# Antibacterial Activities of Soil Fungi Isolated from Monywa University Campus

Khaing Wai Lwin[1]

## Abstract

In this study, the soil samples were collected from 2 different places in Monywa University Campus, Monywa Township, from 2020 to 2021. The soil color, soil type, pH of soil and soil moisture were recorded. Soil moisture (water content) was determined by the method of Pepper and Gerba (2004). A total of 7 fungal strains were isolated from 2 different soil samples by using serial dilution method and Synthetic Low Nutrient Agar Medium (SLNA) proposed by Suto (1999). The antibacterial potentials from each isolated soil fungi were detected by paper disc diffusion method which provided clear zones against 3 test organisms. Four kinds of fungi were isolated from soil sample No.1. Three kinds of fungi were isolated from soil sample No.2. Their macroscopical and microscopical characters of isolated soil fungi have been observed. The strain *Botrytis* sp. showed antibacterial activities against *Agrobacterium tumefaciens* and the strain *Mortierella* sp. showed antibacterial activities against *Escherichia coli* in soil sample No.1. The strain *Aspergillus* sp. showed antibacterial activities against *Bacillus subtilis* in soil sample No.2. The soil fungi *Curvularia* sp. *Cladosporium sp., Alternaria sp. and Mucor sp.* are not presence the clear zone. The soil fungi *Botrytis* sp., *Mortierella* sp., *Aspergillus* sp. have the presence of clear zone. Thus, the strain *Botrytis* sp.is the best in soil sample No.1 and No.2. Fungi have been studied because of their usefulness in medicine, agriculture and bioremediation.

**Keywords**: soil sample, fungi, antibacterial activities

## Introduction

Soil microbiology is the study of microorganisms in soil, their functions, and how they affect soil properties. Microorganisms in soil are important because they affect the soil structure and fertility. Soil microorganisms can be classified as bacteria, actinomycetes, fungi, algae and protozoa. Fungi are abundant in soil, but bacteria are more abundant. Fungi are important in the soil as food sources for other, larger organisms, pathogens, beneficial symbiotic relationships with plants or other organisms and soil health. Fungi can be split into species based primarily on the size, shape and color of their reproductive spores, which are used to reproduce. Fungi constitute the major proportion of the microbial biomass in soils. Most types of fungi can be found in soil habitats, existing either as free living organisms or in association with plant roots (Atlas, 1998).

Soil is a naturally occurring loose mixture of mineral and organic particles, considered as one of the most suitable environments for microbial growth (Nejad *et al.,* 2013). It still remains the most targets for many researchers in their efforts to discover novel antibiotic. Soil fungi are the major source of other industrially important compounds like enzyme inhibitors, anthelminthic, antitumoragents, insecticides, vitamins, immune-suppressant and immune-modulators (Makut and Owolewa, 2011).

Fungi are small, generally microscopic, eukaryotic, usually filamentous, branched, spore-bearing organisms that lack chlorophyll and have cell walls that contain chitin, cellulose, or both. Most of the 100,000 fungus species known are strictly saprophytic, living on dead organic matter, which they help decompose. Some about 50 species cause diseases in humans, and about as many cause diseases in animals, most of them superficial diseases of the skin. More than 8000 species of fungi, however, can cause diseases in plants (Agrios, 1988).

[1]Demonstrator, Department of Botany, Sagaing University

Fungi are employed to produce a wide range of foods (eg. Bread, mycoprotein), alcoholic beverages (wine, beer), recombinant proteins, vitamins and antibiotics (Kavanagh, 2011).

The main aim and objectives of this paper were to isolate the soil fungi from two different places, to study the macroscopical and microscopical characters of isolated soil fungi and to investigate their antibacterial activities.

## Materials and Methods

### Collection of soil samples

The soil samples were collected from 2 different places at Monywa University Campus. The soil color, soil type and pH of the soil were noted and its water content was determined as shown in Table 1.

**Table 1. Location of Soil Sample Collected Place, Some Physical and Chemical Parameters and Designated Strain Numbers of Isolated Fungi**

| Soil No. | Collected Place | pH | Water content | Soil Color, Soil type | Isolated soil fungi |
|---|---|---|---|---|---|
| S. 1 | N 22° 8' 21"<br>E 95° 9' 56" | 7 | 0.1 g | Gray,<br>Clay | KWL-01A, KWL-01B,<br>KWL-01C, KWL-01D |
| S. 2 | N 22°8' 11"<br>E 95°9' 56" | 7 | 0.2 g | Black,<br>Clay | KWL-02A, KWL-02B,<br>KWL-02C |

S.1 = Soil sample No.1,
S.2 = Soil sample No.2

### Determination of water content of soil sample

10 g of soil sample is taken by a clean Petridish and kept in a hot air oven at 110 ºC for 3−5 hrs and then kept at 60ºC overnight. On the next day, the weight of the soil is measured. The loss of weight is due to evaporation of water from the soil (Pepper and Gerba, 2004).

