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TENTATIVE IDENTIFICATION OF FUNGAL ISOLATES ASSOSIATED WITH *AQUILLARIA* SPP. FROM REMAINING FOREST AREAS IN NUNUKAN REGENCY, NORTH KALIMANTAN

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Abstract: *Aquillaria spp. is one kind of tree that generates agarwood as a non timber forest product. Agarwood is formed due to the pathological process as respond to fungal infection. The research took samples from stem of Aquillaria spp. that formed agarwood naturally, at 4 districts in Nunukan regency: South Nunukan, Lumbis, Krayan Induk and South Krayan. The research aimed to identify the kinds of fungi that able to induce agarwood formation on stem of Aquillaria spp. The 9 fungal isolates were observed for their morphology on PDA and BLA growth media, and identified according to Leslie and Summerell (2006). The result indicated that based on their morphological characters, 9 isolates were identified as Fusarium solani, F. oxysporum, F. lateritium, F. comfactum, and Fusarium spp. Further identification will be done by molecular character using SSU, LSU and ITS primers.*
Keywords: Identification, Fungi, *Fusarium spp., Aquillaria spp.*

I. INTRODUCTION

Agarwood plants is one commodity timber forest products (NTFPs), which has a high economic value, even has many benefits such as: the manufacture of perfume, air freshener, incense, cosmetics and traditional medicine (medicine kidney pain, toothache, rheumatism, pain reduction, power adders and could bidder) (Sudrajat, 2003). Agarwood is also used in rituals and religious ceremonies and beliefs devotional objects such as beads and rosaries (Barden et al, 2000).

The high demand of the world market and the price will aloes aloes have a high enough public interest, both locally and newcomers to exploit massive aloes. As a result, Aquilaria spp. populations in natural forests has declined and even at one time became extinct. To prevent the extinction of the meeting of CITES (The Convention on International Trade in Endangered Species of Wild Flora and Fauna) IX in Florida , USA on 7-18 November 1994 , species of Aquilaria have been included in Appendix II of CITES (Mandang and wiyono, 2002). Such a situation is also occurring in the territory of North Borneo , especially in areas along the Indonesia-Malaysia border countries in the region including KMNP, which is an area of high conservation value . Traditional by gaharu collection of 11 local ethnic communities such as the Kenyah, Punan, Pua, Merap, Bau, Lun Daye, Brusu, Tagel, Abai, Agabag, Tidung, in Nunukan and Malinau, has been done decades ago. Gaharu harvesting on a large scale at the start when Gaharu-Man (search aloes) from Java and Lombok

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entered exploring the woods in North Borneo. They do logging blindly , without regard to whether the tree aloes found containing pig or not . Agarwood extraction activities in North Borneo peaked in 1993-1995 era (Wollenberg , 2003).

Awareness of the importance of saving gaharu-producing trees as one important source of livelihood of tribal people in North Borneo had emerged from some residents. Evidenced by the start of cultivation effort that started about 5-7 years ago. By the time they do the collection of natural aloes, they also take seeds scraped aloes that grow around the tree, then took him to the village to be planted later. In addition, at the time of clearing forests for gardens, they leave tree stands aloes grow naturally they believe not contain pig aloes.

Agarwood trees and require microbial compounds to induce the formation of agarwood. Agarwood tree is formed as a defense reaction against pathogen infection, wounding through the trunk, branches or twigs or other physical effects. Pathogen infection resulted in the release of resin deposited on the wood tissue. Over time the wood tissue will harden and change color to brown to blackish this section becomes heavy and smell fragrant wood found on the roots of plants or agarwood.

Based on the above it is necessary to study the identification of fungal isolates isolates associated with sp Aquillaria of the remaining forest areas in North Borneo Nunukan to restore the status of commodity scarcity or extinction into a flagship product.

II. MATERIALS AND METHODS

Place and Time. Tentative identification of isolates of the fungus research carried out at the Division of Agricultural Biotechnology Research and Development Center for Biotechnology LP2M Hasanuddin University, Makassar. This study was conducted from February 2013.

