MINISTRY OF FORESTRY OF INDONESIA
IN COOPERATION WITH
INTERNATIONAL TROPICAL TIMBER ORGANIZATION

ITTO PD425/06 Rev. 1 (I)

Production and Utilization Technology
for Sustainable Development of Eaglewood (Gaharu)
in Indonesia

Proceeding of Gaharu Workshop

DEVELOPMENT OF GAHARU PRODUCTION TECHNOLOGY

A FOREST COMMUNITY BASED EMPOWERMENT

Edited by:
Maman Turjaman

R & D CENTRE FOR FOREST CONSERVATION AND REHABILITATION
FORESTRY RESEARCH AND DEVELOPMENT AGENCY (FORDA)
MINISTRY OF FORESTRY
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The place and date the report was issued	:	Bogor, July 1, 2011.
Disclaimer	:	Copyright @ 2011 This Proceeding is a part of Program ITTO PD425/06 Rev. 1 (I) : Production and Utilization Technology for Sustainable Development of Eaglewood (Gaharu) in Indonesia
Published by	:	Indonesia's Work Programme for 2011 ITTO PD425/06 Rev.1 (I) R&D Centre for Forest Conservation and Rehabilitation Jalan Gunung Batu No. 5 Bogor, Indonesia Phone :62-251-8633234 Fax :62-251-8638111 E-mail : turjaman@gmail.com
ISBN	:	978-979-3145-80-8
Cover by		Bintoro

Project number	:	PD425/06 Rev. 1 (I)
Host Government	:	Indonesia
Name of the Executing Agency	:	Forestry Research and Development Agency (FORDA)
Project Coordinator		Dr. Ir. Maman Turjaman, DEA
Starting date of the project	:	May 1, 2008
Duration of the project	:	36 months

PREFACE

The first gaharu workshop in 2009 signifies as a dissemination technique which proved effective to provide information for the stakeholders coming from various parties. The topic of first gaharu workshop was “Development of Gaharu Production Technology: A Forest Community Based Empowerment”

This workshop could represent the collection of information about the development of gaharu technology from various parties, such as universities, research institutions, community self-sufficiency institutions, private companies, policy holders, and gaharu practitioners in the field. In other sides, this workshop also offered the current information about gaharu development already achieved by the ITTO PD425/06 Rev.1(I) project. The most current information and invention can be scrutinized technically and discussed in-depth by the workshop participants. The participants were also given a chance to tell their practical experiences in performing gaharu development in each of their own regions.

The conducting of workshop afforded the outputs that brought benefits to the decision makers sticking to the policies on gaharu production in Indonesia. In different views, other stakeholders such as forest-farmer group, privates, gaharu enterprisers, community self-sufficiency community have forwarded some valuable inputs to immediately arrange and compile the master plan about the management of gaharu production in national scale. The gaharu workshop also offered benefits by the establishment of gaharu-communication forum under the name called Indonesia’s Gaharu Forum (IGF) as the informal holding-place between the stakeholders who are interested in gaharu development.

In gaharu workshop, there were a lot of inputs put forward by the participants abiding by their own experience in gaharu development. These inputs become the items which can be very valuable to develop inoculation technology and all the related aspects in the future. Nevertheless, there were some participants whose opinions differed from or did not get along with the workshop theme, as they might have different understanding-views or since the reference they learnt so far was different from the gaharu development currently conducted by the FORDA (Forestry Research Development Agency).

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DEVELOPMENT OF EAGLEWOOD (GAHARU) IN BENGKULU, SUMATERA

By:
Mucharromah[1]

ABSTRACT

Gaharu is a resin product which is produced by particular trees and has a certain high comercial value. This paper presents an insight of gaharu development in Bengkulu province, Sumatera. Indonesia has high diversity of gaharu-producing trees, but the gaharu found in nature is threatened to extinction due to uncontrolled exploitation. Therefore, there is a need to conserve gaharu in nature while maintaining well-managed gaharu production. The community who lives near the forest has long known gaharu and how to harvest them, but the knowledge of gaharu-forming and gaharu induction technology is still limited. Technology transfer and the community's capability development will mantain the perpetuation of natural gaharu and increase the community income by gaharu artificial induction. The gaharu development needs a certain capital and investment. Therefore interference by several parties will fasten the achievement of the development, for instance the government, privates, research and development institutions, and the forest community. Certain organization who facilitates the whole process of gaharu development is necessary in gaharu center region. In this paper, we also include the calculation needed to start gaharu business.

Keywords: gaharu, resin product, high economical value, conservation, management, capability development

I. INTRODUCTION

Gaharu is a forest product which has a high economical value compared to other forest products, therefore has potention to develop. Gaharu development is necessary, specifically to mantain the production continuity and also to conserve gaharu-producing tree diversity in Indonesia. In gaharu development, the community who lives near the forest is an ideal target. They will be able to multiply the roles and function of this development program. From the view of gaharu seedlings material availability, the area around the forest has the highest number of nature gaharu trees. Considering that

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these trees' fruits are recalcitrant, unless by human's interference, the fruits will not disperse too far. From the view of community readiness, generally the community who lives around the forest is already familar with gaharu, even some of them were gaharu collectors. Therefore the knowledge and skill needed to support the development of gaharu industry cluster were already sufficient.

From the view of environment safety and biodiversity, gaharu development around the forest will also support biodiversity safety and forest conservation, considering the community will have income from gaharu development business which is economical prospective. Furthermore, considering that gaharu-producing trees has a certain morphology which has a role in environment protector, such as increasing ground water absorption and retention, strengthening soil, preventing landslide, absorbing CO_2 and producing O_2 which is very vital in supporting life.

Therefore, gaharu development in the area around forest will enforce the function of forest itself, beside the empowerment and the prosperity of the community around the forest. With gaharu's high economical value and increasing world's demand, the development of gaharu is very potential to realize people and nation's welfare, other than preventing natural disaster of draught, shortage of freshwater, landslifde, global warming, polution, and shortage of oxygen. However, gaharu development is not like the development of agricultural plants which yields directly when is well managed. In gaharu-producing trees, gaharu will not be formed if the trees grow smoothly and are not even slightly disturbed. Therefore, the development of gaharu production will not be sufficient only by planting the gaharu-producing trees' seedlings, but also should be provided by development of production technics and production development system, specifically related to production cost which is relatively high.

So far, gaharu productions in Indonesia, many of them, are still taken from nature, therefore known as nature gaharu. Nature gaharu has been known since thousands years ago to be traded to Middle East countries by Indian and Indo-Chinese traders, including from west region of Indonesia or Sumatera and has been highly valued, especially those with super (or higher) quality. Super quality gaharu will release fragrant scent even without being heated or burnt. The form of super quality gaharu varies greatly, some have very hard texture and soft (tidak berserat), shiny black color and heavy as to drawn in water. Meanwhile lower quality gaharu (kemedangan and abuk) needs to be distilled to get the resin and dregs to make *makmul* or *hio* (Chinese insence) for religious rituals. With increasing demands from international market, the trade volume of gaharu is also raised, put the losing of gaharu-forming trees at risk due to cutting down and mincing by people to get the gaharu. This condition cannot be solved unless by performing grand gaharu development, especially in the most potential area: the area around the forest. With this effort, gaharu production in Indonesia will still be abundant and the people who produce it will also be prosperous so then they will be able to mantain the the natural resources diversity and environment safety around them.

II. GAHARU DEVELOPMENT READINESS

A. Production Process-Supporting Human Resources Readiness

Although gaharu has long been one of Indonesian export commodities, public is not really aware of what gaharu is, except the community around forest area who has been involved in searching, cleaning, and trading gaharu. Therefore, they are the ready target group for human resources for gaharu development, especially for post-harvest process; separating gaharu from its white wood. This step progresses very slow, almost like a sculpture-making, hence many skillful labors are needed. With long enough nature gaharu-searching experience, many people around the forest are skillful in cleaning gaharu, making them ready enough to support gaharu development in their region.

B. Production Technology Readiness

Different from other tree products which are always produced as long as the plants are healthy or in other words, production is a function of a healthy plant growth, gaharu can not be obtained from a healthy tree which grew without any disturbance. Most gaharu is found in disturbed trees, naturally by abiotic or biotic factors, or by artificial induction. Abiotic factors can be wind, rain, showery weather, and thunder. Nevertheless gaharu forming by natural abiotic factors is difficult to imitate, hence can not be reliable in industrial production process.

Meanwhile gaharu-forming by biotic factors may be caused by microorganisms infections to plants, other than friction by animal or unintentionally by human. Discovery about microorganism which induce the accumulation of fragrant resin and then form gaharu is the base for discovery of gaharu-forming induction technics which can be used to support gaharu production process in industrial scale. Several researcher groups have succeded in stimulating gaharu-forming by inoculation (Mucharromah *et al.,* 2008a, b, c, d; Santoso *et al.,* 2006, 2008; Kadir, 2009). Nevertheless considering that the application technics can not yet be done by the community, therefore skill training for the preparation of production process is necessary, for instance; inoculation technics or gaharu-forming monitoring technics. In addition to that, inoculant production skill training is also necessary so then production process will run more efficient. With operational support, inoculant production and gaharu-forming by inoculation technics and skills are ready to be passed down to the community in order to support gaharu production development through community empowerment around the forest area.

C. Product Quality Control Readiness

In order to obtain success and continuity of production process, generally, product quality monitoring is necessary. Therefore, preparation of human resources supporting gaharu development also needs to include personnels who are able to identify quality

and to sort or to collect gaharu that has been produced based on quality gradation and purpose. From the shape's view, gaharu is a wood tissue from particular trees (gaharu-producing ones) containing high amount of volatil sesuiterpenoid resin with fragrant scent. The unique and lasting fragrant scent of gaharu that makes it very preferable and aprreciated with very high economical value. Besides that, the tissues that contain eagleweood fragrant resin are only found in parts of the tree in which particular process has occured, such as wounding and followed by pathogen infection through inoculation or by other means that makes the wood tissue has different color, scent, texture, level of hardness, and mass density. These what make gaharu valuable and the value is highly determined by purity and content quality of the resin contained.

In nature gaharu, quality gradation is determined by national quality standard written down in SNI 01-5009.1-1999. In this standard, gaharu quality is sorted to: (a) gubal gaharu, (b) kemedangan, and (c) abu gaharu. These three categories are then further divided into 13 quality classes consisting:

1. Gubal gaharu has three quality marks: (a) main quality = super quality; (b) first quality = AB quality and (c) second quality = super sabah quality;
2. Kemedangan, divided into 7 quality classes: (a) first quality = TGA/TK1 quality; (b) TGB/TK2 quality; (c) TGC/TK3 quality; (d) GD/TK4; (e) TGE/TK5 quality; (f) TGF/TK6 quality and (g) seventh quality = equal to M3 and
3. abu gaharu has three quality classes which are: (a) superior quality; (b) first quality and (c) second quality.

Nonetheless differentiating the quality classes in details is a very difficult job, so in practice, the consumers themselves who determine the gaharu product quality and price. This is an anomaly condition for comodity trading, where the product owner usually determines the price. With the skill of grading, gaharu standard quality and its price will be more guaranteed. For that purpose, human resources training in gaharu-forming development monitoring skills and quality control is crucial.

Nowadays, there are more gaharu-producing trees which are inoculated, especially *Aquilaria* and *Grynops*. Inoculation technics to induce the forming of gaharu has become cheaper and more efficient. In advanced test result of gaharu production technics several months ago in Bengkulu Province, gaharu-forming process has become faster. TGB and TGA of kemedangan quality, which had been previously achieved after 12-18 months after inoculation, was achieved in six months, although most product was still in TGC level of quality (Mucharromah *et al.,* 2008). This inoculation technic success is very prospective to support development of gaharu production in which producing trees were already cultivated in Sumatera and other regions in Indonesia. Thus gaharu development is ready to be implemented, not only around the forest area but also in office, school, and home backyard, as has been done in Bengkulu and surrounding areas. Nevertheless, the gaharu development near the forest area will probably be far more efficient in prosuction process, beside the other benefit of environment safety and the community prosperity.

D. Capital and Institutional Readiness

Indonesia has a great gaharu-producing tree diversity. This is the main capital in making gaharu-producing process easier and cheaper. It was recently reported by Wiriadinata (2008) and Sumarna (2002) that natural gaharu-forming occured in at least 16 species of trees, several genuses belong to Thymelaceae family, one from Leguminoceae family, and one from Euphorbiaceae family.

In nature, not all gaharu-producing trees form gaharu or only form very little gaharu. Gaharu amount produced in forest varies greatly from 0.3 – 14 kg per tree and usually increases when the tree diameter is bigger (MacMahon 1998). Beside that, not all trees form gaharu. This will lead nature gaharu producing process to a higher cost. In gaharu cultivation, gaharu production quantity is highly determined by the number of holes or wounds which was inoculated and the quality is determined by how long time has passed since inoculation until harvest time. The longer time passes after inoculation, the more fragrant resin will be accumulated and the higher gaharu quality will be achieved. Therefore the gaharu production through cultivation and inoculation might be much more efficient than nature-dependent production.

Nonetheless, gaharu development still needs relatively high fund supports although profit-capital ratio is also pretty high, as shown in gaharu inoculation business analysis (Annex 1) and gaharu cultivation (Annex 2). Based on these analysis, gaharu development will be most efficient when the area around forest is still rich in gaharu stands with diameter bigger than 20 cm which can be inoculated to fasten production process and add a start capital for cultivation in broader area in order to achieve gaharu business continuity. Collaboration and commitment from all parties involved in gaharu development is necessary to start this business based on their expertises.

Gaharu-producing trees in field has helped activities such as inoculation effectivity test, production test, inoculation training, monitoring of gaharu-forming and gaharu production process through inoculation. From quality's side of view, gaharu produced through inoculation has not yet achieved the highest quality of nature eagewood; super, double super, and higher qualities. The forming of super quality gaharu, often named super gubal, in gaharu produced through inoculation might be achieved, as research on the development of excellent inoculant is still undergoing. Theoretically, inoculant superiority in inducing gaharu-forming is related to the microorganism species and purity, as shown in microscopic data on gaharu resin deposited in tissue area around the inoculated holes or wounded (Mucharromah and Marantika, 2009).

While other kinds of fungi, especially wood-rot fungus, redegrade deposited gaharu resin and even disrupt the cells, making the gaharu that has been formed destroyed and at at least half-rotted and results in lower quality. The superior inoculant usage and inoculation technics which minimalize contamination will lead to higher quality and more efficient production process, hence lowering the cost.

In gubal-quality eagelwood, fragrant resin accumulation occurs in its maximum, even overflows and covers the cells around. This makes the wood tissues smooth, appeared like agar-covered, and reddish or blackish brown depends on the intesity or resin level it contains. If this quality can be achieved from inoculating the trees in the initial stage of development, the next gaharu production will not need much capital support anymore because this high quality like nature gaharu has a very high value; USD 2,000 – USD 16,000 per kg on the end-consumer level in overseas, hence is able to cover the cost for the further stage of the development.

Nowadays, the quality of gaharu achieved through inoculation is getting better. In some research, results show the quality reached gubal quality in kemedangan B/C level (Santoso *et al*., 2006; Mucharromah and Surya, 2006) or even in B level (Surya, 2008 – personal communication; Mucharromah, *et al.* 2008). Through more innovated inoculation technics, more potential and purer isolates (innoculants), and supported by longer inoculation-to-harvest time, the super gubal quality might be achieved.

Beside the higher level of resin contained, the resin fragrance also determines the quality of eglewood. So far, the nature gaharu's fragrance is softer than eagewood that is achieved through inoculation, probably due to the purity of the resin contained. In microscopic observation, Mucharromah and Marantika (2009) showed that the tissues, which are previously tranparent reddish brown, turned blackish and dissapeared before finally the cells get destroyed after being contaminated by gaharu resin. Therefore in gaharu production process through inoculation, applying aseptic piricipals is necessary in order to limit the contamination probability (Mucharromah *et al.*, 2008).

Gaharu fragrance peculiarity is also determined by the species of the producing-tree and the microorganism as the inoculant, therefore the aroma from the highest quality of gubal gaharu from different regions may vary (Mucharromah *et al.*, 2007). Gaharu-producing trees such as *Aquilaria malaccensis, A. beccariana, A. microcarpa, A. hirta,* and *A. agallocha* which are commonly found in Sumatera, has long been known to produce gaharu that are preferred by consumers around the world. Therefore the the gaharu development around the forest through raising the numbers of *Aquilaria* trees that have already been there and inoculating the old trees to cover the the cost of its rejuvenation will regain the gaharu production potention that Sumatera and other regions in Indonesia once have.

III. GAHARU DEVELOPMENT MODEL IN SUMATERA

Theoretically, fragrant resin accumulation of gaharu has been reported to be stimulated by infection of particular fungi (Mucharromah & Surya, 2006, 2008a,b,c; Santoso *et al*. 2006; Sumarna, 2002). The ability of inoculant fungi in stimulating resin production is also related to resin accumulation level which is netto product from synthesis process minus its degradation, and the resin type and purity (Agrios, 2005; Langenhein, 2004; Mucharromah, 2004). The usage of particular inoculant and purity, assembling of

aseptic technics in inoculant preparation and application, the accuracy of inoculation technics, and labors' skills will greatly affect the production process and product quality. Therfore, in Bengkulu, gaharu development was started with effectivity test of several fungi isolates which are potential in inducing gaharu-forming and then followed by development of excellent inoculant which are still undergoing to upgrade the quality.

After effective inoculant is achieved and the promising inoculant results is shown, collaboration for gaharu development through recultivation and inoculation should be offered to the community who have gaharu-producing trees with diameter >20 cm. This collaboration is including the maintanance and recultivation of the natural seedlings around the stand parents until reaching minimum population of 10 – 100 seedlings per parent tree to be inoculated for gaharu production. So far about more than 10,000 trees were planted around the inoculated parent trees. This collaboration still needs fair enough capital to become an independent gaharu development business, because the harvest and gaharu cleaning or seperation is labor intensive.

Although harvested gaharu delivers selling products, harvest and cleaning processes require a fair capital. Gaharu development can not be done independently, especially capitalless community who only depends on natural resources and skills. Therefore, government's intervention is well expected.

As previously described, gaharu development requires a quite fair capital due to its complex process. Firstly, natural seedlings must be planted and maintained. Then, the trees that are big enough (>20 cm in diameter) will need to be induced by microorganism inoculation to produce gaharu. Gaharu-forming induction is done through inoculate the holes which are made in spiral style throughout the stem with a range 7 – 10 cm horizontally and 12 – 20 cm vertically from the trunk base until the highest reachable shoot tip. This inoculation process needs a certain skill as well as courage and personnels availability with supporting physical condition for field work. Following inoculation is monitoring the trees until harvest time. After totally harvested, a cleaning process to seperate the gaharu from the less resinous tissues. This process is done manually hence it is labor intensive.

For cleaning one kg gaharu through inoculation, generally it requires 4-5 person work day is, so that to produce 270 tonnes of gaharu, as was exported in year 2000s, it will absorb labors as many as 56,000 – 68,000 persons/day. This is a large number and will employ the people around the forest about 280 – 340 persons/year with 200 work days/year. The workforce will also increase due to increasing demand and production capacity. From workforce's view, the workforce needed in handling the cleaning process of nature gaharu and cultivated gaharu are not significantly different, but from environment and labor safety's view, gaharu development through cultivation and inoculation will give more benefits in long term and will be more sustainable, while nature eaglwood will eventually be exhausted. Therefore, the gaharu development should be done seriously, so that the efforts that has been put will result sustainably.

Considering that gaharu production process is more complicated than most of other forest, plantation, or agriculture product commodities, it will be necessary to have an institution whose task is to support the preparation and implementation of the production process in order to develop into independent gaharu industry. This intitution may be simple if the development of eglewood can be implemented like agriculture or cultivated plantation commodities. However, if the eglewood trading is still regulated by quota in which the trading process involves many parties, therefore the gaharu development should also involves these parties.

In practice, the involvement of these parties, if they do not have detail job descriptions or if there are overlapped tasks, will hamper the targeted gaharu development. So far the gaharu development in Bengkulu Province, both privates or universities and community groups, was made simple, with a collaboration contract between land/tree owner(s) and the implementer which could be universitiy, private, or community group(s). Considering that the inoculated trees or planted seedlings are in private land, either backyards or gardens, gaharu development process that has been implemented so far is secured. This security is only due to the stage of development that has not yet reach the trading stage. When the production stage is done, with quota regulation, gaharu trading still requires quantity certification to verify that quota limit has not yet been passed. Although the certification process is simple, but it can be a bothersome. This probably should be simplified in order to achieve gaharu development, considering that the region around the forest, where the gaharu development is undergoing, is usually pretty far from the institution who certify the products.

In gaharu development, University of Bengkulu can play role which benefits all parties. The university can play role in developing gaharu researches such as, founding the gaharu production community around the forest, sophisticating production technics, developing preeminent inoculant for quality improvement, and developing product quality control technics. These roles can be played through research activities and community services which are routinely done by universities with financial support from government or privates.

IV. CONCLUDING REMARKS

1. Gaharu development is a huge program, not only because its products have high commercial value which is potential for the empowerment of the community around the forest but also because it need technology investment and sufficient capital for its success. Therefore gaharu development should be carried out with mature plan for every stage in order to improve the independency of the community around the forest. This is an important thing to do, not only to guarantee the improvement and continuity of gaharu production,but also to secure the forest and biodiversity around it and also to improve the forest capacity in overcoming the danger of natural disaster caused by landslide, flood, drought, pollution, and many others.

2. To make the gaharu production process more efficient and to lower the cost, gaharu developing program needs tto adopt the most efficient and practical model, therefore success can be more guaranteed. It is important to note considering that gaharu forming is not automatically occured in healthy plant unlike other products of agriculture, plantation, or forest.

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Annex 1. Gaharu through inoculation business analysis in Bengkulu

Gaharu through inoculation business analysis					
No	Description				
1	Inoculation time (year)	3			
2	Gaharu stand quantity (stand)	1			
3	Result projection (kg/stand)	20	BC Class		
		30	Kemedangan		
	Result projection total (kg/stand)	50			
4	BC class selling per stand (kg)	60			
5	Powder selling per stand (kg)	100			
				price/unit	Total cost
No	Description	QTY	Unit	(Rp'000)	(Rp'000)
A.	Operational expenses				
	Tree/land purchase expense	1	Stand	100	100
	Inoculant expense	1	Stand	5.000	5.000
	Equipment purchase expense	*1*	Set	90	90
	Stressing agent expense	1	Stand	1.500	1.500
	Inoculation expert expense	1	Stand	200	200
	Labor expense	1	Stand	600	600
	Maintainance expense	3	Year	12	36
	Other operational expense	1	Stand	300	300
	Total operational expense				7.826
A.1.	Harvest & post-harvest expenses				
	Tree-cutting expense	1	Stand	50	50
	Storehouse freight load expense	1	Stand	50	50
	Gaharu cleaning expense	50	Kg	25	1.250
	Packing expense	50	Kg	2	100
	Total harvest & post-harvest expense				1.450
A.2.	Other marketing & common expenses				
	Selling transport expense	50	Kg	5	250
	Selling expense	50	Kg	10	500
	Restribution expense	50	Kg	5	250
	Administration expense	1	Stand	6	6
	Other common expense	50	Kg	0,5	25
	Total marketing & common expense				1.031
	Total Operational Cost				10.307
No	Description				
B.	Income projections				
	BC class selling	60	Kg	2.000	120.000
	Powder selling	100	Kg	5	500
	Total income projection				120.500
C.	Tax/zakat cost	5%	%		6.025
D.	Profit projection				104.168

Source : Calculation results by researcher and CV Gaharu 88 Bengkulu, 2006

Annex 2. Gaharu cultivation business analysis in Bengkulu

Gaharu cultivation business analysis					
No	**Description**				
1	Cultivation time (year)	7			
2	Area (ha)	1			
3	Stand population per ha	1.000			
4	Ratio of injection number (hole)/kg	80			
5	Hole number per stand	160			
6	Projection of harvest yield per stand (kg)	2	(No.5 / No.4)		
7	*Exchange rate IDR*	9.000			
				Price /	Total
				Unit	cost
No	Description	QTY	Unit	(Rp'000)	(Rp'000)
A.	Land acqusition expense				
	Land purchase expense	1	ha	15.000	15.000
	Licensing /certificate /notarial cost	1	letter*	4.000	4.000
	Total land acqusition expense				19.000
B.	Start up cost				
	TBM infrastructure & Utility				
	Guard house	1	Unit	2.000	2.000
	Lighting utility (PLN)	1	Unit	1.000	1.000
	Communication utility	1	Unit	2.000	2.000
	Other utility	1	-	1.000	1.000
	Total start up cost				6.000
A+B	Total cost (recapitulated)				25.000
C	Operational expenses				
C.1.	Tree planting expense				
	Land clearing expense	1	ha	1.000	1.000
	Seedlings purchase expense	1.000	Stand	5	5.000
	Holes making expense	1.000	Stand	1	1.000
	Gaharu tree planting expense	1.000	Stand	0,5	500
	Fertilizer expense	1.000	Stand	5	5.000
	Maintanance & security expense	1	Ha	24.000	24.000
	Total tree planting expense				36.500
C.2.	Inoculation expenses				
	Inoculant making expense	1.000	Stand	20	20.000
No	Description				
	Equipment purchase expense	1	Set	3.000	3.000
	Stressing agent expense	1.000	Stand	10	10.000
	Labor expense	1.000	Stand	5	5.000
	Maintainance expense	1.000	Stand	10	10.000
	Other operational expense	1.000	Stand	1	1.000
	Total inoculation expense				49.000

Gaharu cultivation business analysis					
No	**Description**				
C.3.	Harvest & post-harvest expenses				
	Tree cutting expense	1.000	Stand	5	5.000
	Storehouse freight load expense	1.000	Stand	5	5.000
	Gaharu cleaning expense	2.000	Kg	10	20.000
	Packing expense	2.000	Kg	2	4.000
	Total Harvest & post-harvest expense				34.000
C.4.	Other marketing & common expenses				
	Selling transport expense	2.000	Kg	5	10.000
	Selling expense	2.000	Kg	10	20.000
	Restribution expense	2.000	Kg	20	40.000
	Other common expense	2.000	Kg	0,5	1.000
	Total marketing & common expenses				71.000
	Total operational expenses				190.500
D	Income projections				
	C class selling	2.000	Kg	2.000	4.000.000
	Total income projections				4.000.000
E	Tax/zakat expenses	5%	%		200.000
F	Profit projections				3.609.500

Source : Calculation results by researcher and CV Gaharu 88 Bengkulu, 2006

CHEMICAL STUDY OF EAGLEWOOD (GAHARU) RESULTING FROM INOCULATION OF *Fusarium* sp. on *Aquilaria microcarpa*

by:

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ABSTRACT

Gaharu is highly economy-valued product with enormous vary of utilization. Knowing the content of product we widely used, such as gaharu, is essential, moreover it will provide information of alternative usages as some other new compounds have been revealed, gaharu production development through biotechnology, and else. Chemical analysis were carried out on artificial gaharu produced by inoculating *Fusarium* sp. from some origin to *Aquilaria microcarpa*, which were Bahorok (North Sumatra), Tamiang Layang (Central Kalimantan), Mentawai (West Sumatra) and Seram Island (Maluku). Though quantitatively or infection site area, there was indifferent effect of origins, but it was revealed that there were distinctions in compounds composition and relative concentration. Artificial gaharu produced by inoculating *Fusarium* sp. of Tamiang Layang's origin showed the highest confirmed constituents of gaharu but isolate of Maluku's origin noted to have the highest total concentration of odorant compounds.

Keywords: gaharu, *Fusarium* sp., *A. microcarpa*, chemical analysis

I. INTRODUCTION

Gaharu is a non-timber forest product with high economy value and various market price starts from 300 thousands rupiahs to 25 millions rupiahs for double super quality. This product is produced by several gaharu-producing species in Thymelaeaceae family. Indonesia as one of the biggest gaharu supplier has the highest biodiversity in the world; more than 27 species from 8 genus and 3 families across Sumatra, Kalimantan, Maluku, and Irian (Sumarna, 2005).

Gaharu has high selling value especially from its fragrant resin, named 'scent of God', even though this product usage is not only limited to fragrance. In principle, gaharu

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usages are for medicine, incense, and perfume (Barden *et al.,* 2000). Gaharu incenses are used in beliefs rituals and religious rituals, as fragrances for ritual room and religious objects such as rosario and *tasbih* (Barden *et al*., 2000). Whereas in medical world, gaharu is used as anagesic and anti-inflamatory (Trupti *et al.*, 2007), and useful to overcome various diseases such as toothache, kidney, reumatic, asthma, diarrhea, tumor, diuretic, liver, hepatitist, cancer, smallpox, malaria, tonic for pregnancy and post natal, also used as anti-toxicity, anti-bug, antimicrobes, and digestive and neurotic stimulants (Hayne, 1987; Barden *et al.*, 2000; Adelina, 2004; Suhartono and Mardiatuti, 2002).

Gaharu is a phytoalexin compound which is a secondary metabolites in gaharu trees as a defense mechanism. Healthy gaharu trees never produce fragrant sesquiterpenoid as secondary metabolites (Yuan, referenced in Isnaini 2004). Plants synthesize and accumulate secondary metabolites as responses to particular agent infections, physiological stimulus, or stress (Goodman *et al.*, referenced in Isnaini 2004). Secondary metabolites or plants extractive substances can be effective against plant diseases and pests due to analogy with particular vital component from celluler signals or related to vital enzymes and blocks metabolism pathways (Forestry Commission GIFNFC, 2007). Secondary metabolites on terrace wood can be tree's defense toward distructive agents even though its influence varies depends on the habitat (Hills, 1987). Secondary metabolites concentration also varies between species, tisuues (the highest concentration is in dermal, terrece wood, root, branch base, and wounded tissues), between trees in the same species, inter-species, and seasons (Forestry Commision GIFNFC, 2007).

Information about chemicals that gaharu contains is important in product usage. Gaharu chemicals information will be required in product standard system based on chemicals composition it contains, therefore leads to the uniformity of product quality determination in practice. Gaharu chemical study will be the gate for discovery of novel compounds and novel benefits, the gaharu biosynthesis pathway itself, possibly leading to produce compounds synthetically or expand the compounds utilization with biotechnology, and many other development opportunities. Nevertheless, efforts in continous research are to be taken in order to discover the unknown.

II. CHEMISTRY ANALYSIS OF GAHARU RESULTED THROUGH INOCULATION BY ISOLATES FROM SEVERAL SOURCES

In this research, gaharu chemistry analysis was done with pyrolisis GCMS analysis using Shimadzu GCMS-QP2010 apparatus. Helium was used as carrier gas (0.8 mL/min) which was equipped with DB-5 MS capilary column (60 mm x 0.25 mm, film was 0.25 µm thick), and was operated with electron impac (EI) mode at 70 eV and ion source temparature at 200^{0}C. Chromatography conditions are as follows; column oven temperature at 50 ^{0}C, and injection temperature at 280 ^{0}C. Injection was done in split mode which is isothermal at 50 ^{0}C for 5 minutes, and then was increased until 280 ^{0}C for

30 minutes, and was held at this temperature until minute 60. Compound identification was carried based on retention and MS analysis.

Chemical component analysis was done for gaharu resulted through inoculation of *Fusarium* sp. isolates originated from Bahorok, Central Kalimantan, Tamiang Layang, Mentawai, and Maluku. Infection area measurement was done 6 months after inoculation, whereas chemical analysis was carried for ± 1 year old samples.

Figure 1 presents *Fusarium* sp. infection area on *A. microcarpa* stems. Although descriptively Bahorok originated isolate seemed to cause widest infection area, statistically isolate origines did not significantly affect the infection area on these gaharu-producing trees.

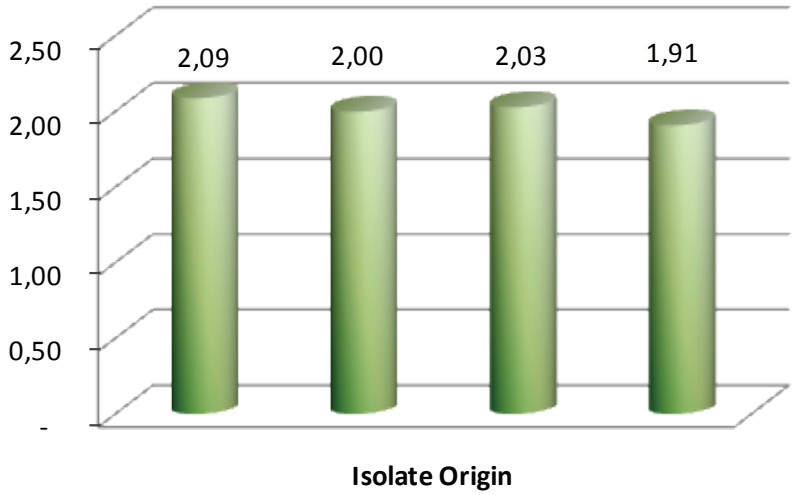

Figure 1. The infection length on *A. microcarpa* stems 6 months after inoculation with isolate origins as differentiator

The insignificant effect of isolate origins to infection area probably was due to the same genus of *Fusarium* sp., and to be mentioned that none of the isolates originated from Carita, where the research was carried. Although at the beginning after inoculation, each isolate shows different speed of infection according to its virulance, but after a while, they did not significantly affect the infection area.

Even though the isolate origins did not significantly affect to infection area, the chemical component analysis showed difference. Table 1 presents chemical component analysis with py-GCMS to gaharu samples one year after inoculation. In this table, the analysed samples are samples with 5 cm and 20 cm injection range.