### Preparation of Soil Suspension

10 g of soil sample is added to 90 ml of sterilized water. The flask is shaken vigorously to ensure thorough mixing. Serial dilutions are made by transferring 1 ml of suspension to 9 ml of sterilized water in a culture tube to obtain soil suspension of $10^{-1}$ dilution. This process is repeated until $10^{-10}$ dilution is obtained (Pepper and Gerba, 2004). These soils suspension were used for the isolation of soil fungi.

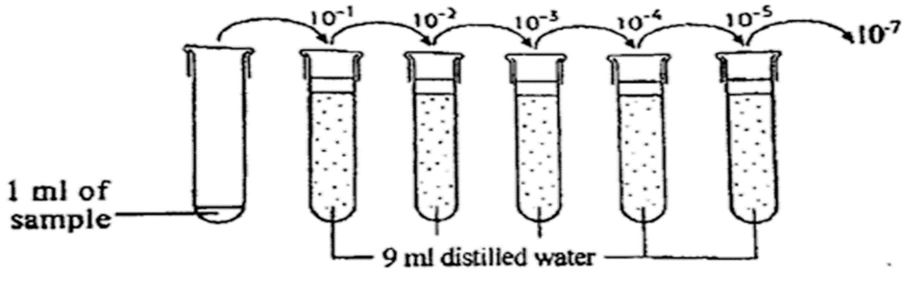


Figure 1. Making a Serial Dilution of Soil Sample (Pepper and Gerba, 2004)

**Isolation of Soil Fungi**

SLNA medium was used for the isolation of fungi (Suto, 1999).

**SLNA medium (Synthetic Low Nutrient Agar medium)**

| | |
|---|---|
| Glucose | 0.2 g |
| Sucrose | 0.2 g |
| $K_2HPO_4$ | 0.2 g |
| $MgSO_4.7H_2O$ | 0.5 g |
| $KNO_3$ | 0.1 g |
| KCL | 0.5 g |
| Agar | 1.8 – 2.0 g |
| Distilled water | 100 ml |
| pH | 6.5 |

(After autoclaving, penicillin 0.8g was added to the medium for fungi)

**Macroscopical and Microscopical Characters**

**Macroscopical Characters of Isolated Fungi**

The PGA medium was utilized for the macroscopical characters of isolated fungi. The fungus was inoculated onto the PGA medium for seven days.

**PGA medium (Potato Glucose Agar medium)**

| | |
|---|---|
| Potato powder | 0.5 g |
| Glucose | 2.0 g |
| $K_2HPO_4$ | 0.5 g |
| Agar | 1.8 – 2.0 g |
| Distilled water | 100 ml |
| pH | 6.5 |

(After autoclaving, penicillin 0.8g was added to the medium for fungi)

**Microscopical Characters of Isolated Fungi**

The WGA medium was utilized for the microscopical characters of fungi. The fungus was inoculated onto the WGA medium for 10 days.

**WGA medium (Water Glucose Agar medium)**

| | |
|---|---|
| Glucoses | 1.6 – 1.8 g |
| Agar | 1.8 – 2.0 g |
| Distilled water | 100 ml |
| pH | 6.5 |

(After autoclaving, penicillin 0.8g was added to the medium for fungi)

**Screening of Antibacterial activities by Paper Disc Diffusion Assay**

The screening of antibacterial activities was carried out by the method of Applied Microbiology Lab, Hokkaido University, Japan, 1998. The isolated soil fungi were grown on PGA agar medium and were inoculated into seed medium and then incubated for 3 days at 27°C. Seed culture (5 ml) is transferred to the identical fermentation medium. The fermentation was carried out for 7 days. After the end of fermentation, the fermented broth (20 µl) was used to check the antibacterial activity against test organisms by paper disc diffusion assay. Paper disc having 8.41 mm was utilized for antibacterial assays.

The assay medium (Glucose 10.0g, Polypeptone 3.0g, $KNO_3$ 1.0g, Agar 18.0g, Distilled water 1000 ml, pH 6.5 - 7.0) was used for the antibacterial activity test. One percent of test organism was added to assay medium, then poured into plates. After solidification, paper discs impregnated with samples (fermented broth) were applied on the agar plates and the plates were incubated for 24–36 hrs at 28° to 30°C. Clear zones (inhibitory zones) surrounding the test discs indicate the presence of bioactive metabolites which inhibit the growth of test organisms.