Equipment and Materials. The tools used are: laminar air flow, oven, microscope camera, petri dish, etc. autoclaf. While the materials used are: fungal isolates obtained from the stem Aquillaria who have demonstrated the formation of agarwood naturally in various districts in Nunukan (See Table 1). Media used for propagation and establishment of reproductive structures and morphology of the fungus is PDA (potato-dextrose-agar) and media BLA (Banana Leaf Agar).

Preparation Phase: The initial stage is to consider sterilization, both tools and materials to be used. Sterilization of media for planting and rejuvenation isolates performed using an autoclave with saturated water vapor pressure of 1 atm for 15 minutes at 121 º C. Sterilization of instruments such as needle inoculation is done by burning or by first soaked in 70% alcohol.

Purification isolates. All materials obtained from surface sterilized stem spp Aquillaria first, then grown on PDA, and incubated at room temperature. After a few days fungal isolates were grown, grown back on PDA to produce pure isolates.

Isolates rejuvenation. Isolates Rejuvenation aims to spur the formation of the reproductive structure / morphology of fungi. Media used to isolate rejuvenation is: PDA media. PDA that has been sterilized and poured into a petri dish, allowed to freeze, then isolate the fungus put in media that had been prepared by using a needle ose in Laminar air flow cabinet, cultures were then incubated under timed conditions.

Morphology observation fungus. The morphology of the fungus was observed to be carried through the first rejuvenation. Parent material for rejuvenation using purified isolates. Observation of morphology of fungi transactions are carried out with several stages, namely:

a. Measuring and observing the rate of growth of colonies of fungi and color. Isolates were grown on PDA medium in petri dishes, which were incubated at room temperature and placed in adequate lighting. Diameter of colonies formed was measured vertically, diagonally and horizontally every day for seven days. Color and presence or absence of aerial hyphae is determined on the seventh day. Each treatment will be repeated three times and each replicate contained three units of observation. The data obtained were analyzed statistically using a completely randomized design.
b. Observing the characteristic shape conidiofor. Conidiofor characteristic shape can be observed by slide culture, which is made by taking a small number of fungal colonies on PDA, then placed on an object glass and covered with a cover glass. The next slide is placed on a petri dish culture sterile humidity is maintained, by putting a filter paper

that had been moistened with sterile distilled water. After incubated for 24-72 hours at room temperature, the culture slides were observed under a microscope.

c. Observing the shape makrokonidia and mikrokonidia observed by isolates grown on media BLA. In this natural medium sporodokia structure, the establishment makrokonidia also be observed. BLA made conformed to Nelson et al method (1983), by putting pieces of banana leaf size ± 1cm2 sterilized at a temperature of 121 º C on Petri dishes containing PDA medium. Colony isolates of the fungus was placed among the pieces of banana leaves. Then the cultures were incubated for 10-14 days at room temperature with 100-130 lux illumination. Colonies of fungus that grows on the surface of banana leaf was observed under a microscope.

Phase identification. Identification of isolates by Nelson et al identification key (1983), John F. Leslie and Brett A. Summerell (2006). Determination of colony color refers to the A Mycological Colour Chart (Rayner, 1970).

Table 1. Observed Isolates list

No	Code	Location of Origin
1	NKS	South Nunukan
2	LM	Lumbis
3	KR	KrayanInduk
4	KRS	South Krayan

III. RESULTS AND DISCUSSION

Morphological characters aerial mycelium, colony color, and diameter of colonies of Fusarium spp isolates from four districts in Nunukan highly variable (see Table 2). Morphological characters of *Fusarium* spp differences due to the differences in the origin of the isolates.

