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Aquatic Microfungi Biodiversity in the Highland Lake of Telaga Warna, Bogor

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PREFACE

Pusat Penelitian Lingkungan *Hidup* - Institut Pertanian *Bogor* (PPLH-IPB) [Center for Environmental Research - Institut Pertanian Bogor (CER-IPB)] was established in *1976.* One of the Center's goal is to develop policies and concepts for natural resources and environmental management based on ecosystem characteristics, community participation, local community tradition, economic justice, and global environmental change.

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We are pleased to publish Dr. Hefni Effendi and Surantiningsih's paper **Aquatic Microfungi Biodiversity In the Highland Lake of Telaga Warna, Bogor**. **We** also express our gratitude and appreciation to Mr. Hendi Satrio Aji, Dr. Lilik Budi Prasetyo, and Ellyn K. Damayanti, Ph.D.Agr. for their cooperation in proof-reading and lay-outing the manuscript.

Finally, we hope this publication will be valuable and beneficial for those who have interest in Indonesia's natural resource and environmental management.

September *2009,*

Kukuh Murtilaksono
Director

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ABSTRACT

Lake of Telaga Warna situated in highland area of Bogor represents a lake with a mild sorrounding environment. Consequently, the aquatic biodiversity structure of the lake may differ conspicously with that of the the lowland lake. Hence, Lake of Telaga Warna may also contain a high biodiversity.

The objective of this research was to determine the biodiversity of aquatic microfungi in the highland lake of Telaga Warna, and to collect the aquatic microfungi of the highand lake of Telaga Warna.

During dry season it was collected 11 microfungi. During rainy season it was collected 15 microfungi. As much as 24 species of aquatic microfungi were found in Telaga Warna lake. They belong to 9 genus namely: Mucor, Abisidia, Aspergillus, *Peniciliium, Trichoderma, Acremonium,* Chepalosporium, *Monilia,* and Rhizopus. Microfungi Rhizopus stolonifer has the highest growth rate. It attained 90.58 mm diameter within 36 hours, whereas R. cohnii and R. *oryzae* have their diameter 83.21 mm and 88.22 mm, respectively.

Key-words: Microfungi, Telaga Warna

I. INTRODUCTION

1.1. Background

The existence of microorganism in aquatic environment functions as an agent of biodegradation of waste. The microorganisms convert organic substance (dissolved, suspended, or colloid) into a variety of gasses and cell biomass. Biological treatment of waste are actually based on the natural food change occurred in the environment (Molla *et al.* *2001;* Sigee, *2005;* Sawyer and McCarty, *1978).*

The utilization of biological agent particularly microfungi in wastewater biological treatment is not as common as that of bacteria. Similarly, the effort of exploration and exploitation of aquatic microfungi is still also rudimentary. Meanwhile, the potencial source of aquatic microfungi in the tropic either in freshwater or in marine environment is enormous (Coulibaly *et al., 2003;* Fleury, *2007;* Mainwright, *1992).*

Lake Telaga Warna locating in highland area of Bogor represents a lake with a mild sorrounding environment. Consequently, the aquatic biodiversity structure of the lake may differ conspicously with that of the the lowland lake. Lake of Telaga Warna and its viccinity was declared by the government as an conservation area of highland ecosystem. In addition, this area is also famous as a holiday resort due to its mild climate and beautiful scenery.

Since the Telaga Warna and the sorrounding is still intact in term of their natural resources, the biodiversity of this area is also regarded interesting to study. Therefore, the research on aquatic microfungi biodiversity as one of ecosystem component in the lake is performed.

1.2. Objectives of the research

To determine the biodiversity of aquatic microfungi in the highland lake of Telaga Warna. To collect the aquatic microfungi of the higland lake of Telaga Warna. These objectives would be pursued by a series of measures such as: aquatic microfungi sampling, isolation, purification, cultivation, identification, preservation, and collection.

