The Republic of the Union of Myanmar
Ministry of Environmental Conservation and Forestry
Forest Department

Examine on presence fungi from some naturally infected agar wood of some parts of Myanmar

မြန်မာနိုင်ငံဒေသအချို့မှ သဘာဝသစ်မွေးပင်အချို့တွင်ဝင်ရောက်စွဲကပ်နေသော မှိုများကိုလေ့လာစာရင်းပြုစုခြင်း

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2013,August

မြန်မာနိုင်ငံဒေသအချို့မှ သဘာဝသစ်မွေးပင်အချို့တွင်ဝင်ရောက်စွဲကပ်နေသော မှိုများကိုလေ့လာစာရင်းပြုစုခြင်း

ဝေဝေသန်း
သုတေသနအရာရှိ
သစ်တောကာကွယ်ရေးဌာနစု
သစ်တောသုတေသနဌာန

စာတမ်းအကျဉ်း

သစ်မွေးပင်ထဲသို့ သဘာဝအရ (သို့မဟုတ်) ဖန်တီးယူထားသော မှိုဝင်ရောက်စွဲကပ်ရာမှ အပင်အတွင်း ဇီဝနှင့်ဓါတုဖြစ်စဉ်ပြောင်းလဲတုံ့ပြန်မှုများ ဖြစ်ပေါ်လာပြီး သစ်သားအတွင်း သစ်မွေးဆီဖြစ်တည်မှု ရှိလာသည်ဟု ဆိုကြပါသည်။ သဘာဝသစ်မွေးပင်များ လျော့ပါးလာခြင်းကြောင့် ယခုအခါမြန်မာနိုင်ငံ အပအဝင်ဒေသတွင်း၌ သစ်မွေးစိုက်ခင်းများ တည်ထောင်လာကြပါသည်။ မြန်မာကုမ္ပဏီအချို့က သစ်မွေးစိုက်ပင်များသို့ မှိုဝင်ရောက်မှုနှင့် သစ်မွေးဆီဖြစ်တည်မှုကို အားပေးသည့် လှုံ့ဆော်ပစ္စည်း များကို နိုင်ငံရပ်ခြားမှ တင်သွင်းပြီး စမ်းသပ်အသုံးပြုလျက်ရှိပါသည်။ သဘာဝသစ်မွေးပင်မှ အသားစ နမူနာများကို တနင်္သာရီတိုင်းဒေသကြီး မြိတ်မြို့ ဘိုထောင်ကြိုးဝိုင်း အကွက်အမှတ်(၁၇)နှင့် ကချင်ပြည်နယ် မြစ်ကြီးနားမြို့ ကိုယ်ပိုင်ဥယျာဉ်အချို့မှ စုဆောင်း၍ ဓါတ်ခွဲခန်း၌ အဟာရပြင်ပေါ်တွင် မွေးမြူလေ့လာပါသည်။ မှိုမျိုးခွဲခြားခြင်းကို အဏုကြည့် မှန်ပြောင်းဖြင့် လေ့လာကာ မိတ်ဆက်မှိုပညာ (Alexopoulos & Mims. 1979. USA) နှင့် ပုံပါ အထွေထွေမှိုစာအုပ် (Barnett & Hunter. 1972, USA) တို့ကို ကိုးကားခဲ့ပါသည်။ မှိုမျိုးစု ၅ခု (*Alternaria*, *Aspergillus, Culvularia, Fusarium, Leptosphaeria*) ကို မြိတ်မှလည်းကောင်း၊ မျိုးစု(၃)ခု (*Aspergillus, Fusarium, Nectria*) တို့ကို မြစ်ကြီးနားနှင့် တန့်ယန်း သစ်မွေးနမူနာများမှလည်းကောင်း၊ လေ့လာတွေ့ရှိရပါသည်။ ယခုတွေ့ရှိချက် သည် လှုံ့ဆော်ပစ္စည်း ဖန်တီးယူမှုနှင့် သစ်မွေးစိုက်ခင်း အပင်များထဲသို့ လှုံ့ဆော်ပစ္စည်း ထိုးသွင်းခြင်း၌ ထည့်သွင်းစဉ်းစားစရာ အချက်တစ်ခု ဖြစ်လာနိုင်ပါသည်။ နိုင်ငံတကာ ကျွမ်းကျင်ပညာရှင်များဖြင့် ပူးပေါင်းဆောင်ရွက်မည်ဆိုပါက နည်းပညာ၊ စီးပွားဖြစ် ထုတ်လုပ်နိုင်မှု၊ ဈေးကွက် ဖွံ့ဖြိုးမှုတို့နှင့်အတူ မျိုးဆက်သစ် ပညာရှင်များ ကိုလည်း မွေးထုတ်နိုင် ပါမည်။