Table2.Growth of fungus colonies

No	**Code Isolate**	**Origin Locations**	**Morphological characters**		
In thecolonies			**The averagediameter ofcolonies(cm/7 Days)**	**AerialMycelium**	**MediumColorPDA**
1	NKS	South Nunukan	4,75	There are +++	White, Yellow
2	LM	Lumbis	4,40	There are +++	White, Violet
3	KR 1	KrayanInduk 1	1,54	There are +	White, Brown
4	KR 2	KrayanInduk 2	4,04	There are +++	Yellow
5	KRS 1	South Krayan 1	4,74	There are +++	White, Brown
6	KRS 2	South Krayan 2	4,50	There are +++	White, Crem
7	KRS 3	South Krayan 3	4,49	There are+++	White, Violet
8	KRS 4	South Krayan 4	4,58	There are +++	White
9	KRS 5	South Krayan 5	4,46	There are +++	White, orange

Information the relative abundance of aerial mycelium: + Bit, ++ Quite a lot, +++ Many

The morphology of isolates of Fusarium spp character of the District Four. Diameter isolates morphological characters, and color aerial mycelium of Fusarium spp colonies derived from four diverse districts Nunukan can be seen in Table 2, this is due to differences in the origin of the isolates. Fusarium spp colony diameter ranged from 1 to 4.75 cm. Between 4.50 to 4.75 cm diameter isolates (isolates KRS 2, 4 KRS, KRS 1, and NKS), isolates with a diameter between 4.00 to 4.49 cm (2 isolates KR, LM, KRS 3, and KRS 5), and 1.54 cm yang diameternya isolates (isolate KR 1).

Table 3. Character *Fusarium* spp character from four districts

No	Code	Character Histology			
		Macroconidia	Microconidia		
		Number septa	Conidiofor	Abundance	Shape
1	NKS	4-7	Simpel	Many	Elips,oval
2	LM	3-5	Simpel	Many	Elips,sekat
3	KR 1	-	Simpel	Many	Elips,oval
4	KR 2	-	Simpel	Many	Elips,oval
5	KRS 1	4-7	Simpel	Many	Elips
6	KRS 2	5	Simpel	Many	Elips
7	KRS 3	4-7	Simpel	Many	Elips,oval
8	KRS 4	4-7	Simpel	Many	Elips,oval
9	KRS 5	4-7	Simpel	Many	Elips,oval

The diversity of colony diameter of isolates are closely related to the variable speed of growing hyphae. In some isolates of *Fusarium* spp speed of growth is a distinctive character in each isolate. High growth rates can be associated also with the ability virulence isolates, so as to determine the ability of virulence isolates need to be tested on its host.

Character aerial mycelium present in any isolates of *Fusarium* spp, though there are differences between isolates when viewed from the relative abundance of aerial mycelium. Isolates NKS, LM, KR 2, KRS 1, KRS 2, 3 KRS, KRS 4, and KRS 5, while isolates KR 1 shows the abundance aerial mycelium slightly (Table 2).

Fungi grown on light conditions continuously to form a relatively large aerial mycelium, aerial mycelium is formed with a relatively large abundance fototropi mechanism to the presence of light (Irawati, 2004). However, in this study all isolates tested are treated the same light, so the presence of an abundance of aerial meselium due to the character of each isolate.

Colors colony isolates showed a different character. Isolates NKS white yellow, purple white LM isolates, isolates brown KR 1, KR 2 isolates yellow, white chocolate KRS1 isolates, KRS 2 isolates creamy white KRS, KRS 3 isolates white purple, white 4 isolates KRS , and isolates KRS 5 white orange (Figure 1). The diversity found on colony color associated with pigment contained by the cell wall of hyphae. Pigmented fungi are not generally colorless hyaline.