The test organisms used in paper disc diffusion assay were *Agrobacterium tumefaciens* IFO-5431 and *Bacillus subtilis* KY-327, *Escherichia coli* AHU-5436. These test organisms were supported by NITE (2004) (National Institute of technology and Evaluation, Japan) and Faculty of Agriculture, Hokkaido University, Japan for the cooperation researches.

## Results

### Collection of soil samples

The soil sample No.1 was collected at N 22° 8' 21", E 95°9' 56". The color of soil sample No.1 is gray and the soil type is clay. The soil pH is 7 and the water content is 0.1 g. The soil sample No. 2 was collected at N 22° 8' 11", E 95°9 '56". The color of soil sample No.2 is black and the soil type is clay. The soil pH is 7 and the water content is 0.2 g as shown in Table 1.

### Macroscopical character of KWL-01A

The isolated soil fungus was grown on the PGA medium at 25°C or room temperature. After 4 days, the growth of mycelium colony was circular shape, 2.5 cm in diameter and whitish gray colour as shown in Figure 2 (B).

### Microscopical character of KWL-01A

The hyphae are septate. The conidiophore was long, slender and branched bearing clusters of conidia on short sterigmata. The conidia were 1-celled and ovoid as shown in Figure 2 (C).

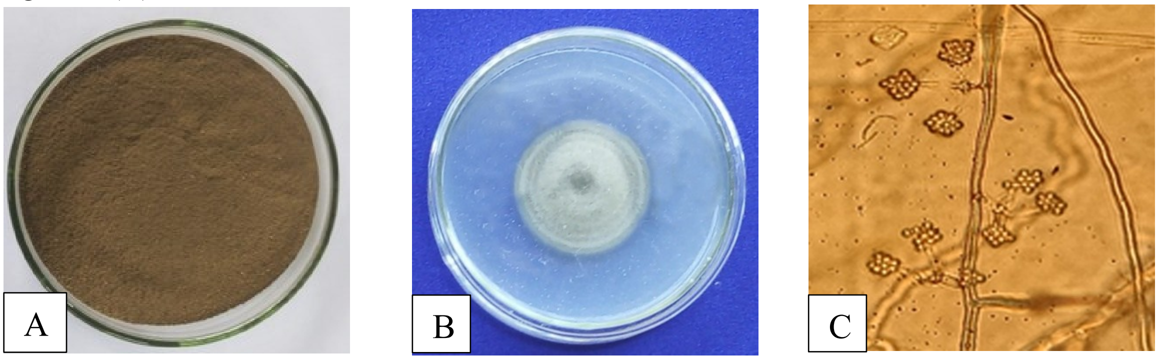


Figure 2. (A) Soil sample No.1
(B) Macroscopical character of KWL-01A
(C) Microscopical character of KWL-01A

### Macroscopical character of KWL-01B

The isolated soil fungus was grown on the PGA medium at 25°C or room temperature. After 5 days, the growth of mycelium colony was filamentous shape, 2.5 cm in diameter and pale pink colour as shown in Figure 3 (B).

### Microscopical character of KWL-01B

The hyphae are aseptate. Sporangiophores hyaline, erect, tapering gradually from base toward apex, simple, bearing sporangia terminally. Sporangia hyaline, globose, lacking columellae, several spores included. Sporangiospores were hyaline, broadly ellipsoidal or subglobose as shown in Figure 3 (C).

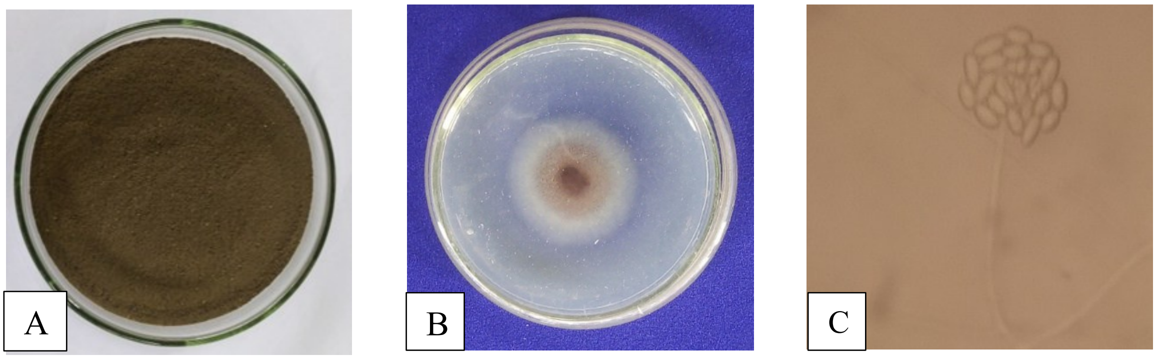


Figure 3. (A) Soil sample No.1
(B) Macroscopical character of KWL-01B
(C) Microscopical character of KWL-01B

