ISOLATION AND MORPHOLOGICAL CHARACTERIZATION OF FUNGI FROM BEIKTHANO ANCIENT CITY AND THEIR ANTIMICROBIAL ACTIVITY

Khin Nilar Oo[1], Zar Zar Yin[2]

Abstract

Soil samples were collected from four different places of Beikthano Ancient city, Taungdwingyi Township, Magway Region. Twenty fungi were isolated from these four different soil samples. Isolation of fungi were undertaken by the chemical treatment method. The soil fungi were isolated by using Low Carbon Agar medium and Malt Extract Agar medium for first culture and Potato Glucose Agar medium for pure culture. The isolated fungi were given as NLF-1 to NLF-20. The surface color of isolated fungi NLF-1 to NLF-20 were white, green, orange white, greenish yellow, yellow, pale brown, black, gray and their reverse color were pale yellow, white, cream, greenish yellow, brown, gray and pink. Antimicrobial activity of isolated fungal strains was evaluated by the agar well diffusion assay with four test organisms. These isolated fugal strains were tested by using 3 days to 10 days old culture. Nine strains showed the antimicrobial activity against *Agrobacterium tumefaciens*, *Aspergillus paraciticus*, *Micrococcus luteus* and *Pseudomonas fluorescens.* Among them, NLF-14 showed the highest antibacterial activity (32.53 mm) on *A. tumefaciens* followed by NLF-7 (29.40 mm) and NLF-16 (29.96 mm) respectively. And then, NLF-14 also showed the highest antifungal activity (32.43 mm) on *A. paraciticus* followed by NLF-9 (31.09 mm) and NLF-6 (30.73 mm). Among all the selected fungi, NLF-12 showed the best inhibition zone (34.85 mm) against *M. luteus.*NLF-6 showed the highest antibacterial activity (30.26 mm) on *P. fluorescens.*

Keywords: Isolation, Soil Fungi, Antimicrobial activity

Introduction

Soils are highly complex system, with many components playing diverse function mainly due to the activity of soil organisms (Goddeyya. G., 2012). Microorganisms live in every part of the biosphere including soil hot spring, seven miles deep in the ocean 40 miles high in the atmosphere and inside rocks for down within the Earth's crust. Soil is the largest source of microorganisms. There are billions to hundreds of billions of soil microorganisms in a mere handful of a typical garden soil.

That single handful might well contain thousands of different species of bacteria, hundreds of different species of fungi and protozoa. Almost all of these countless soil organisms are not only beneficial, but essential to the life giving properties of soil. Microorganisms constitute an important source of biodiversity in soils and are an integral part of terrestrial ecosystems. They contribute to major biological functions such as nutrient and gas cycling, biogeochemical processes and decomposition and transformation of organic matter (Kiran *et al.,* 1999).

The spectrum of antimicrobial activity of the active substance is very important for the competition in nature. In the soil, where most antimicrobial producing microorganisms are found, life is competitive (Lancini *et al.,* 1982).The aim and objectives of this study were to isolate the soil fungi , to study the morphology of isolated fungi and to observe the preliminary study of antimicrobial activities by using four test organisms.

[1] Lecturer, Department of Botany, Magway University
[2] Associate Professor, Department of Botany, Pathein University

Materials and Methods

Collection of Soil Samples

Soil samples were collected from four different places of Beikthano Ancient city, Taungdwingyi Township, Magway Region. Soil samples of four different places were collected during July 2016 to August 2016.

Isolation of fungi from the soil samples

The soil fungi were enumerated by chemical treatment dilution method or media such as (LCA) Low Carbon Agar medium, (MEA) Malt Extract Agar medium and (PGA) Potato Glucose Agar medium.

Table 1 Four different soil samples collected from Beikthano Ancient City

Soil samples No.	Samples collected areas	pH	Soil types	Location
S.I	Gokkone	9.54	Loam	N 19º 59.421' E 95º 24.432'
S.II	Shwe-yaung-taw	6.87	Sandy Loam	N 20º 0.874' E 95º 23.371'
S.III	Nan-twin-taw ya Monastery	9.04	Sandy Loam	N 20º 0.852' E 95º 23.277'
S.IV	Beikthano station	6.80	Sandy Loam	N 19º 59.646' E 95º 23.353'