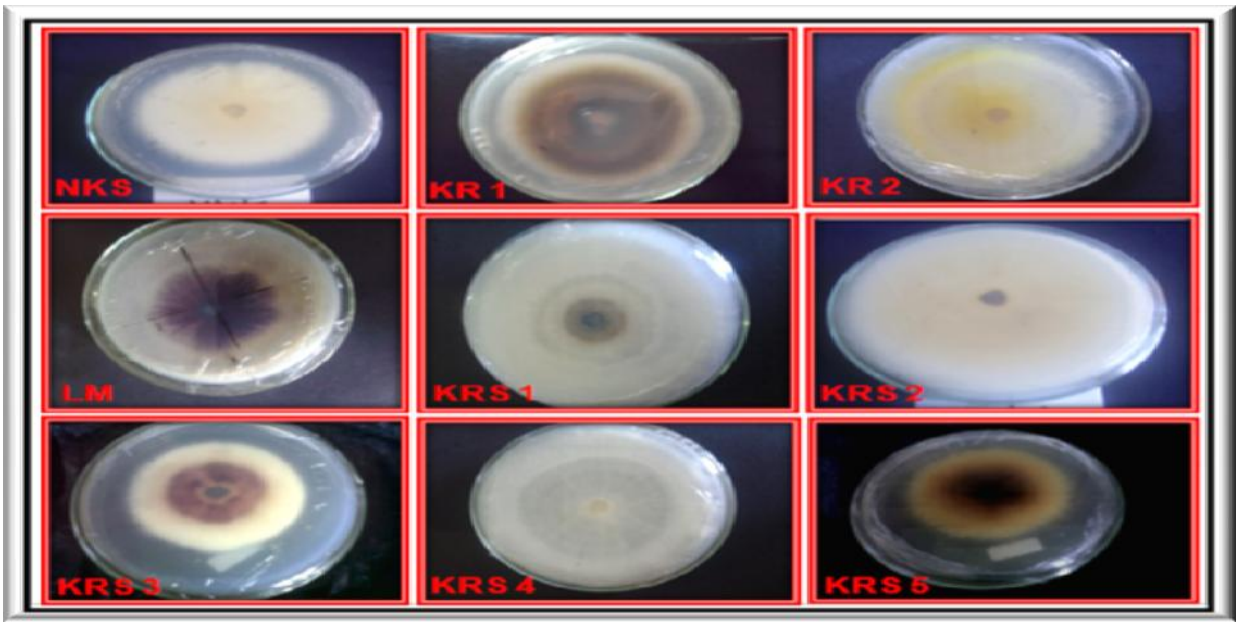

Figure1.Media fungus isolates on PDA (PotataDextrosaAgar)

Macroconydia diversity, and Sporodocya Microconydia *Fusarium* spp isolates. Observations on makrokonidia, mikrokonidia and sporodokia of test isolates showed character and shape macroconydia number of septa, branching conidiophores, shape and abundance microconydia, it can be seen in full by using multiple methods for identification of *Fusarium* spp but true, that is the makrokonidia formed from sporodokia, obtained in cultured fungus on media BLA (Banana Leaf order) with the lighting of 100-130 lux. The growth of the fungus sporulation can be affected by the presence of light. Hawker (1971) reported that some species cultured in a petri dish shows macroconydia production zone in response to light. Light can induce the length and number of septa macroconydia on several species of *Fusarium* spp. Seifert (1996) states that macroconydia be observed from colonies grown on SNA medium (Synthetic Nutrient Agar), CLA (Carnation Leaf Agar), or BLA (Banana Leaf Agar).