**Macroscopical character of KWL-01C**

The isolated soil fungus was grown on the PGA medium at 25°C or room temperature. After 5 days, the growth of mycelium colony was in irregular shape, 3 cm in diameter and gray colour as shown in Figure 4 (B).

**Microscopical character of KWL-01C**

The hyphae are septate. The conidiophore was brown and simple. The conidia was dark, end cells lighter, 3-5-celled, more or less fusiform, typically bent or curved, with one or two of the central cells enlarged as shown in Figure 4 (C).

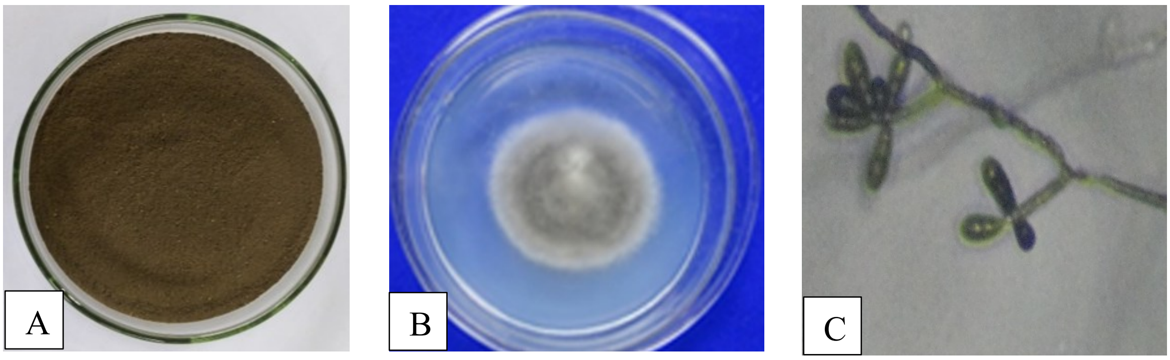


Figure 4. (A) Soil sample No.1
(B) Macroscopical character of KWL-01C
(C) Microscopical character of KWL-01C

**Macroscopical character of KWL-01D**

The isolated soil fungus was grown on the PGA medium at 25°C or room temperature. After 5 days, the growth of mycelium colony was filamentous shape, 3.5 cm in diameter and pale white colour as shown in Figure 5 (B).

**Microscopical character of KWL-01D**

The hyphae are septate. The conidiophore was branched variously near the upper or middle portion, clustered or single. The conidia are 1-celled, variable in shape and size, ovoid to cylindrical and irregular shaped as shown in figure 5 (C).

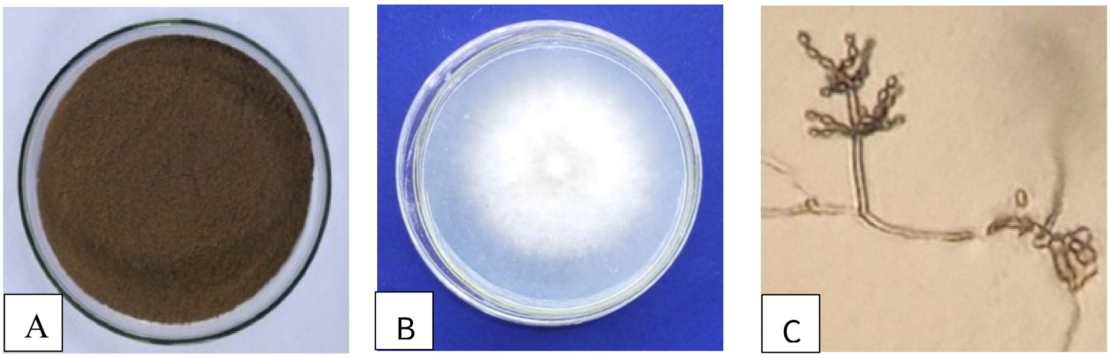


Figure 5. (A) Soil sample No.1

(B) Macroscopical character of KWL-01D

(C) Microscopical character of KWL-01D

**Macroscopical character of KWL-02A**

The isolated soil fungus was grown on the PGA medium at 25°C or room temperature. After 4 days, the growth of mycelium colony was in circular shape, 2 cm in diameter and whitish black colour as shown in Figure 6 (B).