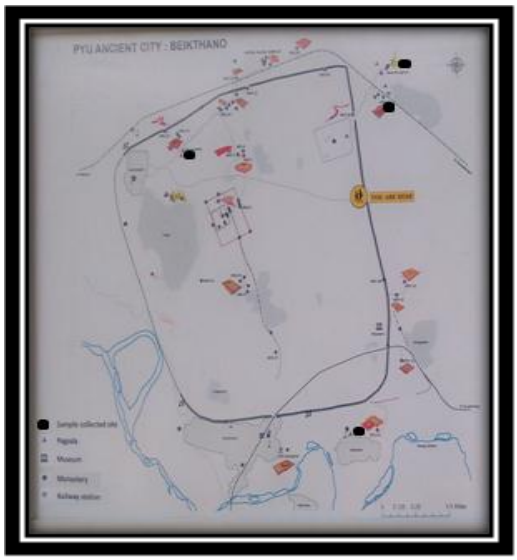

Figure 1 Map of Beikthano Ancient City (Source Beikthano Museum)

Soil isolation method

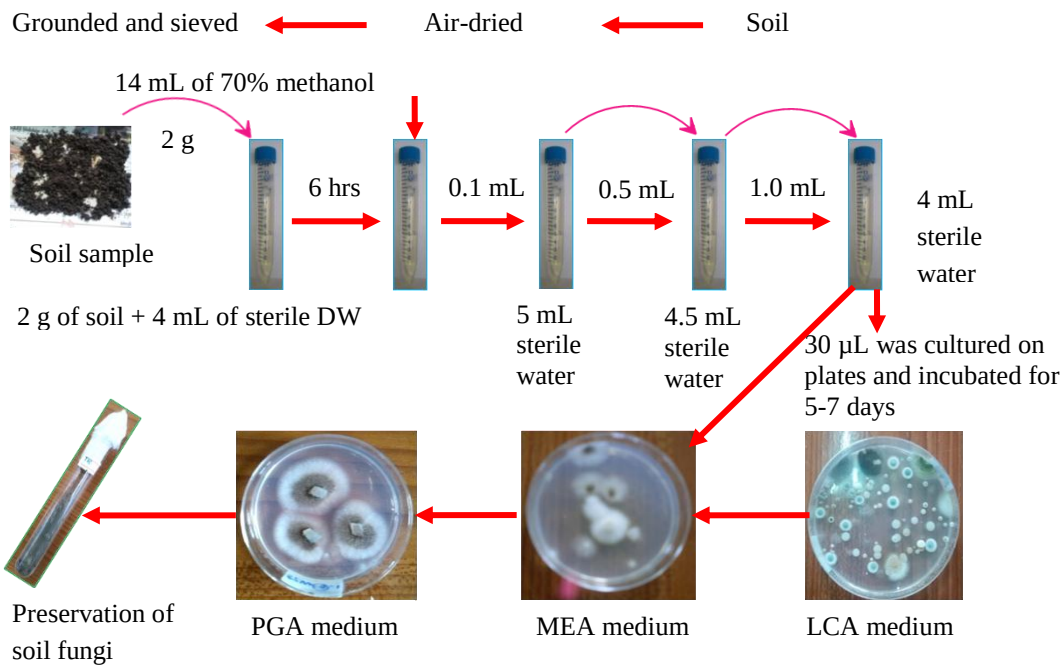

Figure 2 Isolation procedure for fungi from soil samples

Chemical treatment dilution method (Phay & Yamamura, 2005)

- Soil was air-dried at room temperature
- Grounded and sieved
- 2 g of the sieved soil is put into test tube
- 4 ml of sterilized distilled water is also put into the tube containing soil
- 14 ml of 70% of Methanol solution is then added into the tube containing soil suspension and shaken for 1 minute and dilution with sterile water
- Culture on LCA medium and MEA medium

Media Used for the Isolation of Fungi (Ando, 2004)

Low Carbon Agar (LCA) medium

Components per liter (for first culture)

Glucose	2 g
Sucrose	2 g
K_2HPO4	1 g
$MgSO_4.7H_2O$	0.5 g
KNO_3	1 g
KCL	0.5 g
Agar	18 g
pH	6.5

After autoclaving chloramphenicol was added into the medium.

Potato Glucose Agar (PGA) medium

Components per liter

(for transfer culture)

Potato	20 g
Agar	18 g
Glucose	2 g
pH	6.5
DW	1000 ml

After autoclaving chloramphenicol was added into the medium.