Examination of Fungi Species found in naturally infected Agar Wood from Some Localities of Myanmar

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Abstract

In these decades, agar wood plantations are initiated in the Asia as well as in Myanmar. Because of over exploitation of natural resource that makes the stock of agar tree becoming reduced. Oleoresin of the wood is valued for medicine and incense. When agar tree is damaged either naturally or artificially, fungus enters the tree. The fungal infection progresses, which results the tree produces aromatic resin in the heartwood as a response to the attack. In the natural forest, only about 7% of the trees was infected by the fungus. The paper listing fungi from naturally infected agar wood would support to make stimulate agents for inoculation. Samples collections were carried out in the Bodaung Reserve Forest, Compartment 17, Myaik, wood samples from Boakpyin, Tanninthayi Region; and in the private garden of Myitkyina, Kachin State and wood sample from Tant Yunn, Shan State. Fungi from sample woods were isolated in the Forest Pathology laboratory, FRI and identified by Introductory Mycology (Alexopoulos & Mims. 1979. USA) and Illustrated Genera of Imperfect Fungi (Barnett & Hunter. 1972, USA). The symptoms was found that infected pieces of agar wood were grey and dark in color, smell and oily; at the initial stage infection appears as brown streaks in the agar wood tissue. Five fungi genus (*Alternaria*, *Aspergillus, Culvularia, Fusarium, Leptosphaeria,*) from Myeik and Boakpyinn; three fungi genus (*Aspergillus, Fusarium, Nectria*) from Myitkyina and Tant Yunn were observed from the agar wood samples. The findings are considerable and providing the making of stimulate agent for inoculation to agar wood oleoresin production. International cooperation is necessary for the systematic technologies, commercial production, and marketing with expertise to nurture young generation of future.

Key Words: fungi, identification, inoculation, infection, oleoresin, stimulate agents, symptoms

Contents

	Page
စာတမ်းအကျဉ်း	i
Abstract	ii
Introduction	1
Literature Review	1
Objectives	2
Materials and Methods	3
Results	3
Discussions and Conclusions	5
Acknowledgement	6
References	7
Table 1	4
Table 2	5
Appendix 1	8
Appendix 2	11

1. Introduction

Aquilaria tree is an evergreen tree belongs to the family Thymelaeaceae (Kress *et.al,* 2003). It bears white flowers that are sweet scent. FRI, Yezin (2009) reported that 12 species were found in Asia and, according to The Rainforest Project Foundation, 2002, fifteen species of *Aquilaria* trees are known**.** *Aquilaria malaccensis* Lamk. DC.Prod. syn. *A. agallocha* Roxb. is growing naturally in Myanmar listed in the literatures. Agar tree does not thrive at all in water-logged condition, paddy field and swamp but any soil are favorable to agar tree. The tree needs sunshine except the seedlings and young trees, they prefer the shade (Pojanagaroon S, Kaewrak , 2010).

When the fungus damages agar tree either naturally or artificially, the fungal infection progresses, the tree produces a dark aromatic resin in response to the attack, which result resin embedded in heartwood. In the natural forest, only about 7% of the trees are infected by the fungus (Li, Tana,1998).