**Microscopical character of KWL-02A**

The hyphae are septate. The conidiophore was upright, simple, terminating in a globose to elliptical swelling, bearing phialides at the apex or radiating from the filiform surface. The conidia are 1-celled, spherical, produced basipetally as shown in Figure 6 (C).

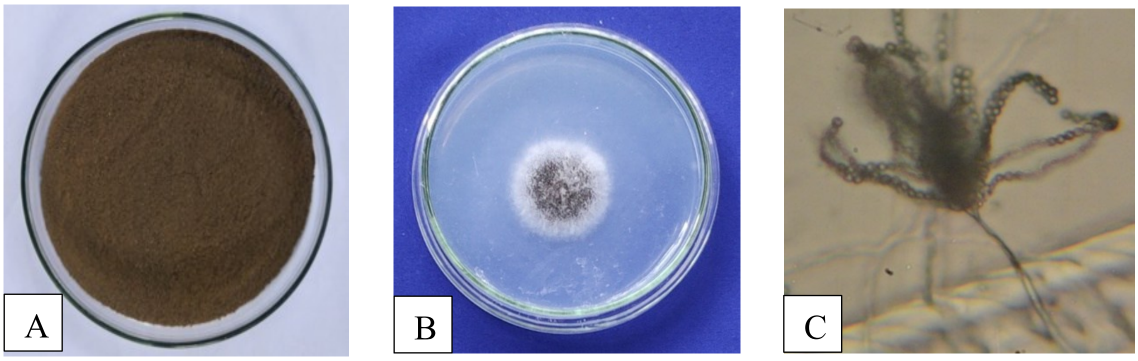


Figure 6. (A) Soil sample No.2

(B) Macroscopical character of KWL-02A

(C) Microscopical character of KWL-02A

**Macroscopical character of KWL-02B**

The isolated soil fungus was grown on the PGA medium at 25°C or room temperature. After 7 days, the growth of mycelium colony was circular shape, 4 cm in diameter and whitish brown colour as shown in Figure 7 (B).

**Microscopical character of KWL-02B**

The hyphae are septate. The conidiophore was dark, simple, rather short, typically bearing a simple or branched chain of conidia. The conidia were dark, typically with both cross and longitudinal septa; variously shaped as shown in Figure 7 (C).

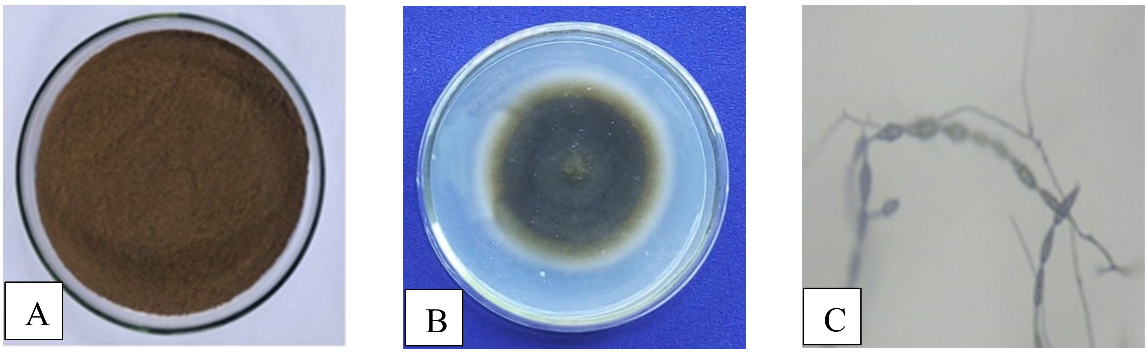


Figure 7. (A) Soil sample No.2
(B) Macroscopical character of KWL-02B
(C) Microscopical character of KWL-02B

**Macroscopical cahracters of KWL-02C**

The isolated soil fungus was grown on the PGA medium at 25°C or room temperature. After 7 days, the growth of mycelium colony was filamentous shape, 7 cm in diameter and whitish pale colour as shown in Figure 8 (B).

**Microscopical characters of KWL-02C**

The hyphae are coarse, coenocytic and branched. The sporangiophore bears with globular sporangium. The spores are 1-celled and rounded in Figure 8 (C).