Malt Extract Agar (MEA) medium
Components per liter (for first culture)

Malt Extract	20 g
Agar	20 g
Glucose	20 g
Peptone	1.0 g
pH	6.5
DW	1000 ml

After autoclaving chloramphenicol was added into the medium.

Medium used Antimicrobial activity test (Ando, 2004)
(Seed Medium)

Glucose	15 g
Yeast extract	3 g
Polypeptone	2 g
K_2HPO_4	0.01 g
DW	1000 ml

Medium used Antimicrobial activity test (Ando, 2004)
(Fermentation Medium)

Glucose	10 g
Yeast extract	3 g
Polypeptone	2 g
K_2HPO_4	0.01 g
DW	1000 ml

(Assay Medium)

Glucose	5 g
Polypeptone	2 g
Agar	16 g
DW	1000 ml

Isolated strains were subjected with antimicrobial activities by agar well method. Cork borer was used to make the wells (8 mm in diameter) in the autoclaved basal antimicrobial test-medium. Wells impregnated with 3-10 days old culture fermented broth (20 µl) were incubated at room temperature for 24-48 hours. After 24-48 hours of incubation, the clear zones were measured. Therefore, the diameter of clear zones has observed as potent activity shown by respective strain. Clear zones surrounding the test wells indicated the presence of antimicrobial activities which inhibit the growth of the test organisms selectively (Collins, 1965).

Table 2 Test organisms used in antimicrobial activities (NITE, 2004)

Sr No	Test organisms	Infections
1	*Agrobacterium tumefaciens* NITE 09678	Plant disease
2	*Aspergillus paraciticus* IFO 5123	Fruit disease
3	*Micrococcus luteus* NITE 83297	Skin disease
4	*Pseudomonas fluorescens* IFO 94307	Rice disease

Results

Isolation of fungi from soil samples

In the investigation, 20 fungi were isolated from the four different soil samples of BeikthanoAcient city, Taungdwingyi Township, Magway Region. Isolated fungi NLF-1 to NLF-5 were collected from Gokkone village, NLF-6 to NLF-15 from Shwe-yaung-taw, NLF-11 to

NLF-15 from Nan-twin-taw-ya Monastery and NLF-16 to NL-20 were collected from Beikthano station. These results were shown in Table 3 and Fig. 3-4.

The isolated fungi were designated as NLF-1 to NLF-20. The surface color of isolated fungi NLF-1, 3, 17 and 20 were white and their reverse colors were white, pink, pale yellow and yellow. The surface color of isolated fungi NLF-2 is greenish yellow and reverse color was cream. The surface color of isolated fungi NLF-4, 5, 8, 10 and 18 were green and their reverse color were white, pale orange, pale yellow, cream and black green. The surface color of isolated fungi NLF-6 and 9 were pale orange and their reverse color were white and greenish yellow. The surface color of isolated fungi NLF-7 was black and its reverse color was gray.

The surface color of isolated fungi NLF-11 and 13 were pale brown and their reverse color were brown and cream. The surface color of isolated fungi NLF-12 was gray and its reverse color was gray. The isolated fungi NLF-14 was greenish white and its reverse color was cream. The surface color of isolated fungi NLF-15 was yellowish brown and its reverse color was brownish white. The surface color of isolated fungi NLF-16 and 19 were brownish yellow and their reverse color were brownish orange.

Table 3 Isolated fungi from soil samples

Soil samples No.	LCA Medium	MEA Medium
S.I	NLF-1, NLF-2	NLF-3, NLF-4, NLF-5
S.II	NLF-6	NLF-7, NLF-8, NLF-9, NLF-10
S.III	NLF-11, NLF-12	NLF-13, NLF-14, NLF-15
S.IV	NLF-16, NLF-17	NLF-18, NLF-19, NLF-20

Table 4 Morphological character of isolated fungi

No.	Isolated Fungi	Character	
		Surface color	Reverse color
1	NLF-1	White	White
2	NLF-2	Greenish yellow	Cream
3	NLF-3	White	Pink
4	NLF-4	Green	White
5	NLF-5	Green	Pale orange
6	NLF-6	Pale Orange	White
7	NLF-7	Black	Gray
8	NLF-8	Green	Pale yellow
9	NLF-9	Pale Orange	Greenish yellow
10	NLF-10	Green	Cream
11	NLF-11	Pale brown	Brown
12	NLF-12	Gray	Gray