Table 1. Components in gaharu resulted through inoculation of *Fusarium* sp. to *A. microcarpa*

Compound name	Relative Concentration (%)							
	Bo		Kt		Me		Mu	
	5 cm	20 cm	5 cm	20 cm	5 cm	20 cm	5 cm	20 cm
A. Aromatic compounds identified as gaharu constituent								
4-(2'-Methyl-3'-butenyl)azulene	0.09	0.06	0.49	-	0.07	-	0.09	-
2,5-DIMETHOXY-4-ETHYLBENZALDEHYDE	-	0.08	-	0.08	-	0.10	-	-
2-Hydroxy-4-methylbenzaldehyde	0.09	0.08	-	0.06	-	-	-	-
4-Ethoxy-3-methoxybenzaldehyde	-	-	-	0.21	-	-	-	-
4-METHYL-2,5-DIMETHOXYBENZALDEHYDE	4.35	2.37	3.66	1.52	4.65	1.45	4.42	4.60
Benzaldehyde, 2,4-dihydroxy	0.42	0.30	-	-	-	-	-	0.25
Benzaldehyde, 2,4-dimethoxy- (CAS) 2,4-Dimethoxybenzaldehyde	-	-	-	0.22	-	-	0.11	-
Benzaldehyde, 3,4-dihydroxy- (CAS) 3,4 Dihydroxybenzaldehyde	-	-	0.32	0.29	0.26	-	0.24	0.28
Benzaldehyde, 3-hydroxy- (CAS) m-Hydroxybenzaldehyde	-	0.37	-	-	0.39	-	-	0.29
Benzaldehyde, 4,6-dimethoxy-2,3-dimethyl- (CAS) 2,4-Dimethoxy-5,6-dimethyl	-	-	0.36	-	-	-	-	-
Benzaldehyde, 4-[[4-(acetyloxy)-3,5-dimethoxyphenyl]methoxy]-3-methoxy	-	-	0.37	-	-	0.54	0.48	-
Benzaldehyde, 4-hydroxy- (CAS) p-Hydroxybenzaldehyde	-	-	-	-	0.43	0.23	0.44	-
1,2-benzenedicarboxylic acid, diisooctyl ester (CAS) Isooctyl phthalate	-	0.07	-	0.12	-	-	-	-
2-Butanone, 4-phenyl- (CAS) Benzylacetone	0.24	-	-	0.41	-	0.53	-	-
2-Butanone, 3,3-dimethyl- (CAS) 3,3-Dimethyl-2-butanone	-	0.04	-	0.04	-	-	0.05	-
2-Butanone, 3-phenyl- (CAS)	-	-	-	-	-	-	0.15	-
4H-1-Benzopyran-4-one, 2-(3,4-dihydroxyphenyl)-7-(.beta.-D-glucopyranosyl)	-	-	-	0.05	-	-	-	-
4H-1-Benzopyran-4-one, 2-methyl- (CAS) 2-Methylchromone	-	-	-	0.18	-	-	-	-
4H-1-Benzopyran-4-one, 5,7-dihydroxy-2-methyl- (CAS) 2-Methyl-5,7-dihydroxy	-	0.06	-	0.36	-	-	0.34	-
4H-1-Benzopyran-4-one, 6-dihydroxy-2-methyl- (CAS) 6-Hydroxy-2-methylchromone	-	-	-	0.46	-	-	-	-
2-Coumaranone	-	-	-	-	-	-	0.28	-
.gamma.-Eudesmol	-	0.04	-	-	-	-	-	-
Hexadecanoic acid, 2-(octadecyloxy)-, tetradecyl ester (CAS) TETRADECYL	-	-	-	-	-	-	-	0.03
Hexadecanoic acid, methyl ester (CAS) Methyl palmitate	-	-	-	-	-	-	0.05	-
2,4-Hexadienedioic acid, 3,4-diethyl-, dimethyl ester, (Z,Z)- (CAS) CIS.CIS.D	-	-	-	-	0.69	-	0.85	-
2,4-Hexadienedioic acid, 3-methyl-4-propyl-, dimethyl ester, (Z,E)- (CAS)	-	0.12	-	0.16	-	0.09	0.17	-
.alpha.-humulene	-	-	-	-	-	0.11	-	-

Compound name	Relative Concentration (%)							
	Bo		Kt		Me		Mu	
	5 cm	20 cm	5 cm	20 cm	5 cm	20 cm	5 cm	20 cm
1-Naphthalenol, 1,2,3,4-tetrahydro- (CAS) 1-Tetralol	-	-	-	-	-	0.07	-	-
1-Ethynyl-3,4-dihydro-2-naphthalenecarbadehyde	-	0.08	-	-	-	-	-	-
Phenol, 2,6-dimethoxy- (CAS) 2,6-Dimethoxyphenol	2.94	3.37	2.74	3.67	3.11	3.05	4.22	2.83
Phenol, 3,4-dimethoxy- (CAS) 3,4-dimethoxyphenol	0.25	0.33	0.33	0.40	0.24	0.42	0.40	0.22
Benzenepropanoic acid, methyl ester (CAS) Methyl hydrocinnamate	-	-	-	0.25	-	-	-	-
Propanoic acid, 3-(2-propynyloxy)-, ethyl ester (CAS) ETHYL 3-PROPARGYL	-	0.28	-	0.24	-	-	-	0.12
Propanoic acid, anhydride (CAS) Propionic anhydride	-	1.31	1.02	0.60	-	-	0.44	-
Propanoic acid, ethenyl ester (CAS) vinyl propionate	0.04	-	-	-	-	-	-	-
CYCLOPENTANEPROPANOIC ACID, 1-ACETYL-2,2-DIMETHYL-, METHYL	3.86	-	-	4.25	0.12	-	-	-
Benzenepropanoic acid (CAS) Phenylpropionic acid	-	-	-	2.74	-	-	-	-
3,4,5,6,7,8-HEXAHYDRO-2H-CHROMENE	-	-	0.20	-	-	-	-	-
1,2,3,4,4A,5,6,8A-OCTAHYDRO-NAPHTHALENE	-	-	-	-	-	-	0.58	-
Total	**12.28**	**8.95**	**9.49**	**16.30**	**9.95**	**6.59**	**13.30**	**8.62**
Mean for both injection range	10.61		12.89		8.27		10.96	
B. Aromatic compounds which are pyrolysed from wood parts								
4H-Pyran-4-one, 3-Hydroxy-2-methyl- (CAS) Maltol	0.14	0.17	0.17	0.21	0.19	0.29	0.14	0.27
4H-Pyran-4-one, 5-Hydroxy-2-methyl- (CAS) 5-hydroxy-2-methyl-4H-pyran-4-one	0.66	-	0.18	0.22	-	-	-	0.20
2-Propanone, 1-(acetyloxy)- (CAS) Acetol acetate	0.12	-	-	0.15	-	0.15	0.17	-
2-Propanone, 1-hydroxy- (CAS) Acetol	5.57	4.99	3.55	4.26	6.94	3.84	5.87	6.17
Ethanone, 1-(4-hydroxy-3,5-dimethoxyphenyl)- (CAS) Acetosyringone	0.50	0.58	0.66	0.67	0.56	0.38	0.49	0.65
ACETOVANILLONE	-	-	-	1.03	-	0.49	-	-
Ethanone, 1-(4-hydroxy-3-methoxyphenyl)- (CAS) Acetovanillone	-	-	0.46	-	-	-	0.74	0.83
1,2-Benzenediol (CAS) Pyrocathecol	-	-	-	-	-	-	2.20	-
1,2-benzenediol, 3-methyl- (CAS) 3-methylpyrocathecol	0.66	0.58	0.20	1.17	0.19	0.66	0.79	0.28
3-Methoxy-pyrocathecol	1.43	1.69	1.06	2.01	1.13	1.37	1.70	1.14
4-METHYL CATHECOL	1.90	0.46	0.19	-	0.24	-	-	-
Phenol, 2-methyl- (CAS) o-Cresol	-	-	-	-	-	-	-	0.18
Phenol, 3-methyl- (CAS) m-Cresol	0.27	0.30	0.71	0.18	-	0.45	0.31	-
Phenol, 4-methyl- (CAS) p –Cresol	-	-	-	0.57	0.11	-	-	-
Phenol, 2-methoxy- (CAS) Guaiacol	1.57	1.92	1.82	2.08	1.82	2.19	-	1.36
Phenol, 2-methoxy-4-propyl- (CAS) 5-PROPYL-GUAIACOL	0.18	0.23	-	-	0.13	0.15	-	0.11

Compound name	Relative Concentration (%)							
	Bo		Kt		Me		Mu	
	5 cm	20 cm	5 cm	20 cm	5 cm	20 cm	5 cm	20 cm
Phenol, 3-methoxy- (CAS) m-Guaiacol	-	-	-	0.22	-	0.12	0.14	-
Phenol, 4-ethyl-2-methoxy- (CAS) p-Ethylguaiacol	0.39	0.52	0.40	0.35	0.34	0.35	0.50	0.35
Phenol (CAS) Izal	-	-	0.89	1.07	-	0.87	0.36	-
Total	**13.37**	**11.43**	**10.28**	**14.18**	**11.63**	**11.31**	**13.40**	**11.54**
Mean for both injecion range	12.40		12.23		11.47		12.47	
C. Components with other odorant characters which have not yet mentioned as gaharu constituent								
Ascaridole	-	-	-	-	-	-	2.39	-
2H-Pyran-2-one, 6-ethyltetrahydro- (CAS) 6-ETHYL-.DELTA.-VALEROLACTONE	-	-	-	-	-	-	0.14	-
Oxacycloheptadec-8-en-2-one (CAS) Ambrettolide	0.05	-	0.82	0.52	-	0.64	-	-
Oxacycloheptadecan-2-one (CAS) Dihydroambrettolide	0.06	-	0.64	0.16	-	-	-	-
Benzoic acid, 3,4,5-trimethoxy-, methyl ester (CAS) 3,4,5-Trimethoxybenzoic	-	-	-	-	-	-	0.03	
Benzoic acid, 4-(methylamino)-	0.24	-	-	-	-	-	-	-
Benzoic acid, 4-ethenyl-, methyl ester (CAS) METHYL 4-VINYLBENZOATE	-	0.07	-	-	-	-	-	-
.beta.-bisabolene	-	-	-	-	-	0.51	-	-
2-Butanone (CAS) Mehtyl ethyl ketone	0.78	0.66	0.98	0.53	1.23	0.55	1.66	2.31
Butyric acid, m-nitrophenyl ester (CAS) m-Nitrophenyl butyrate	-	-	-	0.09	-	-	-	-
Carveol, dihydro-, cis-	0.85	-	-	-	-	0.76	0.61	-
Cholestane-3,6,7-triol, (3.beta.,5.alpha.,6.beta.,7.beta.)- (CAS)	-	-	-	-	-	0.07	-	-
2,5-furandione, 3-methyl- (CAS) Citraconic anhydride	-	-	-	-	-	0.03	-	-
Citronellyl acetate	-	-	-	-	-	-	-	0.20
.beta.-Cyclocitral	-	-	-	-	-	-	0.24	-
Cyclopentanone, dimethylhydrazone (CAS) Cyclopentanone dimethylhydrazone	-	-	-	-	-	0.26	-	-
Cyclopropyl carbinol	4.95	6.45	0.65	3.93	4.99	4.17	4.38	4.93
Cyclopentanone (CAS) Dumasin	-	-	0.32	-	-	-	-	-
1-Eicosanol (CAS) n-Eicosanol	0.33	-	1.93	0.70	-	1.61	-	-
TRANS-ISOELEMICIN	-	0.04	-	-	-	-	-	-
Ethanone, 1-(2,5-dihydroxyphenyl)- (CAS) Quinacetophenone	-	-	-	0.42	-	-	-	-
Phenol, 2-methoxy-4-(1-propenyl)- (CAS) Isoeugenol	-	-	-	-	-	-	0.28	-
Phenol, 2-methoxy-4-(1-propenyl)-, (E)- (CAS) (E)-isoeugenol	0.98	1.14	1.25	1.45	1.30	0.71	1.38	0.84
Phenol, 2-methoxy-4-(2-propenyl)- (CAS) Eugenol	-	0.12	0.22	0.22	-	1.67	-	-
5-BUTYL-2-VALERYLFURAN	-	-	-	-	-	0.33	-	-
2(3H)-Furanone, 3-acetyldihydro- (CAS) 2-acetylbutyrolactone	-	-	-	-	-	-	-	0.20

Compound name	Relative Concentration (%)							
	Bo		Kt		Me		Mu	
	5 cm	20 cm	5 cm	20 cm	5 cm	20 cm	5 cm	20 cm
2(5H)-Furanone, 5,5-dimethyl- (CAS) 4,4-Dimethylbut-2-enolide	-	-	0.18	-	-		-	-
2(5H)-Furanone, 5-methyl- (identity?) (CAS) 2-Penten-4-olide	-	-	-	-	-	0.09	-	-
2(3H)-Furanone, 5-hexyldihydro- (CAS) 4-decanolide	-	-	-	-	-	0.93	-	-
2-Furancarboxaldehyde (CAS) Furfural	0.60	0.31	0.56	0.28	0.74	0.56	0.40	0.75
2-Furanmethanol (CAS) Furfuryl alcohol	0.54	0.54	0.26	0.70	1.09	0.98	0.54	1.03
2-Furanmethanol, tetrahydro- (CAS) Tetrahydrofurfuryl alcohol	-	-	-	-	-	0.13	0.23	-
2-Heptanol, acetate (CAS) 2-HEPTYL ACETATE	0.35	-	0.63	0.25	-	-	-	0.30
2-Heptanone, 3-methyl- (CAS) 3-Methyl-2-heptanone	-	0.55	-	-	-	-	-	-
Hexanoic acid, 1-methylethyl ester (CAS) Isopropyl hexanoate	-	0.14	-	-	-	0.11	-	0.06
3-Hexenoic acid	-	-	0.21	-	-	-	-	-
1H-Indole (CAS) Indole	0.64	-	0.77	0.65	-	0.51	0.18	-
1H-Indole, 2-methyl- (CAS) 2-methylindole	-	-	-	-	-	0.48	-	-
6-Nitro-5-hydroxy-1,2-dimethylindole	-	-	-	0.03	-	-	0.04	0.02
Indolizine (CAS) Indolizin	-	-	0.59	-	-	-	0.36	-
Ionol 2	-	0.03	-	-	-	-	-	-
3-pentanone CAS) Diethyl ketone	0.71	-	-	-	-	-	-	-
1-Penten-3-one (CAS) Ethyl vinyl ketone	0.39	0.38	-	0.69	-	-	-	-
.GAMMA.HEXALACTONE	0.65	-	0.66	-	-	-	0.76	-
3,5-Dihydrodecanoic acid .delta.-lactone	-	0.31	-	-	0.09	-	-	-
Muskolactone	-	-	-	-	-	0.21	-	-
L-isoleucine, N-acetyl- (CAS) N-Acetyl-L-isoleucine	-	-	-	-	-	0.70	-	-
5,7-dimethoxy-2-methylindan-1-one	-	0.04	-	-	-	-	-	0.04
Lineolone	-	-	0.13	-	-	-	-	-
METHYL MALONIC ACID	-	-	-	-	-	0.11	-	-
p-Menthane-2-one-1,3,3-d3 (CAS)	-	-	-	-	-	0.89	-	-
2,6,6-TRIDEUTERIO-O-MENTHONE	-	-	0.19	0.22	-	-	-	-
Benzene, 1-methoxy-4-methyl- (CAS) p-methylanisole	-	-	-	0.20	-	-	-	-
NEROLIDOL ISOMER	-	-	-	-	-	-	-	0.15
4-Nonanol, 4-methyl- (CAS) 4-methyl-4-nonanol	-	-	-	-	-	0.21	-	-
2,5-Norbornanediol (CAS) 2,5-DIHYDROXYNORBORNANE	-	-	-	-	-	-	0.13	-
Piperidine, 1-nitroso- (CAS) NITROSOPIPERIDINE	-	0.89	-	-	0.65	-	0.57	-
PIPERIDINE, 1-(1-METHYLPENTYL)-	-	-	-	-	-	0.48	-	-
3-(2,5-DIMETHOXY-PHENYL)-PROPIONIC ACID	0.50	-	0.86	0.43	3.56	2.34	3.29	0.50

Compound name	Relative Concentration (%)							
	Bo		Kt		Me		Mu	
	5 cm	20 cm	5 cm	20 cm	5 cm	20 cm	5 cm	20 cm
3-PHENYL-PROPIONIC ACID ISOPROPYL ESTER	-	2.45	-	-	-	-	-	-
2-PROPYNOIC ACID	-	3.17	-	-	-	-	-	5.93
9H-Purine, 6-methyl-9-(trimethylsilyl)- (CAS) 6-METHYLPURINE, 9-TRIMETHYLSILYL	-	-	-	0.01	-	-	-	-
1,3-Benzenediol, 4-ethyl- (CAS) 4-Ethylresorcinol	-	-	-	-	-	-	0.42	-
1,3-Benzenediol, 5-methyl- (CAS) Orcinol	0.21	0.19	-	0.20	0.13	0.04	0.21	0.15
Benzaldehyde, 4-hydroxy-3,5-dimethoxy- (CAS) Syringaldehyde	0.47	0.57	0.52	0.52	0.58		0.50	0.58
(E)-2-hydroxy-4'-phenylstilbene	-	-	-	-	-	0.09	-	-
1-TRICOSENE	0.11	-	-	-	-	-	-	-
Benzaldehyde, 3,4-dimethoxy- (CAS) Vanillin methyl ether	-	-	-	-	-	-	0.97	-
Benzaldehyde, 4-hydroxy-3-methoxy- (CAS) Vanillin	0.38	0.40	0.50	0.52	0.59	0.52	0.45	0.45
Benzeneacetic acid, .alpha.-hydroxy-2-methoxy- (CAS) 2-methoxymandelic acid	-	-	-	-	-	-	0.01	-
Benzeneacetic acid, 4-hydroxy-3-methoxy- (CAS) Homovanillic acid	-	-	-	-	0.20	0.11	0.21	-
ISO-VELLERAL	-	-	-	0.02	-	-	-	-
Benzenemethanol, 3,4-dimethoxy- (CAS) Veratryl alcohol	-	0.10	-	-	-	-	-	-
2-Butanone, 4-(4-hydroxy-3-methoxyphenyl)- (CAS) Zingerone	-	-	-	0.63	-	-	-	-
Ethanone, 1-(2-furanyl)- (CAS) 2-Acetyfuran	-	0.14	-	0.12	-	-	0.12	-
2-ACETYL FURAN	-	-	-	-	-	0.23	-	-
2(3H)-Furanone (CAS) .alpha.-Furanone	-	0.27	-	0.23	-	-	0.32	-
2(3H)-Furanone, 5-methyl- (CAS) 5-Methyl-2-oxo-2,3-DIHYDROFURAN	-	0.06	-	-	-	-	-	-
2(3H)-Furanone, hexahydro-3-methylene- (CAS) 6-HYDROXYCYCLO	-	-	-	-	-	-	0.15	-
2(5H)-FURANONE	0.36	1.72	1.70	1.61	2.48	1.77	1.55	2.96
2,5-DIMETHYL-3(2H)FURANONE	-	0.04	-	-	-	-	-	-
2-ET HYL-4-HYDROXY-5-METHYL-3(2H)FURANONE	-	-	-	0.17	0.14	0.13	-	0.13
2-HYDROXY-5-METHYL-2(5H)-FURANONE	-	-	-	-	-	-	0.28	-
3-HYDROXY-5-METHYL-2(5H)-FURANONE	-	0.39	-	-	-	-	-	0.26
5-HYDROXYMETHYL-DIHYDRO-FURAN-2-ONE	1.23	1.65	-	1.30	1.02	1.33	-	1.18
HYDROXY DIMETHYL FURANONE	0.81	-	-	-	-	-	-	0.87
2-(Acetyloxy)-1-[2-(acetyloxy0-2-(3-furanyl)ethyl]-5a-[(acetyloxy)methyl]hexah	-	-	-	-	-	0.06	-	-
2-Methoxy-4-methylphenol	-	-	0.95	-	-	-	-	-
Phenol, 2,6-dimethoxy-4-(2-propenyl)- (CAS) 4-allyl-2,6-dimethoxyphenol	2.52	3.13	2.05	3.06	2.80	2.10	3.17	2.23
9H-Xanthen-9-one, 1,3-dihydroxy-6-methoxy-8-methyl- (CAS) 6-O-METHYL-	-	-	-	-	0.06	-	-	-

Compound name	Relative Concentration (%)							
	Bo		Kt		Me		Mu	
	5 cm	20 cm	5 cm	20 cm	5 cm	20 cm	5 cm	20 cm
Xanthosine (CAS) Xanthine riboside	-	-	-	-	-	0.23	-	0.29
Total	**18.70**	**25.93**	**17.57**	**19.86**	**21.62**	**27.12**	**25.95**	**26.33**
Mean for both injection range	22.31		18.71		24.37		26.14	
Total	**44.34**	**46.30**	**37.33**	**50.34**	**43.19**	**45.01**	**52.65**	**46.48**
Total mean for both injection range	45.32		43.83		44.10		49.56	

Note: Bo = Bahorok, Kt = Central Kalimantan Tamiang Layang, Me = Mentawai, Mu = Maluku

Reference: FAO (2008); Abrishami *et al.* (2002); Rho *et al.* (2007); Fotouhi *et al.* (2008); Sheikholeslam & Weeks (1987); Baker *et al.* (2004); Hua *et al.* (2001); Azah *et al.* (2008); International flavor and fragrance, Inc (2008); Castro *et al.* (2002); Lynd-Shiveley (2004); ChemYQ (2008); Rossi *et al.* (2007); Koeduka *et al.* (2006); Zaika *et al.* (2004); Valentines *et al.* (2005); The Good Scent Company (2008); Bunke & schatkowski (1997); Pedroso *et al.* (2008); Wikipedia encyclopedia Online (2008).

Table 1 was divided into 3 groups, A) gaharu constituent group which was identified previously by researchers, B) chemicals with odorant characters group originated from pyrolysis of wood parts such as cellulose and lignin, C) unconfirmed gaharu constituent chemicals with odorant characters.

The A group from Table 1, without differentiating injection range showed that the highest relative concentration accumulation of confirmed constituent (Yagura *et al.,* 2003; Bhuiyan *et al.,*2009; Pojanagaroon & Kaewrak, 2006; Burfield,2005; Tamuli, 2005; Alkhathlan *et al.,* 2005; Konishi, 2002; Nor Azhah *et al*., 2008) happened to isolates from Central Kalimantan Tamiang Layang for 12.89 %, followed by Maluku (10.96 %), Bahorok (10.61 %), and Mentawai (8.27%). Quantitatively and qualitatively for confirmed chemical components, isolate from Tamiang Layang (Central Kalimantan) gave the best artificial gaharu result as shown in relatively higher infection area and highest confirmed gaharu compounds accumulation.

Table 1 was shown that the B group is a group for compounds with odorant characters which was resulted from pyrolyisis of cellulose and lignin. This fact was shown beacause gaharu was commonly used as incense which produce fragrant aroma only when the resin-containing wood is burnt. The presence of odorant compounds from pyrolysis of wood parts probably has roles in the whole fragrance produced from burning gaharu incense. In other words, since the incense releases fragrant aroma when it is burnt, the presence of B group compounds can not be ignored despite that they are not the true gaharu resin constituent. For this odorant group which is generated from wood parts pyrolysis, the highest relative concentration was achieved by Maluku originated isolate (12.47 %), followed by Bahorok (12.40 %), Tamiang Layang (12.23 %), and Mentawai (11.47 %). This concentration difference probably was affected by the concentration of cellulose and lignin from the stem parts that were taken as samples. The effects from the compounds from this group to gaharu fragrance need further investigation.

As in the C group, the highest relative concentration accumulation was achieved by Maluku isolate (26.14 %), Mentawai (24.37 %), Bahorok (22.31 %), and last by Tamiang Layang isolate (18.71 %). The same order was also applied to the total of relative concentration for odorant-character components; Maluku, followed by Mentawai, Bahorok, and Tamiang Layang. Nevertheless, the contribution of odorant-character components for gaharu fragrance needs closer observation.

For confirmed gaharu components (the A group), generally the 5 cm injection range resulted higher accumulation concentration, unless for isolate from Tamiang Layang (Central Kalimantan) which showed higher accumulation concentration in 20 cm injection range (Table 4). Generally, the A group, the 5 cm and 20 cm injection range showed accumulation relative concentration 11.25 % and 10.11 % respectively. For the B group, accumulation for the 5 cm and 20 cm injection range was 12.17 % and 12.11 % respectively. These numbers are not far different because probably the wood components were relatively the same in the tree samples which were in the same age and grew under realtively same condition.

The total accumulation for odorant-character components showed that the 20 cm injection range treatment (52.59 %) resulted higher relative concentration than the 5 cm injcetion range treatment (50.23 %). The same order was also shown in relative accumulation concentration in the C group compounds, 24.81 % and 20.96 % respectively for 20 cm and 5 cm injection range treatments.

With more space between injections, the compounds formation ran relatively slowere as shown in less infection area. Nevertheless, this process might give more time and opportunity for particular compounds to be synthesized or accumulated, therefore resulted in relatively higher concentration. On the other side, with less space between injections where infections occured faster and more massive, the other odorant-character compounds was produced but the accumulation might not high enough when observation was carried. Further study is to be done to observe the development or changes that happen as time pass after inoculation.

The py-GCMS analysis results also showed the presence of compounds that were mentioned previously in other researches as defence compounds. Some of these components even also has fragrance characteristics which are known as essential oil constituent and have been used comercially in fragrance and perfume industry, such as vanillin, euginol (Cowan, 1999; Rhodes, 2008; Koeduka *et al.,* 2006), 4H-pyran-4-one compound and its derivats (Abrishami *et al.,* 2002; Rho *et al.,* 2007; Fotouhi *et al*., 2008), benzoic acid (NBCI, 2008), cyclopentane derivats (Wikipedia, 2008), syringaldehyde (Pedroso *et al.,* 2008), dumasin (Chem, 2008), and elimicin (Rossi *et al.*, 2007).

Eugenol and isoeugenol are used in vanillin production which are vital ingredients in fragrance industry (Cowan, 1999). Eugenol, isoeugenol, metileugenol, and isometileugenol are the four fenilpropanoid compounds from 12 volatile compounds which have been known responsible for sweet scent in *Clarkia breweri* (Rhodes, 2008). Whereas coniferyl

alcohol is the intermediate product in eugenol and isoeugenol biosynthesis (Cowan, 1999), and guaiacol is the intermediate in eugenol and vanillin synthesis (Li and Rosazza, 2000).

Table 2. Components in gaharu resulted through inoculation of *Fusarium* sp. originated from various regions to *A. microcarpa* which have important odorant characteristics

Component name	Information
Ambrettolide	This compound has musk, fruit, and flower scent characters (International Flavor and Fragrance, Inc., 2008)
Ambrox	*Ambrox* has odorant character amber type and also is anti-inflamatory which has potential in medical industry (Castro *et al.,* 2002).
Valerolactone	This compound has herbal scent which has been used in fragrance and perfume industry (Wikipedia Online, 2008).
Ketoisophorone	*Ketoisophorone* releases sweet scents of wood, tea, and tobacco leaves (The Good Scent Company, 2008).
Maltol	This component presents caramel scent and is used for sweet scent in fragrance, also used as flavor enhancer and aroma in breads and cakes (Wikipedia Online, 2008).
Indole	This compound in low concentration presents flowery scents and is constituent in various flowery scents and perfume. Indole is the main constituent in jasmine oil and since the jasmine oil is expensive, the syntheticly product was made using indole (Wikipedia Online, 2008).
Isolongifolen	*Isolongifolene* is a useful ingredient in odorant and perfume oil (Bunke & Schatkowski, 1997).
Limonene	*Limonene* is a terpen with flower and fruit scent. Limonene is monoterpenoid which is used as botanical insecticides, as also in cosmetic compound and flavorung for its citrus scent. Geraniol and limonen is also used as herbal medication and constituent in various herbals (Wikipedia Online, 2008; The Good Scent Company, 2008; Mann *et al*., 1994; Blake, 2004).
Cadinene	This compound presents in essential oil constituent in various plants (Wikipedia Online, 2008).
Dumasin	Also known as cyclopentanone which has mint scent. It is a fragrance, medication, and pesticide materials (ChemYQ, 2008).
Benzylacetone	*benzylacetone* has sweet flowery scent which is abundant attractant component in flowers, also found as volatile components in cocoa (Wikipedia Online, 2008).
Azulene	*Azulene* is very often found in essential oil in Asteraceae family plants and has scent and blue color in its oil and extracts (Lynd-Shiveley, 2004).

Acetosiringon compounds was also tracked in all gaharu resulted from inoculations of all five isolates used in this research, where this compound is phenolic which is produced by plants as a natural response of wounding (Sheikholeslam and Weeks 1986). In Hua (2001), it was mentioned that the acetosiringon concentration raised ten times when plant's active tissues are wounded. Acetosiringon is a bioactive compound in plant-microbe interaction which accelerate pathogen

presence detection by plants, where the concentration of this compound is raised in plants as microbes concentration increases (Baker *et al.,* 2004).

Table 3. Compounds listed as in several references were known as defense mechanism in particular plants and were detected in gaharu resulted through inoculation

Compound	Information
Eugenol	Bacteriostatic toward fungi and bacteria (Cowan 1999). Eugenol is used in perfume, essential oil, and medicine production. This compound is used to produce isoeugenol which is required in vanillin synthesis; which is essential in medicine, fragrance, and perfume industry. Eugenol and isoeugenol is derivated from lignin precursor; ferulate acid or coniferil alcohol (Rhodes, 2008).
Coniferyl alcohol	A phytoalexin type defense compound; belongs to fenylpropanoid group, for example is the one found in *Linum usitiltissimum* (Sengbusch, 2008).
Guaiacol	An intermediate in eugenol and vanillin synthesis; also used as antiseptic and parasiticide compound (Li & Rosazza, 2000).
Catecol and pyrogalol	A hydroxylated phenol which is toxic toward microorganisms. The position and amount of hydroxyl group in phenol group are thought to be realted with its relative toxicity toward microorganisms, where the toxicity increases at higher hydroxylation (Cowan, 1999).
Veratrol	A dimetil eter compound from pyrocatecol. Both compounds and their derivatives are used as antiseptic, expectorant, sedative, deodorant, and parasiticides agents (Wikipedia, 2008a). The resveratrol constituent which is derivated from *p*-hydroxycinamate acid and 2 unit malonate have antimicrobial activity (Torssel, 1983; p:144).

III. CONCLUDING REMARKS

1. *Fusarium* sp. inoculation to *A. microcarpa* stems results can be analysed quantitatively and qualitatively through infection area and chemical components approaches wich reflect the quantity and quality of gaharu that was formed.
2. In artificial gaharu formed through *Fusarium* sp. inoculation to *A. microcarpa,* previously identified as gaharu constituent compounds were found and several other compounds that have odorant characteristics and comercially are used in perfumery and flavoring industry.
3. Although statistically isolate origins did not show significant difference for infection area 6 months after inoculation, isolate origins made differences in gaharu compounds concentrations. Generally, inoculation of *Fusarium* sp. from Tamiang Layang (Central Kalimantan) resulted higher concentration of confirmed gaharu constituent compounds,

whereas Maluku originated isolate resulted relatively higher total concentration for odorant-character compounds.

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GAHARU-PRODUCING TREE INDUCTION TECHNOLOGY

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ABSTRACT

Gaharu is formed as an gaharu producing-tree responsed to particular factors which are the plant physiology and fungal infection. Fungi isolates which are potential to induce gaharu-forming have been isolated from various regions. This activity was carried in order to provide information about the diversity of isolates that have been collected. Wood samples were taken from several locations, from cultivated plants as well as nature (Java, Sumatera, Kalimantan, Sulawesi, and Maluku). Isolation, purification, and cultivation were done with adding standard medium, while qualification was carried with observing *Aquilaria malaccensis* and *A. microcarpa* characteristics. Cultured isolates on (Potato Dextrose Agar) PDA medium were incubated in room temperature for seven days. Isoalates that have been collected include *Fusarium solani* (Mart), Appell and Walenw, *F. sambunicum*, and *F. tricinctum*. Inoculation of four isolates of *Fusarium* to *Aquilaria microcarpa* was carried in KHDTK Carita, Banten. Inoculation of Gorontalo-originated *Fusarium* to *Aquilaria microcarpa* stems caused the largest and fastest infection compared to *Fusarium* originated from West Sumatera, West Kalimantan, or Jambi in 2-6 months.

Keywords :gaharu, induction technology, *Aquilaria* spp., *Fusarium* spp.

I. INTRODUCTION

Gaharu, which is a comercial product which has a highly economical value, is actually a resin deposit which is accumulated in wood tissue as a reaction toward wounding or pathogene infection. Gaharu has been traded since hundreds years ago. According to Suhartono and Mardiastuti (2002), the trading of this product in Indonesia was first registered in fifth century, and China was reported as the main buyer. In international trading this comodity was known with several names; agarwood, aloeswood, gaharu, gaharu, karas, jinkoh, oudh, and many others. Trading shape varies from chunks, chips, powder, and gaharu oil (Surata and Widyana, 2001). Oil-formed comodity was usually

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achieved by distilation or extraction from low quality chips.

Nowadays, gaharu has a high sale value especially from its fragrant resin which is called 'Scent of God', although the usage of gaharu is not limited to fragrance industry. In principal, gaharu can be used for medicine, incense, and frargrance (Barden *et al.,*2000). Gaharu incense is used in beliefs rituals and religious ceremonies, as room fragrance, and religious accecories such as rosario and *tasbih* (Barden *et al.*, 2000). Meanwhile, in medical industry, gaharu is used as analgesic and anti-inflammatory agent (Trupti *et al.,* 2007) and is known has benefits to cure various diseases like toothache, kidney pain, reumatics, asthma, diarrhea, tumor, diuretic, liver, hepatitist, cancer, smallpox, malaria, tonic for pregnancy and after-labor, and also has anti-toxic, anti-microbes, and neuron and digestive stimulant characteristics (Heyne, 1987; Barden *et al*., 2000; Adelina, 2004; Suhartono and Mardiastuti, 2002).

Researches concerning various aspects related to gaharu have been done for a long time and is still developing. These researches was primely initiated by the nature-dependent gaharu comodity. Due to the high gaharu-collecting activity which was solely dependent to nature, the main genus of gaharu-producing tree, *Gyrinops* and *Aquilaria* were included in Appendix II CITES. Not all gaharu-producing trees contain gaharu which is only synthesized under certain stress conditions. Gaharu forming process requires a long time, in which during the process various levels of quality are formed and at the end of the process, gaharu with highest quality will be achieved (Sumadiwangsa and Harbagung, 2000).

Gaharu-forming is initiated by biotic or abiotic factors. To synthesize gaharu artificially, one of these methods can be used mechanical wounding on the stem, or chemical inducing methods (methyl jasmonic, soybean oil, or brown sugar). Abiotic gaharu forming as mentioned above did not distribute its mechanism to other regions in the tree which are not directly affected by the abiotic factor. On the contrary, gaharu-forming by biotic factor such as fungi or other microbes let the mechanism spread into other region on the tree. Due to the spreading of gaharu-forming mechanism to other tissues, the quality and quantity of the gaharu product would be more satisfying.

II. MATERIALS AND METHODS

A. Materials

Materials that were used in this activity is 21 isolates of *Fusarium* spp. Which were inoculated in Laboratory of Forest Microbiology, R & D Center for Forest Conservation and Rehabilitation, Bogor. The fungi isolates were isolated from *Aquilaria* spp. stems which have shown gaharu-forming naturally. *Aquilaria* spp. stems were taken from various gaharu-producing trees in Java, Kalimantan, Sumatera, Maluku, West Nusa Tenggara, and Sulawesi (Tabel 1).

The medium for growing the fungi was Potato Dextrose Agar (PDA). The inoculation targets of the *Fusarium* spp. isolates were 13 years-old *A. microcarpa* trees. The fungi isolates used in this research were originated from Gorontalo, jambi, West Kalimanta, and Padang (West Sumatera). Inoculation tools were electric drills, 3 mm-sized drills, generator set, and many others.

