 Fruiting body and conidiospores of *L. theobromae* (a) immature, hyaline one called conidiospore (b) mature pigmented spore 91

4.10 Mature, pigmented, two celled conidiospores of *L. theobromae* 92

4.11 Conidio of *L. theobromae* (X 400) 92

4.12 Intervessel pits viewed from inside the vessels, some of the pit apertures available in grooves. (Clone RRIM 703, x1900) 102

4.13 Inner pit aperture. The pit apertures are round to oval. (Clone IAN 873, x2200) 102

4.14 Intervessel pits bordered alternate, viewed from outside the vessels. The outer pit apertures are elongate and the pit aperture is oval. (Clone RRIM 600, x2000) 103

4.15 Inner pit apertures viewed from inside the vessels. Clone RRIM 600 103

4.16 Intervessel pits bordered and alternate, viewed from outside the vessels. (Clone IAN 873, x2500) 104

4.17 Intervessel pits bordered alternate, viewed from outside

	the vessels. The outer pit apertures are elongate. (Clone RRIM 703, x1700)	104
4.18	Intervessel pits bordered alternate, viewed from outside the vessels. The outer pit apertures are elongate. (Clone BPM 9, x1500)	105
4.19	Tangential section of heveawood. Vessel filled with tyloses and crystals. (Clone BPM 9, x220)	105
4.20	Starch grain in the axial parenchyma cells. (Clone IAN 873, x1600)	106
4.21	Tangential longitudinal surface. Starches presence in axial and ray parenchyma cells. (Clone BPM 9, x400)	106
4.22	Crystal in the ray cell. (Clone IAN 873, x1900)	107
4.23	Infected heveawood. Colonisation of vessel by fungal hyphae (X 1,500)	108
4.24	Fungal mycelium of infected heveawood (X 330)	108

LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
AVROS	Algemene Vereniging Rubberplanters Ooskust Sumatra
BPM	Balai Penelitian Medan, Indonesian
BS	British Standard
CPD	Critical of Point Dry
DNMRT	Duncan Multiple Range Test
FA	Ford Acre, Brazil
FRIM	Forest Research Institute of Malaysia
GLM	General Linear Model
IAN	Instituto Agronomico do Norte
LSD	Least significant difference
MA	Malt agar
MC	Moisture content
MDF	Medium density fibreboard
MS	Malaysian Standard
MTIB	Malaysian Timber Industry Board
NaPCP	Sodium pentachlorophenate
NUV	Near ultra violet
OD	Oven-dry
PB	Prang Besar, Malaysia
PDA	Potato dextrose agar
PEC	Per Cent Effective Control

p.s.i	Pound per square inch
RH	Relative humidity
RRIM	Rubber Research Institute of Malaysia
SEM	Scanning electron microscopic
SG	Specific gravity
SPSS	Statistical Package of Social Science
Tjir	Tjirandi, Indonesia

GLOSSARY

Dipping	Involves immersing the timber in the preservative for a short time
Discoloration	Any alteration of the natural colour of wood, which may be the result of weathering, of contact with chemicals or metals or of infection by fungi or moulds, or of other causes
Durability	The natural resistance of heartwood to destruction by wood-destroying organisms in conditions conducive to their growth
Hardwood	Conventionally a term used to denote the timber of trees, mostly broadleaved, and the trees themselves belonging to the botanical group *Angiosperms*
Mold	A woolly or powdery fungal growth that forms on the surface of wood in damp, stagnant atmospheres. Similar growth on other materials are popularly referred to as 'mildews'
Penetration	The depth to which preservative enters the wood
Pentachlorophenate, sodium (sodium PCP)	A salt of pentachlorophenol (PCP) used extensively for sapstain control treatment
Sapstain	A discoloration of timber resulting from the growth of certain fungi that derive their nourishment from the cell contents but do not cause decomposition of the timber. It is principally confined to sapwood. Blue stain is the most common form of sapstain. It is most commonly caused by fungi of the genera Lasiodiplodia and Curvularia
Sapstain control	The application of chemicals to green timber to protect it from sapstain

Sapwood	The outer layers of wood which, in the growing tree, contain living cells and reserve materials (e.g. starch); generally lighter in colour than heartwood though not always clearly differentiated. All sapwood has low natural durability
Stain	Blue, see sapstain
Surface treatment	Any treatment in which a liquid preservative is applied to the surface of timber by brushing, spraying or dipping
Wood preservative	A chemical or mixture of chemicals in a form suitable for application to wood in order to preserve it from attack by wood-destroying organisms

CHAPTER 1

INTRODUCTION

General Background

Malaysia through the Malaysian Timber Industry Board (MTIB) has been successful in promoting both heveawood products and heveawood as a timber in international and local market. Malaysian Timber bulletin (1998) reported about 80% Malaysia's furniture exports, which was expected to reach RM 3.2 billion in 1998 is made from heveawood (Anonymous, 1998). The demand has been increasing for the furniture manufacturing industry and it is estimated that in the next few years there will be an imminent shortfall in heaveawood supply.

In order to sustain adequate supply and maintain the quality of heveawood production, a further step by integrating the heveawood growing and heveawood processing industry has been undertaken with the support of the Malaysian government.

Heveawood processing industry is incorporating wood preservation process as part of its operation. Wood preservation industry has been established in Malaysia since 1900. Since then, this industry gradually grows and become one of the important subjects in the heveawood industry.