II. MATERIALS AND METHODS

Research was performed in the highland lake of Telaga Warna, Bogor. The research was divided into aquatic microfungi sampling, isolation, purification, cultivation, identification, preservation, and collection.

2.1.. Aquatic microfungi sampling and isolation

Sampling was carried out at 12 July 2006 (dry season) and 8 February 2007 (rainy season). A number of substrate which is a host of aquatic microfungi is selected. Those are stone, leaf, twig, aquatic plant, etc. Aquatic microfungi are sampled by an oase needle. Aquatic microfungi are inoculated in a Petri disk containing agar media (potatoes dextrose agar/PDA). Then they are incubated several days at constants room temperature.

2.2. Purification and cultivation

After several days, a variety of aquatic microfungi grow in Petri disk containing agar media. In order to get a single isolate, a purification process follows. The purification is conducted by subculturing the mixed aquatic microfungi isolate into a fresh agar media, by means of selecting one by one isolate. This step is done several times until a single isolate is achieved. Once the single isolate in agar media is obtained, the single isolate is then cultured in a liquid media (potatoes dextrose broth/PDB).

2.3. identification

At the bottom of petri disk is laid a piece of whatman paper. A V-shaped rod glass is put on top of the whatman paper, and covered with object glass, then autoclaved (121 – 145°C) for ±15 minutes. On top of the previously sterilized object glass is poured a tiny volume of PDA. The microfungi are then inoculated in PDA. Put 5 - 7 drops glycerol 10% on the whatman paper. The object glass culture is then inoculated in constant room temperature.

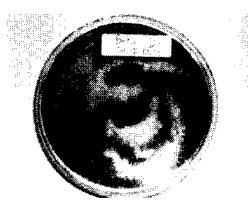
After the microfungi grow, microfungi fungi structure is observed under a microscope. This identification will be conducted for all collected aquatic microfungi. The books for identification are Introduction to tropicall microfungi (Ganjar *et al.*, 1999), A Manual of Soil Fungi (Gilman, 1945), Fungal physiology (Griffin, 1981) as well as Moulds and Filamentous Fungi in Technical Microbiology (Fassatiova, 1986).

2.4. Preservation and collection

After knowing the species of isolated microfungi and the culture, the aquatic microfungi are then preserved by mean of growing them in a slant agar media tube. For long term collection, the preserved aquatic microfungi are stored in a refrigerator (4°C). It is subcultured regularly for maintaining the collection. The overall step of isolation and cultivation are illustrated at Figure 2.1.

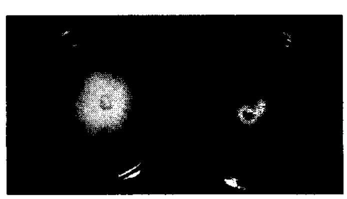
Aquatic microfungi host

↓



A mixed isolate of aquatic microfungi in PDA media

↓



A single isolate of aquatic microfungi in PDA media

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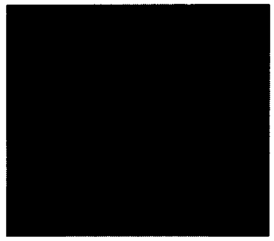


A single isolate aquatic microfungi in PDB media

Figure 2.1. Isolation and purification of aquatic microfungi (Effendi, 2004)

2.5. Growth Observation

All pure isolates of microfungi collected at Telaga Warna lake are cultivated in PDA media. Every 6 hours for 66 hours, their growth are observed. The growth of microfungi is determined by the the increase of colony diameter. Through this growth observation, it is revealed the growth pattern (lag, exponential, desseleration, and stationer phase) of microfungi. The highest and the lowest growth of microfungi are also determined.