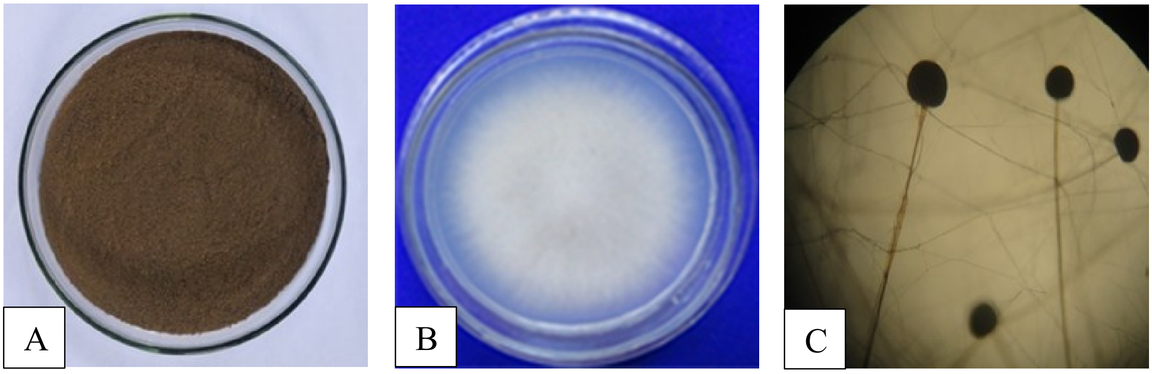


Figure 8. (A) Soil sample No. 2
(B) Macroscopical character of KWL-02C
(C) Microscopical character of KWL-02C

**Screening of Antibacterial activities by Paper Disc Diffusion Assay**

In the study of antibacterial activity, the fungus, strain KWL-01A against the *Agrobacterium tumefaciens* (30.59 mm) , KWL-01B against the *Escherichia coli* (8.70 mm), and the fungus, strain KWL-02A against the *Bacillus subtilis* (13.73 mm) are shown in Figure 9, 10,11 and Table 2 respectively.

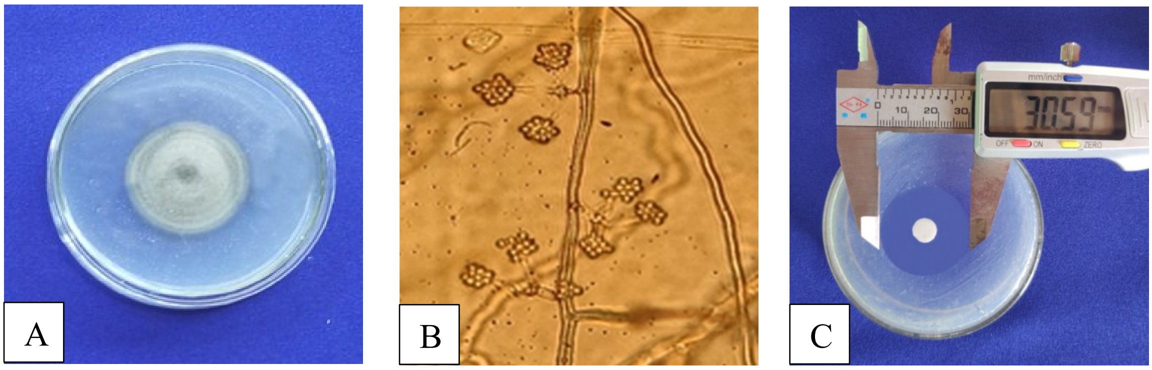

Figure 9. (A) Macroscopical character of KWL-01A
(B) Microscopical character of KWL-01A
(C) The antibacterial activity of isolated fungus, strain KWL-01A against *Agrobacterium tumefaciens* (30.59 mm) (size of paper disc = 8.41 mm)

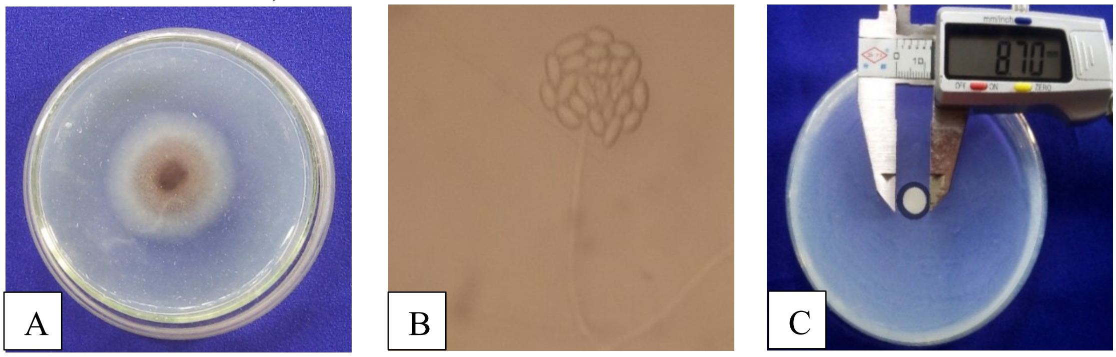


Figure 10. (A) Macroscopical character of KWL-01B
(B) Microscopical character of KWL-01B
(C) The antibacterial activity of isolated fungus, strain KWL-01B against *Escherichia coli* (8.70 mm) (size of paper disc = 8.41 mm)