Over exploitation of natural resource of agar wood make the stock becoming reduced. The oleoresin of the wood is value for medicine and incense. Therefore, in these decades, it has been initiated to grow agar wood producing plants in Asia as well as in Myanmar.

Literature Review

Distribution and habit

The genus Aquilaria is naturally growing in South East Asia. It is a large to medium evergreen tree 15-20 m high sometimes grows up to 40 m in height. Agar plant prefers warm and high humid sub-tropical climate with annual rainfall ranging from 1800-3500 mm **(**70.87 in-137.80 inches). It prefers well drain soils, shallow soils rocky beds on hill slopes and forest environment (TREE CD 1973-99). However, the tree grows in natural forests at an altitude of a few meters to about 1000 meters (3280.84 ft.), and it grows best around 500 meters (1640.42 ft.) (TRP agar wood project, 2002).

The tree can be found naturally in Tanninthayi Region, Kachin State, and Upper Chindwin in Myanmar at Altitude 300 ft-3000 ft, Rain fall 1200 mm- 2000 mm (47.25- 78.74 in.) ranging, and average temperature 18- 32 □C (M M Than & C Doo, 1977).

Symptoms of infected agar wood

While the uninfected wood of the tree is light in color but at the initial stage, infection appears as brown streaks in the tissue and the resin dramatically increases the mass and density of the affected wood. Accumulation of oleoresin goes with the increase of infection area, more oleoresin deposits in the wood and finally changing its color from pale to dark brown or black. Heavy and old age infection may lead to death of the plant (2010 FreePatentsOnline.com); (Pure-Agarwood.com, 2005).

Fungi infection

When the agar wood tree is about 7-10 years old, the fungi attack through holes or injuries trunk of the tree There are different species of fungi found in infected agar wood - Class Ascomycetes, Deuteromycetes, Genera *Aspergillus*, *Diplodia*; Species *Botryodiplodia theobromae; Cytospherae magniferae; Epicoccum granulatum*; and *Phomopsis aquillariae;* (TREECD 1973-99/07).

The literature has mentioned that fungi growing in the wounded Aquilaria tree may cause agar wood formation. Different types of fungi were described including *Phialophora parasitica, Torula* sp., *Aspergillus* sp., *Penicillium* sp., *Fusarium* sp., *Cladosporium* sp., *Epicoccum granulatum, Cylimndrocladium, Sphaeropsis* sp., *Botryodiplodia theobromae, Trichoderma* sp., *Phomopsis* sp., and *Cunninghamella echinulata.* With all of these fungi suggested as a possible cause but definitive research to show was necessary that a fungus is responsible for agarwood to form. (Blanchette, Robert A. 2005).

About 3-6 years needed for the production of resin wood from the tree that undergoes primary infection. A common method in artificial is to inoculate all the trees with the fungus. Infection may also occur due to mechanical or natural injuries on the stem or branches. Once the production of aromatic trunk on agar wood is completion, the tree slowly starts drying up, signaling its readiness to be harvesting. The resinous or infected part of the tree will be heavier than the other parts of the tree (Blanchette, Robert A. 2005).

Fungi, the known stimulators in formation of agar wood were used in the cultivation of agar wood. *Fusarium laseritum* is the faster fungus infection of Aquilaria sp. tree, and can be isolated and inoculated easily into nutrient medium. Thus, this fungus is used by inoculate into the holes on the trunk. One year after inoculation obtained agar wood with the lowest grade of resin (Indian Agar wood, 2010).

Objectives

Myanmar have known about the agar tree, wood and resin since 1977 and some forester studied that was cause of infection by fungi and produced oleoresin and valuable in the world market. Researches of the species; and technologies were more advanced in the other Asian countries such as China, India, Malaysia, Thai, and Vietnam. Identification of the fungi from infected agar wood in forestry of Myanmar is not started yet; nevertheless, the initiate of testing

present fungi from natural infected agar wood can be approached to make artificial agents for production of the oleoresin from the plantation agar tree. In recent years, some Myanmar companies have planted the agar tree and imported stimulate agents from other countries that have conducted inoculation the tree. Some made the stimulate agents with different ingredients, tested the effective inoculation, therefore the fungi identification from naturally infected agar wood would support to make stimulate agents.