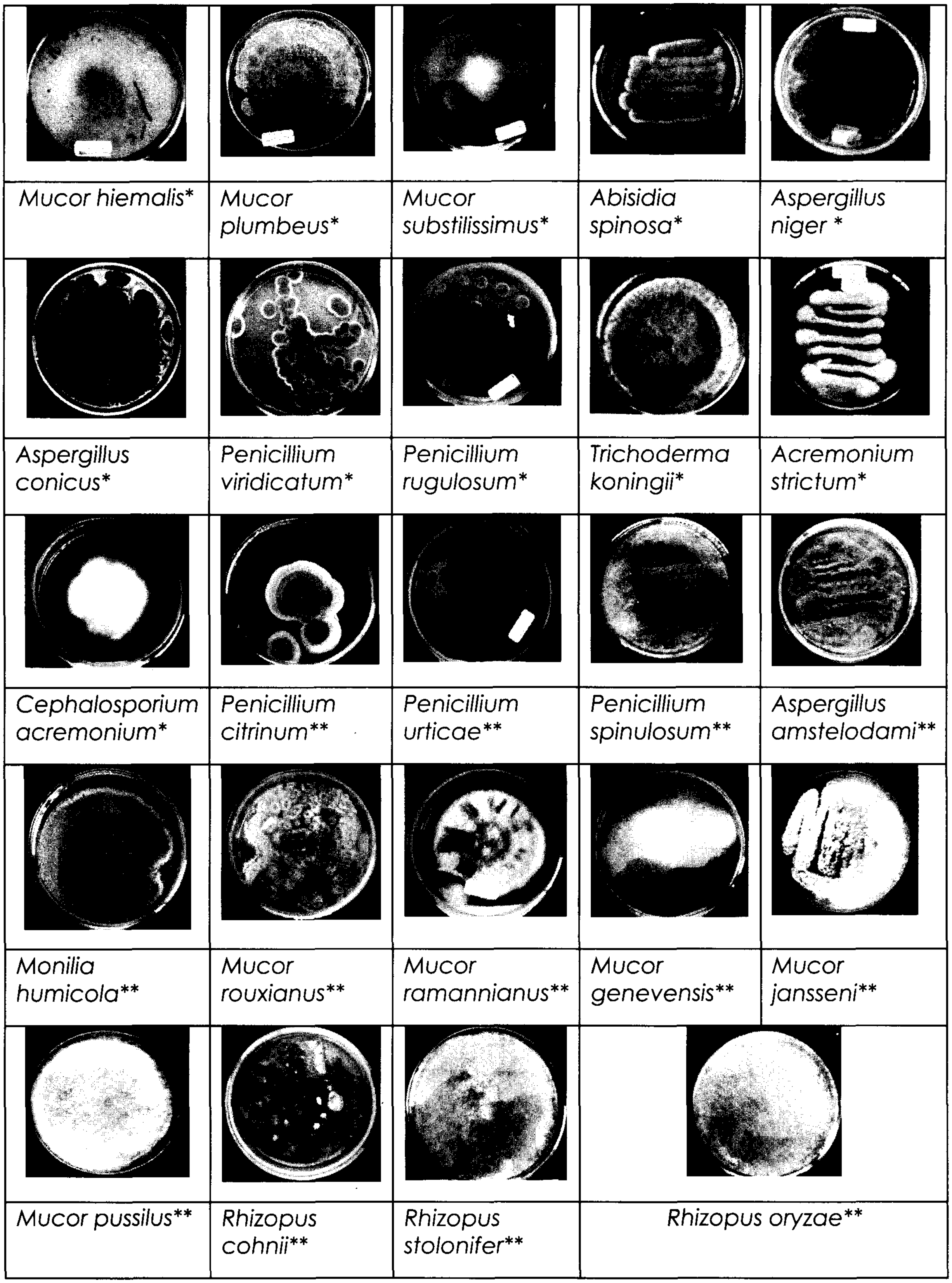
III. RESULTS AND DISCUSSION

Pure isolates of microfungi collected during the research in the lake of Telaga Warna are presented at Table 3.1 and Figure 3.1. it was collected 11 microfungi. Meanwhile at rainy season, 15 microfungi were able to be collected. Among them, two microfungi (Penicillium *rugulosum* and Cephalosporium acremonium) were found either at dry or rainy season. Hence, all together 24 species of aquatic microfungi were collected from Telaga Warna Lake. They belong to 9 genus (Mucor, Abisidia, Aspergillus, Penicillium, *Trichoderma, Acremonium,* Chepalosporium, *Monilia* and Rhizopus).