Table 1. Observed Isolates

No.	Isolate Codes	Origins	No.	Isolate Codes	Origins
1	Ga 1	Kalimantan Tengah	12	Ga 12	Lampung
2	Ga 2	Maluku	13	Ga 13	Bengkulu
3	Ga 3	Sukabumi	14	Ga 14	Bogor
4	Ga 4	Kalsel	15	Ga 15	Mentawai
5	Ga 5	Kaltim	16	Ga 16	Kaltim LK
6	Ga 6	Belitung	17	Ga 17	Kalbar
7	Ga 7	Riau	18	Ga 18	Yanlapa
8	Ga 8	Bengkulu	19	Ga 19	NTB
9	Ga 9	Jambi	20	Ga 20	Kalsel MIC
10	Ga 10	Sumatera Barat	21	Ga 21	Kalteng TL
11	Ga 11	Gorontalo			

B. Methods

Prior to identification, each colonies were grown in PDA medium in petri dishes, and then incubated in room temperature for seven days. Morphology observation was carried under parameter microscope. The observed parameters were colony diameter, horizontally and vertically, colony color, and miselium aerial presence.

The observation for identification also cover the characteristics of macroconidium, microconidium, and the shape of conidiophore. The culture preparation was made by removing a small cut of the fungi isolates using a 5 mm-sized cork borer, placing them each on top of an object glass, and covering them with cover glasses. The slides were then incubated in a closed chamber with mantained moisture (by putting sterile aquadest-wetted cotton inside). After seven days, colonies that have grown on object slides were stained and their the shape and miselium were observed under microscope.

C. Inoculation Technics

1. Inoculation

The sample trees were *A. microcarpa* grown in Carita Research Forest. The Completely Randomized Design (CRD) was used with isolate origins as observed treatments (I), which were *Fusarium* spp. From Gorontalo (II); West Kalimantan (12); Jambi (13), and Padang (14) and also mix of these four isolates (15). Each isolates were inoculated with 3 times as repetitions.

Inoculation was done to all sample trees. Before injection, all the tools were streilized with 70% alcohol to prevent cross-contamination. The drilling was done down to 1/3 of stem diameter, aiming the liquid inoculant would reach to cambium and phloem. One ml of the liquid inoculant was injected to each holes on the stem. The injection holes were keep open for aeration condition for the inoculated microbes.

Figure 1. Drilling on stem of tree sample (A) and isolate injection through the drilling hole (B)

2. Gaharu Observation and Sampling

Infection observation was carried 2 months and 6 months after inoculation by measuring the length of infection on stem surfaces vertically and horizontally. Data collection was done randomly in several injection spots. Infection length value is the mean of the infection length of every holes in one tree.

III. RESULTS AND DISCUSSION

A. The Diversity of *Fusarium* spp. Isolates

1. Morphology Diversity

Aerial miselium morphology character, colony color, and colony diameter of *Fusarium* spp. from different origins varied greatly (Table 2). The diversity of morphology was due to the origins of the isolates.

Table 2. Morphology Diversity of *Fusarium* spp. from various origins

No.	Isolate codes	Origins	Morphology characters		
			Coloby diameter mm/7 days	Aerial miselium	Color on PDA medium
1	Ga-1	Kalteng	61	Yes,+++	White, bright yello
2	Ga-2	Maluku	49	Yes,++	White, bright brown
3	Ga-3	Sukabumi	48	Yes,+	Bright brown
4	Ga-4	Kalsel	50	Yes,++	White
5	Ga-5	Kaltim	45	Yes,++	White
6	Ga-6	Belitung	38	Yes,+	White
7	Ga-7	Riau	59	Yes,++	Cream white
8	Ga-8	Bengkulu	49	Yes,++	White
9	Ga-9	Jambi	59	Yes,+++	Cream white, bright brown
10	Ga-10	Padang	61	Yes,+++	White
11	Ga-11	Gorontalo	58	Yes,+++	Brownish white
12	Ga-12	Lampung	58	Yes,+++	Bony white, pink
13	Ga-13	Bangka	59	Yes,+++	White
14	Ga-14	Bogor	61	Yes,++	White
15	Ga-15	Mentawai	56	No	Brown, yellow, white
16	Ga-16	Kaltim LK	57	Yes,+	White, purple
17	Ga-17	Kalbar	59	Yes,+++	Creamy white
18	Ga-18	Yanlapa	58	Yes,++	White, bright yellow
19	Ga-19	Mataram	52	Yes,++	White
20	Ga-20	Kalsel MIC	50	Yes,++	White, bright yellow
21	Ga-21	Kaltel TL	69	Yes,++	White, creamy

The abundance of aerial miselium: + A little, ++ Fairly present, +++ Abundant

a. Aerial Miselium Presence

Aerial miselium presence is one of character for almost *Fusarium* spp. isolates. Isolate Ga-15 from Mentawai was found without aerial miselium. Although most of the isolates have aerial miselium, its relative abudance differs between isolates. Isolate Ga-1,

Ga-9, Ga-10, Ga-11, Ga-12, Ga-13, and Ga-17 have realtively abundant aerial miselium, whereas Ga-3, Ga-6, and Ga-16 isolates have relatively less abundant aerial miselium.

Irawati (2004) reported that generally the fungi that have been grown in bright light conditon for a long time would grow relatively more aerial miselium. The aerial miselium that has formed is a phototropic mechanism toward light (Irawati 2004). In this research, all isolates were given the same light treatment, the various abundance of aerial miselium was due to isolates' own character.

b. The Color of Colony

Beside the aerial miselium, the diversity among *Fusarium* spp. isolates also cover the color of the colony. Results showed that Ga-4, Ga-5, Ga-6, Ga-8, Ga-10, Ga-13, Ga-14, and Ga-19 have white colonies (Figure 2, 3, and 4). Other than white, several isolates formed white and bright yellow (Ga-1), bright brown (Ga-2), creamy white (Ga-7, Ga-17, and Ga-21). Ga-17 and Ga-21 isolates have similar colony color with Riau-originated *Fusarium* which was identified as *Fusarium solani* (Luciasih 2006).

Fusarium solani is a cosmopolit species with unique characteristic of its elips-shaped microconidium. Ga-10 and Ga-11 isolates formed white colony and peach colony. Ga-18, Ga-19, Ga-20 formed white and bright yellow colonies.

There was also found an isolate with distinctly different colony color from other isolates; which had purple miselium hyphae but was also thin white on the aerial miselium by the edge of the colony. Fungi without pigment generally have hialin color.

c. Colony Diameter

Colony diameter of *Fusarium* spp. was around 30-69 mm. All isolates can be classified into 3 groups, with diameter less than 40 mm (Ga-6), 40-50 mm (Ga-2, Ga-4, Ga-5, Ga-8, and Ga-20), and more than 50 mm (isolat Ga-1, Ga-3, Ga-7, Ga-9, Ga-10, Ga-11, Ga-12, Ga-13, Ga-14, Ga-15, Ga-16, Ga-17, Ga-18, Ga-19 ,and Ga-21) (Table 2, Figure 2, 3, and 4).

The diversity of colony diameter is related to the hyphae's speed of growth which is also highly related to the presence of aerial miselium. The speed of growth is a unique character for each isolates. It is also related to the isolates' virulance. Isolate's virulance can be tested toward its host.

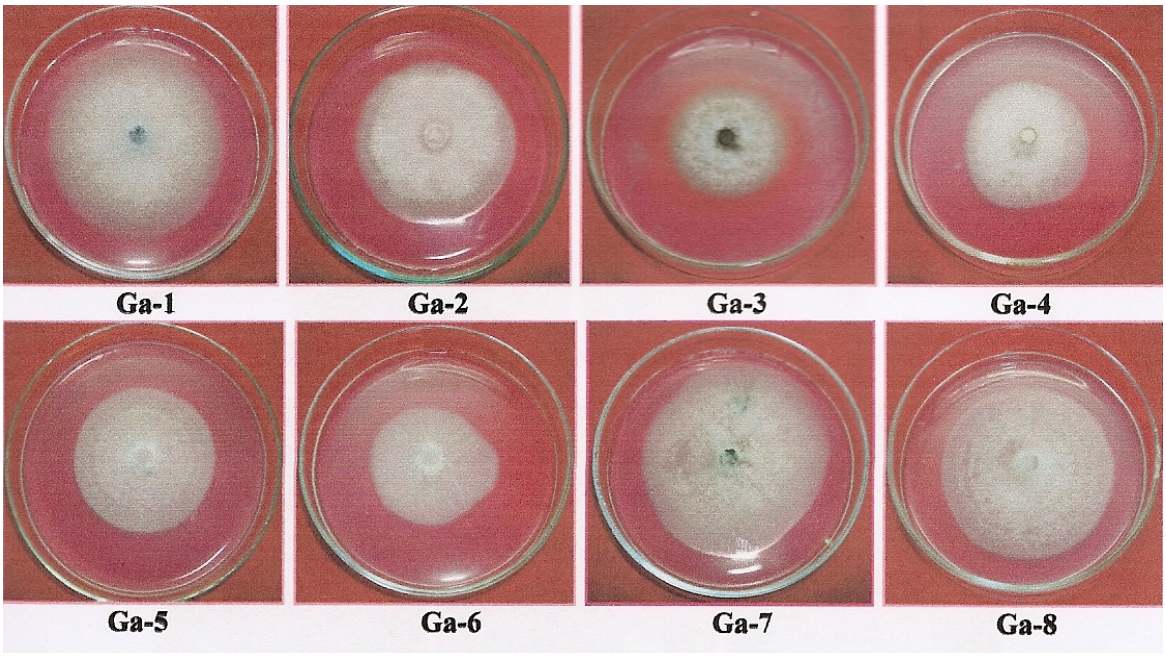

Figure 2. Morphology Diversity of *Fusarium* spp. (isolate Ga-1, Ga-2, Ga-3, Ga-4, Ga-5, Ga-6, Ga-7, and Ga-8) age seven days at PDA media

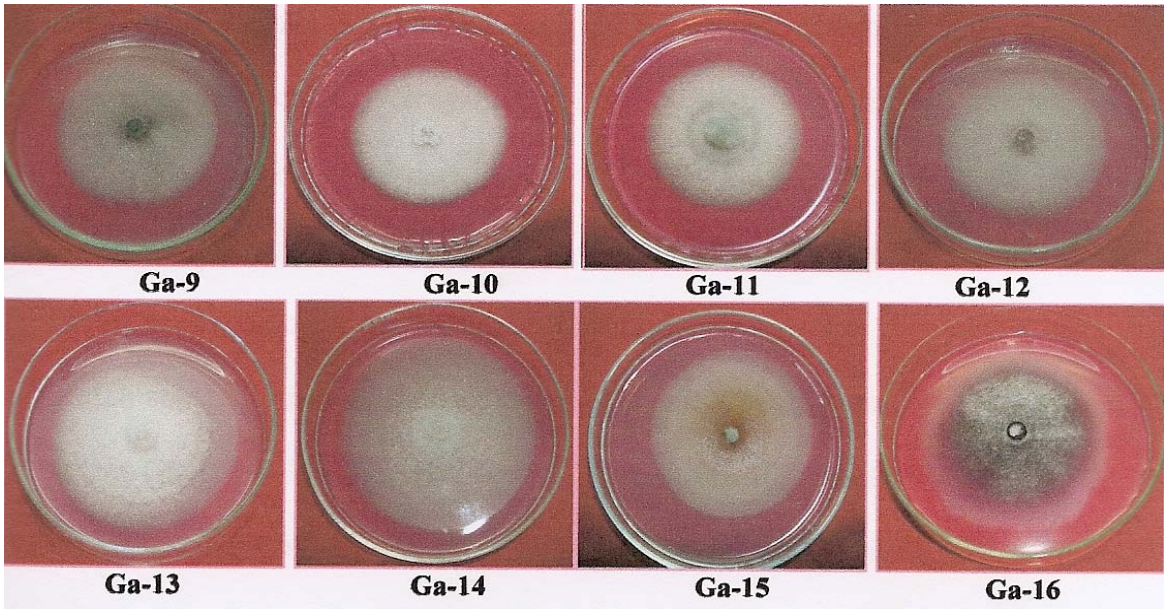

Figure 3. Morphology Diversity of *Fusarium* spp. (isolate Ga-9, Ga-10, Ga-11, Ga-12, Ga-13, Ga-14, Ga-15, and Ga-16) age seven days at PDA media

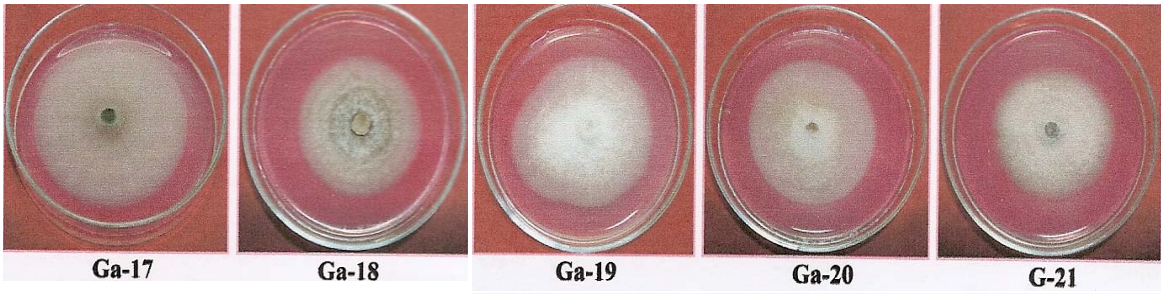

Figure 4. Morphology Diversity of *Fusarium* spp. (isolate Ga-17, Ga-18, Ga-19, Ga-20, and Ga-21) age seven days at PDA media

2. The Diversity of Micro and Macroconidiphore

The *Fusarium* spp. isolates showed various micro and macroconidia characteristics. Observation showed that diversity was seen in the macroconidia septaa number, conidiophore branch, and microconidia abundance.

The dominant macroconidia septa number of 2-3 was possesed by Ga-1, Ga-3, Ga-5, Ga-6, Ga-7, Ga-8, Ga-10, Ga-18, Ga-20, and Ga-21 isolates. But these 10 isolates had different conidiophore branch. Ga-18 and Ga-21 isolates had branched conidiophores; whereas Ga-1, Ga-3, Ga-5, Ga-6, GA-7, Ga-8, and Ga-10 had simple conidiophores (Table 3).

Ga-2 Ga-3, Ga-15 isolates had 4 septa, but all three could be distinguish based on their conidiophore branch and the microconidia shape. Ga-2 had branched conidiophore, and elips-ovale microconidia. Ga-13 had simple conidiophore and ga-15 had branched conidiophore.

Table 3. Diversity of macroconidia characteristics of *Fusarium* spp. from various origins

No.	Isolate Codes	Histological Character			
		Macroconidia	Microconidia		
		Septa number	conidiophore	Abundance	Shape
1	Ga-1	3	Simple	Abundant	Elips
2	Ga-2	4	Branched	Abundant	Elips, oval
3	Ga-3	3	Simple	Abundant	Elips
4	Ga-4	4-7	Simple	Abundant	Elips, oval
5	Ga-5	2	Simple	A few	Elips
6	Ga-6	3	Simple	A few	Elips, oval
7	Ga-7	2	Simple	A few	Elips, oval
8	Ga-8	2	Simple	A few	Elips, oval
9	Ga-9	5	Simple	A few	Elips, with partitions
10	Ga-10	3	Simple	Abundant	Elips, with partitions
11	Ga-11	4	Branched	Abundant	Elips

No.	Isolate Codes	Histological Character			
		Macroconidia	Microconidia		
		Septa number	conidiophore	Abundance	Shape
12	Ga-12	5	Simple	Abundant	Elips
13	Ga-13	4	Simple	A few	Elips
14	Ga-14	7	Simple	A few	Elips
15	Ga-15	4	Branched	Abundant	Elips
16	Ga-16	7	Simple	A few	Elips, 3-partitions
17	Ga-17	5	Branched	A few	Elips
18	Ga-18	3	Branched	Abundant	Elips
19	Ga-19	4	Simple	Abundant	Elips
20	Ga-20	2	Branched	A few	Elips, oval
21	Ga-21	3	Branched	A few	Elips

Microconidia in various *Fusarium* have distinct shape named fusoid, therefore are easy to distinguish from other genus with similar morphology with *Fusarium*. *Fusarium* genera have similarities with ylindrocarpon morhologically, but Booth (1971) distinguished *Cylindrocarpo* apart because its base of conidia was relatively blunt and did not have hock/foot cell which is very clear on *Fusarium* spp.

Ga-12, Ga-14, and Ga-16 isolates had relatively numerous septa, around 5-7 sepat (Table 3). Two out of those three isolates; Ga-12 and Ga-14 had similar conidiophore and microconidia shapes; but these two isolates had different type of macroconidia. Ga-12 had relatively bigger macroconidia compared to Ga-14 (Figure 5 & 6). Ga-14 was different from Ga-16 due to the parted microconidia in Ga-16 (Figure 7).

Luciasih *et al.* (2006) reported species diveristy among 21 isolates of *Fusarium* spp.. Several isolates have been identified to the species level.The identified isolates were *F. smbunicum* (Ga-1), *F. tricinctum* (Ga-2, Ga-3, and Ga-5), and *F. solani* (Ga-4, Ga-6, Ga-7, Ga-8, and Ga-9). Between three species, *F. solani* was the most dominant species, therefore required more attention.

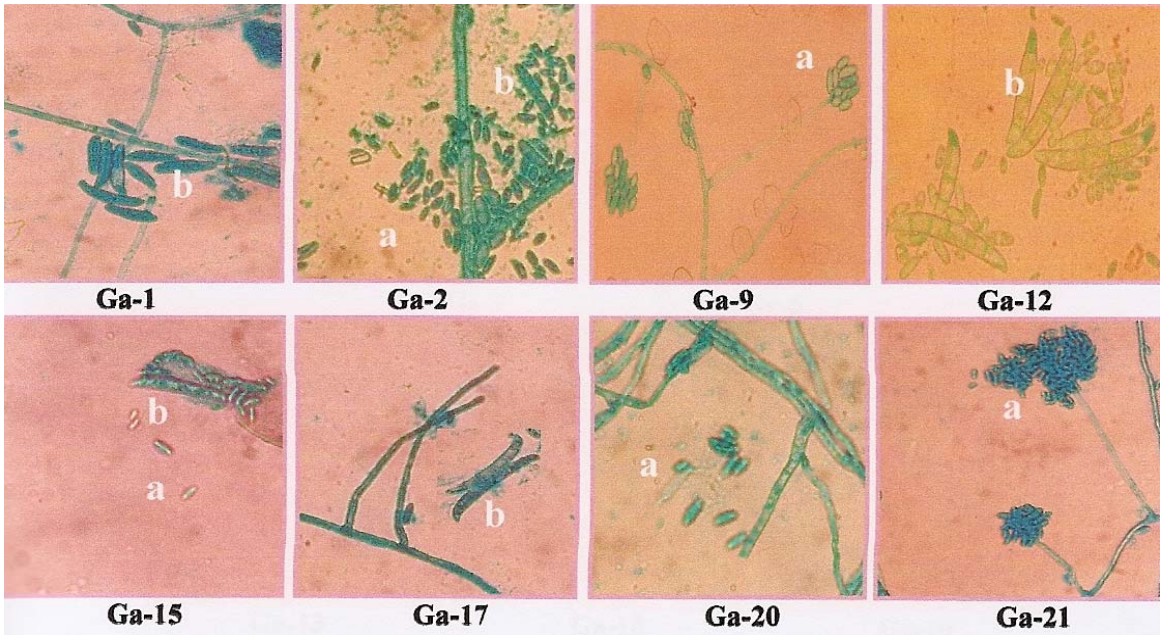

Figure 5. Macroconidia diversity (a) and Microconidia diversity (b) *Fusarium* spp. (isolate Ga-1, Ga- 2, Ga-9, Ga-12, Ga-15, Ga-17, Ga-20, and Ga-21) with zooming in 40x

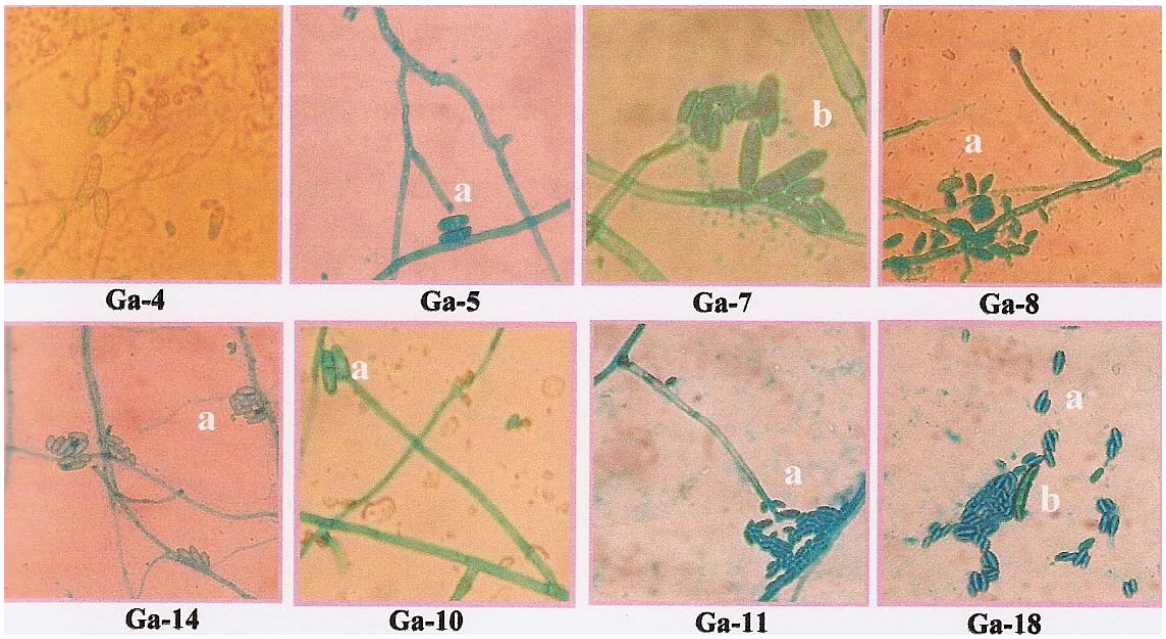

Figure 6. Macroconidia diversity (a) and Microconidia diversity (b) *Fusarium* spp. (isolate Ga-4, Ga-5, GA-7, Ga-8, GA-10, Ga-11, Ga-14, and Ga-18) with zooming in 40x

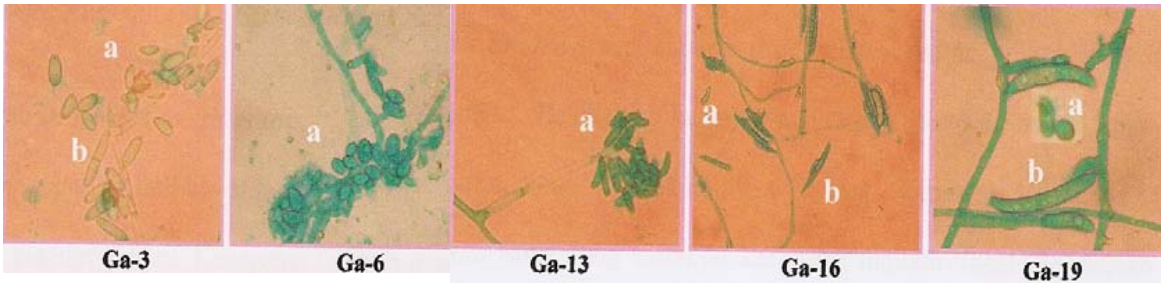

Figure 7. Macroconidia diversity (a) and Microconidia diversity (b) *Fusarium* spp. (isolate Ga-3, Ga-6, Ga-13, Ga-16, and Ga-19) with zooming in 40x

Fusarium solani is different from *F. sambunicum*, for one by its shape and abundance of microconidia. Whereas *F. solani* can be distinguish from *F. tricinctum* by the shape of its macroconidia, also by the relatively bigger and elips-shaped microconidia for *F. solani*.

Cowan (1999) explained that plants had unlimited ability in synthetizing aromatic subtances which were mostly fenolic compounds or its oxygen-subtitute derivatives. Generally, these compounds are secondary metabolites which often have roles in plant's defense mechanisms against microbe, insect, or herbivore attacks.

Gaharu is a phytoalexin compound from gaharu-producing trees as their defense mechanism toward pathogene infection. This resin-contained wood is a secondary metabolite as plant's defense respons. The healthy gaharu-producing trees will never produce sesquiterpenoid as fragrant secondary metabolite (Yuan *in* Isnaini 2004). The plant synthesizes and accumulates secondary metabolites as a response toward infection by certain agents; physiology stimulation or stress condition (Goodman *et al. in* Isnaini 2004).

Secondary metabolites in plants defense system, like phytoantisipin or phytoalexin, play a big role (Verpoorte *et al.,* 2000). Phytoantisipin is an active compound with anti-microbe activity which present in plant, but sometimes its activity is stimulated by wounds. Phytoalexin is an anti-microbial active compound which is produced *de novo* after wounding or infection. The biosynthesis of both compound are stimulated in gene level (Verpoorte *et al.,* 2000; Vidhyasekaran, 2000).

Plants secondary metabolites which are derivated from terpenoid have various functions in plants; like as an anti-microbial agent (sesqui-, di-, and triterpena). Based on the various functions, the expression of the related biosynthesis pathways would be different. There are biosynthesis pathways that are stimulated in gene level after wounding or infection and there are others that occur in compounds level, where the already present compounds are to change enzimatically into active compounds when there is a wound. For instance, certain sesquiterpena biosynthesis in solanaceae is stimulated when there is microbe infection, whereas in other plants, sequiterpenoid biosynthesis is a common expression. In *Morinda citrifolia,* anthraquinone can be found in all area of the plant (Verpoorte, 2000).

The secondary metabolite concentration varies between species, inter-tissues (the highest is in the derm, teras wood, roots, branch base, and wounding tissues), between trees in the same species, inter-species, and is also season-dependent. Tropical and sub-tropical species usually contain higher extractive amount than temperate trees (Forestry Comission GIFNFC, 2007).

Secondary metabolites in wood can be called as extractive compounds. The extractive compounds which consist various components have important roles in defense against fungi and insect attacks, producing scents, flavors, and color of the wood. The extractive compounds in teras wood can be tree defense against distructive agents even though the influence varies in different habitats (Hills, 1987). Rowell (1984) stated that one of the role of extractive compounds was as tree defense mechanism toward microbial infection. The plant's secondary metabolites is effective against pathogene agents due to the analogue of certain vital component of the cellular signal or related to vital enzymes and block the metabolism pathways (Forestry Comission GIFNFC, 2007).

B. Stem Infection Analysis

In condition against fungal infection, gaharu-tree responses to mantain and recover itself. Tree resistance will determine the winner between the tree and the pathogene. In order to get gaharu, one would prefer that the pathogene succeed, therefore the desired gaharu product will be produced. Producing certain chemicals is one of the defense mechanism toward pathogenes. Gaharu, identified as sequiterpenoid compound, defense compound of phytoalexin type. The vulnerability of the tree against fungal infection is related to the gaharu production, reflected by the infection area and chemicals components.

In Figure 8 is shown the infection length in *A. microcarpa* stems 2 months and 6 months after inoculation. Two months after inoculation *Fusarium* spp. from Gorontalo showed the highest infection value; 4.13 cm, followed by mix isolates, Padang, West Kalimantan, and lastly from Jambi. Variant analysis result showed that isolates' origins signifficantly affected the infection length. Duncan's further test confirmed that 2 months after inoculation, isolate from Gorontalo caused the most severe infection, followed by the mix isolates (Table 4).

Table 4. Duncan's further test on 2-months infection of inoculation

Isolate origin	Mean value
Jambi	1,857a
Kalimantan Barat	2,223a
Padang	2,297a
Campuran	3,193a
Gorontalo	4,133a

Remark: Value followed by the same character is not completely different at 0,05

Six months after inoculation, the mix isolates caused higher infection than other isolates (Figure 8). At this time, statistically, isolate origins did not significantly affect the infection degree. As in 2 months after inoculation, the isolate from Gorontalo and mix isolates still showed the highest infection.

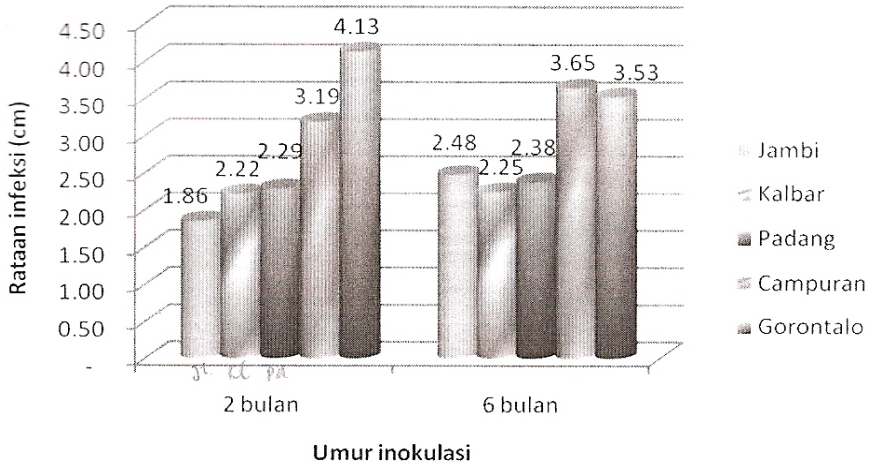

Figure 8. The lenght of infection of A. Microcarpa stem stem

Figure 9 showed the growth of infection length since the second month until the sixth month. Although was still seen as an isolate with the highest infection value, the sixth month infection value barely raised anymore from the second month's value, whereas other isolates showed various raised infection value. Nevertheless, statistically for the sixth month, the isolates' origins did not significantly affect the infection speed (significant value 0.186 at 5%).

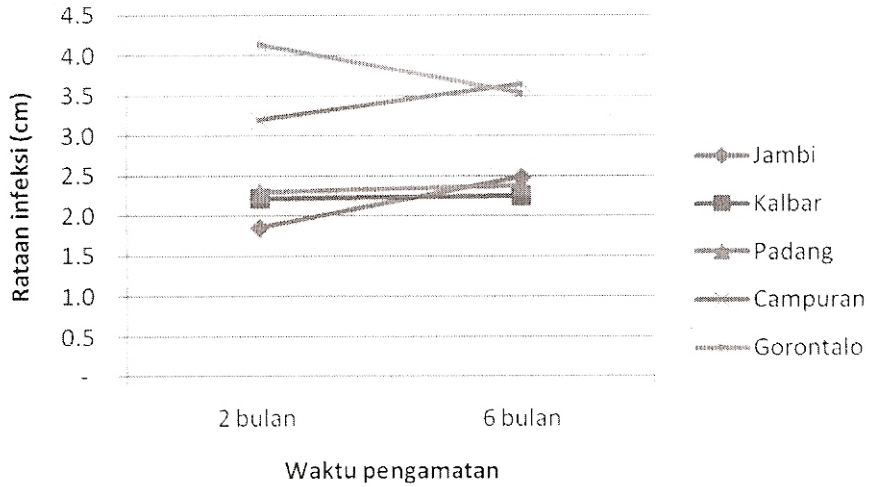

Figure 9. The speed of infection of *A. microcarpa*

The infection development on the sixth month after inoculation showed that the isolates' origins did not significantly affect the infection value anymore. This was probably also related to the uniqueness of each sample trees. Even though, the Isolate from

Gorontalo caused the highest infection, further research is needed to observe the infection speed development for quite some time.

From the infection development results, isolate from Gorontalo caused the highest infection, which means this isolated resulted the highest quantity of gaharu. Despite that the mix isolates showed the highest infection value after 6 months, there is possibility that it was due to the presence of isolate from Gorontalo in the mix.

IV. CONCLUSIONS

1. Morphologically, *Fusarium* spp. isolates were dominated by white colonies, but there were also pink, yellow, and purple colonies. Almost all isolates formed aerial miselium. Histologically, *Fusarium* spp. isolates had macroconidia with 3-4 septa and the microconidia were dominated by elips shape.
2. The growth speed comparison showed that Ga-9, Ga-11, and Ga-17 isolates showed faster growth speed than other isolates.
3. Inoculation of *Fusarium* spp. to *Aquilaria microcarpa* could be analysed quantitatively and qualitatively through infection area and chemical components as reflections of the quality and quantity of formed gaharu.
4. *Fusarium* spp. from Gorontalo caused the highest infection value, therefore this isolate is recommended for large amount desired gaharu production.

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EFFECTIVITY AND INTERACTION BETWEEN *Acremonium* sp. AND *Fusarium* sp. IN FORMATION OF GAHARU CLUMP IN *Aquilaria microcarpa*

By:

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ABSTRACT

Aquilaria microcarpa is one of the trees that produce gaharu. Gaharu is formed as a response to a fungus infection. *Acremonium* sp. and *Fusarium* sp. were the fungus which often used to induce clump formation. Both these fungus were often isolated from one single clump symptom. Interaction between both fungus in clump formation was unknown. Therefore the ability of *Acremonium* sp. and *Fusarium* sp. and their interaction in clump formation were to be studied. Tree trunks of A. microcarpa were drilled and then inoculant 1 (A= *Acremonium* sp. or F= *Fusarium* sp.) was inserted into a sequence of holes and followed by inoculant 2 (F=*Fusarium* sp. or A=*Acremonium* sp). into another sequence of holes with 1 week interval on the same tree trunk. Before the inoculant *Acremonium* was inserted into the holes, the holes were treated with 2% sugar solution. Range between a sequence of holes of inoculant 1 and inoculant 2 was 15 cm. All treatments consisted treatment with single inoculant AA and FF, with double inoculant AF and FA, without inoculant (only drilled =B, drilled and treated with sugar=G), and negative control (K). Range between holes of a pair of treatments was 30 cm. Every treatment was made in 3 different trees. Effectivity and interaction between inoculant were determined by length, width of color-change zone on wood, color level, fragrant level, and precentage of fragrant induction point, and terpenoid compound accumulation. Wood color change level and fragrant level were determined by Liebermann-Burchard method. Observation was carried every month for 4 months. Generally, every treatment caused color change on wood and stimulated wood's fragrant change. Sugar solution caused the symptom of gaharu clump formation supressed. *Acremonium* and *Fusarium* were relatively more effective in stimulating the gaharu clump formation rather than holes-making or sugar solution treatment, especially in inducing fragrance. Double inoculant treatments, especially AF was more effective in inducing fragrance formation than FA and single inoculant. On the other side, inoculant FA was better at other parameters. With 1 week interval, inoculant 1 did not raise resistence to inoculant 2, likewise, inoculant 2 did

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not seem to affect inoculant 1. Terpenoid compound which is classified into triterpenoid was detected in all double treatments and single treatment F. In other treatment, sterol compound was found. The concentration of both compounds were lower than those found in nature gaharu.