- To record infected fungi from natural agar wood of Myanmar
- To produce stimulate agents for inoculation to planted agar tree
- To induce mass production of oleoresin from planted agar tree

Materials and Methods

1). In these experiments, design and layout could not be done in the field for collection of sample agar wood, and laboratory tests, in fact, samples raring and expenses were problems to collect systematically for statistical analysis from natural distribution. Samples were collected from Bodaung Reserve Forest, Compartment 17, Myaik, Tanninthayi Region; wood samples of Boakpyin; and some natural trees of home garden and private small cottage of resin production in Myitkyina, Kachin State.

Laboratory Procedures

2). PDA (Potato Dextrose Agar) nutrient medium was prepared in the Forest Pathology Laboratory, FRI.
3). Infected agar wood samples were washed by H_2O_2 (Sodium Hypo Chloride) and DDW (Double Distilled Water) for disinfectants
4). Culture the fungi (infection symptom appearing agar wood pieces on the PDA plates and observation daily)
5). Emerging fungi isolation into PDA test tubes for pure culture
6). Monitoring spores formation
7). Slide preparation
8). Study microscopic structures
9). Photographic record
10). Identification of the infected fungi (refer to Introductory Mycology (Alexopoulos & Mims. 1979. USA and Illustrated Genera of Imperfect Fungi (Barnett & Hunter. 1972, USA).

Results

Myeik (2001- 2011)

Average Rainfall	164.476 inches
Temperature	21.99 □ C – 31.23 □ C

Altitude 20 ft

Myitkyina (2001-2011)

Average Rainfall 95.99 inches
Temperature 18.8 C – 30.5 C
Altitude 476 ft

Symptoms

At an initial stage of infection appears as brown streaks in the agar wood tissue (Figure 1). Its uninfected wood of the tree is light in color, but infected pieces of agar wood were grey and dark in color, smell and oily; and heavier than uninfected wood (Figure 2).

Figure 1. Fungi infection symptom on agar wood

Figure 2. Dark brown or black agar wood with accumulation of oleoresin

Table 1. Fungi from naturally infected agar wood of Myeik trip

No.	Source	Taxonomic group (Family)	Genera	Fig.	Tree size (ft.)	Age
1.	Myeik, tree 35	Dematiaceae	*Curvularia sp*	5,6	Ht. 25, Girth 1.6	About 7 yrs
2.	Myeik, tree 39		*Curvularia sp.*	7, 8	Ht. 35, Girth 1.5	
3.	Myeik, tree (U ThanMyint)	Tuberculariaceae	*Fusarium sp.*	9, 10	Ht. 65, Girth 3	About 20 -25 yrs
4.		Sphaeropsidaceae	*Leptosphaeria sp.*	11, 12		
5.	Myeik, wood	Dematiaceae/ Moniliaceae	*Alternaria sp.*	13, 14		
6.	Boakpyin, wood	-	*Alternaria sp.*	15, 16		

7.	□	Eurotiaceae/ Moniliaceae	*Aspergillus sp.*	17, 18		
8.	□	Tubercularіaceae	*Fusarium sp.*	19, 20		
9.	Injected fungi, oil	Ascosphaeraceae	*Ascosphaera apis*	21, 22	Universe General Trading Com.Ltd.	

Figures: Appendix 1.