Table 3.1. Microfungi isolates found in Telaga Warna lake.

Dry Season Collection				
No.	Name of Microfungi	Family	Class	Host
1	*Mucor* hiemalis	Mucoraceae	Phycomycetes	Aquatic Plant
2	Mucor *plumbeus*	Mucoraceae	Phycomycetes	Stone
3	Mucor *substilissimus*	Mucoraceae	Phycomycetes	Leaf
4	*Abisidia* spinosa	Mucoraceae	Phycomycetes	Leaf, Root
5	Aspergillus niger	Moniliaceae	Deuteromycetes	Root
6	Aspergillus *conicus*	Moniliaceae	Deuteromycetes	runk, Twig
7	Penicillium viridicatum	Moniliaceae	Deuteromycetes	Root
8	Penicillium rugulosum [a]	Moniliaceae	Deuteromycetes	Twig, Aquatic Plant
9	*Trichoderma* koningii	Moniliaceae	Deuteromycetes	Stone
10	Acremonium *strictum*	Moniliaceae	Deuteromycetes	Leaf
11	Cephalosporium acremonium [b]	Moniliaceae	Deuteromycetes	Aquatic Plant, Twig
Rainy Season Collection				
12	*Penicillium citrinum*	Moniliaceae	Deuteromycetes	Leaf
13	*Penicillium urticae*	Moniliaceae	Deuteromycetes	Stone
14	Penicillium spinulosum	Moniliaceae	Deuteromycetes	Twig
15	Mucor rouxianus	Mucoraceae	Phycomycetes	Leaf
16	Mucor *ramannianus*	Mucoraceae	Phycomycetes	Twig
17	Mucor genevensis	Mucoraceae	Phycomycetes	Twig
18	Mucor jansseni	Mucoraceae	Phycomycetes	Stone
19	Mucor *pussilus*	Mucoraceae	Phycomycetes	Stone
20	*Aspergillus* amstelodami	Moniliaceae	Deuteromycetes	Leaf
21	*Monilia* humicola	Moniliaceae	Deuteromycetes	Stone
22	Rhizopus cohnii	Mucoraceae	Phycomycetes	Twig
23	Rhizopus *stolonifer*	Mucoraceae	Phycomycetes	Stone
24	Rhizopus *oryzae*	Mucoraceae	Phycomycetes	Leaf, Aquatic Plant

Note: a and b were found at dry and rainy season



* Dry season collection

** Rainy season collection

Figure 3.1. Microfungi colonies found in Telaga Warna lake

All aquatic microfungi isolated from Telaga Warna have different growth rate. Observations on 24 microfungi, isolated during dry and rainy season, for 66 hours with interval of 6 hours showed different growth pattern (Figure3.2 and 3.3).

