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RESPONSE OF *AQUILARIA MICROCARPA* TO TWO SPECIES OF *FUSARIUM* UNDER TWO DIFFERENT CULTIVATION SYSTEMS

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AKHSAN N, MARDJI D & SUTISNA M. 2015. Response of *Aquilaria microcarpa* to two species of *Fusarium* under two different cultivation systems. *Aquilaria microcarpa* was inoculated with two types of *Fusarium* sp. and planted under two different planting systems in Sebuhu Modern Village, Sebuhi District and Bukit Rava Village, Tenggarong Seberang District, Kutai Regency. The research was designed by 2 × 2 × 12 factorial using randomised blocks consisting of (1) cultivation system, namely, mixed and monoculture and (2) variant of *Fusarium* spp., namely, *Fusarium* isolated from East Kalimantan and *Fusarium* isolated from Bogor. *Fusarium* isolates from East Kalimantan were *F. oxysporum* and those from Bogor, *F. solani*. After one month of inoculation, 100% holes were infected with *F. oxysporum* and *F. solani*, indicated by the infection area around the hole, either in monoculture or mixed system. The monoculture system showed better result than the mixed system and *F. oxysporum* showed the best widespread symptoms of infection (31.43 cm^2), infected wood colour (brown = 1.53), wood aroma (1.50 = good smell/aromatic). The widest infection area was observed in the combination of monoculture system and *F. oxysporum*.

Keywords: Gaharu, aromatic, mixed, monoculture, wood colour, infection

INTRODUCTION

Gaharu is precipitated resin that accumulates in certain woody tissue as a result of tree reaction caused by pathogen infection induced physically or mechanically. Gaharu is defined as a kind of wood with specific shape and colour. It contains mastic. Gaharu generally occurs in the family Thymelaeaceae. Gaharu wood has high economical value because of its aromatic resin. It is used as perfume, cosmetic ingredients, for healing and spiritual purposes (Surata & Widnyana 2001).

Gaharu supply is dependent on nature. This results in indiscriminate wounding of trees in search for gaharu. Due to lack of supervision and public knowledge of the characteristics of trees containing gaharu as well as their dwindling number in the forest, gaharu-producing trees of *Gyrinops* and *Aquilaria* species have been grouped in Appendix II of CITES (Convention on International Trade in Endangered Species of Wild Fauna and Flora) clusters in 1994 as rare. Not all plants produce gaharu wood. Gaharu formation takes a long time (Sumadiwangsa & Harbagung 2000).

A healthy wood doesn't produce sesquiterpenoid as secondary metabolites known as aromatic gaharu wood (Novriyanti 2009). Gaharu wood is a phytoalexin compound, which becomes secondary metabolite of *Aquilaria* tree, as a defence mechanism to disturbance. Therefore, not all *Aquilaria* trees produce gaharu. However, its formation can be engineered. *Aquilaria microcarpa* is a tree on the Kalimantan island, particularly in East Kalimantan which produces gaharu wood.

Fusarium is one of the pathogens that can be isolated from *Aquilaria* trees that produce gaharu. More than one species of *Fusarium* have been isolated. *Fusarium* inoculation causes disease or disturbance to the *Aquilaria* tree. The tree reacts by producing secondary metabolites. Some produce secondary metabolites but some don't. Secondary metabolite in plants are influenced by season, genera, species, trees

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Fusarium is one of the pathogens that can be isolated from *Aquilaria* trees that produce gaharu. More than one species of *Fusarium* have been isolated. *Fusarium* inoculation causes disease or disturbance to the *Aquilaria* tree. The tree reacts by producing secondary metabolites. Some produce secondary metabolites but some don't. Secondary metabolite in plants are influenced by season, genera, species, trees

within species, part (trunk, branch and root) and external factors (biotic and abiotic) (Hills 1987, Anonymous 2007).