Table 2. Fungi from infected agar wood of Myitkyina trip and wood from Tant Yunn

No.	Source	**Taxonomic group**	**Genera**	**Fig.**	**Tree size**	**Age**
1.	Myitkyina, tree	Eurotiaceae/ Moniliaceae	*Aspergillus sp.*	25, 26	Ht. 40, Girth 3.5	About 20-23 yrs
2.	Myitkyina, tree	Tubercularіaceae	*Fusarium sp.*	27, 28	□	□
3.	Tant Yunn, wood	Nectriaceae	*Nectria sp.*	29, 30		

Figures: Appendix 2.

Discussions and Conclusions

The symptom of agar wood infection in Myanmar was similar to the literatures cited.

In the present fungi list table 1 & 2, two fungi species *Aspergillus sp*. and *Fusarium sp*. were the same as the literatures (TREECD 1973-99/07) (Blanchette, Robert A. 2005). The results cannot be concluded definitely that these fungi existing are certainly inducing primary factor to produce resin of agar wood, that would be associated with possibility some other factors. According to the literatures, important factors are not only fungi but also other factors such as environmental conditions, host and natural consequence happenings are included

Injected oil from Mawkun Company and Universe General Trading Com.Ltd of Myeik was isolated in the laboratory, it was fungi *Ascosphaera apis.* The fungus was not found in the lists of naturally infected fungi genera (Table 1 & 2). However, the injected fungus was included in fungi division Ascomycota (Blanchette, Robert A. 2005). It is possible, the fungus can be observed in the wood of planted agar tree after inoculation. However, the trees could not be induced by the inoculated fungi in the short term; therefore, the wood from inoculated tree could not be studied of fungi observation whether the agar tree was induced or not; and time is necessary about 3 years or more after inoculation to induce the tree by the fungus.

The climatic conditions were found in 2001- 2011 in Myeik were average rainfall 164.476 inches; temperature 21.99 °C – 31.23 °C and altitude 20 ft. In the Myitkyina, average rainfall 95.99 inches; temperature 18.8 °C – 30.5 °C and altitude 476 ft were recorded. The conditions were not too difference with literatures except altitude and rainfall of Myeik.

Researcher have criticized such lab experiments had so unnatural conditions, much more valid would be results obtained by a study of behavior e.g. temperature, moisture, and light at the time the fungus sporulation on its natural substratum. The doubt on the certain characteristics used in limiting taxa of the fungi. Reference of previous research of Myanmar was lack and advanced tools & technologies for identification of fungi are inadequate in the department.

Root rot disease of a planted agar tree (3 years old) outbreak in Myitkyina in 2011 (Wai Wai Than 2011, Departmental Report), it was caused by fungi *Fusarium* and roots of the tree were dried and died by the fungi blocking in xylem. In the list of presence fungi (table 1 & 2), *Fusarium* were found in the infected agar wood thus observations have a contrast the fungi infected in roots to be die the tree, however, the fungi *Fusarium* is possible agent to oleoresin producing from tested agar tree by response chemical reaction. In the literatures, *Fusarium laseritum* is the faster fungus infection of agar tree, and can be isolated and inoculated easily into medium. However, the tree survival is not possible if any fungi infected its roots.

Nevertheless, it is satisfied that the findings are considerable and providing the making of stimulator fungi agents for inoculation to agar wood oleoresin production.

Although the mechanisms are difficult to understanding, plant Physiologist and Mycologist need to study the mechanisms such as fungi infection, observation of fungi, chemical reaction, and tree response by using advanced tools & technologies in the future. International cooperation is also necessary supporters in the systematic technologies, commercial production and marketing with expertise to nurture a young generation.

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References:

Barnett & Hunter. 1972. Illustrated Genera of Imperfect Fungi. Burgess Publishing Company, Minnesota. Third Edition. USA

Blanchette, Robert A. 2005. United States Patent 6848211. 2007. Cultivated agarwood . Google.

C.J. Alexopoulos & C.W. Mims. 1979. Introductory Mycology. Third Edition. John Wiley and Sons, Inc. NY. USA.

FRI. Yezin. 2009. Departmental report of Thit-hmwe, Botany and Tree Improvement Section.