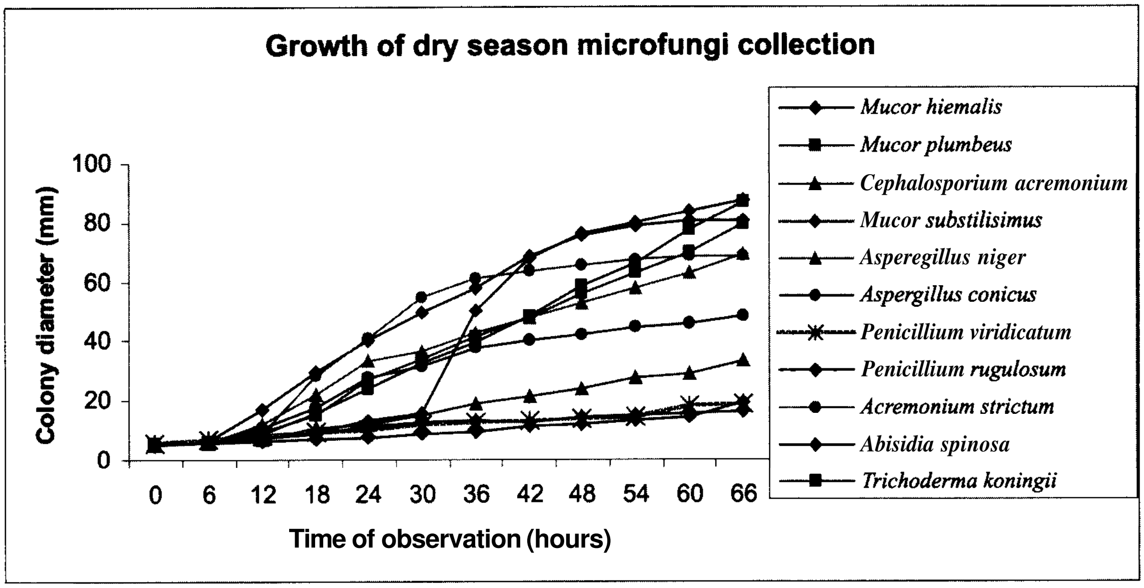


Figure 3.2. Growth of aquatic microfungi isolated at dry season

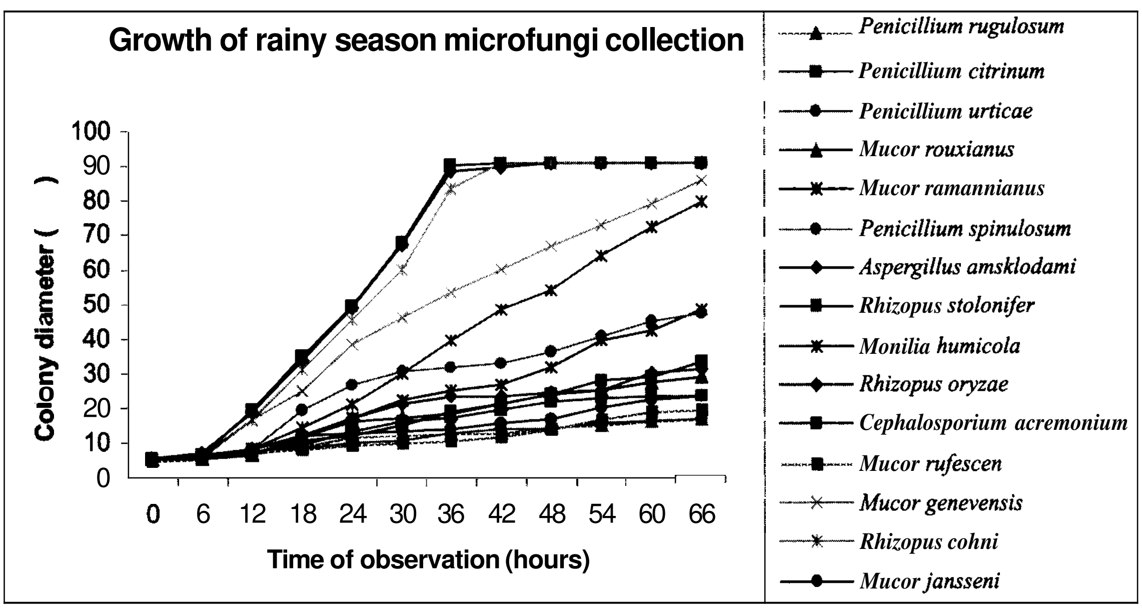


Figure 3.3. Growth of aquatic microfungi isolated at rainy season

Colony growth of microfungi was observed from the development of colony diameter on agar media at Petri disk. The growth was classified as slow, medium, and quick. From the dry season collection, microfungi that showed the biggest colony until 66 hours observation was Mucor substilissimus (87,21 mm) and *Trichoderma* koningii (86,48 mm). Exponential growth of M. substilissimus occurred at 30 - 48 hours observation. Moore-Landecker (1972) stated that significant cell multiplication (colony diameter development) indicated exponential growth phase. This phase is important in the growth cycle of microfungi.

Exponential growth of Acremonium *strictum* lasted at 12 - 36 hours observation. Meanwhile exponential growth of M. hiemalis occurred at 12 - 48 hours. This fact denoted that each microfungi have different exponential growth. From dry season microfungi collection, the growth of microfungi could be divided into quick, medium, and slow growth (Table 3.2).

Table 3.2. The growth of dry season microfungi collection

Quick Growth (61 - 100 mm diameter)	**Medium Growth (30 - 60 mm diameter)**	**Slow Growth (<30 mm diameter)**
1. Mucor hiemalis	1. Aspergillus conicus	1. *Penicillium* rugulosum
2. Mucor *plumbeus*		2. Penicillium viridicatum
3. Mucor substilisimus		3. Abisidia spinosa
4. Trichoderma koningii		4. Cephalosporium acremonium
5. Aspergillus *stric*tum		
6. Aspergillus niger		

P. rugulosum, P. viridicaturn, Abisidia spinosa and C. acremonium are slow growth microfungi of dry season collection. Lag growth phase for these four microfungi lasted until the end ob observation. Other dry season microfungi collection has lag phase within 6 hours. In the lag phase the growth is extremely low. Within 12 hours the exponential growth phase of these microfungi initiated. This meant that these microfungi were able to immediately adapt themselves to the artificial environment with artificial culture media and multiply quickly during the exponential growth phase.