In order to sustain gaharu production and to reduce dependency on natural gaharu, proper cultivation system and infection by *Fusarium* are needed. Cultivation systems developed to increase the potential of *Aquilaria* tree to depend on soil conditions and regions were (1) monoculture system, i.e. system implemented in the form of monoculture plantations and (2) farming system, i.e. system implemented to create diversification in the form of intercrops, crop edges and intercropping with *Aquilaria* trees (Sumarna 2009). The objectives of this study were to determine the reaction of *Aquilaria* trees infected with two *Fusarium* spp. under two different cultivation systems.

MATERIALS AND METHODS

This study was conducted from April 2010 till April 2011. The study was conducted in Sebulu Modern Village, Sebulu District and Bukit Raya Village, Tenggarong Seberang District, Kutai Kartanegara regency. The experiment was designed according to factorial randomised block (2 × 2 with 12 replications). The treatments were: (1) cultivation system, i.e. monoculture and mixed as well as (2) types of fungus, i.e. *Fusarium* from East Kalimantan and *Fusarium* from Bogor.

The two *Fusarium* species were taken from gaharu and grown in potato dextrose agar (PDA) and carnation leaf agar (CLA) and identified. The PDA medium was used to see the colour of fungal colonies and aerial hyphae, while the CLA medium was used to see macroconidia, microconidia and clamidospora of *Fusarium*. The *Fusarium* spp. were identified using *Fusarium* identification guide books (Leslie & Summerell 2006, Salleh 2008). *Aquilaria* trees with trunk diameters 10 cm were selected. Borehole points were made 20 cm from the soil surface with grid having four horizontal holes (1 cm diameter/hole). The first and second rows of holes were inoculated with *Fusarium* from East Kalimantan, while the third and fourth rows were inoculated with *Fusarium* from Bogor. Each tree contained 40 holes—20 holes inoculated with *Fusarium* from East Kalimantan and 20, from Bogor. The experiments were conducted in two fields with different cultivation systems, namely, mixed system in Bukit Raya Village and monoculture system in Sebulu Modern Village. The parameters studied were infected holes in months after inoculation, widespread symptoms of infection at 1, 3, 6 and 12 months after inoculation, intensity of colour and aroma of wood at 3, 6 and 12 months after inoculation, number of holes healed and weathered at 6 and 12 months after inoculation. Widespread symptoms were determined by Y = PL(0.82) where Y = extensive symptoms, P = vertical length, L = horizontal length and 0.82 = correction factor. Wood colour intensity was determined using a scoring system, whereby 0 = white, 1 = white brown, 2 = brown and 3 = dark brown. The rate of change of the average colour of wood was expressed as means of three observations. The smell of wood was determined by a scoring system, whereby 0 = not fragrant, 1 = somewhat fragrant and 2 = fragrant. Wood was carved around the point of inoculation and organoleptic fragrance was determined from burned wood. Level was expressed as mean of three observations (Rahayu 2010). Data on the number of infected holes, number of holes, number of holes healed and weathered were calculated based on the percentage of infected units. Width, colour and aroma of infected wood were tested using F-test in analysis of variance, followed by least significant test at 5% if the results were significant (Gomez & Gomez 1995).

RESULTS AND DISCUSSION

Research sites

At Bukit Raya Village and Sebulu Modern Village, *Aquilaria* trees were planted under different cultivation systems. Table 1 shows that the mixed system implements intensive cultivation by planting vanilla as owners expect returns other than that from the cultivation of *Aquilaria*. Fertilisation and weeding around vanilla plants affected growth of *Aquilaria* and teak trees. The growth of *Aquilaria* tree in Bukit Raya Village showed higher tree increment than that of Sebulu Modern Village. The canopy of *Aquilaria* was relatively wide, reaching another tree and vanilla plant, causing microhumidity of 80–100%.