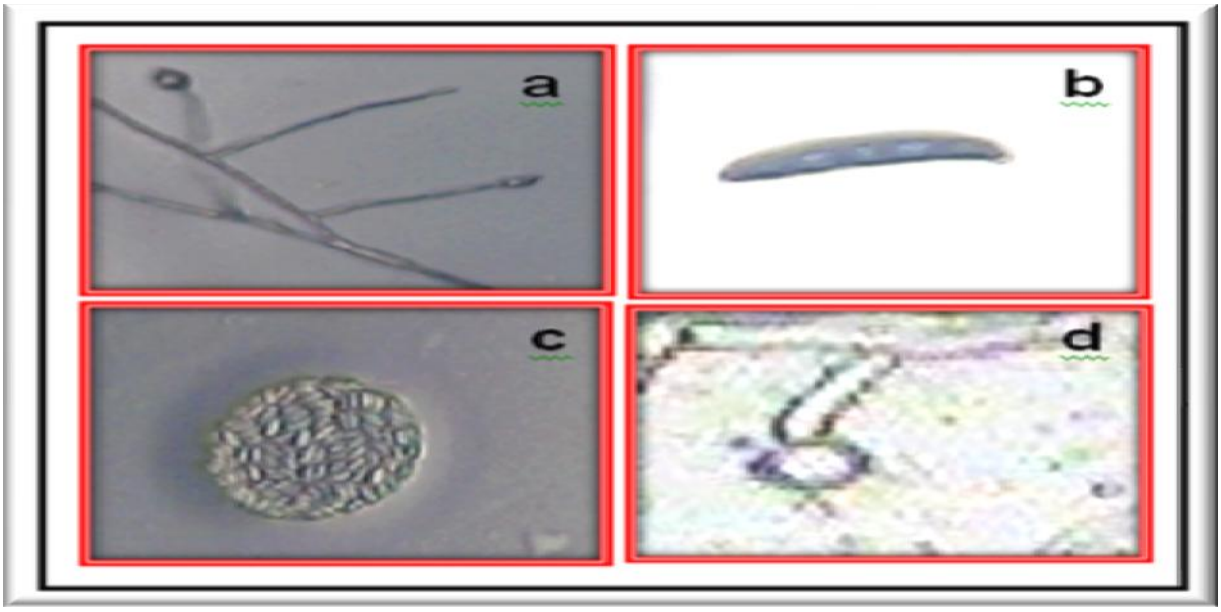

Figure 3. Histology of ***Fusarium solani* (Mart) Appel & Wollenweber emend, Snyder & Hansen)** a. Sporodocya, b. Macroconidia, c. Microconidia, and d. Clamydospora

The number of septa macroconidia 4-7 isolates possessed by NKS, KRS 1, 3 KRS, KRS KRS 4 and 5. The number of septa 3-5 owned by LM and KRS 2 isolates, whereas isolates KR 1 and KR 2 there have been no suspected macroconidia, macroconidia formation process is hampered due to lack prevalence lighting during incubation under the lights. Conidiophores all isolates have simple shapes, elliptical and oval forms microconidianya.

Microconidia on various *Fusarium* spp crescent-shaped (fusoid) so easily distinguished from other genera that have similar characteristics Fusarium. Isolat NKS, KRS 1, 3 KRS, KRS KRS 4 and 5 are isolates that have a relatively large number of septa ranged 4-7 (Table 3). One of the five isolates that KRS 1 isolates are similar in shape but different in form conidiophores with microconidia and macroconidianya with the four isolates. KRS 1 isolates have microconidia elliptical shape, while the isolates NKS, KRS 3, 4 and KRS KRS 5 elliptical oval shape. KRS 1 isolates macroconidianya long thin sporodocya longer, and slightly curved, slightly twisted apikalnya cells as a hook (hook/beak), notched basal cells (Figure 6). Isolates NKS, KRS 3, KRS 4 and KRS 5 Macroconidia apikal cell shape and slightly rounded blunt, always abundant in sporodocya the long form. Basal cells are usually notched or rounded edges (Figure 3).