Keywords: Terpenoid compound, *Aquilaria microcarpa, Acremonium, Fusarium*

I. INTRODUCTION

Gaharu is one of non timber forest products (NTFPs) commodity which is produced by several species of gaharu trees (*Aquilaria* sp., Thymelaeaceae). The clump formation process in gaharu trees is still investigated. According to Nobuchi and Siripatanadilok (1991), gaharu clump was thought to be formed through fungus infection. Various specieses of *Fusarium* such as *F. oxysporum, F. bulbigenium,* and *F. lateritium* have been isolated by Santoso (1996). In addition, Rahayu *et al.* (1999) stated that several isolates of *Acremonium* sp. from gaharu clumps of *Gyrinops versteegii* and *A. malaccensis* were able to induce symptom of clumps formation in 2 year-old gaharu trees (*A. crassna, A. malaccensis, A. microcarpa*). In gaharu clumps formed through fungus induction, oleoresin compound was detected (Prema & Bhattacharyya, 1962). Rahayu *et al.* (2007) and Rahayu (2008) also stated that *Acremonium* sp. stimulated wood color change and the formation of terpenoid compound. Therefore, wood color change and the existance of terpenoid compound was selected as indicators of effectivity and interaction between inoculants in clump formation.

Acremonium sp. and *Fusarium* sp. were often obtained from one clump symptom. Infection mechanisms of both fungus in one infection location have not yet studied. Whereas, according to Sticher *et al.,* 1997, in several cases of fungus infection in plants, the infection of the first fungus might raise resistance called Systemic Acquired Resistance (SAR) toward the infection of the next fungus. For instance, Caruso and Kuc (1977) stated that infection of *F. oxysporum* f.sp. *cucumerinum* had raised SAR of watermelon plants toward infection of *Colletotrichum lagenarium*. Liu *et al.* (1995) also found SAR process raised by *Pseudomonas lachrymans* infection in cucumber toward *F. oxysporum.* Using double infection by *Fusarium* sp. and *Acremonium* sp. as double inducer requires information about SAR occurance raised by *Fusarium* sp. toward *Acromonium* sp. and vise versa. Therefore, this research aimed to study the effectivity and interaction between *Acremonium* sp. and *Fusarium* sp. in clump formation in eglewood trees (*A. microcarpa*).

II. MATERIALS AND METHOD

A. Materials

Materials and equipments used in this research are 13 year-old *A. microcarpa* trees in Hutan Penelitian (Forest for Reseach) Carita, Banten, *Acremonium* sp. isolate IPBCC 07.525 (IPBCC collection, Department of Biology, Faculty of Mathematics and Naturan Science, Bogor Agricultural University), and *Fusarium* sp. originated from *Aquilaria* sp. in Padang (Forest Microbiology Laboratory, Forest and Nature Conservation Research and Development Centre), 2% sugar solution, alcohol, aquades, drill, 4 mm-sized brace and bit, gage, pelet materials and its printer.

B. Methods

1. Inoculant Making

Acremonium sp. and *Fusarium* sp. were replanted on potato dextrose agar (Difco) and incubated at room temperature for seven days. These cultures were then used as inuculant sources for making inoculant. *Acremonium* sp. was grown on sawdust medium for two weeks, and then formed into 4 x 40 mm pellets. *Fusarium* sp. was grown in 300 liquid medium and incubated for three weeks in shaker incubator.

2. Test of Effectivity and Interaction between *Acremonium* sp. and *Fusarium* sp.

Firstly, a sequence of holes were made around the main stem (started from 0.5–1m above the soil) with 4 mm brace and bit, with maximum hole depth equals to 1/3 stem diameter. Range between holes in a sequence was about 5 cm. Into these sequences of holes, inoculant 1 was inserted until it filled the holes. One week later, the trees were drilled again, vertically 15 cm apart from the previous sequence of holes. Into these sequence of holes, inoculant 2 was inserted. Inoculant pair (FA or AF) was a set of treatment. Range between treatment set in 1 tree was ± 30 cm. For inoculant in pellet form, 2 % sugar solution was added into the holes before inoculant insertion. Tree stems without any treatment (K), only-drilled stems (B), drilled and treated with 2 % sugar solution stems (G), and single treatment stems (treated with only *Acremonium* sp. (AA) or only *Fusarium* sp. (FF)) were used as comparisons. Observation was carried every 1 month for 4 months.

Effectivity and interaction were measured through clump formation symptom development around induction area. Stem color change and fragrance formation are the indicators for clump formation. The stem around the holes were peeled, and then the stem color change was measured horizontally and vertically. The area which showed stem color change from white into blackish brown was chiselled and taken to laboratory for

further observation. Color change was observed in 10 points for every tree. Wood color change level was determined based in score system (0 = white, 1 = brownish white, 2 = brown, 3 = blackish brown). Wood color change level was presented in average (mean) from observation result from 3 respondents.

Fragrant level was determined based on score system (0 = not fragrant, 1 = a little fragrant, 2 = fragrant). Wood around the inoculation point was chiselled, and then was observed the fragrant organoleptically when the wood is burnt. Fragrance was stated in fragrant level and precentage of induction points with a little fragrant and very fragrant category. Fragrant level was presented in the average (mean) score form 3 respondents.

3. Terpenoid Compound Detection

Terpenoid compound was detected with Lieberman-Burchard methode (Harborne, 1987). After the observation of fragrant level, wood samples which had color change were seperated from the healthy ones. Color-changed 0.4 g wood was soaked in 5 ml hot absolute ethanol, and then was filtered on sterile Petri dish and was evaporated until it became dry (until yellowish deposits formed). On the deposits, 1 ml concentrated diethyl ether was added, homogenized, and then transferred into sterile reaction tube, and then 3 drops of anhydrous acetic acid and concentrated H_2SO_4 was added. Color change into red or purple shows triterpenoid compound was contained (Harboune, 1987). Absolute ethanol of 5 ml was added into the solution, then the absorbance was measured with spectrophotometer in λ 268 nm.

4. Data Analyses

Observation result data (width and length of color change zone, color change level, and fragrant level) was analyzed with SAS 9.1 version using Completely Randomized Design (CRD) (Rancangan Acak Lengkap, RAL) one factor with time and F test at α = 5%. When significant influenced by observed treatment, every treatment degree would then be compared using further test *Duncan* at 5 % degree.

III. RESULTS

A. Inoculant Effectivity in Inducing Gaharu Clump Formation Symptom

Generally every treatment caused wood color change and stimulated wood fragrant change (Table 1). Sugar treatment suppressed gaharu clump formation symptom. Effectivity of single or double inoculant of *Acremonium* and *Fusarium* were relatively higher in stimulating gaharu clump symptom formation than other induction methods. As a single inoculant, A and F had relatively similar effectivity. Clump formation symptom due to double inoculant also tended to be not significantly different from its single inoculants. Based on percentage of induction points in fragrant category, double inoculant was

more effective. Between double inoculants, AF was more effective in inducing fragrance formation than FA and single inoculant. While for other parameters, AF was better.

Wood color altered from white into brown or blackish brown (Figure 1). Inoculant treatment did not affect length and width of color change zone. However, the highest length of color change zone occured on wood which was treated with double inoculants FA and AF respectively. Whereas color change level was affectred by inoculant. The highest color change level was achieved in FA treated woods and was significantly different from other treatments.

Table 1. Gaharu clump symptom formation by single and double fungus inoculation

Treatment		Mean*					
		Wood color change			Fragrant (score)	Indoction point for a little fragrant (%)	Induction for fragrant (%)
		Length (cm)	Width (cm)	Color (score)			
Single inoculant	AA	2,54ab	0,82a	1,90b	0,63ab	34,37	1,39
	FF	3,14a	0,94a	1,45c	0,62ab	31,07	0,00
Double inoculant	AF	3,20a	0,87a	1,75b	0,70a	39,55	6,24
	FA	3,30a	0,83a	2,18a	0,59ab	20,12	4,16
Positive control	G	1,86b	0,55b	1,02d	0,38c	10,41	0,00
	B	2,87ab	0,73ab	1,16d	0,47bc	11,11	0,00
Negative control	K	0,00c	0,00c	0,00e	0,00d	0,00	0,00

* from 3 repetitions except in widht and length, means are from 5 repetitions, different letter on numbers in the same column shows significantly different for Duncan test at = 0.05.

Inoculant treatment had no significant effect on fragrance formation. Different from wood color change, the highest fragrant level was achieved on wood which was treated with double inoculant AF. Based on the *mean*, the fragrant level score of inoculant treatments belonged to not fragrant category. Nevertheless, inoculant treatments increased the percentage of fragrance induction points. Even the single inoculant AA and double inoculants placed the induction points in fragrant category (Table 1).

Figure 1. Wood color change with different darkness level from (a) the lowest level to (d) the highest level.

Induction period affected all clump formation parameters except for color change zone length (Table 2). Generally, the highest parameter score for clump formation occured on the second month, except for color change level. On the second month after induction, color intensity tended to increase, but the intensity of wood color on the 4th month was relatively the same with the one on the third month.

Table 2. Influence of induction period toward gaharu clump formation symptom

Month	Mean*					
	Wood color change			Fragrant (score)	Induction points of a little fragrant (%)	Induction point of fragrant (%)
	Length (cm)	Width (cm)	Level (score)			
1	2,46ab	0,68a	0,83c	0,32c	8,43	0,00
2	2,58a	0,71a	1,24b	0,64a	39,47	0,00
3	2,32ab	0,65b	1,67a	0,51b	17,45	0,00
4	2,26b	0,65b	1,65a	0,36c	18,45	6,74

* from 3 repetitions except in widht and length, means are from 5 repetitions, different letter on numbers in the same column shows significantly different for Duncan test at = 0.05.

B. Interaction between Inoculant 1 and Inoculant 2

Generally inoculant 1 did not raise tree's resistance toward inoculant 2 (Table 3). Inoculation of F before inoculation of A did not affect clump symptom formation on A point including fragrance formation. Inoculant F presence tended to increase the wood color change response due to inoculation of A. Likewise, inoculation of A before F did not affect clump symptom formation on F point, except that color on F became darker and the pecentage of induction points of fragrant relatively higher compared to the ones treated with its single inoculant. Double inoculants AF and FA resulted 8.33% of induction points for fragrant category.

Table 3. Influence of inoculant 1 toward inoculant 2 in gaharu clump formation symptom

Treatment	Mean*					
	Wood color change			Fragrant (score)	Induction point of a little fragrant (%)	Induction point of fragrant (%)
	Length (cm)	Width (cm)	Color (score)			
FAa	2,73abc	0,71bcd	2,15ab	0,60ab	26,37	8,33
AAa	1,96bc	0,66bcd	1,82bc	0,63ab	36,11	0,00
AFf	2,52abc	0,83abcd	1,57cd	0,70a	40,27	8,33
FFf	2,61abc	0,75abcd	1,36de	0,62ab	27,78	0,00
GGg	1,75c	0,53d	1,02e	0,38c	0,00	0,00
BBb	2,40abc	0,65cd	1,13e	0,47bc	0,00	0,00
KKk	0,00e	0,00e	0,00f	0,00d	0,00	0,00

* from 3 repetitions except in widht and length, means are from 5 repetitions, different letter on numbers in the same columnshows significantly different for Duncan test at = 0.05.

Table 4. Influence of secondary infections by different fungus from primary infection fungus

Treatment	Mean*					
	Wood color change			Fragrant (score)	Induction point of a little fragrant (%)	Induction point of fragrant (%)
	Length (cm)	Width (cm)	Color (score)			
FFF	3,68ab	0,99ab	1,53cd	0,62ab	34,37	0,00
FAF	3,87a	0,95abc	2,22a	0,50bc	13,89	0,00
AAA	3,13abc	0,96abc	1,98ab	0,63ab	32,63	2,78
AFA	3,88a	1,06a	1,93ab	0,70a	38,86	4,15
GGG	1,98bc	0,56d	1,01e	0,38c	20,83	0,00
BBB	3,35abc	0,80abcd	1,20de	0,47bc	13,89	0,00
KKK	0,00d	0,00e	0,00f	0,00d	0,00	0,00

* from 3 repetitions except in widht and length, means are from 5 repetitions, different letter on numbers in the same column shows significantly different for Duncan test at = 0.05.

Secondary infection did not consistantly affect the primery infection (Tabel 4). Inoculation of F before inculation of A tended to not affect clump symptom formation including fragrant level, except for wood color change. Color intensity on F induction point was better than its single treatment. Second infection by F also tended not to affect clump formation symptom and fragrant level.

Secondary infection by the same fungus did not affect clump symptom formation (Table 5). Nevertheless, generally the parameter scores for clump symptom on secondary infection points were lower than the primary infection points. Inoculant A and F have relatively same potention in inducing fragrance formation.

Table 5. Influence of secondary infection by the same fungus which infected primarily

Treatment	Mean*					
	Length (cm)	Width (cm)	Color (score)	Fragrant (score)	Induction point of a little fragrant (%)	Induction point of fragrant (%)
AAA	3,13abc	0,96abc	1,98ab	0,63ab	32,63	2,78
AAa	1,96bc	0,66bcd	1,82bc	0,63ab	36,11	0,00
FFF	3,68ab	0,99ab	1,53cd	0,62ab	34,37	0,00
FFf	2,61abc	0,75abcd	1,36de	0,61ab	27,78	0,00

* from 3 repetitions except in widht and length, means are from 5 repetitions, different letter on numbers in the same column shows significantly different for Duncan test at = 0.05.

C. Compound Formation

Terpenoid compound was detected in all treatments. In FF single and double treatment, red color was formed, indicating triterpenoid compound. Red color on gaharu oil was used as comparison to triterpenoid consisted due to treatments. In wood extracts of B, G, and inoculant A, green color was formed. This green color showed sterol compound was contained (Harborne, 1987). Meanwhile on K, color was not formed (transparent). This showed that in K, triterpenoid or sterol compound was not found.

Triterpenoid compound in color change zone varied in every treatment 4 months after induction (Table 6). Generally, absorbance values of terpenoid extracts from treatments were less than of gaharu oil (0.813) as a comporison.

Table 6. Absorbance values of color-changed gaharu extracts

Treatment	Month			
	1	2	3	4
K	0	0	0	0
G	0,29*	0,24*	0,34*	0,14*
B	0,12*	0,22*	0,39*	0,45*
AF A	0,20**	0,06*	0,25*	0,12**
AF F	0,20**	0,05*	0,23**	0,11*
FA F	0,12**	0,11**	0,21*	0,06*
FA A	0,12**	0,19**	0,23**	0,23*
AA	0,15*	0,20*	0,27*	0,40*
FF	0,14**	0,06*	0,25**	0,15*

* Green colored deposits; ** Brownish red colored deposits

Besides that, generally absorbance values of double inoculant treatments were almost the same as single inoculants (Table 6). This showed that double inoculants were not effective in increasing terpenoid compound contained.

Inoculant AF or FA also did not affect the terpenoid contained. This indicated that inoculant 2 treatment did not affect the absorbance value of inoculant 1. Likewise, inoculant 1 treatment did not affect the absorbance value of inoculant 1. On the third month after inoculation, on samples from single inoculation FF treatment, red deposit was formed and has a relatively high absorbance value. This showed a relatively high concentration of triterpenoid (Table 6).

IV. DISCUSSION

A. Induction Effectivity

The trees that have been given treatments started to show less fitness since 1 month after inoculation. Less fitness was shown in chlorosis leaves on the first and second

branch from induction hole area, and then these leaves fell. Generally single inoculant caused chlorosis on leaves on two closest brances from induction holes. Whereas on double inoculant treated trees, chlorosis occured on three closest branch from inoculation holes. Different from leaves on treated trees, leaves on trees as controls did not have chlorosis on them until the end of observation. Two months after inoculation, the number of chlorosis leaves was not different from the previous observation on one month after inoculation, but the chlorosis has covered almost the whole leaves.

Chlorosis might be related to nutrient availability. Nutrient availability was disturbed because of the obstructed distribution route due to drilling. Besides that, the inoculant itself might be the cause of the chlorosis. Caruso & Kuc (1977) stated that *Colletotrichum lagenarium* had caused chlorosis on watermelon and muskmelon leaves. The trees suffered more when worms attacked. The tree shoot became leafless. The decreasing leaf number drastically might hamper photosynthesis process because leaves are the main domain for photosynthesis. Photosynthates as the carbon source for antimicrobial secondary metabolites synthesis might be hamperd because probably the carbon source would be prioritized for new bud formation. In the end, clump formation symptom was hampered.

Wood color change occured in all treatments. Wounding, sugar treatment, and *Acremonnium* sp. and *Fusarium* sp. inoculation caused wood color change from white into darker. According to Braithwaite (2007) *Acremonium* sp. and *Fusarium* sp. were associated with wood color change symptom and dicline on *Quercus* sp. in New Zealand. Previously, Walker *et al.* (1997) also stated that wood color change into brown (browning) might be caused of pathogen attack (fungus) and physical distruction. Wood color change on gaharu might indicate the presence of gaharu compounds. This was supported by Rahayu and Situmorang (2006) who stated that color change from white into blackish brown was the early symptom of gaharu compound formation.

Sugar solution treatment (G) supressed gaharu clump formation symptom development. This is because of the sugar will immediately be used by tree for curing process rather than be used by the fungus. According to Nobuchi and Siripatanadilok (1991), wood color change into brown appeared after the cells lost starch after wounding.

Incubation period tended to influence all parameters in gaharu clump symptom. The longer the incubation period is, the darker wood color would be achieved. Meanwhile for the other parameters, the highest value was achieved two months after inoculation. Most likely this phenomenon was related to the trees' decreasing fitness which started twomonths after inoculation.

Based on the precentage of induction points of fragrant, double inoculants are better than single inoculants or other induction way. This proved that fragrance is a specific response toward disturbance form (Rahayu *et al.,* 2007).

Fragrance was started to be detected two months after inoculation and was decreasing afterward. Fragrance was part of the gaharu compounds (Rahayu *et al.,*

2007). Fragrance is a volatile compound therfore most likely belongs to sesquiterpenoid compounds. Nevertheless isopentenyl pirophosphate metabolism as terpenoid synthesis precursor (McGarvey and Croteau, 1995) might not stop at sesquiterpenoid product, but it might enter further metabolic pathway. In this research, triterpenoid and sterol compounds were also detected. This indicated that terpenoid metabolism might go on and end at products beside sesquiterpenoid when harvested.

The fragrance and fragrant frequency in double inoculant AF treatment were relatively higher then other treatments. However, wood color intensity was lower than double inoculant FA. This showed that the fragrance produced might not always be in proportion to wood color intensity. In accordance to what Rahayu *et al.* (1999) stated that gaharu fragrance synthesis was not always followed by wood color change.

Generally inoculant 1 did not raise tree resistance toward inoculant 2. This is different from research results by Krokone *et al.* (1999) which proved that inoculation of *Heterobasidion annosum* which was followed by *Ceratocystis polonica* supressed blue-stain symptom formation in Norway speuce trees (*Picea abies*). Most likely this was due to different pathologic activity. *Acremonium* and *Fusarium* are known to cause stem-rot or stem cancer in woody trees. *Heterobasidion* also cause stem rot but *C. polonica* cause only blue-stain and do not cause stem-rot. *Heterobasidion* might stimulated trees to synthesize phytoalexin compounds which is anti *C. polinica.* Another possibility is that inoculation period between inoculant 1 and 2 was only 1 week. Krokene *et al.* (1999) stated that in Norway spruce (*P. Abies*), SAR was formed 3 weeks after *H. Annosum* infection.

B. Gaharu Compound Formation

Acremonium sp. and *Fusarium* sp. in single or double inoculants forms were able to stimulate gaharu tree to produce terpenoid compounds. Paine *et al.* (1997) stated that fungus attack toward trees would stimulate the tree to synthesize terpenoid compounds as tree's defense. On previous research, Putri (2007) also stated that *Acremonium* sp. treatment on *A. crassna* proved to be able to stimulate the terpenoid compound synthesis.

In this research, triterpenoid was started to be detected one month after inoculation. Treterpenoid was detected in single inoculant FF treatment and double inoculant treatments which was shown in the presence of red deposits on Lieberman-Burchard test. Meanwhile on B, G, and single inoculant AA treatments, green deposits were formed. This green color indicated that sterol compounds was found. Harborne (1987) stated that sterol belongs to terpenoid compounds.

V. CONCLUSION

All inoculated trees had less fitness one month after inoculation. Double inoculants, especially AF, were more effective than single inoculants in stimulating fragrance synthesis. Induction by inoculant 1 one week after inoculant 2 did not raise tree resistance toward the

second inoculant. All inoculants, except single inoculant A stimulated tree to synthesize triterpenoid.

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TRIAL FOR GENERATIVE AND VEGETATIVE PRODUCTION OF GAHARU (EAGLEWOOD) PLANTING STOCKS

By:

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ABSTRACT

Gaharu is one of the reliable and superior trees, particularly for development of people plantation forest. R & D Centre for Forest Conservation and Rehabilitation, with the support of Project of ITTO PD 256 prepared science and technology needed for the aspect of planting stocks production and fungi injection for gaharu stimulation. In the development of science and technology for gaharu planting stocks production, research had been conducted concerning the effect of storage duration on seed germination, which was related to the recalcitrant seed property. Research on gaharu cutting was also conducted to learn the ideal condition for gaharu propagation with cutting, in relation with program of gaharu clonal development. Duration and condition seed storage were influential on gaharu seed germination. Gaharu seed germination decreased from 82% in the initial germination to 42% after 8 weeks storage in room temperature condition. Storage of gaharu seed in refrigerator decreased germination percentage of seeds which had been stored for 8 weeks to 24%. Propagation by cutting on media comprising mixture of coconut rind powder and rice husk with ratio of 1:1, and twice a week watering, produced the best growth percentage of 69%.

Keywords : gaharu, generative, vegetative, planting stocks.

I. INTRODUCTION

Development of gaharu plants is generally not intended for producing wood, but instead, it is intended to produce gaharu resin which is formed from the plant's respond toward microbe, particulary the fungi of *Fusarium* sp*, Cylindrocarpon* sp*, Trichoderma* sp*, Pythium* sp*, Phialophora* sp and *Popullaria* sp (Santoso *et al*, 2007; Daijo & Oller, 2001; Parman *et al,* 1996; Sidiyasa & Suharti, 1987). In nature, less than 5% of gaharu tree population produce the gaharu, and if the gaharu is formed, the amount is usually less than 10% of the wood biomass of the infected tree. However, natural gaharu

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could achieve highest quality (superior class) whose price could reached Rp 30 million per kilogram. Because of the very high price potency, exploitation of natural gaharu is conducted without proper consideration of its sustainability. As a result, population of gaharu species decline rapidly, so that this species is included in *Appendix* II CITES (Santoso *et al*, 2007). As a consequence, in formal trade, gaharu should be produced from trees resulting from culture, not from nature.

Gaharu culture requires input in the form of science and technology to optimize growth and production of gaharu resin. Such scientific and technological support are in the form of planting stocks production and injection of gaharu stimulant. Discussion in this paper is focused on the aspect of planting stocks production or propagation of gaharu. Propagation of gaharu could be conducted either vegetatively or generatively (Hou, 1960). Gaharu plant has recalcitrant seeds which germinate rapidly and could not be stored for a long time (Roberts and King, 1980). The practice of vegetative propagation and tree improvement had the potency to produce gaharu clone planting stocks which possess superiority in terms of growth and productivity of gaharu resin.

Project of ITTO PD 425/06 attempted to develop and apply science and technology in gaharu planting in order that the growth and productivity of gaharu resin is high. However, information on science and technology of gaharu seeds and vegetative propagation is still very limited. This paper present results of research in the aspect of seed technology and propagation of gaharu from cuttings.

II. MATERIALS AND METHODS

A. Generative Propagation

Testing in generative propagation was conducted on seeds and uprooted natural seedlings (wildlings) of gaharu. Gaharu seeds which were used in the test of seed storage and germination were mixture of *Aquilaria microcarpa* and *A. Malacensis* originating from Sukabumi. Treatments in test of gaharu seed storage were storage durations (0, 2, 4, 6 and 8 weeks) and storage temperatures (25.4-26.1^{o}C and 4.9-6.5^{o}C). Testing was conducted by completely randomized design with 5 replications.

Uprooted seedlings which were used in the test of storage duration of uprooted seedlings, were mixture of *Aquilaria microcarpa* and *A. malaccensis*. Treatments in test of storage duration of uprooted seedlings were three storage durations (1, 2 and 3 days of storage) and conditon of transplanting (inside cover house and without cover house). Testing was conducted by using completely randomized experimental design with five replications.

B. Vegetative Propagation

Technique of vegetative propagation which was used in this study was the use of shoot cutting. The research was conducted in greenhouses which used mist cooling

of KOFFCO system (Sakai and Subiakto, 2007; Subiakto and Sakai, 2007). Cutting materials used in this testing were *A. malaccensis*. Planting stocks production test using gaharu cutting was conducted in three stages. Treatment in the first step used routine procedure, namely the use of media in the form of mixture of coconut rind powder and rice husk with ratio of 2:1. Watering was conducted 2 times a week. In the second test, treatment of watering intensity was reduced to once a week with media of rice husk charcoal. In the third test, the media used mixture of coconut rind powder and rice husk with ratio of 1:1 and watering intensity of 1 time in the first month, 2 times in the second month and 3 times in third month.

III. RESULT AND DISCUSSION

A. Generative Propagation

Gaharu seeds are categorized as recalcitrant, so they should be soon germinated. Seed storage test was conducted to learn on how long the gaharu seed can be stored. Results of seed germination test from the two storage conditions are presented in Table 1 and Table 2. Storage condition (room condition and in refrigerator condition) did not significantly affect seed germination (P Anova=0,0993). On the other hand, storage period, affected seed germination percentage (P Anova = <0,0001).

Technically, germination of gaharu seed is easy to be conducted, germination medium could be in the form of rice husk charcoal or zeolite. In this testing, the germination medium used was rice husk charcoal. In species of recalcitrant seeds such as meranti, seed sowing was conducted after the fruit ripened and fell down. In gaharu species, storage at room condition for 2 months could still produced seedlings with success rate of 48%.

Germination is usually started at second week and the percent of successful planting stocks was calculated at sixth week after sowing. In Table 2, it can be seen that there was decrease between germination percentage and planting stocks success percentage. The decrease tended to be greater if the seeds were stored for longer duration. Therefore, for obtaining high percentage of successful planting stocks, sowing (germination) should be conducted soon after fruit harvesting.

Table 1. Germination percentage from results of seed germination test

Storage duration (period)	Room condition	Refrigerator
Direct	82%	-
2 weeks	69%	69%
4 weeks	77%	69%
6 weeks	56%	61%
8 weeks	48%	24%

Table 2. Percentage of succesful planting stocks (6 weeks after sowing) from the results of seed storage test

Storage duration (period)	Room condition	Refrigerator
Direct	74%	-
2 weeks	50%	54%
4 weeks	64%	58%
6 weeks	37%	48%
8 weeks	29%	9%

Generative propagation could also be conducted by using planting stocks obtained as uprooted seedlings occuring under the mother plants. In the planting test of uprooted seedlings, gaharu seedlings with height of 7 cm, whose cotyledon have fallen down, were used. Results of planting test of the uprooted seedlings are presented in Table 3. The use of cover house increased signficantly the growth percentage of uprooted seedlings (P Anova = <0,0001).

Table 3. Growth percentage of uprooted seedlings from test of storage and planting condition of the uprooted seedlings

Storage duration (period)	With cover house	Without cover house
0 day	80 %	40 %
1 day	76 %	46 %
2 days	87 %	24 %
3 days	76 %	38 %

Generally, uprooted seedlings which still have cotyledon, could be directly planted in plastic pot without using cover house. However, if the cotyledons have fallen down, the planting of uprooted seedlings should pass through the stage of cover house usage. Cover house could be made from transparent PVC plastic. The cover house should be tight, to maintain humidity inside the cover house at level above 95%. Results of this test proved that high humidity inside the cover house affected the planting success of uprooted seedlings. Storage of uprooted seedlings for three days could still give sufficienty good results (76%) if the planting used cover house.

B. Vegetative Propagation

Production test of gaharu cutting was conducted by using technology of KOFFCO system developed by Agency for Forestry Research and Development (*Badan Litbang Kehutanan*) and Komatsu (Sakai and Subiakto, 2007; Subiakto & Sakai, 2007). This technology regulates environmental condition, namely light, humidity, temperature and

media at optimum level for growth (Sakai *et al*. 2002). Results of production test of gaharu cutting are presented in Table 4.

Table 4. Rooting percentage of cutting from a series of production test of cutting

Research stage	Species	Treatment	Rooting percentage
1	*A. crassna*	Wihout pot-tray, watering 3 times a week, media of cocopeat : rice husk= 2:1	40%
1	*A. crassna*	With pot-tray, watering 3 times a week, media of cocopeat : rice husk = 2:1	42%
1	*A. microcarpa*	Without pot-tray, watering 3 times a week, media of cocopeat : rice husk = 2:1	44%
1	*A. microcarpa*	With pot-tray, watering 3 times a week, media of cocopeat : rice husk = 2:1	47%
2	Mixture of *A. crassna* and *A. microcarpa*	Media of burnt rice husk, watering 1 time a week	17%
2	Mixture of *A. crassna* and *A. microcarpa*	Media of sand, watering 1 time a week	31%
2	Mixture of *A. crassna* and *A. microcarpa*	Media of zeolite, watering 1 time a week	55%
3	Mixture of *A. crassna* and *A. microcarpa*	Media of cocopeat : rice husk = 1:1, watering 1 time a week	53%
3	Mixture of *A. crassna* and *A. microcarpa*	Media of cocopeat : rice husk = 1:1, watering 2 times a week	69%
3	Mixture of *A. crassna* and *A. microcarpa*	Media of cocopeat : rice husk = 1:1, watering 3 times a week	49%

Production test of cutting was conducted in three stages of research. In the first stage, standard procedure was used for cutting production. The treatments were types (kinds) and container of cutting planting (sowing). Average percentage of success rate of cutting at first stage test ranged between 40%-47%. Cutting production is assessed as being able to be applied economically if the success rate reach 70% (Subiakto & Sakai, 2007). Treatment species did not show significant differences (P Anova=0.6600) in percent of cutting success rate. Also, the planting container did not show significant differences (P Anova=0.8276) in percent of cutting success rate. Considering that cutting success rate was still below 70%, then there was further test being performed.

In the second stage test, watering was reduced to one time a week, whereas the tested treatments were types (kinds) of media (rice husk charcoal, sand and zeolite). Test results showed that the media had significant effect (P Anova = 0,0083) on percent of succes of cutting. The best medium was zeolite, but the rooting percentage was still below 70%. Zeolite is a medium with good porosity and was not grown over with fungi or algae. Because zeolite is a medium which is heavy and relatively expensive, then there is a need to try other media which possess porosity level which is relatively similar with that of zeolite.

In the third test, the media being used was mixture between cocopeat and rice husk which had been sterilized. The tested treatments were level of watering (1 time a week, 2 times a week and 3 times a week). Test results showed that at confidence level of 5%, watering had significant effect on rooting percentage of cutting (P Anova = 0,0210). The best watering level was two times a week with rooting percentage of 69%. Effect of watering was making the media be saturated with water and increase the growth of fungi, including the rotting fungi.

IV. CONCLUSION

1. The best germination percentage was obtained from seeds which were directly sown after fruit harvesting. However, by anticipating decrease in germination capacity, the seeds could still be stored for two months. Gaharu seeds do not need to be stored in refrigerator. The seeds could be properly stored in room condition. Planting of uprooted seedlings using cover house, produced better growth percentage as compared to those which did not use cover house.
2. The best medium for gaharu cutting was mixture between coconut rind powder (*cocopeat*) and rice husk with ratio of 1:1. The best watering was conducted twice a week. Propagation with gaharu cutting was conducted in greenhouse with KOFFCO system.

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APPLICATION OF PHYTOHORMONE-PRODUCING RHIZOBACTERIA TO IMPROVE THE GROWTH OF *Aquilaria* sp. SEEDLINGS IN THE NURSERY

By:

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Abstract

Gaharu or aloewood or agarwood is resinous wood found mainly in the genus of *Aquilaria*. Gaharu is formed through a unique pathological process initiated with infection of fungi on the wood tissue. Gaharu has many uses, i.e. incense in religious ceremony, perfume additive, medicine, and cultural activities. In response to overexploitation of gaharu-producing trees that has threatened their existence, genera or *Aquilaria* and *Gyrinops* have been enlisted in Appendix II since October 2004. It is therefore crucial to sustain the existence of gaharu-producing species and to accelerate regeneration of gaharu-producing trees for commercial use. This study was aimed at investigating the effect of plant growth promoting rhizobacteria (PGPR) in accelerating the growth of gaharu-producing seedlings in the nursery. The PGPR have been previously tested in vitro for their phytohormone production from which nine isolates along with one additional isolate of interest were selected for this study. Inoculation accelerated height growth of seedlings up to 5 months after inoculation. *Burkholderia* sp. CK28 and *Chromobacterium* sp. CK8 gave consistent effect on height growth acceleration. Percentage of height increase over non-inoculated control seedlings ranges from 12,2 to 38, 7%, five months after inoculation. No significant effect was observed for the following months and after seedlings were transplanted in the field. Height was the most inoculation-affected parameter which made it reliable for observation of inoculation effect. No significant difference was observed for diameter, total dry weight, shoot/root ratio, and seed quality index. Dual inoculation with mycorhizal fungi may extent the effectiveness of microbial effect on growth.

Keywords: *Aquilaria*, Rhizobacteria, inoculation, growth, nursery.

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I. INTRODUCTION

Gaharu is a resin-contained wood with high comercial value due to its usages as dupa, additive component of fragrance, and essential oil for religious, cultural, and even daily activities. In nature, gaharu hunting has been done aggresively and imprudent. Gaharu-producing trees which were found with small holes named as 'ant holes' were cut down and its gaharu was harvested. This way of gaharu hunting threatened the preservation of gaharu in its natural habitat. In order to prevent the gaharu-producing tree from extinction, since November 1994, *Aquilaria* and *Gyrinops*, 2 genera of the most important gaharu-producing trees which belong to Thymelaeceae family (Ordo: Myrtales and Class: Magnoliopsida) have been put into the CITES list (The Convention on the International Trade in Endangered Species of Wild Flora and Fauna), Appendix II. TRAFFIC-CITES-CoP 13 Prop 49 (2004) noted that there are 24 specieses including *Aquilaria* genus and seven specieses that belong to *Gyrinops* genus. Both genuses were found grew naturally in at least 12 countries including Bangladesh, Butan, Cambodia, Indonesia, Lao PRD, Malaysia, Myanmar, Philipines, Thailand, Viendam, and Papua New Guinea (Barden *et al. in* Gunn *et al. ,*2004).

Gaharu is formed through a pathogenicity process where particular pathogenic fungus infects particular tree and as a response toward the pathogene attack, the tree synthesizes secondary metabolites or resin compounds which is fragrant when it is burnt. Aside from two genuses mentioned above, this unique product was also found in several other genuses; *Aetoxylon, Enkleia, Phaleria, Wikstroemia,* and *Gonystylus.*

Gaharu found in nature is getting hard to find. To mantain the availability of gaharu products and the preservation of gaharu-producing trees, gaharu-producing trees cultivation is required. Cultivated gaharu is expected to fulfill the demand of gaharu to be exported to the users' countries. Cultivation is the main key in increasing the threatened gaharu production.