Table 1 Characteristics of research fields in Bukit Raya Village and Sebulu Modern Village

Characteristic	Village	
	Bukit Raya	Sebulu Modern
Cultivation system	Mixed	Monoculture
Spacing	3 m × 3 m	3 m × 3 m
History of land used	Secondary forest	Banana farm
Type of stand	*Aquilaria* tree Teak Vanilla	*Aquilaria* tree
Fertilisation	Twice a year	Once, 4 months after planting
Weeding	Every month	Once a year
Age stand	± 5 years	± 5 years
Tree increment	± 14.35 cm	± 10.21 cm
Humidity	80–100%	60–80%
Rainfall during the study	2912 mm year^{-1}	2426.3 mm year^{-1}

Identification of *Fusarium*

The morphologies of *Fusarium* from East Kalimantan and Bogor are shown in Figure 1 and Table 2. *Fusarium* from East Kalimantan was identified as *F. oxysporum* and that from Bogor was *F. solani* based on Leslie and Summerell (2006) and Salleh (2008).

Infected hole

Infection could be observed from visible symptoms on the stem under the bark. The surface colour changed from creamy white to light brown, then dark brown. Observation of the monoculture system for 1 month after inoculation indicated that all holes inoculated with *F. oxysporum* and *F. solani* had symptoms (100%). This meant that *Fusarium* inoculation on *Aquilaria* stems successfully infected the trees. Similar observation was seen in *Aquilaria* trees under mixed system (100% infection). Infections by *F. oxysporum* and *F. solani* are shown in Figure 2. Infection will occur if there is a match between pathogenic and host genes. Infection showed compatibility between the pathogen genes (Semangun 2001).

Widespread symptom

Widespread symptoms of infection after inoculation on all treatments are shown in Figure 3. Observations at 1 month and 3 months after inoculation showed increasing symptoms, then decline at 6 and 12 months in mixed system, *F. oxysporum* and its combinations. The extensive development of symptoms indicated that the tree was making effort to build defence over time. Resistance occurred in the form of inhibition of fungal colonisation in trees inoculated with *F. oxysporum* and tissue recovery in trees inoculated with *F. solani*. Resilience and vulnerability are not permanent but are strongly influenced by environmental conditions. However, pathogen possesses virulence ranging from relatively harmless to rapidly destroying tissues. Pathogen virulence, age and condition of the tree as well as environmental conditions influence host and pathogens (Semangun 2001).

As seen in Figure 3, extensive infection increased after 3 months and then decreased during the infection period in both cultivation systems with monoculture showing more extensive infection than mixed. Monoculture planting system which was not intensive showed the best widespread symptoms compared with mixed system. Plants require nutrients for growth. Soil naturally provides nutrients to plants but may not be available in optimum quantity. Without fertiliser or with little fertiliser, plants do not grow well. In addition, competition with weeds will make nutrients less available to plants. In monoculture system, cultivation of plants is not intensive, causing poor growth or unhealthy plants. Infected trees would show more severe symptoms than plants