George Watt. 1889. A dictionary of the economic products of India. Calcutta. Superintendent of Government printing, India.

Hoat Linn. 2004. Thit-hmwe Bin. Forestry Journal. FD.

Indian Agarwood. 2010. All Rights Reserved.

Kyaw Moe Aung. 2010. Agarwood or Eaglewood. Forestry Journal. Myanmar.

Li, Tana (1998) "Nguỹên Cochinchina: southern Vietnam in the seventeenth and eighteenth centuries" Southeast Asia Program Publications.

Maung Maung Than & C Doo. 1977. Study on Thit-hmwe from Kachin State and Upper Chindwin. FD. Union of Myanmar.

Pojanagaroon S, Kaewrak (2002). Mechanical Methods to Stimulate Aloeswod Formation in *Aquliaria crassna* (Kritsana) trees.

The Rainforest Project Foundation. 2002. TRP agar wood project. The European Commission.

TREECD. 1973-99/07.

W. John. Kress. *et.al.* 2003. A Checklist of the Trees, Shrubs, Herbs, and Climbers of Myanmar. Smithsonian Institution. USA.

Wai Wai Than. 2011. Departmental Report of Root Rot Disease on Agar Tree in Myitkyina. FRI, Yezin, Myanmar

www. 2005. Agarwood.com

www. 2010. FreePatentsOnline.com

www. 2010. oudh.co.uk.

Appendix 1

Specimen Collection in Myeik

Fig 3. Bodaung Reserve Forest,
Compartment 17, tree no. 35, 39 .
Myeik

Results from lab test

Myeik

Fig 5. Fungus *Curvularia sp.*in test tube (Myeik)

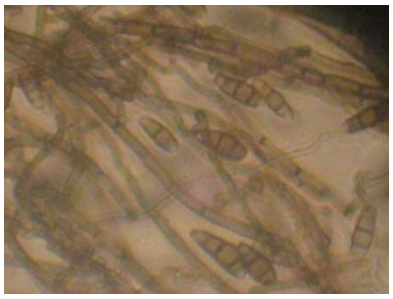

Fig 6.Microscopic structure fungus *Curvularia sp*. (Myeik)

Fig 7. Fungus *Curvularia sp.*in test tube (Myeik)

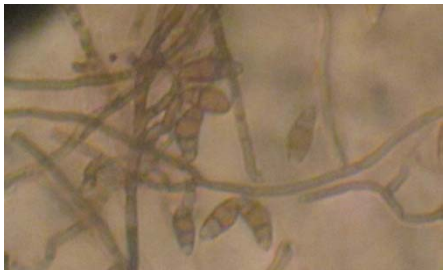

Fig 8. Microscopic structure of Fungus *Curvularia sp*. (Myeik)

Fig 9. Fungus *Fusarium sp.* in test tube (Myeik)

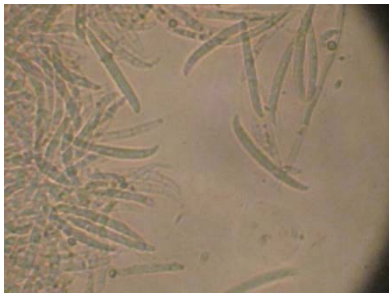

Fig 10. Microscopic structure of Fungus *Fusarium sp.* (Myeik)

Fig 11. Fungus *Leptosphaeria sp.* in test tube (Myeik)

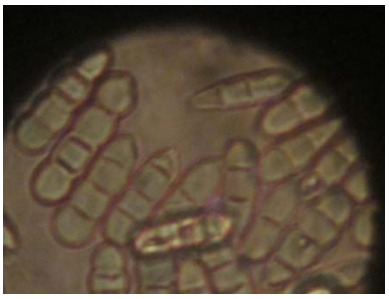

Fig 12. Microscopic structure of Fungus *Leptosphaeria sp.* (agar tree owner U Than Myint) (Myeik)

Fig 13. Fungus *Alternaria sp.* in test tube (Myeik)