Cultivation of gaharu-poducing trees is highly related to the availability of high quality seedlings. Different from agriculture commodity where it is planted directly in the field, forestry seedlings preparation is carried in the nursury. The efforts to improve the seedlings quality in nursery can be done through fertilizing, using high quality seeds, and inoculating growth-promoting microbes such as plant growth-promoting rhizobacteria (PGPR). PGPR term was used for bacteria with the availability to support plant growth through various mechanisms, directly or undirectly (Glick, 1995; Kokalis-Burelle *et al.*, 2006). These mechanisms includes phytohormones production, phosphate mineralization or solubilization, nitrogene fixation, Fe sequestration by siderophores, mycorrhizae-forming supportive, and soil-through pathogene attack prevention (Garbaye, 1994; Glick, 1995; Lucy *et al*., 2004). Among these mechanisms, phytohormones production gained a lot interest because the applications of bacteria with this quality were reported to increse the production of the host plant continously (Narula *et al.* 2006). Narula *et al.* (2006) stated that in application of nitrogen-fixing bacteria to improve plant production, it

was discovered that the nitrogen level did not increse significantly. The plant-growth was improved by other mean, possibly by the phytohormones production by the nitrogen-fixer bacteria. *Azospirillum* sp. which is known as nitrogene-fixer bacteria also produces three kinds of phytohormones; indole acetic acid (IAA), gyberilin (GA), and kinetin. Whereas *Azospirillum chroococcum* was known to produce IAA, GA, and cytokinin (various sources *in* Narula *et al*., 2006). Microorganisms inhabited rhizosphere of various plants generally produce auxin as secondary metabolite as a response to abundant root exudates supply in rhizosphere. Barbieri *et al.* (1986) *in* Ahmad *et al*. (2005) reported that *Azospirillum brazilance* improved the number and length of lateral roots. Meanwhile *Pseudomonas putida* GR12-2 in canola seedlings raised the root length up to three times. It was said that growth hormone-producing bacteria was thought to play important role in promoting plant growth. However, research information about the utilization of phytohormones-producing bacteria for forestry plants in tropical region is still limited until now.

To test this hypothesis, the application test of IAA-producing bacteria in promoting growth of gaharu-producing tree *Aquilaria* sp. seedlings in nursury. In this research, the bacteria were first screened *in vitro* to test their capacity as IAA-producing bacteria.

II. MATERIALS AND METHODS

A. Phytohormones-Producing Bacteria: Identification, *In Vitro* Characterization and Inoculant Preparation

Rhizobacteria were isolated from rhizosphere and seedlings rhizoplane or sapling using a mix of N-free *Winogradsky* mineral with pH range 5.6 - 6.2 which contained 1% sucrose as canrbon source and 0.3% gellan gum as solidifyer (Hashidoko *et al.,* 2002).

This rhizobacteria were then identified by molecular approach using method described in Weisburg *et al.* (1991). DNA sequences were analyzed using BigDye Terminator v3.1 cycle (Applied Biosystems, Foster City, USA) with four choices of primers; 926F (5' AAACTCAAAGGAATTGACGG 3'), 518R (5' GTATTACCGCGGCTGCTGG 3'), 1112F (5' GTCCCGCAACGAGCGCAAC 3'), and/or 1080RM (5' ACGAGCTGACGACA 3'). Sequence homology was traced by using BLASND online DNA database in National Center for Biotechnology Information (NCBI).

The early selection for rhizobacteria *in vitro* was carried to understand its capability in producing phytohormones (Indole Acetic Acid) through qualitative and quantitative characterization. Qualitative characterization was done using modified Brick *et al.* (1991) colorimetric method as follows: rhizobacteria were grown in modified Winogradsky's agar medium (MWA) in which 100mg/L L-tryptophan ($C_{11}H_{12}N_2O_2$) was added. Right after rhizobacteria was inoculated, 0.45 µm pore size and 47 mm diametre nitrocellulose membrane was laid on top of the agar. The media were then incubated in dark at 28 ^{0}C. After 3 days, the membranes were removed and piled on 55m in diametre No.2 filter papers (Advantec, Tokyo Roshi Kaisha Ltd, Tokyo, Japan) which were previously soaked

in Slakowski solution. Color changes were observed after 30 minutes. Rhizobacteria which produce IAA would form red halo ring around the colony. Color intensities were then grouped into pink, red, and dark red. Whereas IAA quantitative characterization was carried using Narula (2004) method. The rhizobacteria which produced pink to dark red halo ring were then proceed to IAA quantitative test. Rhizobacteria were grown in liquid Winogradsky (MW) medium in which 100mg/L L-tryptophan was added and incubated at 28 ^{0}C in dark static condition for 7 days. Salkowski solution were then added ton the rhizobacteria culture supernatant. After half an hour, color were read at $A_{665nm.}$ Rhizobacteria which had positive reaction with Salkowski solution were then tested to see its ability in promoting the root growth of *Vigna radiata* as test plant. It turned out that futher test with *V. Radiata* did not show positive corelation between red color intensity and plant growth rate (height and total root length). The lack of specific corelation indeicated that the red color intensity was not the indication of produced IAA level quantity, but presented the variation of indole compound derivates which were converted from L-tryptophan. Glickmann and Dessaux (1995) stated that Salkowsky solution gave positive respond not only toward auxin (IAA), but also toward other indol piruvic acid and indoleacetamide.

From the three preliminary tests to select IAA-producing bacteria, nine bacteria were choosen (table 1). Additional one mycorrhiza association-promoting bacteria isolate was also used; *Chromobacterium* sp. CK8 because *Aquilaria* sp. was known to associate with arbuscule mycorrrhizal fungi, to understand the bacteria's ability in supporting the formation of mycorrhizal association in *Aquilaria* sp. seedlings.

Table 1. Phytohormones-producing PGPR which were used as inoculant

Host	Substrate	Stadium	Source location	Bacterial Strain	SubClass	IAA *colorimetric* analysis result
Dipterocarpus sp.	Rizhoplane	Sapling ~1 yr	Nyaru Menteng	*Stenotrophomonas* sp. CK34	Proteobacteria	Red
Hopea sp.	Rhizoplane	Sapling ~1 yr	Nyaru Menteng	*Bacillus* sp. CK41	Bacilli	Pink
S. teysmanniana	Rhizoplane	Sapling ~1 yr	Nyaru Menteng	*Azospirillum* sp. CK26	Proteobacteria	Pink
S. teysmanniana	Rhizoplane	Sapling ~1 yr	Nyaru Menteng	*Burkholderia* sp. CK28 (DQ195889)	Proteobacteria	Faint pink
				Burkholderia sp. CK59 (DQ195914)	Proteobacteria	Faint pink
Dipterocarpus sp.	Rhizoplane	Sapling ~1yr	Nyaru Menteng	*Serratia* sp. CK67	Proteobacteria	Faint pink
S. teysmanniana	Rhizoplane	Seedling~ 6 months	Nyaru Menteng	NI CK53		Dark red
				NI CK54		Dark red
S. balangeran	Rhizoplane	Sapling ~1 month	Pembibitan UP	NI CK 61		Pink
S. parviflora	Rhizoplane	Sapling ~1.5 year	Nyaru Menteng	*Chromobacterium* sp. CK8 (DQ195926)	Proteobacteria	*

Note: S: *Shorea*; H: *Hopea*, NI: not yet identified; UP: University of Palangkaraya, * additional isolate due to its mycorrhization helper characteristics, IAA : indol acetic acid

a. b.

Figure 1. The red color that formed around the colony after reaction with Salkowski reagent occured (a): Color forming on ntrocellulose membrane 3 days after incubationa; (b): Color formation in liquid medium. NICK53 bacteria which formed dark red color compared to media control without bacteria.

B. Inoculation of Phytohormone-producing Bacteria to *Aquilaria* sp.

Bacteria cells were grown in liquid medium of MW + 100 mg/L L-tryptophan and incubated at 28ºC. After 3 days, the bacteria culture were thickened by adding 0.5% gellan gum for 30 minutes. Inoculation was done on 4 week-old seedlings bby soaking the seedlings in bacterial suspension for 30 minutes. The seedlings were then planted in polybag which contained 500 g unsterilyzed soil medium. While planting, 1 mL of bacteria suspension was spread around roots area. Seedlings were grown in greenhouse and wateres everyday with tap water. Observations were carried toward height, diameter, and biomass dry weight.

C. Experimental Design and Data Analysis

Thisresearch used completely randomized design with single factor which was 10 bacteria isolates, each were repeated for 10 times per treatment. Data was analyzed statistically with analysis of variance using SPSS®version 10.0 program (SPSS Inc., Chicago, USA). Significantly different data was further tested with Least Significant Difference to group the not significantly different treatments. The parameters observed to see seedlings' response toward inoculation were height, diameter, total dy weight, seedling quality index, and incresed growth precentage.

Precentage analysis of increased growth was done as follows:

$$\% \text{ increase} = \frac{\text{Inoculated seedlings growth} - \text{Control seedlings growth}}{\text{Control seedlings growth}}$$

III. RESULTS AND DISCUSSION

Aquilaria sp. seedlings showed various responses toward phytohormones\-producing bacteria (Figure 2). Phytohormone-producing bacteria gave positive, netral, or negative influences toward plant growth compared to uninoculated plants (negative

control). Plant responses were observed through plants' height and diameter every month. Phytohormone-producing bacteria showed significant effect toward plant height 1-5 months after inoculation ($P < 0.05$). Two bacteria isolates; *Burkholderia* sp. CK28 (DQ195889, β Proteobacteria) and *Chromobacterium* sp. CK8 (DQ195926, b Proteobacteria) were the most consistent isolats in giving the most effective influence in increasing the height growth for 5 months after inoculation (Figure 2). Both bacteria were originated from less than 1 year-old *S. teysmanniana* rhizoplane and less than 1.5 year-old *S. parviflora* from Nyaru Menteng, Middle Kalimantan arboretum.

Aquilaria sp. seedlings growth was promoted 12.2 – 38.7 % more than uninoculated seedlings 5 months after inoculation. All the inoculated seedlings segnificantly had higher height growth than control plants through LSD analysis.

Diameter growth did not show consistent responses toward inoculation (Table 2). Similar response was also reported by Sitepu *et al.* (2007) that *Shorea selanica* seedlingd diameter response toward PGPR inoculation were not consistent. It was also mentioned that forest plants grow much slower than agricultural plants. Therefore, on the early stadium in nursury, the height is a reliable parameter to observe seedlings response toward growth-promoting microbe inoculation. In thick forest habitat with layers of canopies, seedlings which grow in forest floor need to grow tall quickly to compete with other seedlings around to get light for good growth.

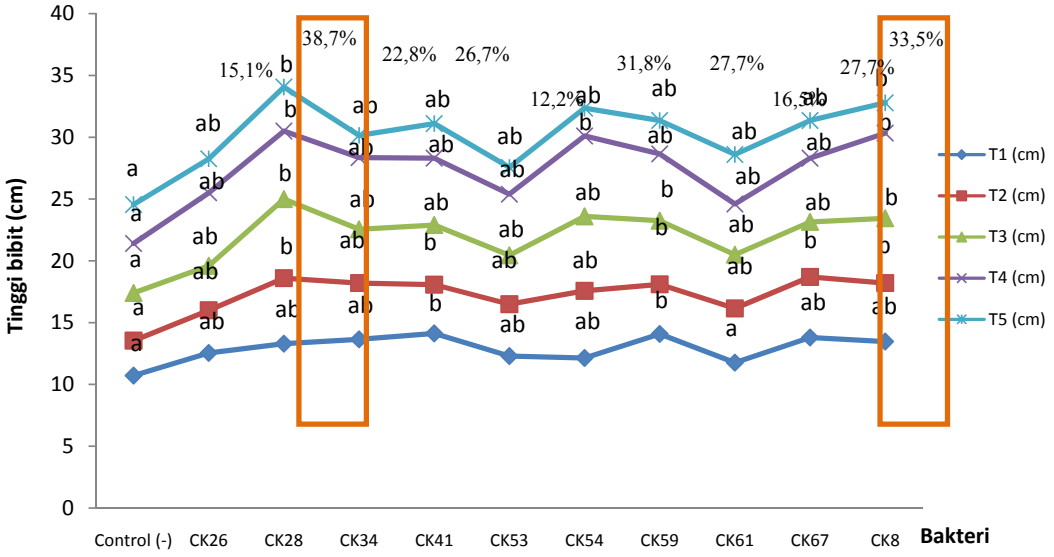

Figure 2. PGPR influence on height growth of *Aquilaria* sp. seedlings up to 5 months after inoculation. The values shown are growth rate compared to control seedlings.

Inoculation did not significantly affect the height growth, total dry weight, shooot-root ratio, and seedlings quality index six months after inoculation (Figure 3 & 4). Inoculation also did not significantly affect the growth after the seedlings were relocated to the field, the seedlings tended to grow slowly (Table 2). *Aquilaria* sp. seedlings were planted under

meranti trees in Dramaga Research Forest.

Table 2. Analysis of variance on measured growth parameter

Parameter	Analysis of variance					
	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6
Diameter (mm)	nd	nd	*	*	nd	nd
Height (cm)	*	*	*	*	*	nd
Shoot Dry Weight (g)	nd					
Root Dry Weight (g)	nd					
Total Dry Weight (g)	nd					
Shoot/Root Ratio	nd					
Seedlings Quality Index	nd					

Note: nd: not significantly different at 0.05 level test; *: significantly different at 0.05 level test.

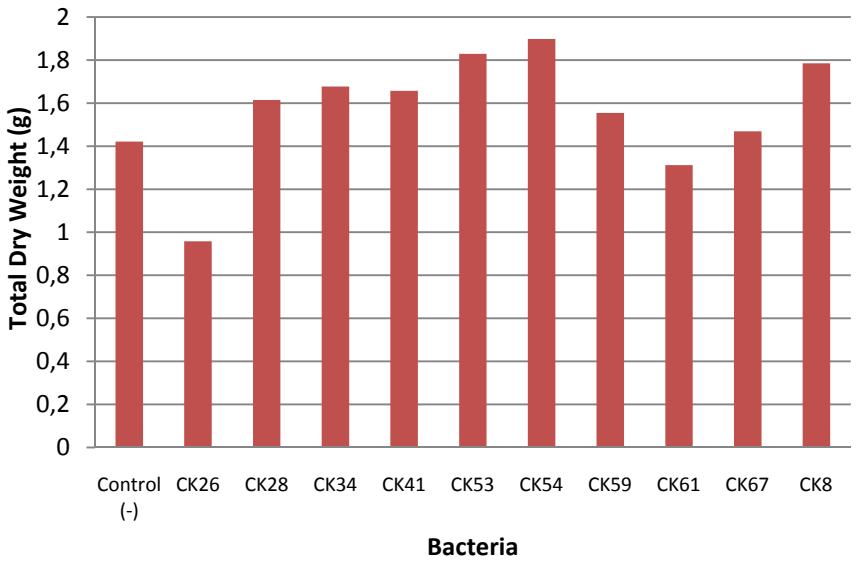

Figure 3. Total dry weight of the inoculated *Aquilaria* sp. seedlings

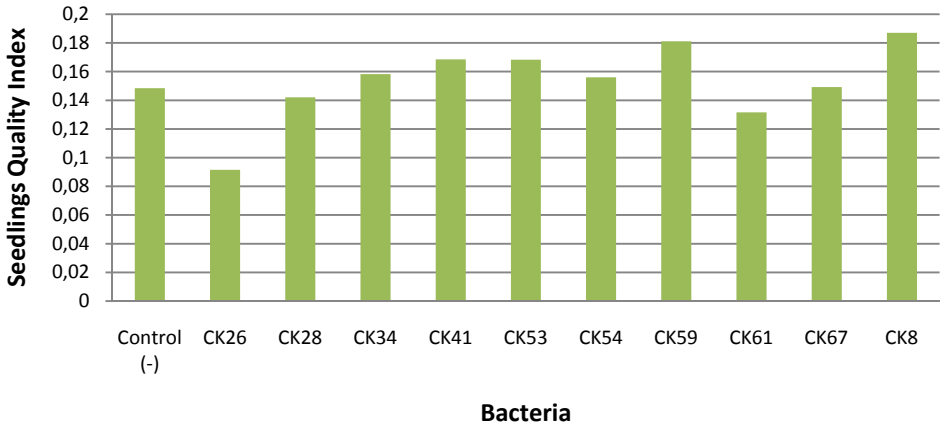

Figure 4. Inoculated *Aquilaria* sp. seedlings quality index

The lack of response toward bacteria inboculation six months after inoculation was explained as follows: the soil as a growth medium and *Aquilaria* sp. seedlings were not sterilized prior to inoculation, therefore the existed microbes in the soil freely interacted with the inoculated bacteria. The lack of response on the sixth month and futher probably was due to the infection of mycorrhizal fungi which were naturally in the soil and water, although the natural mycorrhizal colonization analysis were not done. Mycorrhizal fungi were reported to take effects seven months after inoculation on dipterocarps; *Shorea leprosula, Shorea acuminata, Hopea odorata,* and *Shorea pinanga* (Lee, 1990; Yazid *et. al.,* 1994; Turjaman *et al.,* 2005). On *Aquilaria* sp. seedlings in this research, mycorrrhiza took effects earlier; six months after inoculation. Certain bacteria had role in stimulating the formation of mycorrhizal association between mycorrhizal fungi and the host plant. One of the two most effective inoculants; *Chromobacterium* sp. CK8 was tested *in vitro* previously to promote the growth of ectomycorrhiza *Laccaria* sp. miselium growth. Poole *et al.* (2001) reported that *Paenibacillus* sp., *Burkholderia* sp., dan *Rhodococcus* sp. bacteria stimulated the ectomycorrhizal colonization on lateral roots growth stage between *Laccaria rufus* and *Pinus sylvestris.* While *Paenibacillus monteilii* and *Paenibacillus resinovorans* promoted symbiosis between *Pisolithus alba* and *Acacia holosericea* where *P. monteilii* increased the fungi biomass in the soil (Founoune *et al.,* 2002). Research carried by Enebak *et al.* (1998) on loblolly and slash pine seedlings reported that PGPR inoculation increased the stands' biomass. Indirect effect form PGPR inoculation in form of mycorrhizal association formation (called as mycorrhizal helper bacteria, MHB) was also reported. *Pseudomonas fluorescense* BBc6R8 promoted symbiosis between *Laccaria bicolor* S238N-Douglas fir (*Pseudotsuga menziesii*) and the MHB took effect most effectively when the mycorhizal fungi were grown not in the optimal condition (Garbaye, 1994; Brule *et al.,* 2001).

To understand whether this phenomenon also applied to *Aquilaria* sp. and whether the previously mentioned hypothesis was right, further test to observe the effect of double inoculation between arbuscule mycorrhizal fungi and bacteria on promoting seedlings'

growth in nursery and field is to be done. Research carried by Kashyap *et al.* (2004) showed that double inoculation of arbuscular mycorrhizal (AM) fungi and *Azotobacter* bacteria in addition of indole butyrate acid had significantly increased sapling survival rate of *Morus alba* (Moraceae) which were planted in high salinity condition of 25-50 %. In this case, seedlings with microbes had increased endurance toward extreme condition.

In this research, the approach was to screen the bacteria *in vitro* before testing on target plant in nursery. *In vitro* test was a practical method, especially in screening large number of isolates before further tests. By *in vitro* test, there were nine indole-producing bacteria which were then further test on *Aquilaria* sp. seedlings. The *Aquilaria* sp. seedlings growth response toward inoculation revealed one effective indole-producing bacteria; *Burkholderia* sp. CK28 which produced pink color on colorimetric test.

IV. CONCLUSION

Aquilaria sp. seedlings showed various responses toward the inoculation of phytohormones-producing bacteria. The inoculation increased the *Aquilaria* sp. seedlings' height right after inoculation for five months in a row. The height increase varied from 12,2-38,7% compared to the uninoculated seedlings. *Burkholderia* sp. CK28 and *Chromobacterium* sp. CK8 are two isolates which were consistantly promoted the height growth. Further test on double inoculation with arbuscule mycorhizal (AM) fungi is necessary to carry to understand the microbes which have role in promoting seedlings growth on the next stage in nursery before being moved to the field.

Acknowledgments

Writers would like to thank Ahmad Yani and Zaenal who helped the measuring and nursuring the plants in Dramaga Research Forest.

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APPLICATION OF ARBUSCULAR MYCORRHIZAL FUNGI IN FOUR SPECIES OF *Aquilaria*

By:

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ABSTRACT

The scarcity of natural gaharu (agarwood) production is due to excessive exploitation in Indonesian tropical natural forest. The sustainability of mother trees which produce gaharu is disturbed due to many activities of felling the trees, so that there is a threat of extinction, particularly for species of *Aquilaria.* Afterwards, the availability of natural regeneration seeds which produce gaharu, become also limited. The main problems addressed in this research is the slow growth of *Aquilaria*, either in the nursery or in the field, due to acid soil condition and nutrient deficiency. The use of arbuscular mycorrhizal (AM) fungi is possible to help the initial growth of *Aquilaria* species in the acid soils. The objective of this research was determining the effect of several AM fungi species on *Aquilaria* species, either in the nursery or in the field. Species of *Aquilaria* used in this reserach were *Aquilaria malaccensis*, *A. crassna*, *A. microcarpa* and A*. beccariana*. Species of AM fungi being used in this study were *Entrophospora* sp., *Gigaspora decipiens*, *Glomus clarum*, *Glomus* sp. ZEA, and *Glomus* sp. ACA. This research used completely randomized experimental design with 30 replications. Parameters observed in this research were AM fungi colonization, heigth, diameter, dry weight , fresh weight, seedling survival rate, and absorption of N and P in plant tissue. Research results showed that AM fungi colonization was formed in the root of *Aquilaria* species, after six months being inoculated in greenhouse condition. The use of AM fungi could increase all growth parameters and nutrient absorption in species of *Aquilaria.* Species *Entrophospora* sp. was very effective to be used for increasing the growth and nutrient absorption in species of *A. malaccensis*, *A. crassna* and *A. microcarpa*. *A. beccariana* prefer to have partner and is very effective with *G. clarum* to increase growth and nutirent absorption of N and P. According to the results of this researh, the use of AM fungi could help the regeneration of *Aquilaria* species, either

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at seedling stage or at the field. The use of effective AM fungi is recommended for accelerating the growth of *Aquilaria* species, starting from nursery condition. Availability of AM fungi inoculum at the user level, and socialization for its use, should be pursued so that AM fungi utilization become effective and efficient.

Key words : Application, *Aquilari*a, AM fungi, inoculation.

I. INTRODUCTION

Gyrinops and *Gonystylus* are included in CITES (*Convention on the International Trade in Endangered Specie*s) *Appendix* II (CITES 2005). Species of *Aquilaria* are generally found mostly in primary and secondary forests of lowlands in Indonesia, Papua New Guinea, Thailand, Malaysia, Vietnam, India, Bangladesh, Bhutan, Myanmar, China, Cambodia and Philippines. These species constitute the main source of gaharu wood (a kind of wood which has fragrant resin) which is included in the highest rank of non wood forest product group which has high economic value, originating from tropical forest. Gaharu product is usually used as baic ingredients of perfume, incence, traditional medicine and other commercial products (Eurling and Gravendeel 2005). However, the population of *Aquilaria* species is decreasing in nature, and it is difficult to arrange protection for this genus, and regulating the production sustainability of this natural gaharu production.

Availability of soil nutrients constitutes a limiting factor for initial growth during planting of forest trees in degraded forest land (Santiago *et al*., 2002). In the initial stage, growth of *Aquilaria* species are often slow, because in general, condition of tropical forest land in Indonesia is deficient of nutrients, mainly N and P. At present, reforestation activity produces hundreds of million of forest planting stocks each year. The use of vigorous forest planting stocks is needed very much in reforestation activities. In fact, many planting stocks are made with low quality results and tend to suffer nutrient defficiency, and finally they undergo high mortality rate when they have been planted in the field.

There have been many reports in international journal concerning the importance of utilization of arbuscular mycorrhical fungi in various forest plant species for helping reforestation activities. Arbuscular Mycorrhizal (AM) Fungi have been tested with significant results for the growth of species *Leucaena leucocephala* (Michelsen and Rosendahl, 1990), *Parkia biglobosa*, *Tamarindus indica*, *Zizyphus mauritiana* (Guissou *et al,* 1998), *Sesbania aegyptiaca* and *S. grandiflora* (Giri and Mukerji, 2004), 11 species of *Eucalyptus* (Adjoud *et al*., 1996), and *Tectona grandis* (Rajan *et al.*, 2000). According to literature study, there have been no reports of inoculation test of arbuscular mycorrhizal fungi in species of *Aquilaria*. The objective of this research was determining the effect of arbuscular mycorhizal fungi on *Aquilaria*, either in nursery or in the field.

II. MATERIALS AND METHODS

A. Seed handling and Germination

Seeds of *Aquilaria crassna* were obtained from Dramaga (Bogor), those of *A. malaccensis* were collected from Gudang village (Bangka island), *A. microcarpa* from Mianas village (West Kalimantan), and *A. beccariana* from Sanggau (West Kalimantan). All seeds of *Aquilaria* spp. were soaked for two hours, and afterwards were sterilized with sodium hypochlorite (5%) for five minutes. After sterilization, the seeds were washed several times with water until being clean. Seeds of *Aquilaria* spp. were germinated in plastic box containing zeolite media. Seeds of *Aquilaria* spp. started to germinate 21 days after sowing date.

B. Nursery Media

Soil materials from Ultisol soil type were taken from research forest Haurbentes (Jasinga) and were then stored in green house. The nursery media were sieved with sieve diameter of five mm. The pH of the media was 4.7, available P (Bray-1) was 0.17 mg kg^{-1} and total N (*Kjeldahl*) was 1.7 mg kg^{-1}. Afterwards, the nursery media were sterilized at temperature of 121 ^{o}C for 30 minutes.

C. Inoculum of Arbuscular Mycorrhiza

Arbuscular mycorrhizal (AM) fungi species *G. decipiens, G. clarum, Glomus sp.* ZEA and *Glomus* sp. ACA were isolated from village of Kalampangan, Palangkaraya, Central Kalimantan through culture pot technique. Culture pot technique was initiated with single spore technique. Host which was used for propagating the AM fungi was *Pueraria javanica*. Plastic pot was filled with strerile zeolite and added with 5 g of each species of AM fungi. Afterwards, seeds of *P. javanica* which had been six days old were planted in the plastic pots. Pots were arranged in iron shelves in green house, and were raised for 90 days. Spores, external hyphae, and roots which were colonized by each species of AM fungi, were examined under microscope.

D. AMF inoculation

Polybags (size of 15 cm x 10 cm) were each filled with 500 g sterile soil. Inoculation of AM fungi was given as much as 5 g for each pot and was placed near the roots of *Aquilaria* spp seedling. Uninoculated seedlings of the four species of *Aquilaria* served as control. Results of preliminary research showed that the use of sterile inoculum did not produce efffect on the growth of *Aquilaria* spp. Seedlings were raised and watered every day in greenhouse condition and were observed for 6 months. Temperature in the greenhouse ranged between 26 ^{o}C and 35 ^{o}C and the relative humidity between 80 % - 90%. Disturbing weeds and pests were monitored everyday.

E. Growth parameter

Inoculation experiment on Aquilaria species consisted of the following treatments (a) control (without inoculum); (b) *Entrophospora* sp.; (c) *G. decipiens*; (d) *G. clarum* and (e) *Glomus* sp. ZEA; (f) *Glomus* sp. ACA. The experiment was arranged in completely randomized design with (CRD) with 30 replications. The parameters observed were height, diameter and survival of seedlings. After reaching six months of age, there were harvesting of shoots and roots of Aquilaria seedlings. All samples were dried in oven of 70°C temperature for three days. Analysis of N and P for seedling tissues were conducted with method of *semi-micro Kjeldahl* and *vanadomolybdate-yellow assay* (Olsen and Sommers 1982). In the field, experiment was conducted only on species of *A. beccariana* with the same Completely Randomized Design. The experiment was conducted in KHDTK (Forest Territory with Special Purposes) Dramaga under the shade of *Gmelina arborea* stand. The parameters observed in the field study were height and diameter of *A. beccariana* which have been monitored for two years.

F. Colonization of Arbuscular Myccorrhizae

Roots of each species of Aquilaria species were washed to get rid of soil particles which were still attached. Roots were washed with 100 g l^{-1} KOH for one hour, acidified in HCl solution and were given color with 500 mg l^{-1} tryphan blue in lactoglycerol (Brundrett *et al.,* 1996). Afterwards, the roots were washed in 50% glycerol, and 100 segments of root, measuring one cm eacx, was observed under compound microscope with 200x magnification. Counting of mycorrhiza colonization was conducted by using system of scoring of presence and absence of AMF structure (McGonigle *et al*., 1990).

G. Statistical Analysis

Statistical analysis used ANOVA with software *StatView* 5.0 (*Abacus Concepts*). Further statistical analysis used test of Least Significant Difference (LSD) if the F value was significant.

III. RESULTS

Five species of AM fungi were very effective in colonizing root system of *A. crassna*, *A. malaccensis*, *A. microcarpa* and *A. beccariana* after six months being inoculated in greenhose condition. There were no significant differences between the five kinds of AM fungi in colonizing the roots of four species of *Aquilaria*. Colonization of AMF could increase growth parameters height, stem diameter, dry weight, fresh weigth and survival rate of Aquilaria seedlings in nursery (Table 1.). In species of *A. crassna*, *A. malaccensis* and *A. microcarpa*, the use of AM fungi *Entrophospora* sp. was more effective in increasing growth as compared with other kinds of AM fungi. Particularly for AM fungi *G. clarum*, this was very effective in increasing growth parameter in species

A. beccariana. Uninoculated seedlings were colonized by unidentified AM fungi (1-10%), but could not affect the growth of four species of Aquilaria. Colonization of AM fungi was able increase absorbtion of N and P in the tissue of four Aquilaria species, as compared with uninoculated seedlings (Table 2.). This increase in N and P absorption gave influence in increasing growth parameters of four species of *Aquilaria.* In the field, planting was conducted only for species *A. beccariana* at two years after inoculation by AM fungi. Research results in the field condition showed that species *G. clarum* was more effective in increasing the growth of *A. beccariana* as compared with control and other kinds of AM fungi which had been tried.

Table 1. Colonization of arbuscular mycorrhizae and growth of *Aquilaria* species, after six months under greenhouse condition

Treatments	Coloni-zation	Height	Dia-meter	Fresh weight	Dry weight		Survival rate	
	AM	(cm)	(mm)	Shoot (g)	Root (g)	Shoot (g)	Root (g)	(%)
A. crassna								
Control	4a*	20.90 a	2.9 a	0.68a	1.06a	0.33a	0.13a	70
Entrophospora sp.	73b	46.14 c	5.4 c	12.58b	5.72b	3.82b	1.35b	100
G. decipiens	63b	29.58 b	4.1 b	11.64b	7.36b	3.26b	1.56b	100
G. clarum	78b	32.43 b	4.4 b	8.82b	4.3b	0.86a	0.27a	100
Glomus sp. ZEA	78b	38.94 c	4.7 b	9.92b	4.54b	2.99b	1.01b	87
Glomus sp. ACA	59b	24.60 a	3.7 a	13.46b	6.94b	4.19b	1.52b	100
A. malaccensis								
Control	1a	16.43a	2.28a	1.46a	0.52a	0.41a	0.18a	73
Entrophospora sp.	97b	25.97c	3.88c	4.68c	2.24c	1.44c	0.48c	100
G. decipiens	88b	21.91b	3.02b	2.92b	1.20b	0.88b	0.27b	100
G. clarum	83b	19.96b	2.94b	2.90b	1.28b	1.95c	0.78c	97
Glomus sp. ZEA	84b	22.33b	3.26b	2.62b	1.38b	0.79b	0.27b	90
Glomus sp. ACA	86b	21.30b	3.12b	2.74b	1.22b	0.89b	0.26b	93
A. microcarpa								
Control	2a	13.39a	2.23a	0.75a	0.34a	0.23a	0.09a	67
Entrophospora sp.	97b	24.74d	3.89c	4.32c	2.29c	1.31c	0.37b	100
G. decipiens	88b	21.99c	3.67c	3.87c	3.41d	1.44c	0.57c	97
Glomus clarum	83b	20.28c	3.58c	3.46c	1.55b	0.95b	0.30b	93
Glomus sp. ZEA	85b	17.24b	2.84b	2.24b	1.08b	0.64b	0.24b	87
Glomus sp. ACA	87b	18.09b	2.98b	2.70b	1.23b	0.76b	0.28b	90
A. beccariana								
Control	10a	15.40a	1.90a	0.30a	0.10a	0.09a	0.02a	73
Entrophospora sp.	85b	19.20b	2.37b	5.46e	2.54c	1.76c	0.78c	100
G. decipiens	71b	32.18d	3.94c	4.74d	1.64b	1.59c	0.41b	100
Glomus clarum	79b	45.30e	5.02d	6.74f	2.82d	2.30d	0.91d	100
Glomus sp. ZEA	61b	32.03d	3.75c	3.14b	1.38c	0.97b	0.36b	100
Glomus sp. ACA	84b	26.24c	3.53c	3.84c	1.20b	1.19b	0.28b	100

*Figures with the same letter are not significantly different ($P<0.05$)

Table 2. Content of N and P in *Aquilaria* species, after six months of inoculation by several species of arbuscular mycorrhizal (AM) fungi

Treatment	N Concentrations (mg/g)	N Content (mg/plant)	P Concentrations (mg/g)	P Content (mg/plant)
A. crassna				
Control	7.9 ± 0.1a*	2.6 ± 0.6a	0.78 ± 0.02a	0.26 ± 0.06a
Entrophospora sp.	9.8 ± 0.1c	37.7 ± 4.3d	1.42 ± 0.03e	5.4 ± 0.6d
G. decipiens	8.2 ± 0.2a	26.7 ± 4.1c	0.85 ± 0.02b	2.8 ± 0.5c
G. clarum	8.7 ± 0.2b	7.4 ± 1.0b	0.95 ± 0.02c	0.82 ± 0.14b
Glomus sp. ZEA	8.7 ± 0.1b	25.8 ± 3.6c	0.96 ± 0.03c	2.85 ± 0.41c
Glomus sp. ACA	10.8 ± 0.2d	45.9 ± 9.6d	1.22 ± 0.02d	5.14 ± 1.0d
A. malaccensis				
Control	8.6 ± 0.2a	3.49 ± 0.5a	0.65 ± 0.02a	0.26 ± 0.04a
Entrophospora sp.	12.1 ± 0.1d	17.28 ± 2.0c	0.73 ± 0.01b	1.06 ± 0.15d
G. decipiens	10.7 ± 0.1c	9.02 ± 0.7b	0.85 ± 0.01c	0.75 ± 0.07c
G. clarum	10.4 ± 0.1b	20.5 ± 3.3c	0.72 ± 0.02b	1.60 ± 0.20e
Glomus sp. ZEA	11.1 ± 0.2c	8.8 ± 0.9b	0.77 ± 0.03b	0.6 ± 0.07b
Glomus sp. ACA	10.9 ± 0.2c	9.7 ± 1.8b	1.04 ± 0.03d	0.92 ± 0.17c
A. microcarpa				
Control	7.8 ± 0.1a	1.02 ± 0.07a	0.65 ± 0.02a	0.08 ± 0.01a
Entrophospora sp.	9.6 ± 0.2c	16.9 ± 1.5d	1.12 ± 0.03d	1.97 ± 0.18d
G. decipiens	9.6 ± 0.1c	11.7 ± 0.9c	0.86 ± 0.01c	1.20 ± 0.18c
G. clarum	9.3 ± 0.1c	8.3 ± 0.4b	0.78 ± 0.02b	0.70 ± 0.03b
Glomus sp. ZEA	9.4 ± 0.1c	9.17 ± 1.35b	0.77 ± 0.03b	0.75 ± 0.12b
Glomus sp. ACA	8.9 ± 0.2b	8.28 ± 0.40b	0.77 ± 0.02b	0.9 ± 0.1b
A. beccariana				
Control	6.0 ± 0.1a	5.02 ± 0.07a	0.40 ± 0.02a	0.10 ± 0.01a
Entrophospora sp.	9.9 ± 0.2c	10.2 ± 1.0c	0.98 ± 0.02d	0.87 ± 0.20d
G. decipiens	10.6 ± 0.1c	11.8 ± 0.8c	0.89 ± 0.03c	1.25 ± 0.21c
G. clarum	11.3 ± 0.d	12.5 ± 0.4d	1.11 ± 0.02e	1.95 ± 0.03e
Glomus sp. ZEA	9.4 ± 0.1b	9.17 ± 1.35b	0.77 ± 0.03b	0.75 ± 0.12b
Glomus sp. ACA	8.8 ± 0.2b	9.28 ± 0.40b	0.97 ± 0.02d	1.04 ± 0.1c

*Figures with the same letter are not significantly different ($P<0.05$).