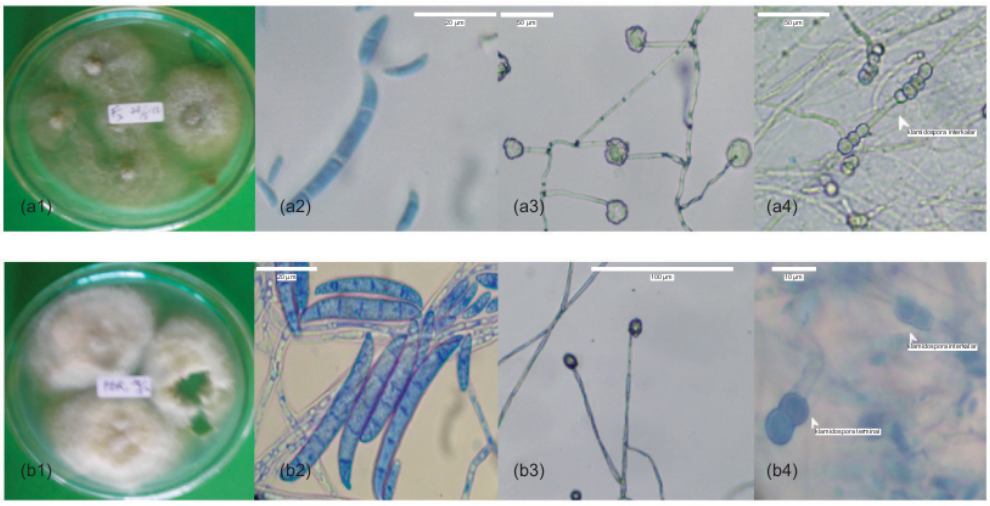

Figure 1 (a1) *Fusarium oxysporum* colonies—white purplish, (a2) macroconidia with three bulkheads and microconidia (400×), (a3) false head in-situ, short monopialid (100×), (a4) clamidospora intercalar (100×); (b1) *F. solani* colonies—white beige, (b2) macroconidia with five bulkheads and microconidia (400×), (b3) false head in-situ, long monopialid (100×), (b4) paired clamidospora (400×)

Table 2 Morphology of *Fusarium oxysporum* and *Fusarium solani* on PDA and CLA media

Morphology	*Fusarium oxysporum*	*Fusarium solani*
Colour of PDA colony	White purplish	White beige
Growth type of aerial hyphae in PDA medium	Evenly thin, insulated hyphae, hyphae firmly attached to the medium	Thick, somewhat wavy, insulated hyphae, aerial hyphae are not attached to the medium
Macroconidia in CLA medium	Much less, generally three bulkhead, there are also four bulkhead	Very much, generally five bulkhead, there are three to six bulkhead
Microconidia in CLA medium	Oval, elliptical, kidney, generally not insulated Short monopialid	Oval, ellipsoid, fusiform, many insulated Long monopialid
Clamidospora in CLA medium	Formed, terminal and intercalar	Formed, terminal and intercalar

PDA = Potato dextose agar, CLA = carnation leaf agar

with vigour. In mixed system, fertilisation and weeding are done intensively, so nutrients are available for maximum plant growth. Plants with vigour have endurance. Colonisation of pathogens in plant tissue inhibit plant growth. The states of the environment (nutrient, soil pH, temperature, humidity and light) often determine whether disease will develop or not (Agrios 1996). Infection by *Fusarium* is greater when there is low levels of nitrogen but high potassium. Pathogens tend to evolve more rapidly in calcium-deficient plants. This happens when the calcium ion doesn't move because phosphate and magnesium ion levels are high (Kranz et al. 1977).

Similar tendency was evident using *F. oxysporum* isolate but not in *F. solani*. It seemed that *F. oxysporum* had more effect on *Aquilaria* trees. This indicates that *F. oxysporum* has higher virulence than *F. solani*. Therefore, *Fusarium* colonisation in infected tissues quickly expanded.

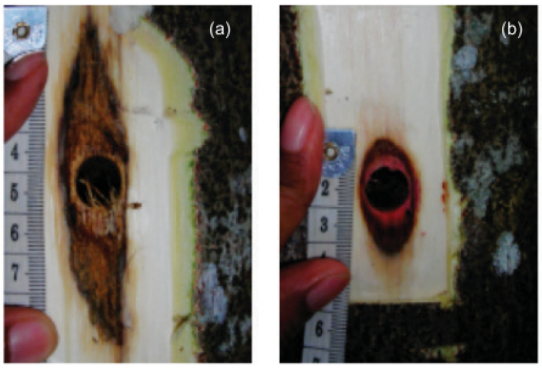

Figure 2 Brown symptoms around the hole after 1 month of being inoculated with (a) *Fusarium oxysporum* and (b) *Fusarium solani*