Fig 14. Microscopic structure of Fungu *Alternaria sp* (Myeik)

Fig 15. Fungus *Alternaria sp.* in media plate (Boakpyin)

Fig 16. Microscopic structure of Fungus*Alternaria sp.*(Boakpyin)

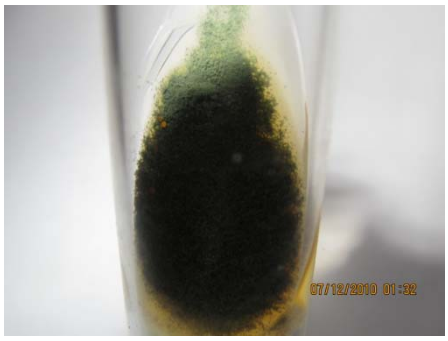

Fig 17. Fungus *Aspergillus sp.*in test tube (Boakpyin)

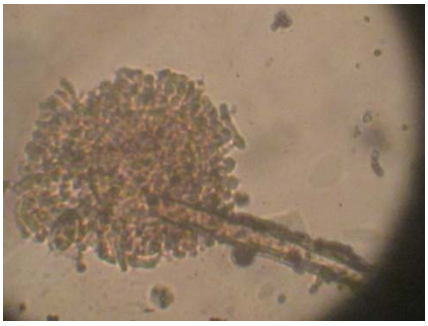

Fig 18. Microscopic structure of Fungus*Aspergillus sp.*(Boakpyin)

Fig 19. Fungus *Fusarium sp* in test tube (Boakpyin)

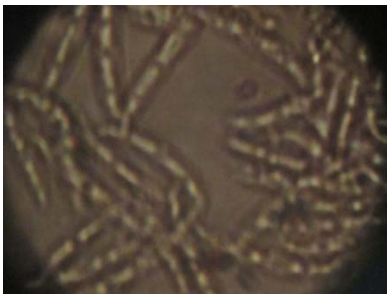

Fig 20. Microscopic structure of Fungus *Fusarium sp*

Fig 21.Injected fungi *Ascosphaera apis* in test tube

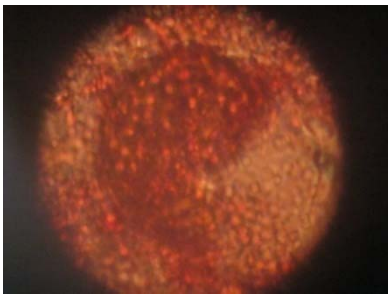

Fig 22. Microscopic structure of Spore balls and spores of *Ascosphaera apis*

Myitkyina

Fig 23. Agar tree selection in Myitkyina

Fig 24. Sample Collection in Myitkyina

Fig 25. Fungus *Aspergillus sp.* in test tube (Myitkyina)

Fig 26. Microscopic structure of Fungus *Aspergillus sp.*

Fig 27. Fungus *Fusarium sp* in test tube (Myitkyina)

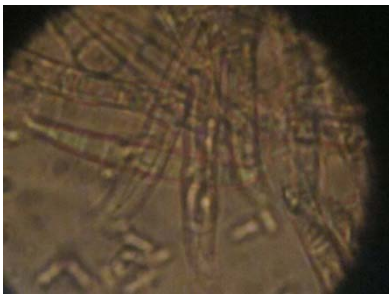

Fig 28. Microscopic structure of Fungus *Fusarium sp*

Tant Yunn

Fig 29. Agar wood sample from Taunt Yunn

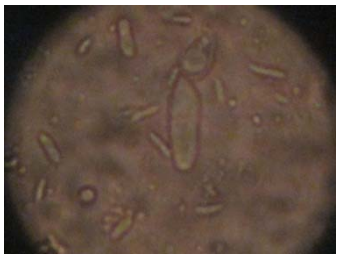

Fig 30. Microscopic structure of Fungus *Nectria sp.* (Taunt Yunn)