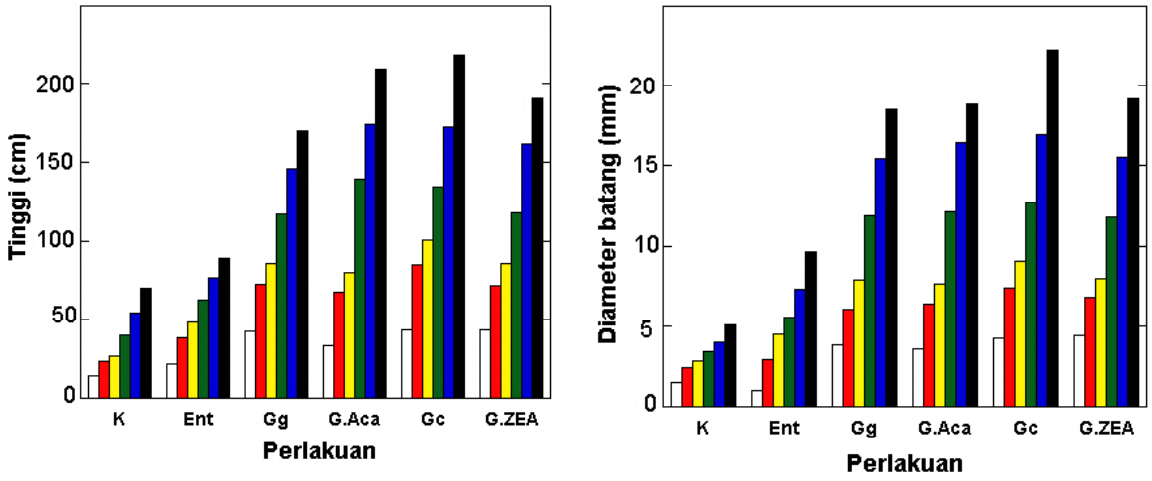

Figure 1. Height and diameter growth of gaharu producing trees *Aquilaria beccariana* after two years being planted in the field. K = Control; Ent = *Entrophospora* sp.; Gg = *G. decipiens*; G.Aca = *Glomus* sp. ACA; Gc = *G.clarum*; G.ZEA = *Glomus* sp. ZEA.

IV. DISCUSSION

Results of this research gave a very important information in the utilization of AM fungi inoculum on species of *Aquilaria* spp. Sustainable regeneration of *Aquilaria* species could be assisted by AM fungi technology starting from the nursery. Effective use of AM fungi could increase growth of Aquilaria to a highly significant extent, so that biomass of gaharu producing trees would increase, which imply that gaharu product resulring from induction, which will be harvested, will increase in yield. This research was in agreement with the previous researches, which was concerned with utilization of AM fungi for 11 species of *Eucalyptus* spp. (Adjoud *et al.* 1996), 17 species of leguminous plants (Duponnois *et al*., 2001) and *Sesbania aegyptiaca* and *S. grandiflora* (Giri dan Mukerji 2004). In the previous research results by Santoso *et al.* (2007), it was shown that colonization of AMF which occured in planting stocks of *A. microcarpa* was started before week – 7 after inoculation. Research on AM fungi utilization for tropical tree species (Muthukumar *et al*., 2001) and particulary for tree species of *Aquilaria* showed that there is possibility that AM fungi inoculum could reduce the need for chemical fertilizer in the nursery. Although calculation on the benefit and cost of AM fungi utilization has not been tested in this research, it could be shown with no doubt that AM fungi could reduce the use of chemical fertilizer in the supply of planting stocks for producing gaharu. Afterwards, mechanism of the use of AM fungi as growth accelerator for *Aquilaria* species in acid soil and in soils with very low population of AM fungi should become one of the important consideration.

Planting pattern of *Aquillaria* species with *agroforestry* system could help very much in accelerating the availability of gaharu producing trees in Indonesia. In principle the

mixing of plant species was conducted to protect the growth of *Aquilaria* seedlings in the first and second years, from the scorching sun light. Species of *Aquilaria* which has been colonized by AM fungi would have relation with tree root system of other species, so that nutrient requirements for gaharu growth could be fulfilled. Tree species which are recommended to serve as admixture with gaharu producing trees are rubber, oil palm, sengon, gmelina, melinjo, jengkol and several other species of fruit trees.

In conclusion, the use of AM fungi on species of *Aquilaria* was highly significant in accelerating the initial growth in the nursery and in the field. Species of AM fungi *Entrophospora* sp. was very effective in accelerating the growth of plants and nuttient absorption in species of *A. malaccensis*, *A. crassna* and *A. microcarpa*. Particularly for gaharu producing species *A. beccariana*, this tree species prefer the AM fungi species *G. clarum* for accelerating plant growth, and improve nutrient absorption in the nursery and in the field. The use of effective AM fungi species is recommended for accelerating n the growth of *Aquilaria* species, starting from the nursery condition. Availability of AM fungi inoculum at the level of user and socialization of its use, should be pursued, to make the use of AM fungi be effective and efficient.

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PESTS THAT ATTACK GAHARU-YIELDING PLANTS

By:

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ABSTRACT

Gaharu or eaglewood or agarwood is non-wood forest product. There are about 27 tree species that can produce gaharu in Indonesia, i.e. *Aquilaria* spp., *Gyrinops* spp., *Aetoxylon* spp., and *Gonystylus* spp. These species exist in the forests in Sumatra, Kalimantan, and Papua, but they are threatened due to overexploitation. Thus, farrmers begin to plant them in monoculture is a small or a big-scale and outside their natural habitat. However, monoculture is generally susceptible to pest and disease attack. Pest has been found attacking gaharu plantations in several locations in Indonesia, included of leaf eater *Heortia vitessoides*. This pest has become increasingly important as it can cause severe damage and kill plants. Several control measures were investigated: a) short term controls with a mechanical measure by a routine collection of the larvae or eggs of the pest from infested plants and; a chemical measure using contact or systemic insecticides that contains natural enemies, parasite or predator, e.g. entomopathogenic microorganism (e,g. *Beavureia bassiana* or *Bacillus thuringiensis*); and silviculture techniques. Our recent study also showed that *Oecophylla smaradgina* may be used as a potential predator for protecting plants against pest attack.

Keywords : Gaharu, *Heortia vittessoides*, pest control.

I. INTRODUCTION

The plants that yield gaharu, which exist in Indonesia, in the number reach 27 species, some of them are quite potential for such gaharu production, among others: *Aqularia* spp., *Aetoxylontallum* spp., *Gyrinops* spp., and *Gonysstylus* spp. The utilization of gaharu in Indonesia by the community particularly in the island regions, such as Sumatera and Kalimnatan island has taken place since hundred years ago. Traditionally, gaharu is used as incense ingredients for religious rituals, fragrance (convenient-smell) for human body or rooms, cosmetics, and simple drugs/medicine.

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Gaharu products are nowadays very demanded by gaharu seekers, due to their expensive prices, where the price of super gaharu can reach Rp. 40 million/kg. Due to such quite-high price, this tempts the gaharu seekers more intensive to acquire it. Currently, the gaharu seekers have focused on finding it in Papua island, where its natural potency (including also gaharu) there is still quite high compared to those in Kalimantan and Suamtera islands. With the growing scarce of the gaharu-yielding plants in the field, and induced with its high prices, then the forestry researchers, foresters, and ordinary community begin domesticating or cultivating the gaharu-yielding plants outside their native habitats. At present, quite a lot of farmers as well as town people begin cultivating the gaharu-yielding plants in small-scale endeavor beginning from just several trees to thousands of trees.

The cultivating of gaharu-yielding plants with monoculture system and situated outside their native habitats is usually vulnerable to the pest and disease attacks. Beginning two years ago, there have been growing numerously the centers for gaharu-yielding plants, which suffered the attacks by leaf pests, called *Heortia vitessoides* Moore. The center site for those gaharu plants, which were attacked by such pests and had been reported occurred in Forest Area for Special Purpose (FASP) of consecutively Carita (in 2008), Sanggau (2007), Mataram (2009), etc.

II. PESTS INFLICTED BY Heortia vitessoides

A. Symptoms of Attack

The attack symptom in the initial stages is noticeable on the surface of leaves (of gaharu-host plants), which has been eaten by the first-stage instar larvae, thereby leaving the leaves with only their bones. In the further stadium, those larvae begin attacking the leaves on the higher part of the stems, causing the individual plants to become less-leafy.

Moths lay down their eggs on the lower surface of the young leaves at gaharu stems near the soil surface.

B. Life Cycle

1. Eggs

Moths lay down their eggs that are yellowish white in color, and soon become greenish yellow in cluster shape, which stick to the lower surface of young leaves at the stems close to the soil surface. The female imago produce as many as 350-500 eggs, and about 10 days afterwards those eggs will hatch.

2. Larvae

The larvae, i.e. *H. vitesssoides* during the first stage appear pale yellow in color, and in the next stage become yellowish green. These larvae consist of 5 stages (phases) that last for 23 days. The larvae at the last phases will become the so-called pupae, which terminate their eating activities and the larvae move down to the soil surface and become pupae.

3. Pupae

The last-phased larvae before developing to pupae will terminate their eating activities and move down to the soil surface with the aid of silky threads as generated by those larvae. The larvae will further envelop themselves using soil grains or small-sized rotten leaves or twigs (or other vegetation litters) falling down from their host trees on the soil surface, assisted by their silky threads. The phase of pupae usually last for 8 days.

4. Moth

The adult insects or the so-called moth usually become active during the night. The female moth can lay down as many as 350-500 eggs. The moth phase usually take place for 4 days.

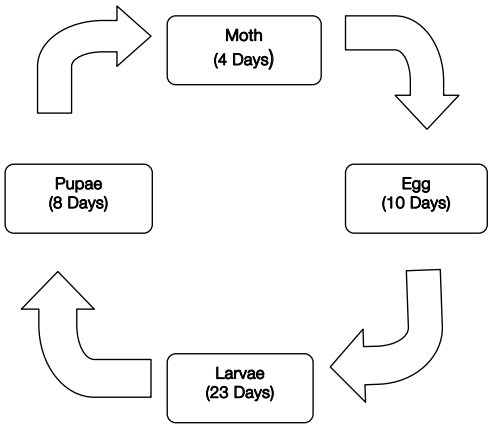

Figure 1. The life cycle of pests (*Heortia vitessoides*) that attack gaharu-yielding plants

III. CONTROLLING STRATEGY

A. Short Term

1. Mechanic Means

The controlling that uses the mechanic means seems very simple, popularly adopted among the farmer levels in that the larvae or their eggs that exist on the gaharu-yielding

plants are manually picked and then discarded. The controlling in this way is easy to implement particularly on the nursery or seedlings with 2-year age, in that their plants can still be assessed by men who are standing without using tools.

2. Chemical Means

The chemical means can be done using insecticides on touch, systemic, or insecticides that contain microorganisms such as *Beauveria bassiana* or *Bacillus thuringiensis*. Since these pests eat-up leaves, and consequently their host individual trees become less-leafy, it is suggested that the spraying of insecticide is combined with the application of fertilizer, particularly green (leaf) fertilizers such as gandasil, growmore, etc. to stimulate the growth of new shoots (buds).

3. Vegetation Control

The vegetation control presents the fairly simple means and can be done by the farmers themselves by taking the materials already available near or surrounding the cultivation site for gaharu plants.

B. Middle Term

Big-sized Red-colored Ants as Predator

These ants (*Oecophylla smaradigna*) represents insects easily found in villages, living on plants that release nectar such as jackfruit trees, *Nephelium lappaceum* trees, *Gnetum gnemon* trees, *Durio zibethinus* trees, etc. The seeking of these ants that have queen signifies as one of the successful factors to develop the population of those insects (ants) in the long term.

C. Long Term

1. Natural Enemy

Natural enemies can be parasites as well as predators of the insects (i.e. *Heortia vitessoides*) that destroy or eat the leaves of gaharu-yielding plants. Introducing these enemies signifies one of the controlling manners expectedly very effective in the long term.

2. Sylviculture Techniques

The control of pests using these techniques presents one of the manners already integrated in the cultivating of particularly plants, and this manner regarded as already popular among the farmers.

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THE ENVIRONMENTAL CHARACTERISTICS OF KANDANGAN SITE FOR GAHARU PLANTATION PROJECT

By :
Erry Purnomo[1] and Maman Turjaman[2]

ABSTRACT

A field study to characterized the site for growing and inoculation gaharu has been carried out. The characterization included location, climate, soil properties and plant species. The selected sites were distributed in regencies, namely, Hulu Sungai Selatan and Hulu Sungai Tengah. The annual total rainfall in the area under study was 2361 mm. The rainy season began in October and ease in June. In general, the soil in each site was considered very poor. The number plant species were varied from site to site. It is recommended that application of compost is needed to get good growth of eagle wood.

Keywords: gaharu, environmental characteristic, inoculation, plantation.

I. INTRODUCTION

Eaglewood (gaharu) plays an important role in gaining foreign exchange and as a source of income for people living in around and inside the forest in Indonesia. However, at the mean time, its production has declined rapidly, due to lack of technology and limited dissemination of the inoculation technology. If no serious action to be taken, gaharu production would not be sustained. As a consequence, pressure on the natural forest will increase significantly. Activities of the project include cultivation technique, plantation trial plot, inoculum's production, artificial inducement and training for forest dweller.

The most important benefits of the proposed project are increasing welfare of forest dwellers and local farmers, and boost foreign exchange earning that contributes to local and national income. This proposal is aimed at introducing inoculation technology to forest communities living in and around on the forest area. The inoculation technology will accelerate and promote gaharu productivity in the natural forest. Dissemination of the technology will be carried out by establishing sample plots in two places, i.e. South

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Kalimantan and a forestry research site in Banten province, covering a total area of 50 hectares. It is expected that artificial inoculums in large scale will enhance local people knowledge about inoculation process and eventually would improve communities' welfare and reduce the pressure on the forest. The method to accomplish this objective will be carried out through several activities, covering reviewing on existing literature and conducting field survey of gaharu species, potency, distribution and cultivation; identifying selected susceptible gaharu stands, and selecting, developing and implementing several prospecting inoculums for artificial inducement; evaluating basic properties of gaharu stands and characterizing and evaluating gaharu product; evaluating and developing the existing inoculation engineering technique; establishing demonstration plots, training and conducting workshops.

The most important benefits of the proposed project are increasing welfare of forest dwellers and local farmers by using community based forest management model (CBFM), and boost foreign exchange earning that contributes to local and national income. This study is also very important in terms of its contribution to the achievements of sustainable forest management in Indonesia.

The aims of this study is :

1. To analyse and evaluate soil properties under existing gaharu stands in Kandangan District.
2. To recommend soil management practices to obtain a good establishment of gaharu in new area.

II. MATERIALS AND METHODS

Site

Distribution of selected sites for the project can be seen in Figure 1. The selected site were use for growing eagle wood and inoculation. The sites located in Banjar, Hulu Sungai Selatan (HSS) and Hulu Sungai Tengah (HST). There were 18 and 5 sites for growing the eagle wood and inoculation activities, respectively. Location-wise, 14 sites would be used for newly planted eagle wood trees and 9 sites for inoculationt

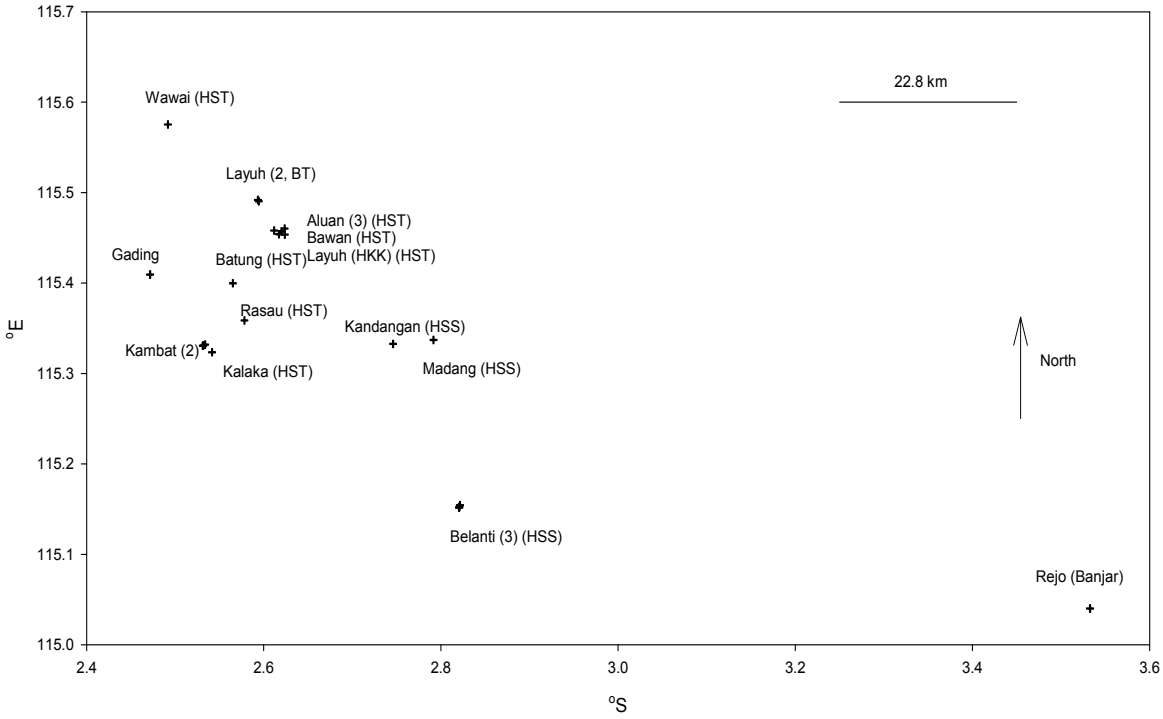

Figure 1. The selected study sites

III. RESULTS AND DISCUSSION

A. Climatic Characters

The average rainfall, air temperature and relative humidity for the last 9 years are shown in Figure 2. The average annual rainfall in the study area was 2361.72 mm. The rainfall distribution can be observed in Figure 2a. The rainy season commenced in October and ended in July each year. A significant low rainfall occurred in the period of July-September. The pattern of air temperature and relative humidity are shown in Figure 2b and 2c, respectively.

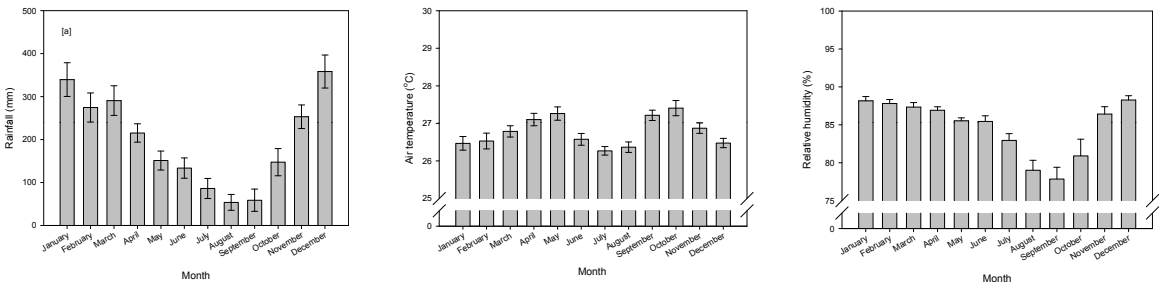

Figure 2. The rainfall, air temperature and relative humidity for the last 9 years.

A strong relationship between rainfall and relative humidity (Apendix 1a). As the rainfall increased up to 200 mm, the relative humidity increased, significantly. The effect of rainfall on relative humidity eased after rainfall of 200 mm. A poor correlation between rainfall and air temperature was observed if all rainfall data were included. However, if the rainfall was less than 150 mm, it had no association with the air temperature (Appendix 1b).

B. Soil Properties

The soil properties of each site are presented in Figures…Soil properties measures were particle fraction analysis, the content of total carbon (C), Total nitrogen (N), total potassium (K) and total phosphorus (P), soil pH, electric conductivity (EC), cation exchange capacity (CEC), and CO_2 evolution. The particle fraction analysis (Figure 3) shows that all soil samples dominated by slit fraction, followed by clay and sand fractions. If applicable, level of status of each soil property will be made available as categorized by Djaenuddin *et al*. (1994).

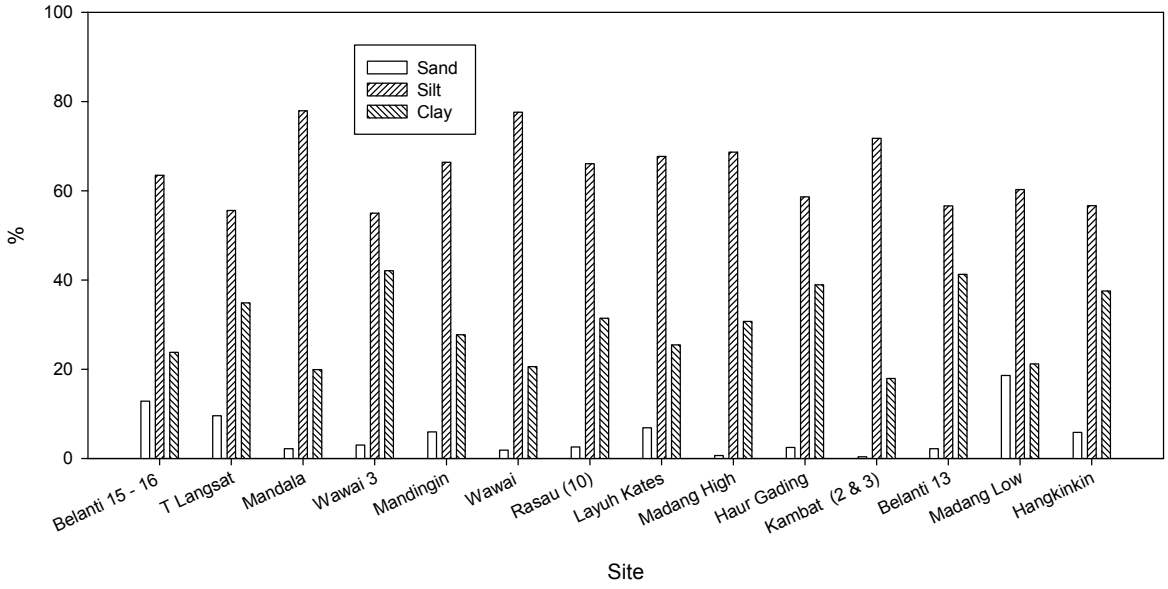

Figure 3. Particle fraction analysis of each soil

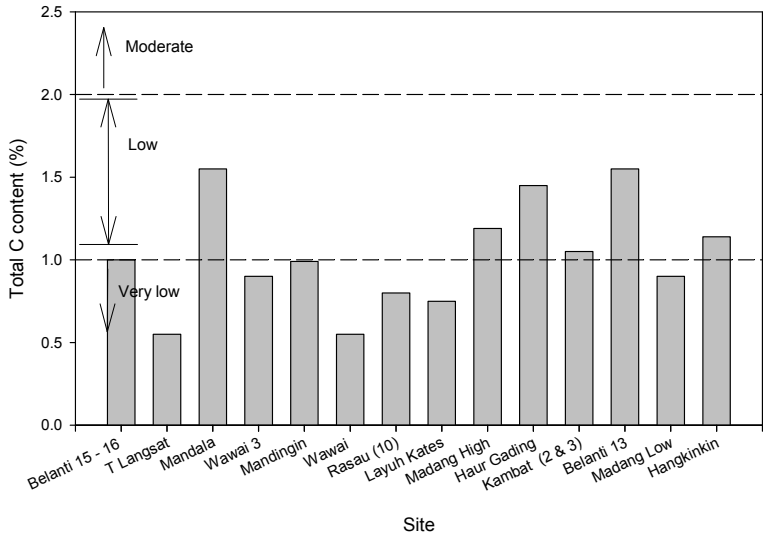

Figure 4. The total soil C content for each site.

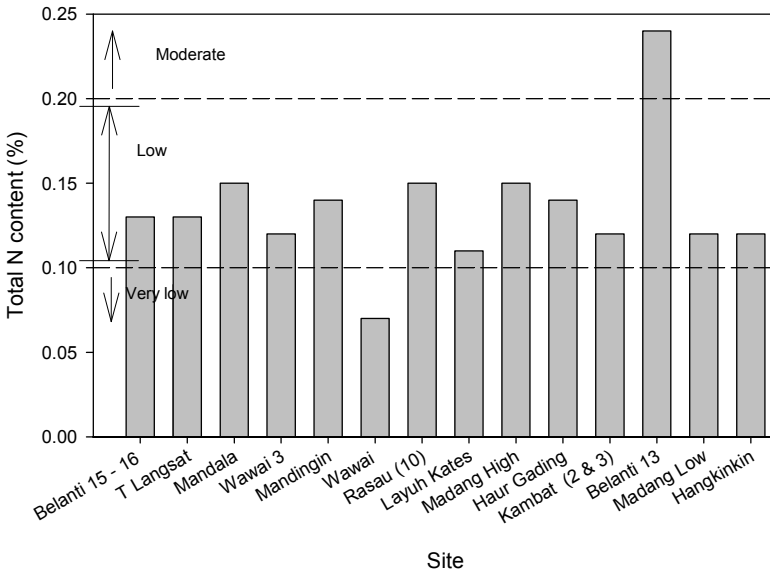

Figure 5. The total N of soil for each site.

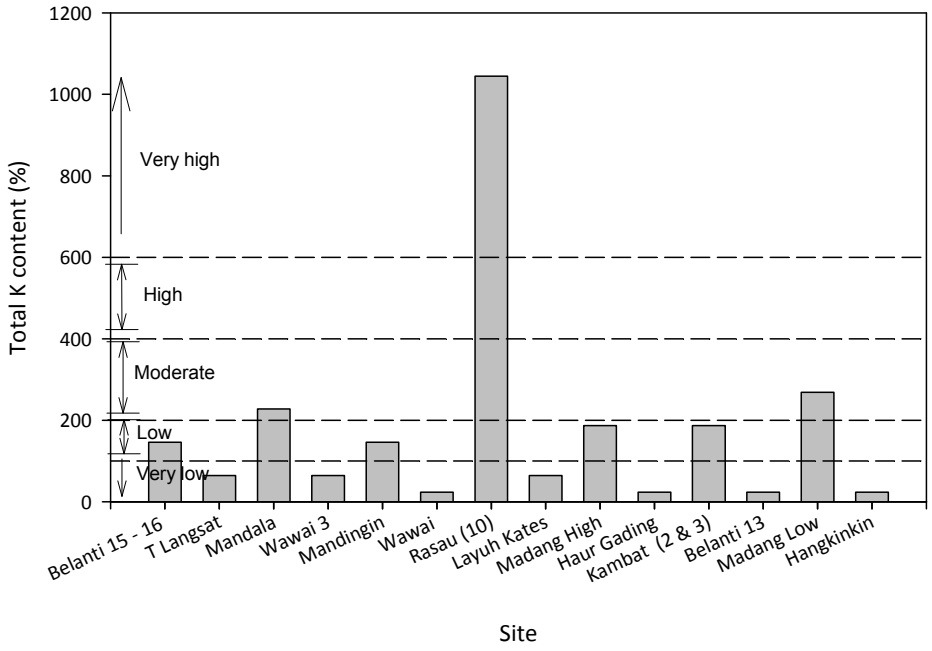

Figure 6. Total K content of soil for each site.

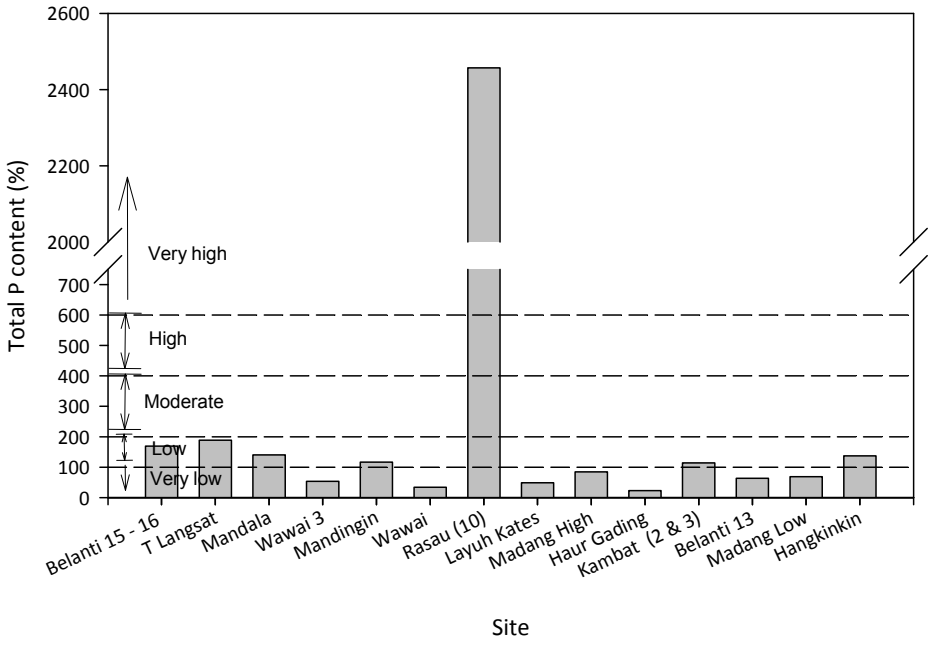

Figure 7. Total P content of soil for each site.

The category range of total C total content was very low to low. (Figure 4) Most of the selected sites contained very low C, only 5 sites had low C. The N content of the soils (Figure 5) was generally low. It was found that Wawai and Belanti 13 sites had very low and moderate levels of N content, respectively. The low level of C and N content confirms the low level of organic matter content of the soil.

The K and P contents of the soils from all sites are demonstrated in Figures 6.

Most of the soil classified as very low to low level of K concentration. Two sites, namely, Mandala and madang Low had K content of moderate level. One site (Rasau 10) had a very high K level.

Most of total P content of the soils was categorized as very low to low. Only one site (Rasau 10) was categorized as very high (Figure 7). It can be concluded that the selected sites need fertilization of P and K to improve the level status.

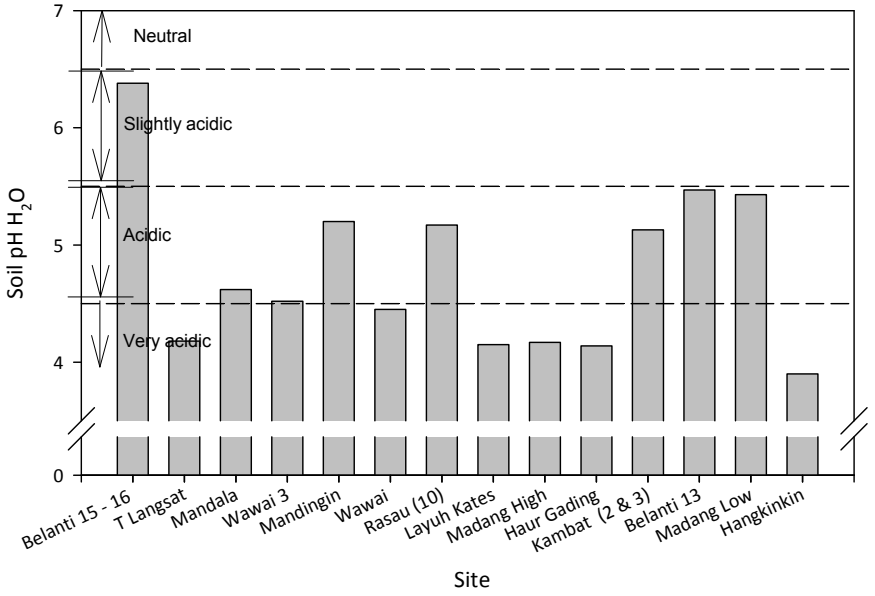

Figure 8. Soil pH for each site

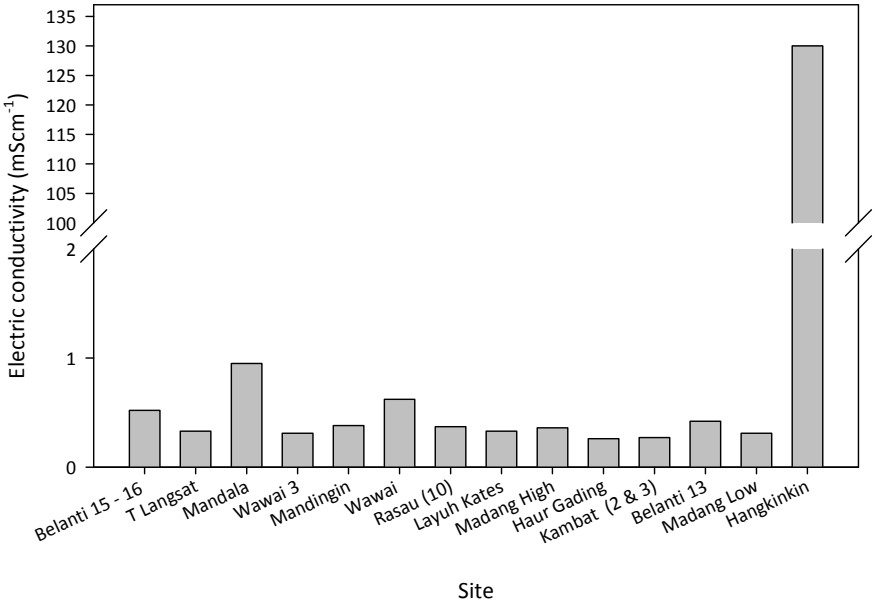

Figure 9. Electrical conductivity readings for soil each site

Almost all the soil pH of the selected soils was fallen into very acidic to acidic

category. Only one site (Belanti 13) had a slightly acidic value (Figure 8). For EC reading, except for Hangkinkin site, all soils had EC below 1 mS cm^{-1} (Figure 9). The low EC readings may be associated with the far distance from the shore. The low EC readings indicate the absence of salinity problem.