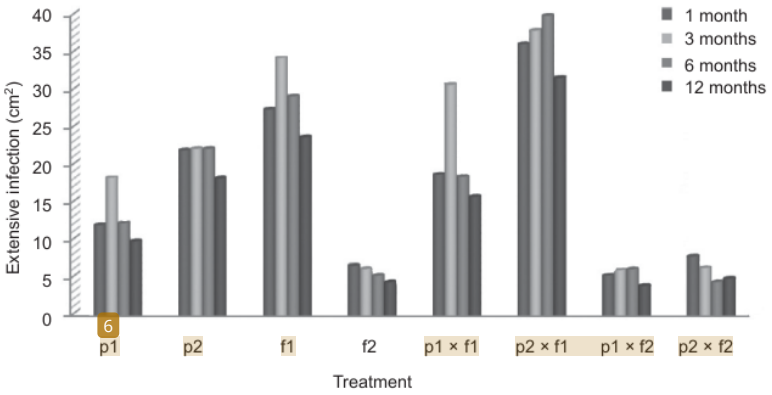

Figure 3 Extensive infection due to treatment of (f1) *Fusarium oxysporum* and (f2) *Fusarium solani* inoculations, and (p1) mixed and (p2) monoculture cultivation systems, and their combinations (p1 × f1), (p2 × f1), (p1 × f2), (p2 × f2) on *Aquilaria microcarpa* at 1, 3, 6 and 12 months after inoculation

The interaction between mixed system and *F. oxysporum* gave more extensive infection on *Aquilaria* trees. This is because the vigour of the plants is not too good and the high virulence of pathogens is able to worsen the infection. Disease occurs when there are susceptible plants, virulent pathogens and appropriate environment. Pathogens will react while host plants. Environments such as humidity and soil nutrients affect both host plants and pathogens (Semangun 2001).

Colour of symptomatic wood

The colour of symptomatic wood was pure white 1 month after inoculation with *F. oxysporum* and *F. solani*. At 3 and 6 months after inoculation, woody tissue changed to light brown. Colour of wood became browner at 12 months after inoculation, especially with *F. oxysporum* but not with *F. solani*, whereby the wood healed and the colour returned to white (Figure 4). At 1 month after inoculation, globule on wood

tissue began to break and secreted mastic which was similar to viscous fluid. Mastic viscous liquid is formed in cells that contain globule, namely, inside phloem, spokes and paratracheal parenchyma. Mastic viscous liquid gradually forms clumps that eventually will overwhelm the cell. When the liquid has coagulated in the cell, the cell will be brown in colour and when solidified, the cell will be black. Mastic will form a boundary line between rotten wood tissue and live tissue. It will block the growth and development of fungus in vertical direction (Mulyaningsih 2005).

Fragrance of symptomatic wood

The scent of gaharu on symptomatic wood were not detected/not fragrant or with score 0 at 1 month after inoculation. Fragrance was evident at 3 months after inoculation with score 1 or somewhat fragrant (Figure 5). At 6 and 12 months after inoculation, fragrance score increased. Figure 5 shows that cultivation system does not affect the rate of fragrance of gaharu wood. *Fusarium* inoculation, cultivation system and *Fusarium* interactions significantly increased the level of fragrance. The most fragrant treatment was *F. oxysporum* and its combinations with mixed and monoculture systems.

Recovered and rotten holes

At 3 months after inoculation, symptoms looked more spacious. At 6 months after inoculation, some holes became narrower and the symptom decreased and tended to heal (Figure 6). Table 3 shows that the mixed system has 9 inoculation holes (75%) in 9 trees to be healed. This was supported by intensive cultivation of *Aquilaria* trees so that whenever damage occurred, rapid healing (recovery) was observed primarily in trees inoculated with *F. solani*. The monoculture system had 3 holes to be healed (25%). At 12 months after inoculation, the mixed system had 9 holes (75%) recovered, which was more than monoculture system. The monoculture system had 7 holes (58%) to be recovered.

The healing or recovery of plants from disease is the plant's defence system. Cork or callus formation in front of the point of infection is a result of stimulation of host cells by substances secreted by pathogen known as tissue defence (histological defence structure) (Agrios 1996) and mechanical active defence (Semangun 2001). The callus inhibits pathogen spread and its secreted toxin.