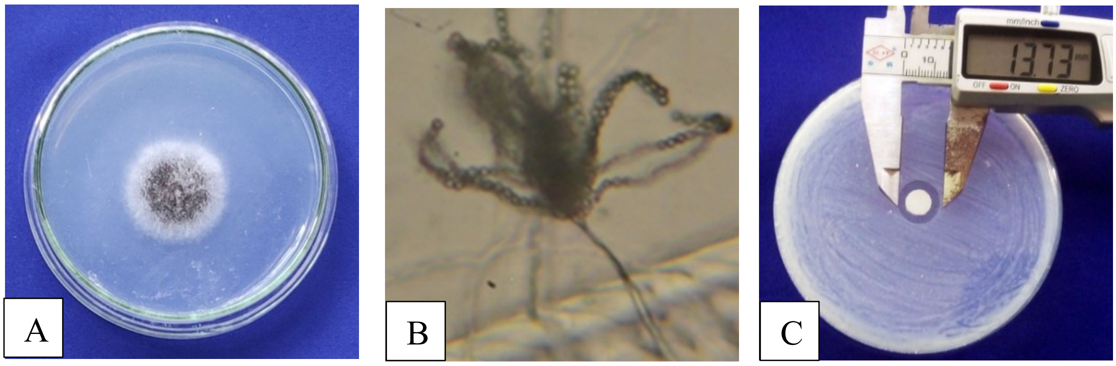


Figure 11. (A) Macroscopical character of KWL-02A
(B) Microscopical character of KWL-02A
(C) The antibacterial activity of isolated fungus, strain KWL-02A against *Bacillus subtilis* (13.73 mm) (size paper disc = 8.41 mm)

**Table 2. Antibacterial activities of isolated soil fungi**

| Types of Soil Fungi | Test Organisms | | |
|---|---|---|---|
| | *Agrobacterium tumefaciens* | *Escherichia coli* | *Bacillus subtilis* |
| *Botrytis* sp.(KWL-01A) | 30.59 mm | - | - |
| *Mortierella* sp. (KWL-01B) | - | 8.70 mm | - |
| *Curvularia* sp. (KWL-01C) | - | - | - |
| *Cladosporium sp.* (KWL-01D) | - | - | - |
| *Aspergillus* sp. (KWL-02 A) | - | 13.73 mm | - |
| *Alternaria sp.* (KWL-02 B) | - | - | - |
| *Mucor sp.* (KWL-02 C) | - | - | - |

## Discussion and Conclusion

In this study, seven fungi species were isolated from 2 different soil samples. The soil samples were collected from Monywa University Campus. The collected soil samples were determined for soil colour, soil type, the soil pH and the soil moisture (water content). Four kinds of fungi were isolated from soil sample No.1. Three kinds of fungi were isolated from soil sample No.2. In the present study, their macroscopical and microscopical characters of isolated fungi and their antibacterial activities have been undertaken.

These macroscopical and microscopical characters of isolated soil fungi were identified based on the reference keys shown in Barnett (1956) and Watanabe (2002). According to Barnett (1956), the *Botrytis* sp. are parasitic, causing “gray mold” of many plants, or saprophytic. The *Curvularia* species were parasitic or saprophytic. Watanabe (2002) stated that the *Mortierella* sp. was occurred as soil fungi. In the present study these observations are in agreement with Barnett (1956) and Watanabe (2002).

Barnett (1956) stated that *Alternaria* species are parasitic or saprophytic on plant material. According to Vashishta and Sinha (2008), *Mucor* is a microbial genus of approximately 6 species of molds found in soil, digestive systems, plant surfaces, rotten vegetable matter.

According to Agrios (1988)*, Aspergillus* was causing rots of stored seeds. Most of the decay or deterioration of grains and legumes after harvest, that is, during storage of transits, is caused by several species of the fungus *Aspergillus*. Barnett (1956) showed that *Aspergillus* containing many species, saprophytic on a wide variety of substrata and a few are parasitic species. Therefore, these observations of research are in agreement with Barnett (1956).