During the rainy season, Rhizophus stolonifer reached the biggest colony (90.58 mm diameter) for 36 hours, whereas R. cohnii and R. *oryzae* have their diameter 83.21 mm and 88.22 mm, respectively. The lag phase of Genus Rhizophus is the quickest among others. They took only 6 hours. Exponential growth phase of this genus was attained at 6 - 36 hours observation, followed by deceleration (cell multiplication is not as active as that of exponential growth phase) and stationer (dear and life cell are balance) of this genus. The growth of rainy season microfungi collection is presented at Table 3.3.

Table 3.3. The growth of rainy season microfungi collection.

Quick Growth (61 - 100 mm diameter)	Medium Growth (30 - 60 mm diameter)	Slow Growth (<30 mm diameter)
I. *Rhizopus stolonifer*	I. *Penicillium spinulosum*	I. *Cephalosporium acremonium*
2. *R. oryzae*	2. *Mucor rouxianus*	2. *Aspergillus amsklodami*
3. *R. Cohni*		3. *Mucor rouxianus*
4. *Monilia humicola*		4. *M. Jansseni*
5. *Mucor genevensis*		5. *M. rufescen*
		6. *Penicuillium rugulosum*
		7. *P. urticae*
		8. *P. citrinum*

IV. CONCLUSION

During dry season it was collected 11 microfungi. During rainy season it was collected 15 microfungi. 24 species of aquatic microfungi were found in Telaga Wama lake. They belong to 9 genus namely: Mucor, Abisidia, Aspergillus, *Peniciliium, Trichoderma,* Acremoniurn, Chepalosporium, Monilia and Rhizopus. Rhizopus stolonifer has the highest growth rate. It attained 90.58 mm diameter within 36 hours, whereas *R. cohnii* and *R. oryzae* have their diameter 83.21 mm and 88.22 mm, respectively.

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