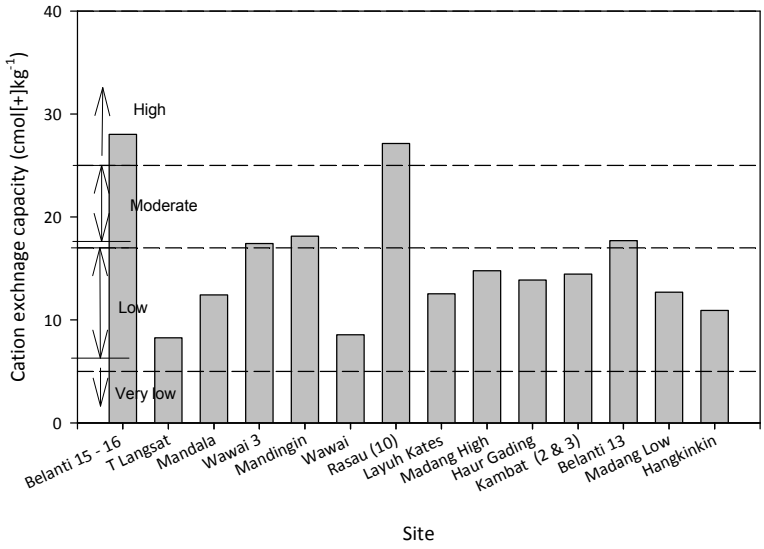

Figure 10. Cation exchange capacity of soil for each site.

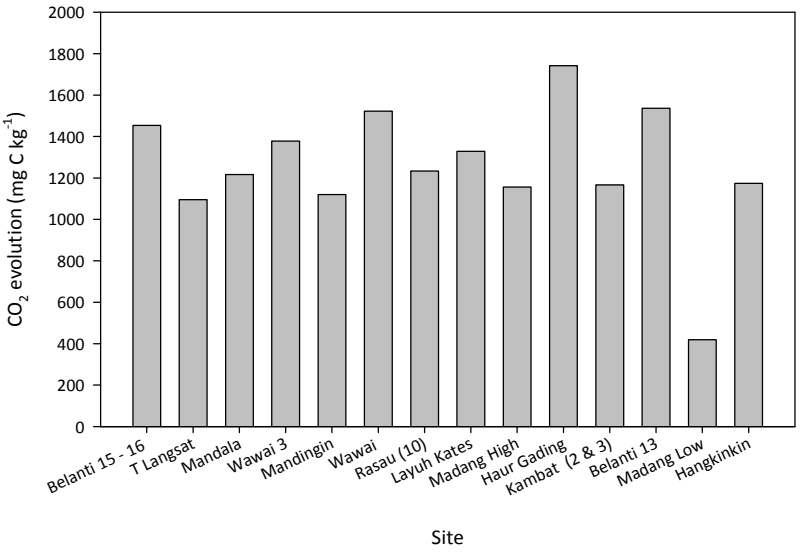

Figure 11. CO_2 evolution from soil from each site.

The CEC of the soils were commonly low (Figure 10). There were 3 sites and 2 sites had CEC of moderate and high, respectively. The low CEC indicates a low storage cation capacity and results in prone to cation leaching.

The CO_2 evolution as an indication microbial activity was similar site-wise

(Figure 11). Except, at Madang Low, it was observed that the microbial was lower than the other sites.

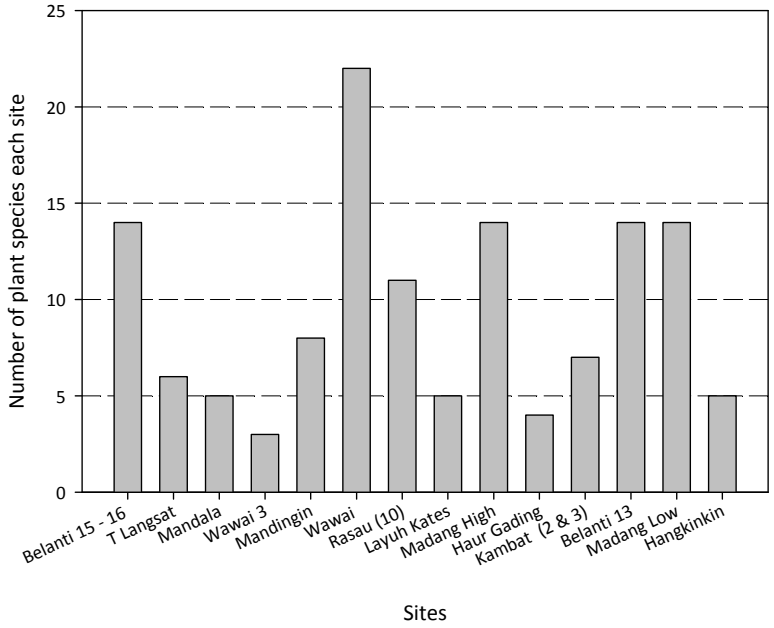

Figure 12. The number of plant species found in each site.

C. Number of Plant Species

It was observed that the number of plant species was varied from site to site (Figure 12). At 5 sites, there were 3-5 plant species. The other 8 sites had 5-14 plant species and one site had 22 plant species.

CONCLUSION

The selected sites were distributed in regencies, namely, Hulu Sungai Selatan and Hulu Sungai Tengah. The annual total rainfall in the area under study was 2361 mm. The rainy season began in October and ease in June. In general, the soil in each site was considered very poor. The number plant species were varied from site to site.

RECOMMENDATION

It is recommended that application of compost is needed to get good growth of eagle wood.

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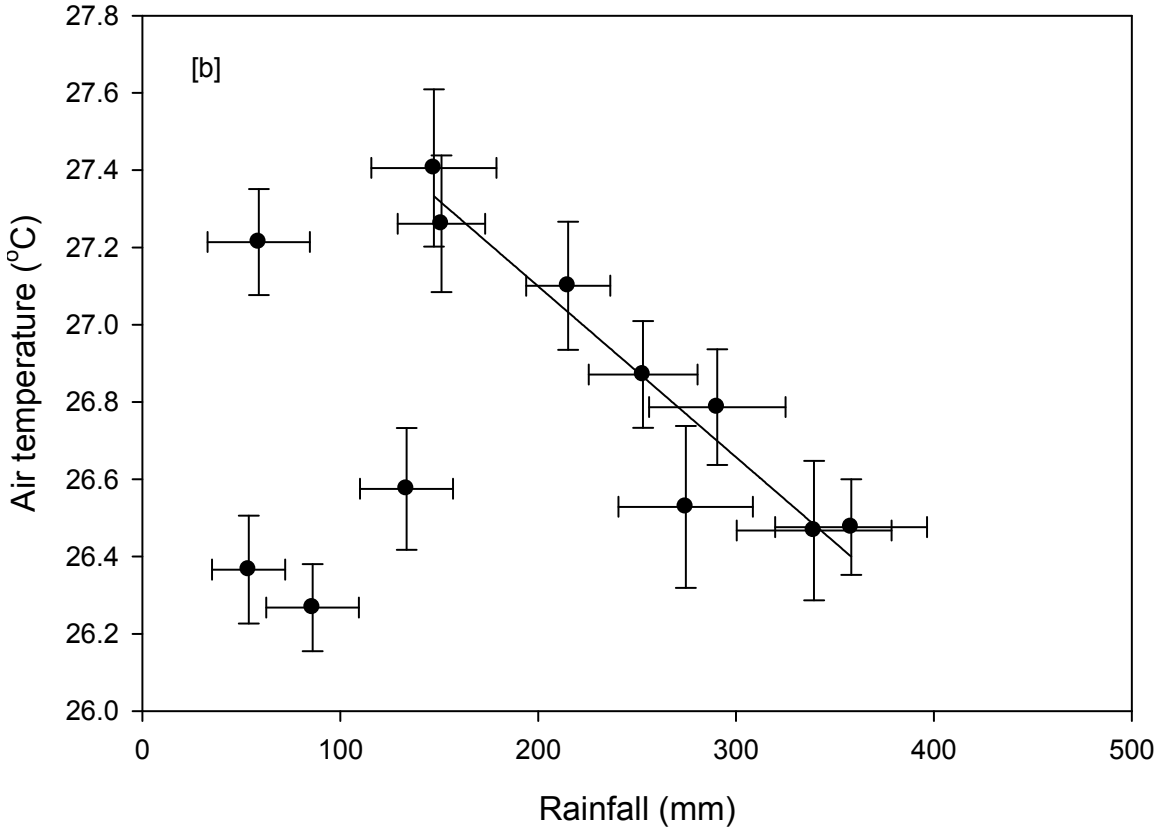
APPENDIX: Relationship between rainfall and relative humidity or air temperature



SOIL PHYSICAL AND CHEMICAL PROPERTIES OF THE GAHARU (*Aquilaria* spp.) STANDS HABITAT IN WEST JAVA

By:

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ABSTRACT

The research aims to collect data and information of gaharu habitat characteristics in forest plantation for support gaharu plantation development in Indonesia. The research was carried out in Carita (Banten), Darmaga (Bogor) and Sukabumi. The observed characteristics include: topography, climate, physical, and chemical characteristics of the soils. Beside that, the underground vegetation were analysed, in order to know the relationship between soil characteristics and underground vegetation composition. Result indicates that gaharu could develop quite favourably in flat to rolling landscape, low to high temperature (20-32°C) , and high rainfall (> 1500 mm/year), hard soil texture (clay), fast drainage, pH about 4,5-5,1, very low to high base saturation (1,2%-78,84%) and low toxic element. The dominant and co-dominant underground species in Carita are jampang (*Panicum disachyum*) and selaginela (*Selaginella plana*), while in Darmaga are pakis (*Dictyopteris irregularis***)** and seuseureuhan (*Piper aduncum*) and in Sukabumi are jampang (*Panicum disachyum*) and rumput pait (*Panicum barbatum*).

Key words: gaharu (*Aquilaria* spp.), land characteristics, forest plantation

I. INTRODUCTION

Gaharu (eaglewood) is one of non timber forest product which plays an important role in gaining foreign exchange and as a source of income people living inside and around the forest in Indonesia. The gaharu is one of important aromatic woods, therefore this non timber forest product is now subject to high rate of commercial extraction. There are several species of trees that produce gaharu. The original gaharu comes from infected trees of tropical species, such as *Aquilaria* spp., *Gonystylus* spp*., and Wikstromeae* spp., *Enkleia* spp., *Aetoxylon* spp., and *Gyrinops* spp. (Chakrabarty *et al*., 1994, Sidiyasa *et al.,* 1986). This research consider two species, that are *Aquilaria*

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crassna and *A.microcarpa*. This genus belongs to the family Thymelaeaceae. Due to the high economic value of this species, their existences should be sustained by doing several efforts. One of the efforts is developing gaharu plantation in several areas. More over, several information concerning gaharu habitat are inventarized, including soil characteristics as well as underground vegetation composition, in order to know the carrying capacity of the land.

Soil is one of the ecosystem components, has an important role as life supporting system, beside water, air and sun energy. Pratiwi and Mulyanto, (2000) and Jenny (1941) said that soil is the result of weathering processes of rocks or parent material by climatic factors and vegetation, and influenced by topographic factors and time. Specific soil characteristics influence the composition of the vegetation down to the type of dominant species (Pratiwi, 1991). Furthermore Pratiwi and Mulyanto (2000) said that the distribution of plants, soil types and the climate (including the microclimates) must be considered as part of the integrated ecosystem. Therefore the variability of vegetation depend on these factors.

According to the above background the aims of this research is to collect data and information of gaharu habitat characteristics in forest plantation in order to support gaharu plantation development in Indonesia. This research was done by making research plots for soil and underground vegetation investigation. It is expected that this information could support the development of gaharu plantation, therefore its exsistence could be sustained as well as increase people income and their prosperity.

II. MATERIALS AND METHODS

A. Location and Research Time

Research were done on September 2008, in Carita, Darmaga and Sukabumi. Administratively, Carita is situated in Pandeglang District, Banten Province, Darmaga in Bogor District, West Java province and Sukabumi in West Java.

The research site of Carita has undulating to mountaneous topography, with A rainfall type (Schmidt and Ferguson, 1951) and annual rainfall is around 3959 mm. The minimum temperature is around 26 ^{o}C and maximum tempera-ture around 32^{o}C. The average humidity between 77% to 85% (Pusat Litbang Hutan dan Konservasi Alam, 2005). The Darmaga research site has flat to undulating topography, with A rainfall type and annual rainfall is around 3600 mm. The minimum temperature is around 24^{o}C and maximum temperature around 30^{o}C The average humidity between 80% to 90%. More over Sukabumi has undulating to hilly topography, with A rainfall type and annual rainfall is around 3000 mm. The minimum temperature is around 20^{o}C and maximum temperature around 25^{o}C. The average humidity between 80% to 90% (Schmidt and Ferguson, 1951).

B. Materials

The soil and underground vegetation were sampled on gaharu plantation in the research areas.

The plots were selected on the basis of the soil map of West Java and Madura, at scale of 1: 500 000 prepared by Lembaga Penelitian Tanah, 1962.

Soil samples were taken from identified horizon in all pedons. Two kind of soil samples were collected: bulk samples for routine physico-chemical analyses, and undisturbed unoriented samples for physical analyses.

The composite soil samples were taken from depth of 0-30 cm; 30-60 cm and >60 cm in each research sites. In every soil depth, soil samples were taken from 20 points which distribute in each horizon. Then soil samples were mixed according its depth. The total composite soil sample from each location are 6 samples (3 for soil physical analyses and 3 for soil chemical analyses). Therefore there are 18 soil samples.

C. Analytical Methods

1. Vegetation Analyses

Underground vegetation analyses were done using the square method (Mueller-Dumbois and Ellenberg, 1974). On every place five transect of 100 m length at distance of 20 m were laid. Every transects is split up in squares of 5 x 5 m, and the distance between the squares is 20 m. Each vegetation individual inside the square was identified like: species, number of individual, and basal area.

2. Physico-Chemical Analyses

a. Routine analyses

The routine physico-chemical analyses were carried out mainly according to the methods described in ”Procedures for collecting samples and methods of analyses for Soil Survey Report No.I” (Soil Conservation Service ,1984) unless otherwise mentioned. All data were reported on the basis of the < 2 mm material (fine earth).

b. Organic Carbon

Determined according to the method of Walkey and Black (Allison, 1965). The involves a wet combustion of the organic matter with a mixture of potassium dichromate and sulphuric acid. After reaction the residual dichromate is titrated against ferrous sulphate.

c. Total Nitrogen

Determined according to the macro Kjedahl method.

d. Available Phosporus

Two gram of air dry soil sample was shaken for 5 minutes with 20 ml pf Bray 1 extracting reagent (0,05 N NH4F and 0,025 N HCl, pH 2,6). The extractable P was determined colorimetrically with Amonium molybdate reagent.

e. CEC

For the CEC of the soil, 1 M NH4OAC pH 7 was used for saturation, and the adsorbed NH^{+} was then displaced by acidified KCl 1 N. After distillation, titration was done with dilute HCl 0,001 N.

f. CEC Sum of Cations

CEC sum was calculated from the exchangeable basic cations and the exchangeable acidity.

g. Exchangeable Bases

Ca, Mg, K and Na were determined in the NH4Oac extraction solution by AAS.

h. Exchangeable Acidity

The acidity ($H^{+} + Al^{3+}$) released upon exchange by an unbuffered KCl solution.

i. Base Saturation

Calculated by dividing the sum of exchangeable Ca, Mg, K, and Na with the CEC (NH4Oact pH 7) and multiplying by 100.

D. Data analyses

Physical and chemical analyses were calculated based on the formula of procedures and standard analyses of every soil characteristics. Soil data were analysed and intepreted in relation with the existing underground vegetation.

From all vegetation data, the frequency, dominancy, density and Important Value (IV) were calculated. The similarity index (SI) was calculated by using Sorensen methode (Mueller-Dumbois and Ellenberg, 1974). For the calculation of SI, the formula is:

$$SI = \frac{2\,w}{a + b}$$

with:
SI = Similarity Index
w = the sum of the smallest IV for the same species which were found in two compared communities (A and B)

a = the sum of IV for all species in community A
b = the sum of IV for all species in community B

III. RESULT AND DISCUSSION

A. Characterization of the studied soil

1. Factors influencing soil formation in the research site.

The research were done in three areas, i.e: Carita (Pandeglang District-Banten Province), Darmaga (Bogor District) and Sukabumi District (West Java Province).

The topography in Carita is undulating to mountaneous, while Darmaga flat to undulating and Sukabumi undulating to hilly.

Parent material of Carita soil is derivated from Danau Volcano,whereas of Darmaga and of Sukabumi are from Salak Volcano and Gede Pangrango Volcano respectively. The volcanic material from these locations has andesitic characteristic. This means that these parent material contain sufficient ferro magnesian minerals and other minerals as sources of base elements. These types of minerals are strongly influence the soil characteristic especially physical and chemical characteristics.

The research site of Carita has undulating to mountaneous topography, with A rainfall type (Schmidt and Ferguson, 1951) and annual rainfall is around 3959 mm. The minimum temperature is around 26 ^{o}C and maximum temperature around 32^{o}C. The average humidity between 77% to 85% (Pusat Litbang Hutan dan Konservasi Alam, 2005). The Darmaga research site has flat to undulating topography, with A rainfall type and annual rainfall is around 3600 mm. The minimum temperature is around 24^{o}C and maximum temperature around 30^{o}C The average humidity between 80% to 90%. More over Sukabumi has undulating to hilly topography, with A rainfall type and annual rainfall is around 3000 mm. The minimum temperature is around 20^{o}C and maximum temperature around 25^{o}C. The average humidity between 80% to 90% (Schmidt and Ferguson, 1951).

The land use in all the research sites are gaharu plantation. In Carita, the species planted is *Aquilaria microcarpa*, with areal is around 5 ha, it was developed since 1998, and the total individual plant are 346. This plantation is mixed with other species, mostly multipurpose trees species such as: pete *(Parkia speciosa)*, melinjo *(Gnetum gnemon)*, nangka *(Artocarpus integra)*, durian *(Durio zibethinus)* etc. The altitude around 100 m above sea level. Both in Darmaga and Sukabumi, the plantations are monoculture, that developed in 1993 and 1999 respectively. The planted species are *Aquilaria crassna* and *A. microcarpa* in Darmaga and *A.microcarpa* in Sukabumi.

2. Soil Properties

a. Physical Properties

The physical characteristic of soils of all site are presented in Table 1,2 and 3. These tables indicate that the soils of all site have relatively same physical characteristics. Data of texture analyses shows that soil in all site have clay texture class. This texture class indicates that dominant soil particle is clay fraction. The implication of this soil characteristic is that water and nutrient retention of soil are relatively good. The clay content data of soil in the soil profiles show that there is clay accumulation. It means that all soil have argilic sub horizon.

The Bulk Density (BD) of soils in all site is less than 1 but more than 0,8. This indicates that the soil are developed from tuff volcanic material.

Since the soils have argilic horizon, they could be classified as Alfisol or Ultisol depend upon the Base Saturation (BS). The soils BS of Carita and Darmaga are less than 50% (see Table 4,5, and 6). Therefore these soils are classified as Ultisol. Since soil of Sukabumi has BS more than 50%, this soil is classified as Alfisol.

Porosity data of all indicate that porosity of the surface horizon is lower than that of underlying horizon. This information indicates there is compaction phenomena due to trampling and probably rain dropped from stem fall.

Table 1. Soil physical properties of Darmaga research.

Depth (cm)	Physical properties	Value	Texture category
0-30	Texture %		
	Sand	8,33	Clay
	Silt	25,1	
	Clay	66,57	
30-60	Sand	8,55	Clay
	Silt	22,1	
	Clay	69,35	
> 60	Sand	6,01	Clay
	Silt	36,51	
	Clay	57,48	
0 cm	Bulk Density	0,9	
30 cm		0,87	
60 cm		0,96	
0 cm	Porosity (%)	63,85	
30 cm		65,86	
60 cm		66,99	

Table 2. Soil physical properties of Carita research site

Depth (cm)	Physical properties	Value	Texture category
0-30	Texture %		
	Sand	8,33	Clay
	Silt	12,59	
	Clay	79,08	
30-60	Sand	6,33	Clay
	Silt	11,98	
	Clay	81,69	
> 60	Sand	5,13	Clay
	Silt	9,09	
	Clay	85,78	
0 cm	Bulk Density	0,93	
30 cm		0,84	
60 cm		0,9	
0 cm	Porosity (%)	64,99	
30 cm		66,21	
60 cm		68,45	

Table 3. Soil physical properties of Sukabumi research site

Depth (cm)	Physical characteristics	Value	Texture category
0-30	Texture %		
	Sand	12,78	Clay
	Silt	18,73	
	Clay	68,49	
30-60	Sand	9,95	Clay
	Silt	5,9	
	Clay	84,15	
> 60	Sand	11,54	Clay
	Silt	26,37	
	Clay	62,09	
0 cm	Bulk Density	0,97	
30 cm		0,86	
60 cm		0,83	
0 cm	Porosity (%)	63,43	
30 cm		67,59	
60 cm		68,75	

b. Chemical properties

The chemical soil properties are: pH of H_2O, N in the ratio 1:1, organic carbon, total N, available P,exchangeable acidity, Cation Exchange Capacity (CEC), exchangeable bases and Base Saturation (BS). The chemical analytical data are presented in Table 4,5 and 6.

The pH H_2O of the material in the research sites profiles is mostly less than 5, except in Sukabumi research site are slightly higher than 5. However these soils are still categorized as acid. Although these soils are developed from andesitic volcanic material that rich in base bearing mineral, because of intensive weathering and leaching the reaction remain acid and base saturation mostly < 100%. This reaction influences the availability of essensial elements.

The essensial elements are element which are needed by plant, and its function can not be replaced by others elements (Pratiwi 2004 and 2005). These elements are categorized as macro nutrient (C,H, O, N, P,K,Ca,Mg dan S) and micro nutrient (Fe,Mn,B,Mo,Cu,Zn,Cl dan Co). Besides the pH, the availaility of the essensial elements are determind by organic matter content and the dynamic processes in the soil profiles.

The organic carbon and total-N content of the soils in the research sites decreases downward. The amount of organic carbon is relatively low in all horizon, but in Carita research site the organic carbon is higher than in Sukabumi and Darmaga research sites. The low content of organic carbon and total – N is related to the low content of organic matter. This agrees to the fact that in Carita there were much more underground vegetation than in Darmaga and Sukabumi research sites. The underground vegetation supplies organic material to the soil. According to Sutanto (1988) the organic matter is also responsible in increasing the CEC by increasing negative charges when the pH increases from natural pH of soil (variable charges). The C/N ratio is high in almost all horizons, particularly in top horizons. It shows that the decomposition of the orgenic matter is not very strong.

The P content in all research sites are very low (< 2). Pratiwi (2004 and 2005) said that this element especially in the top soil has very important function for seedling growth. Others important elements are K, Al^{3+} and H^{+}. In Darmaga K is medium, while in Carita and Sukabumi, are low and high respectively, and

Al^{3+} as well as H^{+} are low to very low in all research sites. Soil with high available Al has toxic characteristic. Therefore there is no danger of Al toxicity in the research area.

The micro nutrient also influence plant growth, but the need are very low. These are Fe,Cu, Zn dan Mn. Elements Fe,Cu and Zn relatively low, while Mn medium to relatively sufficient. Such condition is relatively favourable for plant growth.

The Cation Exchange Capacity (CEC) indicates the soil fertility degree. Soil with high CEC able to adsorb and nutrient availability better than soil with low CEC. The Cation Exchange Capacity (CEC) was determined with a buffer solution NH4Oac pH 7 and the CEC sum of cations is a result of the cations summation (K^{+},Na^{+},Ca^{+2}, Mg^{+2},H^{+}

and Al^{3+}). Table 4,5 and 6 show clearly that CEC NH4oAct pH 7 of all prolifes is strongly higher that the CEC of the sum cations. The higher the CEC means that the area are relatively fertile. From Table 4,5 and 6 indicate that soil in Sukabumi has high Base Saturation (39,35-41,07), while in Darmaga is medium (16,01-17,75) and the lowest in Carita (13,05-15,77). Soil with higher pH generally has higher CEC. This tendency occure in the research sites, whereas the pH of Sukabumi is higher than that of Carita and Darmaga.

The base cation of Sukabumi content is high, while of Darmaga is medium and Carita is low. In all soil horizons of the three sites, the base cation are dominated by calcium and magnesium.

The highest sum of cations in research sites show in Sukabumi research site is highest and the lowest is in Carita. This can be related to the fact that Sukabumi has the highest pH H_2O. The pH seem has relationship with the Base Saturation. There is tendency that the higher pH shows higher Base Saturation.

Table 4. Soil chemical characteristics in Darmaga research site.

Chemical Characteristics	Horizon 1	Horizon 2	Horizon 3
	(0-30 cm)	(30-60 cm)	(>60 cm)
pH H2O 1:1	4,70 (Low)	4,60 (Low)	4,50 (Low)
C org (%)	1,43 (Low)	1,03 (Low)	1,03 (Low)
N-total (%)	0,15 (Low)	0,12 (Low)	0,11(Low)
C/N ratio	9,55	8,58	9,36
P Bray (ppm)	1,7 (Very Low)	1,3 (Very Low)	1,7 (Very Low)
NH4OAc pH 7			
(me/100 gr)			
Ca	5,29 (Medium)	4,17 (Low)	5,32 (Medium)
Mg	1,19 (Medium)	1,09 (Medium)	1,70(Medium)
K	0,44 (Medium)	0,44 (Medium)	0,58 (High)
Na	0,30 (Low)	0,26 (Low)	0,26 (Low)
Exchangeable cation (sum)			
CEC	7,22	5,96	7,6
CEC sum	17,75 (Medium)	16,61 (Medium)	16,99 (Medium)
	11,27	10,48	12,91
KB (%)	40,68 (Medium)	35,88 (Medium)	46,26 (Medium)
KCl			
(me/100 gr)			
Al	3,72 (Very Low)	4,16 (Very Low)	4,90 (Very Low)
H	0,33	0,36	0,41
0,05 N HCl (ppm)			
Fe	2,04	1,8	1,48
Cu	3,44	2,64	2,4
Zn	5,24	4,88	5,28
Mn	85,6	88,01	79,2

Table 5. Soil chemical characteristics in Carita research site.

Chemical Characteristics	Horizon 1	Horizon 2	Horizon 3
	(0-30 cm)	(30-60 cm)	(>60 cm)
pH H2O 1:1	4,60 (Low)	4,50 (Low)	4,60 (Low)
C org (%)	2,31 (Medium)	1,51 (Low)	0,71 (Very Low)
N-total (%)	0,17 (Low)	0,14 (Low)	0,08 (Very Low)
C/N ratio	13,59	10,78	8,88
P Bray (ppm)	1,70 (Very Low)	1,20 (Very Low)	1,20 (Very Low)
NH4OAc pH 7			
(me/100 gr)			
Ca	1,49 (Very Low)	1,01 (Very Low)	1,00 (Very Low)
Mg	0,75 (Low)	0,53 (Low)	0,52 (Low)
K	0,16 (Low)	0,14 (Low)	0,13 (Low)
Na	0,20 (Low)	0,22 (Low)	0,21 (Low)
Exchangeable cation (sum)	2,6	1,9	1,86
CEC			
CEC sum	15,77 (Low)	13,11 (Low)	13,03 (Low)
	8,93	9,79	8,71
KB (%)	16,49 (Very Low)	14,49 (Very Low)	14,27 (Very Low)
KCl			
(me/100 gr)			
Al	5,84 (Low)	7,36 (Low)	6,40 (Low)
H	0,49	0,53	0,45
0,05 N HCl (ppm)			
Fe	1,72	1	1,04
Cu	1,64	1,68	1,52
Zn	3	2,6	2,8
Mn	28,48	17,08	16,4

Table 6. Soil chemical characteristics in Sukabumi research site.

Chemical Characteristics	Horizon 1	Horizon 2	Horizon 3
	(0-30 cm)	(30-60 cm)	(>60 cm)
pH H2O 1:1	5,10 (Low)	5,10 (Low)	4,60 (Low)
C org (%)	1,60 (Low)	2,07 (Medium)	1,01 (Low)
N-total (%)	0,15 (Low)	0,18 (Low)	0,11 (Low)
C/N ratio	10,67	11.50	9,18
P Bray (ppm)	3,90 (Very Low)	3,70 (Very Low)	3,40 (Very Low)
NH4OAc pH 7			
(me/100 gr)			
Ca	16,98 (High)	16,99 (High)	14,64 (High)
Mg	10,52 (Very high)	10,94 (Very high)	10,05 (Very high)
K	0,71 (High)	0,40 (Medium)	0,22 (Low)
Na	0,36 (Medium)	0,43 (Medium)	0,22 (Low)
Echangeable cation (sum)			

Chemical Characteristics	Horizon 1	Horizon 2	Horizon 3
	(0-30 cm)	(30-60 cm)	(>60 cm)
CEC	28,57	28,76	25,15
CEC sum	41,07 (Very high)	36,48 (High)	39,35 (High)
	31,14	31,82	31,97
KB (%)	69,56 (High)	78,84 (Very high)	63,86 (High)
KCl			
(me/100 gr)			
Al	2.32 (Very Low)	2,76 (Very Low)	6,40 (Low)
H	0,25	0,3	0,42
0,05 N HCl (ppm)			
Fe	0,52	0,36	0,32
Cu	1,2	1,12	1,44
Zn	1,4	1,56	1,56
Mn	17	22,12	26,36

B. Vegetation Properties of the Studied Areas

1. General

The vegetation analyses were carried out mainly for underground vegetation in Carita, Darmaga and Sukabumi areas. These areas are gaharu plantation and the dominant tree, sapling and pole stages mainly gaharu. Therefore the vegetation analyses was mainly stressed in underground vegetation.

2. The composition of underground species

The observation shows that in Carita the underground vegetation is higher than that in Sukabumi and Darmaga (Table 7).

Table 7. Total undeground species and its family in the research sites

Research sites	Total Species	Total Family
Carita	30	18
Darmaga	8	16
Sukabumi	6	3

This condition seems due to the difference in plantation system. In Carita the gaharu is mixed with multipurpose trees species, while in Sukabumi and Darmaga the gaharu are planted in monoculture system. The conditions of Carita support some seedling from other species.

3. The dominant underground species

Ecologically, the value of vegetation is defined by the function of the dominant species. The dominant species is species which has the highest important value on vegetation community. The value is a result of the interaction between species with the environmental conditions.

The observations show that the dominant and co-dominat species of each area are different. In Carita the dominant and co-dominant underground species are jampang (*Panicum disachyum*) and selaginela (*Selaginella plana)*, while in Darmaga are pakis (*Dictyopteris irregularis*) and seuseureuhan (*Piper aduncum)* and in Sukabumi are jampang (*Panicum disachyum*) and rumput pait (*Panicum barbatum)* (Table 8,9, and 10). These data indicate that the habitats are ecologically have differences characteristics.

Table 8. Important Value of underground species in Carita

No.	Nama Daerah	Nama Botani	Famili	Kr (%)	Fr (%)	Dr (%)	INP (%)
1.	Jampang	*Panicum disachyum* Linn.	Gramínea	47	8,58	25,7	81,28
2.	Selaginella	*Selaginella plana* Hiern.	Selaginellaceae	14,52	10	32,76	57,28
3.	Harendongmerah	*Melastoma malabathricum* L.	Melastomataceae	5,17	9,99	7,09	22,25
4.	Cingcau	*Cyclea barbata* Miers.	Meraispermaceae	7,88	10	3,82	21,7
5.	Rumput Pait	*Panicum barbatum* Lamk.	Graminae	7,39	5,71	3,71	16,81
6.	Ilat	*Cyperus difformis* Linn.	Cyperaceae	3,69	5,71	0,99	10,39
7.	Parasi	*Curculigo latifolia* Dryand.	Amaryllidiaceae	2,45	4,29	3,09	9,83
8.	Terongan	*Solanum jamaicence* Mill.	Solanaceae	0,98	5,71	2,97	9,66
9.	Hatta	*Coniograma intermedia* Hieron.	Polypodiaceae	0,75	1,43	6,18	8,36
10.	Peletok	*Cecropia peltata* L.	Moraceae	1,23	2,85	2,1	6,18
11.	Paku anam	*Lygodium circinatum* Sw.	Schizophyllaceae	0,98	4,29	0,73	6
12.	Pakis	*Dictyopteris irregularis* Presl.	Polypodiaceae	0,5	1,43	3,09	5,02
13.	Sasahan	*Tetracera indica* L.	Dilleniaceae	0,75	2,86	1,11	4,72
14.	Harendong	*Clidenia hirta* Don.	Melastomaceae	0,49	1,43	0,62	4,57
15.	Kokopian	*Ixora* sp.	Rubiaceae	1,23	2,85	0,48	4,57
16.	Mahoni	*Swietenia macrophylla* King	Meliaceae	0,5	2,85	0,62	4,47
17.	Cacabean	*Morinda bracteosa* Hort.	Rubiaceae	0,25	1,43	1,23	2,91
18.	Alang-alang	*Imperata cylindrica* Linn.	Graminae	0,75	1,43	0,25	2,43
19.	Hawuan	*Elaeocarpus glaber* Blume	Elaeocarpaceae	0,25	1,43	0,62	2,3
20.	Kakacangan	*Stachystarpheta jamaisensis* Vahl.	Verbenaceae	0,25	1,43	0,62	2,3
21.	Pacing	*Tapeinochilus teysmannianus* K.Sch.	Zingiberaceae	0,49	1,43	0,25	2,17
22.	Seuseureuhan	*Piper aduncum* L.	Piperaceae	0,25	1,43	0,37	2,05
23.	Gagajahan	*Panicum montanum* Roxb.	Graminae	0,5	1,43	0,12	2,05
24.	Ki koneng	*Plectronia* sp.	Rubiaceae	0,25	1,43	0,37	2,05
25.	Babadotan	*Ageratum conizoides* Linn.	Compositae	0,25	1,43	0,25	1,93
26.	Pakis Anjing	*Dryopteris dentata* C.Chr.	Polypodiaceae	0,25	1,43	0,25	1,93
27.	Gaharu	*Aquilaria malaccensis* Lamk.	Thymelaeaceae	0,25	1,43	0,25	1,93
28.	Pete	*Parkia speciosa* Hassk.	Leguminosae	0,25	1,43	0,12	1,8

No.	Nama Daerah	Nama Botani	Famili	Kr (%)	Fr (%)	Dr (%)	INP (%)
29.	Kanyere	*Bridelia monoica* L.	Euphorbiaceae	0,25	1,43	0,12	1,8
30.	Cingcanan	*Morinda bracteosa* Hort.	Rubiaceae	0,25	1,43	0,12	1,8
TOTAL				100	100	100	300

Table 9. Important Value of underground species in Darmaga

No.	Nama Daerah	Nama Botani	Famili	Kr (%)	Fr (%)	Dr (%)	INP (%)
1.	Pakis	*Dictyopteris irregularis* Presl.	Polypodaceae	29,41	16,72	28,08	74,21
2.	Seuseureuhan	*Piper aduncum* L.	Piperaceae	11,76	11,03	34,25	57,04
3.	Tales	*Alocasia* sp.	Araceae	5,89	16,72	20,55	43,16
4.	Rumput Pait	*Panicum barbatum* Lamk.	Graminae	17,64	16,72	3,42	37,78
5.	Rumput padi	*Oryza grandulata* Nees.	Gramínea	11,76	16,72	1,71	30,19
6.	Areu	*Micania scandens* Willd.	Compositae	5,89	11,03	5,14	22,06
7.	Babadotan	*Ageratum conizoides* Linn.	Compositae	11,76	5,52	1,71	19
8.	PACINE	*Tapeinochilus teysmannianus* K.Sch.	Zingiberaceae	5,89	5,53	5,14	16,56
TOTAL				100	100	100	300

Table 10. Important Value of underground species in Sukabumi.