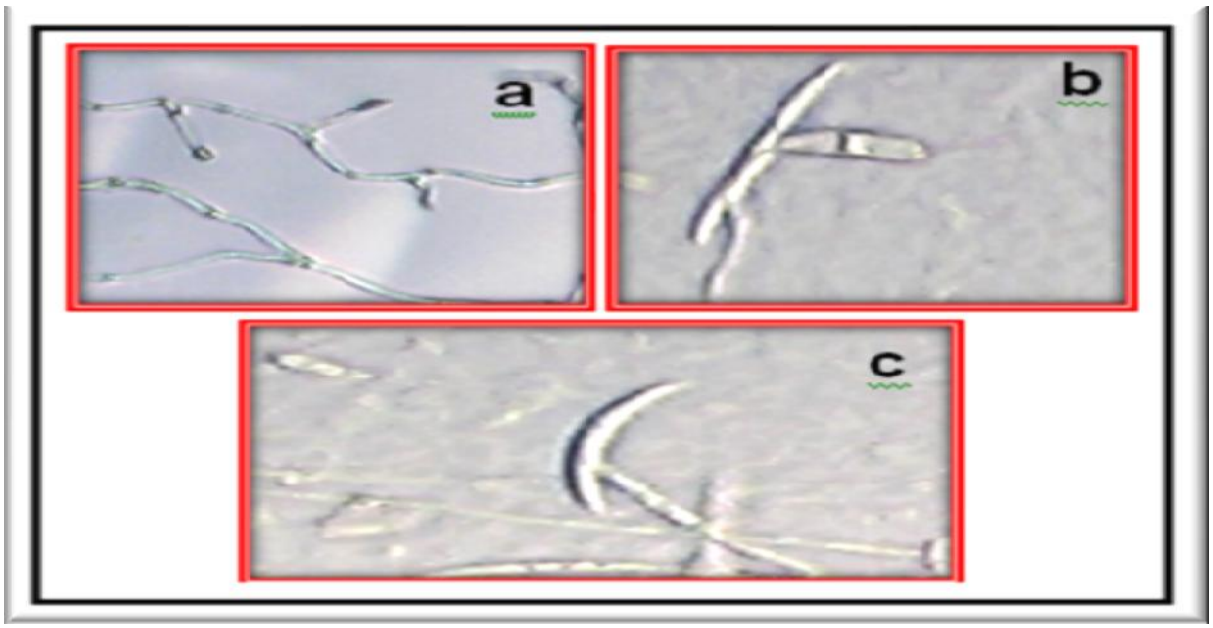

Figure 4. Histology of ***Fusarium oxysporum*** **Schlechtendahl emend, Snyder & Hansen**

a. Sporodochia, b. Microconidia, dan c. Macroconidia

KRS 2 isolates have sporodocya rather short. Microconidianya no. Macroconidia strong and thick-walled, dorsi-ventralnya curved so that the midpoint of macroconidium wide enough. Apikalnya cells elongated and tapering almost taper so it looks like a needle, making distinctive look basal cells (Figure 7).

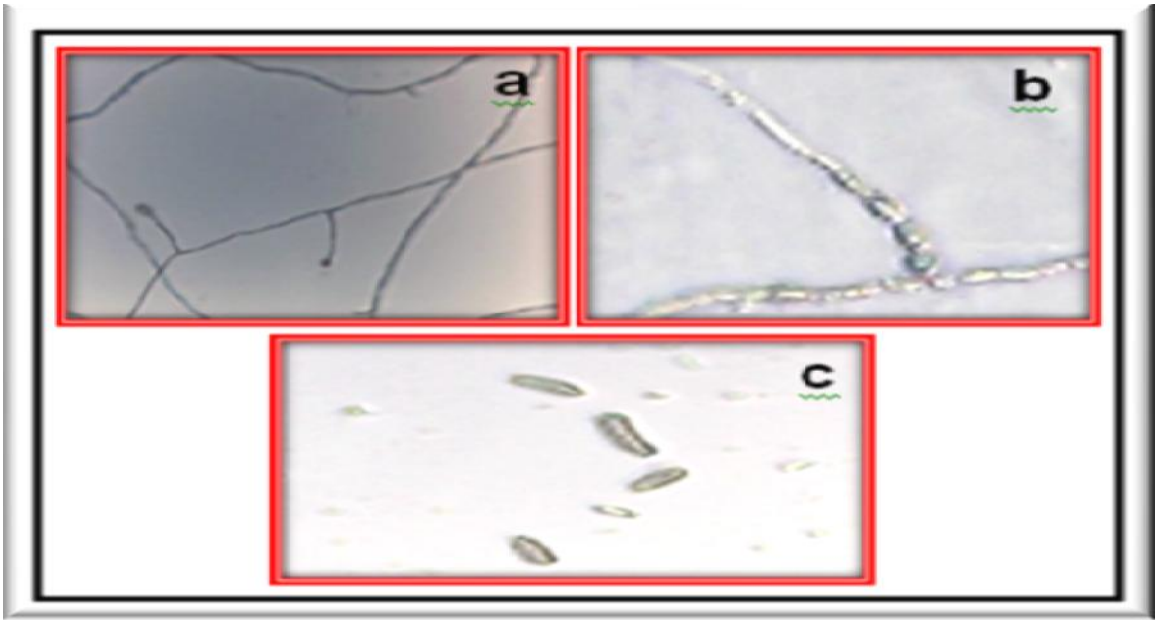

Figure 5. Histology of ***Fusarium*** **sp, a.** Sporodokya, b. Clamydospora, dan c. Microconidia