As shown in Table 3, Figures 6 and 7, rotten holes were also observed at 6 and 12 months after inoculation. This rotten condition is supported by weather data (Figure 8). The location has high amount of rainfall during the study, reaching 2910.5 mm year^{-1} or 242.5 mm month^{-1} in multiple crop system and 2426.3 mm year^{-1} in 202.2 mm month^{-1} in monoculture crop system. Previously both locations had only 171.6 and 164.6 mm month^{-1} of rainfall respectively.

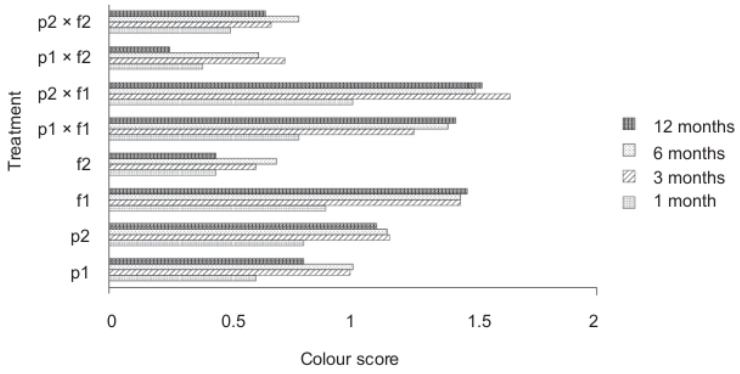

Figure 4 Wood colour caused by infection by (f1) *Fusarium oxysporum* and (f2) *Fusarium solani*, and (p1) mixed and (p2) monoculture cultivation systems, and their combinations (p1 × f1), (p2 × f1), (p1 × f2), (p2 × f2) on *Aquilaria microcarpa* at 3, 6 and 12 months after inoculation

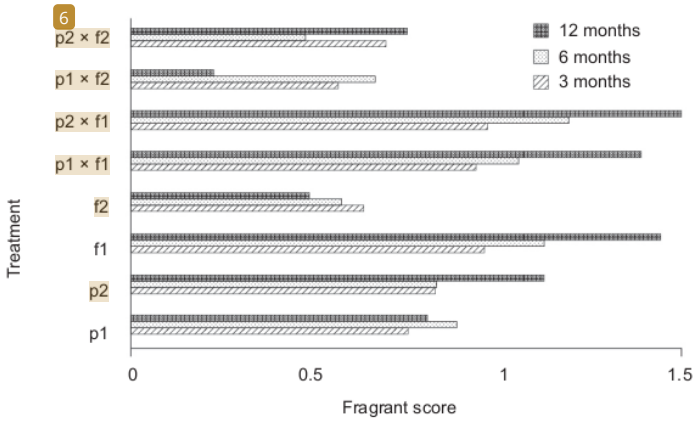

Figure 5 Fragrant score of infected by (f1) *Fusarium oxysporum* and (f2) *Fusarium solani*, and (p1) mixed and (p2) monoculture cultivation systems, and their combinations (p1 × f1), (p2 × f1), (p1 × f2), (p2 × f2) on *Aquilaria microcarpa* at 3, 6 and 12 months after inoculation

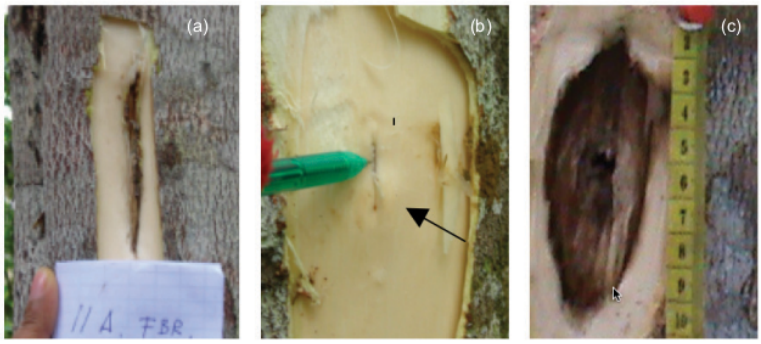

Figure 6 (a and b) Recovered and (c) unrecovered holes

Table 3 Symptom changing in the direction of healing and weathering due to cultivation system and inoculation with *Fusarium* at 6 and 12 months after inoculation