Rivas and Thomas (2005) stated that *Cladosporium* is a genus of fungi including some of the most common indoor and outdoor molds. Many species of *Cladosporium* are commonly found on living and dead plant material. Some species are plant pathogens, others parasitize and other fungi. *Cladosporium* spores are wind-dispersed and they are often extremely abundant in outdoor air. Indoors *Cladosporium* species may grow on surfaces when moisture is present. Barnett (1956) showed that the *Cladosporium* was parasitic on higher plants or saprophytic on plant material.

In the present study, the seven species found in isolated soil fungi were *Botrytis* sp. in strain KWL-01A, *Mortierella* sp. in strain KWL-01B, *Curvularia* sp. in strain KWL-01C, *Cladosporium* sp. in strain KWL-01D, *Aspergillus* sp. in strain KWL-02A, *Alternaria* sp. in strain KWL-02B and *Mucor* sp. in strain KWL-02C. The three species of soil fungi were found the presence of antibacterial activities tests. Thus, *Botrytis* sp. can have the potential to attack the *Agrobacterium tumefaciens* (30.59 mm), *Mortierella* sp. can have the potential to attack the *Escherichia coli* (8.70mm) and *Aspergillus* sp. can have the potential to attack the *Bacillus subtilis* (13.73 mm). *Curvularia* sp. *Cladosporium sp., alternaria sp. and Mucor sp.* can’t have the potential to attack bacteria. In the seven species of isolated soil fungi, *Botrytis* sp. is the best in the antibacterial activities tests.

It is hoped that this study will provide the knowledge of isolation the variety of fungi in different soil study of morphology and their antibacterial activities. In conclusion it is necessary to determine the structure of antibacterial compound and also necessary to perform further investigation concerning the bioassays and application in animals as well as in human beings.

## Acknowledgements

I would like to express my deep gratitude to Rector Dr. Soe Myint Aye, Sagaing University, for his permission to present this research paper. I am greatly indebted to Dr. Phyu Phyu Myint, Prorector, Sagaing University, for her permission to write this paper. I would like to express my thank to Dr. Kyi Kyi Win, Professor and Head, Department of Botany, Sagaing University, for her generous help in this research. Finally, I am also indebted to Dr. Kyaw Lin, Prorector, Meiktila University, for his suggestions on my research paper.

## References

Agrios, G. N. (1988). **Plant pathology**. Academic press, New York.

Atlas, R. M. (1998). **Microbiology: Fundamental and applications**. Macmillan Publishing Company, New York.

Barnett, H. L. (1956). **Illustrated Genera of Imperfect Fungi**. United States of America.
***Cladosporium*** fulvum". Annual Review of Phytopathogen.43:359-436.

Kavanagh, K. (2011). **Biology and Applications**. Department of Biology, National University of Ireland

Makut, M. D., and O. A. Owolewa. (2011). **Antibiotic-producing fungi present in the soil environment of Keffi metropolis, Nasarawa state, Nigeria**. Trakia Journal of Sciences. 9:33-39, Nasarawa State University, Keffi, Nigeria.Maynooth Co. Kildare, Ireland.

Nejad, E. M. A., A. Abtahi., and G. Zareian. (2013). **Evaluation of physical and chemical properties of soils of Doroudzan dam region of Marvdasht province with respect to drainage condictions and elapsed time**.Iron. European Journal of Experimental Biology. 3(5):213-217.

NITE. (2004)."**Medium for fermentation to produce the Metabolites**." National Institute of technology and Evaluation, Faculty of Agriculture, Hokkaido University, Japan.

Pepper, I. L., and C. P. Gerba. (2004). **Environmental microbiology**. Academic Press, New York.

Rivas, S., and C. M. Thomas. (2005). **" Molecular interactions between tomato and the leaf mold pathogen: *Cladosporium fulvum*"**. Annual Review of Phytopathogen.43:359-43.

Suto, M. (1999). **Environmental microbiology**. Academic Press, San Diegos.

Tomita.F.(1998). "**Laboratory Method**". Applied Microbiology Lab, Hokkaido University, Japan.

Vashishta, B. R., and A. K. Sinha. (2008). **Botany for Degree Students Fungi**. S. Chand & Company Ltd. (an ISO 9001:2000 company) Ram Nagar, New Delhi-110 055.

Watanabe, T. (2002). **Pactorial Atlas of Soil and Seed Fungi. Morphologies of Cultured Fungi and Key to Species.** Second Edition. CRC Press LLC, United State of America.