Researches on the isolation and identification of fungi that can infect and induce agarwood have been carried out in various locations in Indonesia. The results of the study stated that the group is a type of fungus Fusarium is often found on the trunk of *Aquilaria* spp. The results of the study

tentative identification of fungal isolates from this study are not much different. Based on the characteristics found stated that the nine isolates studied include the type of *F.solani* (Mart) Appel & Wollenweber Emend, Snyder & Hansen gained from agarwood South Nunukan, South Krayan 3, South Krayan 4, and South Krayan 5.

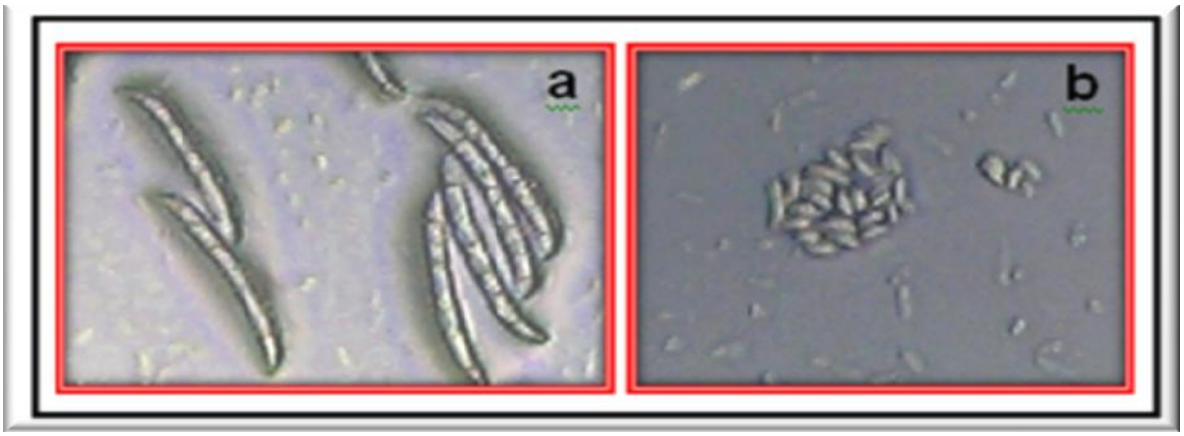

Figure 6. Histology of ***Fusarium lateritium*** **Ness,** a. Macroconidia dan b. Microconidia

Isolates LM has sporodocya shorter, so in some isolates sporodokia sometimes rarely even there. microconidianya much abundance and elliptical, oval. Macroconidianya generally short with medium length, straight and slightly curved, relatively slender thin walled and septa 3-5 with a pointed apikalnya cell shape and curved with a small angle, tapered-shaped basal cells (Figure 4).

F. lateritium Ness obtained from South Krayan 1, further *F. oxysporum* Schlechtendahl Emend, Snyder & Hansen obtained from Lumbis. *F. compactum* (Wollenweber) Gordon found in South Krayan 2. For isolates from districts Krayan Induk 1 and Krayan Induk 2 have not been identified, because of some of the methods used have not found a good macroconidia derived from aerial mycelia. Of PDA and discover new BLA microconidia and sporodocya.