No.	Nama Daerah	Nama Botani	Famili	Kr (%)	Fr (%)	Dr (%)	INP (%)
1.	Jampang	*Panicum distachyum* Linn.	Gramínea	56.56	33.34	50	139,9
2.	Rumput Pait	*Panicum barbatum* Lamk.	Graminae	24,24	16,67	17,87	58,94
3.	Harendong	*Clidenia hirta* Don.	Melastomaceae	4,76	16,67	10,71	32,14
4.	Babadotan	*Ageratum conizoides* Linn.	Compositae	7,74	8,33	14,28	30,35
5.	Kirinyuh	*Euphatorium pallascens* DC.	Compositae	2,38	16,67	3,57	22,62
6.	Alang-alang	*Imperata cilíndrica* Linn.	Graminae	4,16	8,33	3,57	16,06
TOTAL				100	100	100	300

4. The similarity of underground species composition

According to the Similarity Index (SI) of Sorensen (Mueller-Dumbois and Ellenberg, 1974). The composition of underground species is different on every research sites. This is indicated by a low SI value (< 50%) (Table 11).

Table 11. Similarity Index (%) of plant communities at research sites

Location	Carita	Darmaga	Sukabumi
Carita	-	9	35
Darmaga	-	-	9
Sukabumi	-	-	-

This difference in composition is due to the difference of environmental factor such as climate, topography and soil characteristics.

IV. CONCLUSION

1. The soil of three different research sites have relatively the same parent material that are andesitic volcanic materials.
2. Different physical and chemical characteristics of soil in the research sites are related to different stage of weathering process that related to environmental condition of weathering process.
3. Related to weathering state, Carita soil is less fertile than that of Darmaga and Sukabumi. These fertility state is related to stage of weathering process.
4. Soil physical and chemical characteristics of soil in the studies area are support the gaharu plantation.
5. The dominant and co-dominat species of each area are different. In Carita the dominant and co-dominant underground species are jampang (*Panicum disachyum*) and selaginela (*Selaginella plana)*, while in Darmaga are pakis (*Dictyopteris irregularis***)** and seuseureuhan (*Piper aduncum)* and in Sukabumi are jampang (*Panicum disachyum*) and rumput pait (*Panicum barbatum).*
6. The composition of underground species is also different on every research sites as indicated by SI < 50%. This difference in composition is due to the difference of environmental factor such as climate, topography and soil characteristics.

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COMMUNITY BASED FOREST MANAGEMENT(CBFM) USING PROFIT SHARING SYSTEM IN GAHARU PLANTATION ESTABLISHMENT

(a case study in KHDTK Carita-Pandeglang, Banten)

By:
Sri Suharti[1]

ABSTRACT

Forestry sector has an important role in development program in Indonesia. However, during its development, forestry program always deals with several problems both technical and non technical including social community conflicts. The situation gives an indication that community's right and interest in forestry development process besed on sustainable principles need to be taken into consideration shrewdly. One alternative solution that could be done to accommodate rehabilitation of forest function in one hand and fulfilling local community's needs on the other hand especially in areas prone to land encroachment and illegal logging is by promoting participatory forest rehabilitation through Community Forest Based Management (CBFM) approach. CBFM is deemed to be the suitable approach in such areas since CBFM is implemented by involving forest surrounding community in forest management. Forest management would be successful if all stakeholders involved are willing to cooperate and allocate space, time, benefit, right and obligation based on powering, promoting and benefiting each other principles. Collaboration research of gaharu trees plantation establishment through profit sharing system in KHDTK Carita is intended to implement forest land rehabilitaion by increasing land productivity through growing trees with high economic value hence it could inrease people's income as well. Gaharu trees are selected as it has high economic value beside it still could grow well under tree stands with limited light intensity (< 70%). The research was done by using field observation, informal discussion with related stakeholders (Perhutani state owned forest, Banten Forestry Service, personnel of KHDTK Carita, gaharu trader, etc) and followed by Focus Group Discussion/FGD with the people who are going to involve in the research collaboration (40 people). The result of the research shows that in general people's response towards plan of research collaboration with gaharu plantation in the area is very positive. Candidates of participants try to understand every item of the collaboration principles written down in draft of understanding including its risks and consequences. Main principles written

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down in the understanding draft of collaboration research are sustainability and its economic feasibility during period of contract (mutualistic advanteges based on inputs contributed by each stakeholder in order to achieve collaborative objectives i.e social, economic and ecology). After having several in depth discussions with all stakeholders involved especially candidates of participants, draft of collaboration memorandum successfully formulated including right and obligation, reward and punishment and profit sharing system when gaharu trees already produce. Formulation of memory of understanding (MOU) is written down in the document draft.

Keywords: Land rehabilitation, community income, CBFM, KHDTK Carita

I. INTRODUCTION

Forestry program development always deals with several problems both technical and non technical including social community conflicts. The situation indicates that community's right and interest in forestry development process besed on sustainable principles need to be taken into consideration shrewdly. This then clarifies that community involvement is urged in all phases of sustainable forestry development based on Ministry of Forestry (MOF) decree No. 31 (year 2001) concerning community forestry.

To anticipate the situation, since the last two decades, Government of Indonesia (GOI) has developed several programs both preventive (conservation) and curative (rehabilitation). Those programs have the main objectives to increase land productivity and maintain forest land sustainability and also to strengthen bargaining position and welfare of community living surrounding forest area. However, so far the program has not provided satisfying results to overcome the problem of forest degradation in Indonesia.

One alternative that could be taken to conquer the situation and accommodate forest land rehabilitaion in one hand and fulfilling community need on the other hand in areas prone to land encroachment and illegal logging like KHDTK Carita (Forest area with special puroose) is through implementation of community based forest management (CBFM). CBFM is deemed to be the suitable approach since it is implemented by involving forest surrounding community in forest management. Forest management would be successful if all stakeholders involved are willing to cooperate and allocate space, time, benefit, right and obligation based on empowering, promoting and benefiting each other principles.

Collaboration research of gaharu trees plantation establishment through profit sharing system in KHDTK Carita is intended to implement forest land rehabilitaion by increasing land productivity through growing trees with high economic value hence it could inrease people's income as well. Gaharu is selected as its price has been very high and it is now potentially threatened with extinction due to habitat destruction and

unsustainable harvesting many species of gaharu.

Gaharu is a fragrant resinous wood coming from trees belonging to the genera *Aquilaria*, *Gyrinops* and *Gonystylus*. When these trees are injured, damaged or infected, for example by insects or fungal disease, they produce a brown resin in reaction to the wound. This resin can help protect the tree from further infection so it is considered to be a kind of defense mechanism or immune response (Squidoo, 2008).

Demand for gaharu far exceeds supply and consequently during recent years there has been a boom in planting gaharu trees on farms and in plantations, especially in South East Asia. (Squidoo, 2008).

Gaharu trees grow naturally in South and Southeast Asia. It has many names including agarwood, aloeswood, gaharu (Indonesia), ood, oudh, oodh (Arabic), chen xiang (Chinese), pau d'aguila (Portuguese), bois d'aigle (French)and adlerholz (German). *Aquilaria* trees which produce gaharu are now protected in most countries and the collection of eglewood is illegal from natural forests. International agreements, such as CITES (the Convention on International Trade in Endangered Species of Wild Fauna and Flora), accepted by 169 countries, is designed to ensure trade in agarwood products from wild trees does not threaten the survival of *Aquilaria*. Despite these efforts eglewood products from illegally cut trees continues to be sold and unknowing consumers create a demand that helps to destroy the last old growth *Aquilaria* trees in existence (Blanchette, 2006).

The objectives of collaboration research with profit sharing system in KHDTK Carita are to promote land rehabilitation through increasing forest land productivity with eagelwood plantation establishment that has high economic value while increase community welfare living surrounding KHDTK Carita forest area. Furthermore, eagelwood is selected since its growth requirements are suitable with biophysical condition of KHDTK Carita (it could grow well under tree stands with limited light intensity).

II. MATERIALS AND METHODS

The research in Community Based Forest Management in gaharu plantation using profit sharing system was conducted at part of area plot No 21 at KHDTK Carita – Pandeglang, Banten. Total area of research plot is 24 ha. The research was done by involving local community (who formerly cultivate seasonal crops, multipurpose trees/ MPTS and fruit trees in the research area) to plant gaharu trees in their cultivated land. People who are going to participate in the research collaboration come from Sindang Laut Village (especially from Longok and Pasir angin sub village).

Process of establishing plot demonstration of gaharu trees in KHDTK Carita was initiated by intensive discussion and approach with candidates of participants in order to investigate prospect of community participation in plantation establishment. After having sufficient description about prospect of community participation in plot establishment, next process is formulation of technical plan and design through several in depth

discussions. By owning this series of in depth discussions, it is expected that candidates of participants would really understand about the purpose of the research collaboration which eventually could increase their active participation in gaharu plantation.

Method used in the research was field observation, interviews and discussion with related stakeholders (Perhutani state owned forest, Banten forestry service, personnel of KHDTK Carita and gaharu trader). Subsequently, it will be followed by Focus Group Discussion (FGD) by using Participatory Rural Appraisal (PRA) approach (Sulaeman, 1995). Main target of the research is all stakeholders involving in KHDTK Carita management and candidates of participants from local community (40 people). Focus of the discussions were to gain better understanding about main principles of research collaboration including right and obligation, reward and punishment and also profit sharing system which is going to be applied when gaharu tree already produce.

Data obtained from the research would be analysed descriptively (Singarimbun and Sofian, 1982).

III. RESULTS AND DISCUSSION

Total forest area of Banten province is 206,852.44 Ha consisting of production forest, protection forest and conservation forest. In 2003, Ministry of Forestry through MOF Decree No 290 and 291 declared that limited production forest in Carita, Pandeglang regency, Banten province with 3000 ha total area has been decided to become forest area with special purpose (KHDTK). The area which was formerly managed by Perhutani state owned forest had been handed over to Forestry Research and Development Agency (FORDA). Administratively, KHDTK Carita with total area 3000 ha is located in RPH Carita and RPH Pasauran area.

Based on field observation and intensive discussion with related stakeholders in KHDTK Carita, it was found that majority of the area has been encroached by surrounding community (> 70%). Considering trend of development, it seems that the encroachment tends to increase and even more intense from time to time. Underlying factors behind this situation are increase of population, limited job opportunities and limited skill and knowledge of the people in the area. Actually, close location with Carita beach provides other sources of income for the people in Carita (selling local handicraft, being tourist guide, renting beach game tools for guests, etc). However, since tourists (both domestic and foreign tourists) only come on weekend or holidays, people still have plenty of unused time outside those days. The situation then in turn direct people to utilize KHDTK Carita forest area (which is located at the boundary of surrounding villages) as alternative place to gain additional income.

In Government regulation (PP) No. 6 (year 2007) concerning forest arrangement and forest management and use plan article 17 verse 1 it is mentioned that forest land use has the main objective to gain optimal, fair and sustainable benefits of forest product and service. Forest use based on verse 1 of the regulation could be done through (i)

utilization of the area; (ii) utilization of environmental service; (iii) utilization of both timber and non timber product and (iv) collecting timber and non timber forest product. Subsequently, in article 18, it is also stated that forest utilization could be done at all forest area including (i) conservation forest, except natural reserve area, wilderness zone and core zone; (ii) protection forest and (iii) production forest. Considering the situation where forest land was already occupied by local community, it is necessary to have alternative solution to prevent from further forest degradation while accomodating community's needs as well. One alternative to accommodate those two interests is by involving local community in forest management. Involving local community in forest management is intended to accommodate change of paradigm in forest management that has shifted towards community's interest. Hence forest management with former paradigm "timber management" that only focussed on financial benefit for holding company has to be left behind. New paradigm in forest management places environmental protection and ecosystem sustainability aspect at first priority and economic aspect at second priority. Therefore, since 2000 forest management in Indonesia has been using holistic/comprehensive approach that put forest as a unit of ecosystem and utilize all potential resources in it for the sake of community welfare.

Collaboration research with local community through development of gaharu tree plantation establishment in KHDTK Carita is application of new forest management paradigm. Research result shows that people's involvement in forest management could be appropriate solution to preserve KHDTK Carita forest in one hand and empowering surrounding community on the other hand.

In order to learn about prospect of community participation in gaharu plantation establishment, several process are carried out i.e:

1. Introduction about gaharu tree including its growth requirements, cultivation techniques, its morphology and appereance of gaharu after produced and then followed by formulation of mutual understanding about several principles of CBFM.
2. Formulation of mutual objective which is going to be achieved from the collaboration.
3. Intensive discussion with related stakeholders such as personnel of KHDTK Carita, key persons of local community, Perhutani state owned forest and gaharu trader. From the discussion, it can be assumed that gaharu plantation establishment has a good prospect to be developed in the area. Subsequently, by considering biophysical condition of the area, social economic condition of the people and accessibility of the people to KHDTK Carita, it is decided to to involve community from Sindang Laut Village in the research collaboration.
4. Based from initial information, more in depth discussion with key persons from Sindang Laut village was carried out on July 11, 2008 at KHDTK Carita headquarters. There were 4 researchers, 3 KHDTK personnels and 6 key persons from Sindang Laut village attended the discussion. In the discussion, the purpose of the collaboration is introduced including initial description about right and obligation of each party involved.

Process of discussion worked well and dynamically based on mutual advanteges and sustainable principles. Initial description about research collaboration was also formulated. Subsequently, initial formulation about research collaboration was further evaluated by all stakeholder involved.

5. From initial formulation of collaboration, draft of memory of understanding then was composed in more detail. The draft comprises not only objective and techniques of collaboration, right and obligation of the parties involved (R&D Centre for Forest Conservation and Rehabilitation/RDCFCR at one side and community of Sindang Laut Village at the other side), but also explain further about status of the research site, profit sharing system, reward and punishment and risk and consequences if something unexpected occurs.

From the initial evaluation about prospect of community participation in research collaboration of gaharu plantation establishment, it can be perceived that pople of Sindang Laut village is very interested to be involved in the collaboration. Based on the information before, technical plan of the collaboration which is written down in the draft of MOU start to be formulated more detail.

Formulation of MOU draft between Forest and Nature Conservation Research and Development Centre at one side with candidates of participants from Sindang Laut Village on the other side was done based on following principles:

1. Collaboration is economically feasible during contract period.
2. There are mutual objective that is going to be attained
3. There is mutual and fair arrangement based on contribution of each parties involved to reach mutual objective
4. There is mutual understanding about risk and consequences in the collaboration

Main aim of the collaboration is to increase forest land productivity through cultivation of high economic value trees and increase of local people's income surrounding KHDTK Carita forest area (especially community of Sindang Laut village involving in the collaboration) which its dependency upon forest is relatively high. After mutual agreement on those basic principles has been reached, points of MOU draft start to be formulated and then the draft then was discussed again with all stakeholders involved. The discussion was carried out on September 11, 2008 and attended by personnel of KHDTK Carita (3 persons), RDCFCR researchers (8 pesons) and candidates of participants of community (34 persons). From the discussion, draft then was further evaluated and finally reach ending agreement with several minor revision. Draft of MOU then again was further discussed among MOU committee and after several amendments and correction final fraft of MOU was successfully formulated. The final draft of MOU will be signed by those two parties (RDCFCR) and Sindang Laut community). Draft of the MOU could be seen at annex of this report.

IV. CONCLUDING REMARKS

Based on evaluation of prospect of research collaboration (community participation and process of mutual agreement) in gaharu plantation establishment, it can be concluded that:

1. Collaboration research to promote gaharu trees in KHDTK Carita where majority of the area has been occupied by local people has become one alternative solution to preserve KHDTK forest, increase land productivity and community's income.
2. Response of local community towards gaharu plantation establishment is quite positive, this can be seen from their efforts in understanding each part of MOU.
3. Main principles persist in the collaboration are sustainability and economic feasibility based on contribution of each stakeholder involved in the collaboration.

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Annex 1.

PERJANJIAN KERJASAMA
PENGELOLAAN SUMBERDAYA HUTAN BERSAMA MASYARAKAT (PHBM)
MELALUI SISTIM BAGI HASIL PENANAMAN POHON GAHARU
PADA PETAK 21 DI KHDTK CARITA – PANDEGLANG, BANTEN

Pada hari ini ..... tanggal .....bulan ..... tahun ..... bertempat di Desa Sindang Laut, Kecamatan Carita, Kabupaten Pandeglang, kami yang bertanda tangan di bawah ini:

1. Ir. Sulistyo A. Siran, MSc., Kepala Bidang Pelayanan dan Evaluasi Penelitian pada Pusat Penelitian dan Pengembangan Hutan dan Konservasi Alam, Badan Litbang Kehutanan, Departemen Kehutanan, dalam hal ini bertindak untuk dan atas nama Pusat Penelitian dan Pengembangan Hutan dan Konservasi Alam, selanjutnya disebut PIHAK PERTAMA

2. Ustad Djafar, Ketua Kelompok Tani Hutan Giri Wisata Lestari, warga Desa Sindang Laut, Kecamatan Carita, Kabupaten Pandeglang, bertindak untuk dan atas nama Kelompok Tani Hutan Giri Wisata Lestari, selanjutnya disebut PIHAK KEDUA.

Dalam rangka penelitian Pengelolaan Sumberdaya Hutan Bersama Masyarakat Melalui Sistim Bagi Hasil Penanaman Gaharu pada sebagian Petak 21 seluas kurang lebih 40 hektar di dalam Kawasan Hutan Dengan Tujuan Khusus (KHDTK)/Hutan Penelitian (HP) Carita, maka PIHAK PERTAMA, dan PIHAK KEDUA sepakat untuk mengikatkan diri dalam Perjanjian Kerjasama Pengelolaan Hutan dengan ketentuan sebagaimana diatur dalam pasal-pasal dan ayat-ayat berikut:

Pasal 1
DASAR PERJANJIAN KERJASAMA

1. Surat Keputusan Menteri Kehutanan No. 456/Menhut-VII/2004 tentang Lima Kebijakan Prioritas Bidang Kehutanan dalam Program Pembangunan Nasional Indonesia Kabinet Indonesia Bersatu.
2. Surat Keputusan Menteri Kehutanan No. 290/Kpts-II/2003 tanggal 26 Agustus 2003 tentang penunjukan kawasan hutan dengan tujuan khusus seluas ± 3000 (tiga ribu) hektar yang terletak di Kecamatan Labuan, Kabupaten Pandeglang, Propinsi Banten sebagai Hutan Penelitian Carita.
3. Surat Keputusan Menteri Kehutanan No. 291/Kpts-II/2003 tanggal 26 Agustus 2003 tentang penggunaan kawasan hutan.
4. Surat Keputusan Kepala Badan Penelitian dan Pengembangan Kehutanan No. 68/ Kpts/VIII/2004 tentang pembentukan tim penyusun rencana pengelolaan Hutan Penelitian Carita.
5. Surat Keputusan Kepala Badan Penelitian dan Pengembangan Kehutanan No. SK. 90/kpts/VIII/2007 tentang Penunjukan Penanggung jawab Pengelolaan Kawasan Hutan Dengan Tujuan Khusus (KHDTK) lingkup Badan Litbang Kehutanan.

Pasal 2
TUJUAN

Mengoptimalkan fungsi dan manfaat KHDTK Carita untuk menjamin kelestarian sumberdaya hutan dan kesejahteraan masyarakat dengan menerapkan ilmu pengetahuan dan teknologi bidang kehutanan, melalui:

1. Aplikasi konsep Pengelolaan Hutan Berbasis Masyarakat dalam rangka mewujudkan pengelolaan hutan lestari dan masyarakat sejahtera.
2. Memberikan kesempatan kepada masyarakat di sekitar Hutan Penelitian Carita untuk berpartisipasi dan berperan aktif dalam pengelolaan hutan sekaligus sebagai upaya meningkatkan kesejahteraan mereka.

Pasal 3
OBYEK PERJANJIAN

1. Plot uji coba seluas kurang lebih 40 ha pada petak 21 di Kawasan Hutan Penelitian Carita.
2. Tanaman (pohon) hutan dan tanaman pohon lainnya serta tanaman pertanian yang ditanam di lokasi sebagaimana tersebut pada pasal 3 ayat 1 yang merupakan kesepakatan para pihak.

Pasal 4
HAK DAN KEWAJIBAN PARA PIHAK

PIHAK PERTAMA berkewajiban:

1. Mengikutsertakan PIHAK KEDUA dalam kegiatan kerjasama penelitian “Pengelolaan Sumberdaya Hutan Bersama Masyarakat Melalui Sistem Bagi Hasil Penanaman Gaharu” dan memberi kesempatan kepada PIHAK KEDUA untuk mengambil manfaat dari tanaman bawah tahan naungan dan tanaman buah-buahan dan atau serbaguna di kawasan hutan sebagaimana tersebut pada pasal 3 ayat 1.
2. Menyediakan biaya bagi PIHAK KEDUA untuk melakukan kegiatan budidaya penanaman pohon gaharu meliputi biaya kegiatan penanaman (biaya upah dan bibit tanaman gaharu) pada petak 21 dengan jumlah tanaman ± 15.000 (lima belas ribu batang).
3. Melakukan pembinaan teknis budidaya tanaman gaharu kepada PIHAK KEDUA minimal satu kali setahun sejak tahun 2008 sampai tahun 2011.
4. Menyediakan jamur pembentuk gaharu untuk kegiatan inokulasi/penyuntikan tanaman gaharu pada petak 21 sebanyak 25% dari jumlah total tanaman gaharu PIHAK KEDUA (masing-masing penggarap).
5. Membantu mencarikan investor untuk bekerjasama menyediakan produksi jamur pembentuk gaharu untuk kegiatan inokulasi/penyuntikan tanaman gaharu untuk 75% tanaman gaharu lainnya.
6. Memberikan pelatihan budidaya gaharu serta pemanenan gaharu (paket training gaharu) yang akan diadakan paling lambat pada tahun 2010 kepada PIHAK KEDUA.
7. Bersama-sama PIHAK KEDUA melakukan kegiatan inokulasi/penyuntikan tanaman gaharu pada petak 21 sebanyak 25% dari jumlah total tanaman gaharu masing-masing penggarap setelah tanaman gaharu berumur ≥ 5 (lebih tua atau berumur lima

tahun)
8. Memberi informasi mengenai segala bentuk kegiatan dan kebijakan pengelolaan hutan di lokasi kerjasama kepada PIHAK KEDUA.
9. Bersama PIHAK KEDUA menjaga keamanan kawasan hutan dan memelihara sumberdaya hutan di lokasi kerjasama sebagaimana tersebut pada pasal 3 ayat 1 guna kelestarian fungsi dan manfaat hutan.
10. Melaporkan setiap tindakan pelanggaran hukum yang terjadi kepada pihak yang berwenang.

PIHAK PERTAMA berhak:

1. Melakukan pengamatan dan pengukuran pertumbuhan tanaman gaharu dan tanaman hutan lainnya yang ditanam di lokasi kerjasama serta melakukan pengamatan dan pengukuran kondisi biofisik dan sosial ekonomi .
2. Melakukan pemeliharaan (penyiangan, pemupukan, pemberantasan hama penyakit, penyulaman, pemangkasan dan penjarangan) terhadap tanaman gaharu di lokasi kerjasama sepanjang untuk keperluan penelitian.
3. Melakukan penebangan di areal kerjasama sepanjang untuk keperluan penelitian.
4. Memperoleh laporan pelaksanaan kegiatan yang dilakukan PIHAK KEDUA
5. Memperoleh informasi dari PIHAK KEDUA mengenai segala sesuatu yang berkaitan dengan perkembangan kondisi tanaman gaharu dan tanaman hutan lainnya serta tanaman pertanian yang menjadi objek kerjasama.
6. Memperoleh laporan dari PIHAK KEDUA mengenai segala bentuk kejadian dan pelanggaran hukum yang terjadi dalam kawasan hutan yang menjadi objek kerjasama.

PIHAK KEDUA berkewajiban:

1. Memelihara dan menjaga keamanan tanaman (pohon) gaharu dan tanaman hutan lainnya (memberi pupuk organik (kompos), memberantas gulma, hama dan penyakit yang mengganggu pertumbuhan tanaman gaharu) sampai tanaman gaharu dipanen.
2. Memelihara dan mengamankan sumberdaya hutan pada kawasan hutan di lokasi kerjasama sebagaimana tersebut pada pasal 3 ayat 1 guna kelestarian fungsi dan manfaat hutan.
3. Bersama-sama PIHAK PERTAMA melakukan pemantauan dan penilaian terhadap keberhasilan tanaman gaharu dan tanaman hutan lainnya secara periodik.
4. Mengikuti aturan teknis dan kaidah konservasi yang berlaku di dalam pengelolaan kawasan Hutan Penelitian Carita dan menjaga kelestarian hutan.
5. Melaporkan setiap tindakan pelanggaran hukum yang terjadi kepada PIHAK PERTAMA.
6. Melaporkan setiap kejadian seperti serangan hama/penyakit tanaman, kebakaran, atau bencana alam yang mengakibatkan kerusakan sumberdaya hutan baik pada tanaman gaharu atau tanaman lainnya di areal kerjasama kepada PIHAK PERTAMA.

PIHAK KEDUA berhak:

1. Memperoleh informasi mengenai segala bentuk kegiatan dan kebijakan pengelolaan sumberdaya hutan di lokasi kerjasama dari PIHAK PERTAMA.
2. Mendapat pembinaan dan bimbingan teknis budidaya tanaman gaharu pada petak 21 di areal KHDTK Carita dari PIHAK PERTAMA minimal sebanyak 1 (satu) kali dalam setahun sejak tahun 2008 sampai dengan tahun 2011.

3. Mendapatkan jamur pembentuk gaharu untuk kegiatan inokulasi/penyuntikan tanaman gaharu pada petak 21 sebanyak 25% dari jumlah total tanaman gaharu masing-masing penggarap dari PIHAK PERTAMA.
4. Mendapatkan bantuan dari PIHAK PERTAMA untuk mencarikan investor untuk bekerjasama menyediakan produksi obat jamur untuk kegiatan inokulasi/penyuntikan tanaman gaharu untuk 75% tanaman gaharu lainnya.
5. Mendapatkan pelatihan teknis budidaya gaharu (paket training gaharu) dari PIHAK PERTAMA yang diadakan paling lambat pada tahun 2010.
6. Bersama-sama PIHAK PERTAMA melakukan kegiatan inokulasi/penyuntikan tanaman gaharu pada petak 21 sebanyak 25% dari jumlah total tanaman gaharu masing-masing penggarap setelah tanaman gaharu berumur ≥ 5 (lebih tua atau berumur lima tahun).

Pasal 5
SISTIM PENANAMAN DAN JENIS TANAMAN

Pengaturan penanaman pada lokasi kerjasama didasarkan pada kaidah-kaidah konservasi, antara lain:

- Sistem penanaman gaharu yang menyangkut pola dan kerapatan tanaman ditentukan dan disepakati oleh KEDUA BELAH PIHAK dan mengikuti kaidah-kaidah konservasi lahan.
- Jenis tanaman gaharu yang ditanam adalah Aquilaria spp. yang disisipkan pada tanaman yang telah ada, seperti meranti, kapur, cengkeh, melinjo, dan lain-lain.
- KEDUA BELAH PIHAK tidak diperkenankan untuk menambah atau mengurangi jenis yang ditanam kecuali yang telah disepakati KEDUA BELAH PIHAK.

Pasal 6
HAK PEMANFAATAN

1. Kawasan hutan yang menjadi obyek perjanjian kerjasama ini adalah kawasan hutan negara dan tidak dapat dibebani hak perorangan/badan dalam arti dimiliki dan diperjualbelikan.
2. PIHAK KEDUA tidak diperkenankan memindahtangankan lahan kerjasama kepada pihak lain. Dalam hal petani penggarap meninggal dunia atau mengundurkan diri, maka kewenangan pengelolaan lahan garapan secara otomatis akan kembali ke tangan PIHAK PERTAMA.
3. PIHAK PERTAMA DAN PIHAK KEDUA tidak diperkenankan menjadikan lahan kerjasama sebagaimana tersebut pada pasal 3 ayat 1 sebagai jaminan atau agunan dalam suatu transaksi dengan pihak manapun.

Pasal 7
BAGI HASIL

Dalam pelaksanaan kerjasama ini, para pihak telah menyepakati proporsi dan mekanisme berbagi output dari hasil tanaman gaharu dan hasil hutan lainnya, sebagai berikut:

1. PIHAK KEDUA berhak memanen dan memanfaatkan hasil tanaman bawah tahan naungan, tanaman buah-buahan dan/atau tanaman serbaguna yang berada di areal lahan garapan masing-masing.
2. PIHAK PERTAMA dan PIHAK KEDUA memperoleh hasil tanaman gaharu yang ditanam dan dipelihara di lokasi kerjasama dengan proporsi masing-masing 35 % untuk PIHAK PERTAMA dan 60% untuk PIHAK KEDUA
3. Selain PIHAK PERTAMA dan PIHAK KEDUA, sebagian hasil tanaman gaharu akan diberikan kepada Desa Sindang Laut sebesar 2,5% dan LMDH (kelompok) 2,5%.
4. Jika pada saat pemanenan tanaman gaharu ternyata ada tanaman yang mati/ hilang/tidak/belum menghasilkan, maka resiko akan ditanggung bersama sehingga perhitungan bagi hasil pada saat panen ditentukan dengan rumus sebagai berikut:

$$P_{akhir} = \frac{\sum \text{tan total} - \sum \text{tan mati} \times P_{awal}}{\sum \text{tan total}}$$

Ket: P_{akhir} : Proporsi bagi hasil tanaman gaharu yang diterima masing-masing pihak jika ada tanaman yang mati/hilang/tidak/belum menghasilkan

P_{awal} : Proporsi bagi hasil tanaman gaharu sesuai kesepakatan yang tertuang dalam perjanjian kerjasama ini

5. Pelaksanaan pemanenan hasil tanaman gaharu dilakukan secara bersama antara PIHAK PERTAMA dan PIHAK KEDUA dan diberikan dalam bentuk nilai nominal hasil penjualan setelah dikurangi biaya-biaya sarana produksi yang dikeluarkan sesuai hasil kesepakatan yang tertuang dalam perjanjian ini.

Pasal 8
JANGKA WAKTU PERJANJIAN

1. Untuk menjamin adanya kemanfaatan dan kepastian hukum para pihak, jangka waktu perjanjian kerjasama PHBM gaharu berlaku selama 5 (lima) tahun sejak ditandatangani perjanjian ini dan berakhir pada …….November 2013. Perjanjian kerjasama tersebut juga akan berlaku sepanjang petani penggarap menggarap lahan hutan di lokasi kerjasama yang ditunjukkan dengan adanya aktivitas budidaya tanaman di lokasi kerjasama, meliputi penanaman, pemeliharaan tanaman serta pemanfaatan hasil.
2. Perjanjian kerjasama pengelolaan hutan ini akan dievaluasi setiap 1 (satu) tahun.
3. Setelah masa kerjasama ini berakhir, perjanjian kerjasama dapat diperpanjang dengan mempertimbangkan kondisi dan aturan yang berlaku pada saat perpanjangan perjanjian kerjasama.
4. Jika setelah masa kerjasama ini berakhir tidak dilakukan perpanjangan, maka seluruh tanaman yang ada di lokasi kerjasama sebagaimana disebutkan pada pasal 3 ayat 1 harus dikembalikan kepada negara.

Pasal 9
SANGSI DAN PENGHARGAAN

1. Apabila PIHAK KEDUA tidak memenuhi kesepakatan sesuai pasal 6 ayat 2 dan ayat 3 maka hak garapnya akan dicabut.
2. Apabila PIHAK KEDUA tidak memenuhi kesepakatan sesuai pasal 7 ayat 2,3,4 dan 5 dan pasal 8 ayat 4 maka hak garapnya akan dicabut.
3. Jika PIHAK PERTAMA tidak dapat memenuhi kewajiban sesuai pasal 4 maka PIHAK PERTAMA tidak berhak mendapatkan hasil keuntungan sebagaimana ditetapkan pada pasal 7.
4. Apabila lahan garapan tidak dikelola dengan baik, maka PIHAK KEDUA akan mendapat sangsi berupa:
   - teguran/peringatan secara lisan
   - teguran/peringatan tertulis sebanyak-banyaknya 3 (tiga) kali
   - pemutusan perjanjian kerjasama secara sepihak

Pasal 10
KEADAAN MEMAKSA

Masing-masing pihak dibebaskan dari tanggung jawab, dan tidak akan saling menyalahkan atau menuntut, apabila terjadi penundaan atau terhalangnya pelaksanaan pekerjaan, baik sebagian maupun seluruhnya yang disebabkan oleh:

1. Peristiwa Force majeur seperti bencana alam, peperangan dan kerusakan yang tidak disengaja oleh KEDUA BELAH PIHAK.
2. Keadaan seperti pada ayat (1) pasal ini harus dapat dibuktikan sesuai dengan ketentuan yang berlaku dan dapat disetujui oleh kedua belah pihak dengan diketahui oleh aparat yang berwenang setempat.

Pasal 11
PERSELISIHAN

1. Setiap perselisihan yang timbul akan diselesaikan secara musyawarah dan mufakat.
2. Apabila tidak dicapai mufakat, maka akan diselesaikan melalui Pengadilan Negeri Kabupaten Pandeglang.

Pasal 12
LAIN-LAIN

1. Ketentuan perubahan perjanjian ini dapat diadakan melalui kesepakatan bersama dan dituangkan dalam Adendum Perjanjian.
2. Perjanjian kerjasama ini dilampiri dengan daftar nama petani penggarap Hutan Penelitian Carita pada petak 21 berikut luas garapannya dan peta sketsa seperti disebutkan dalam pasal 3 ayat 1 yang merupakan satu kesatuan dan tidak terpisahkan dengan surat perjanjian kerjasama ini.

3. Perjanjian kerjasama ini dibuat dalam rangkap lima, masing-masing bermaterai cukup dan mempunyai kekuatan hukum yang sama.

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Proceeding of Gaharu Workshop

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ISBN 978-979-3145-80-8