Planting system	Symptom changing	6 months after inoculation				12 months after inoculation			
		F. oxysporum		*F. solani*		*F. oxysforum*		*F. solani*	
		Σ tree	%	Σ tree	%	Σ tree	%	Σ tree	%
Mixed	Recovery			9	75			9	75
	Rotten					1	8		
Monoculture	Recovery			3	25			7	58
	Rotten	1	8	1	8	1	8	1	8

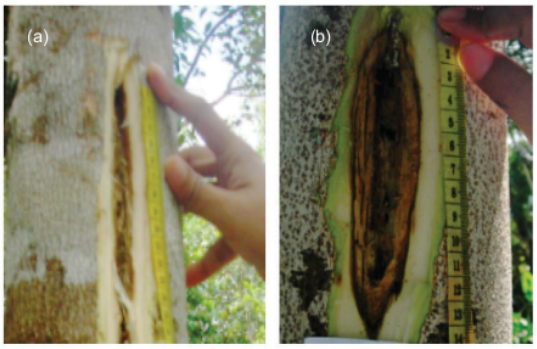

Figure 7 (a) Inoculation holes which experienced weathering and (b) infection symptoms

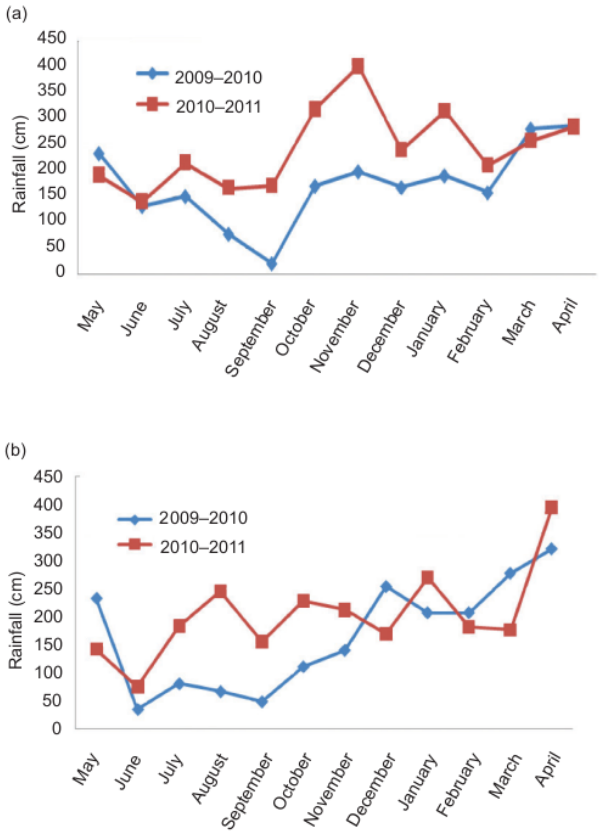

Figure 8 The average rainfall per month in (a) Tenggarong Seberang and in (b) Sebulu

The presence of vanilla plant and teak in mixed system among *Aquilaria* tree could cause micro air humidity to be higher than in the monoculture system, reaching 100%. High humidity can support the growth of rot fungi.

CONCLUSIONS

Aquilaria trees had more reaction with *Fusarium* inoculation especially with *F. oxysporum* in mixed cultivation system. The reaction could be widespread from 6 to 12 months after inoculation. This finding can be applied to produce gaharu in cultivation or farming system combined with other plants in order to create microclimate and humidity which can affect *Aquilaria* trees.

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