Fusarium spp fungus is one that has a very broad distribution with a diverse kind. Backhouse et al. (2001) stated that there Fusarium abundance in every part of the world except the extreme, so that one strain with another strain is relatively difficult to distinguish. There are four species of Aquilaria spp were identified, namely *F. solani*, *F. lateritium*, *F. oxysporum* and *F. compactum*. Among the four species of *F. solani* were most commonly found. *F. solani* has microkonidia generally elliptical in shape, then F.*solani* can be distinguished by F. lateritium based characters leaner macroconidia to *F. lateritium*. While *F. oxysporum* has sporodocya shorter than sporodocya *F. solani*. *F. compactum* has macroconidia the ventral or dorsal part slightly curved than the middle of the macroconidium.

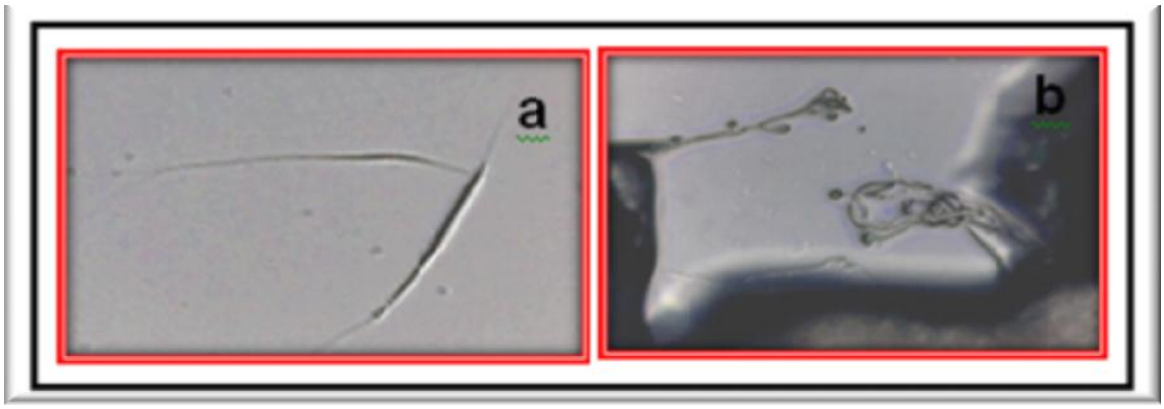

Figure 7. Histology of ***Fusarium compactum*** **(Wollenweber) Gordon, a.** Macroconidia dan b. Sporodochia

According Agustini et al (2006) most species of Fusarium is a fungus that is cosmopolitan, so as to distinguish Fusarium species is complex, because of the variation found in a species very large. Fusarium host plants that could be helpful in the identification of pathogenic Fusarium especially for, but for the saprophyte or weak pathogen require thorough observation. Type *F. solani* can be said to be an important pathogen in plants one on *Aquilaria* spp . This strain is found in many places because it is cosmopolitan. Booth (1971), Nelson et al. (1983) reported *F. solani, F. oxysporum*, and *F. lateritium* is a cosmopolitan species, though some of the species of this group which is a saprophyte. Several studies that have been done previously reported the presence of *F. solani* on agarwood -producing plants. Research Sidiyasa and suharti (1987) states that different kinds of fungi such as *Diplodia* sp., *Pythium* sp and F. *solani* was instrumental in the formation of agarwood resin. Umboh et al (2000) using the fungus *F. oxyforum , F solani , Scyttallidium* sp, *Libertella* sp, and *Trichoderma* sp . To spur formation in *Aquilaria malaccensis* sapwood and *A. crassna* pierre ex LaConte .

Then *F. solani* also has morphological characteristics that are quite varied, from some of the author/authors interpret *F. solani* with slightly different characteristics, so sometimes equally F. solani but there are differences in morphologic properties. While the differences between strains of Fusarium allegedly found an association with location, time of sampling, as well as the ecological condition of each location. This relates to a very broad habitat Fusarium.

IV. CONCLUSION

Based on a tentative identification of fungi isolated from four districts in Nunukan found four species of Fusarium that is *F. solani* (Mart) Appel & Wollenw, *F.lateritium* Ness, *F.oxysporum* Schlechtendahl Emend, Snyder & Hansen, *F.compactum* (Wollenweber) Gordon.

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