No.	Isolated Fungi	Character	
		Surface color	Reverse color
13	NLF-13	Pale brown	Cream
14	NLF-14	Greenish white	Cream
15	NLF-15	Yellowish brown	Brownish white
16	NLF-16	Brownish yellow	Yellow
17	NLF-17	White	Pale yellow
18	NLF-18	Green	Black green
19	NLF-19	Brownish yellow	Brownish orange
20	NLF-20	White	Yellow

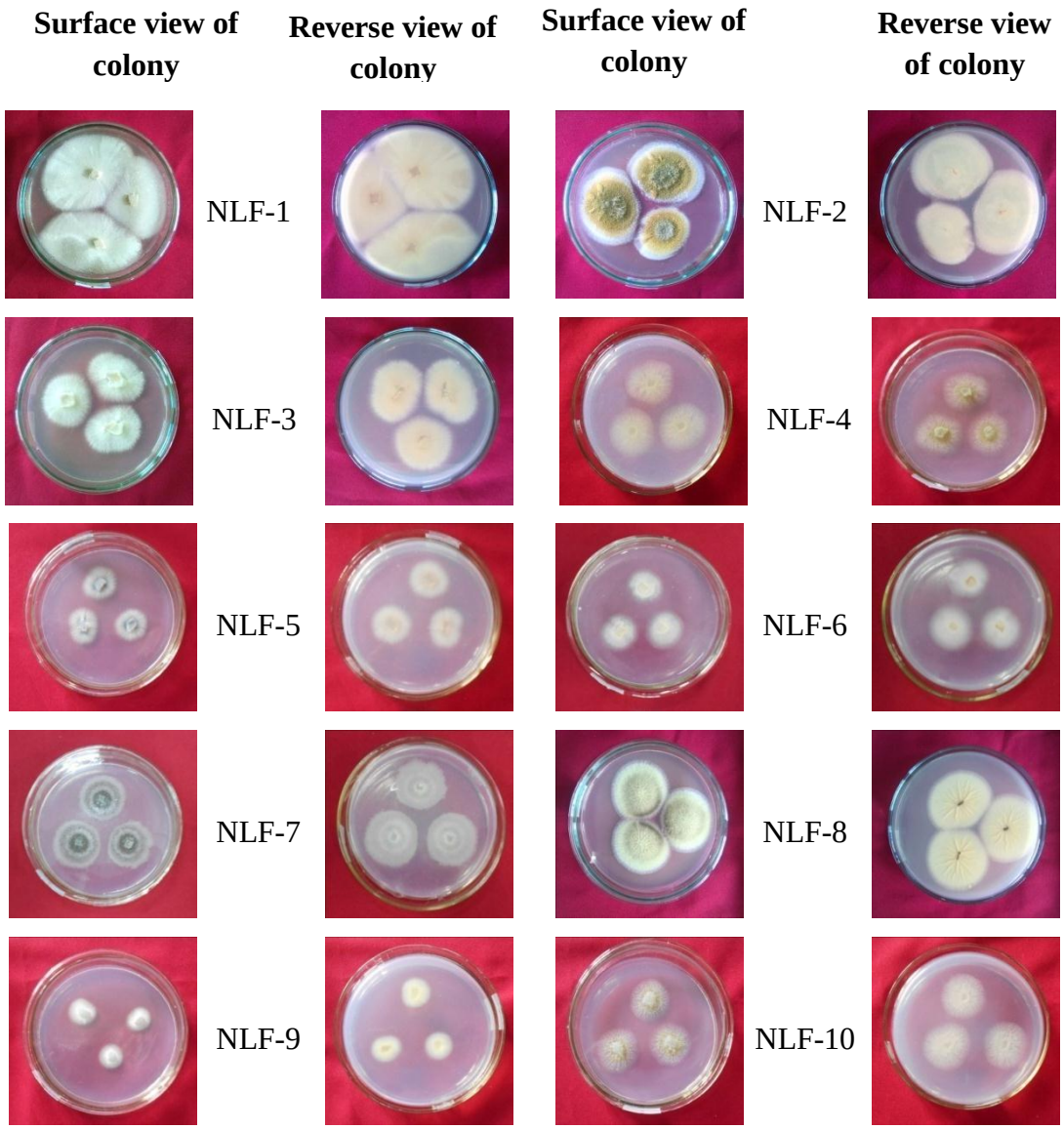

Figure 3 Morphological characters of isolated fungi NLF-1 to NLF-10 (5-days)

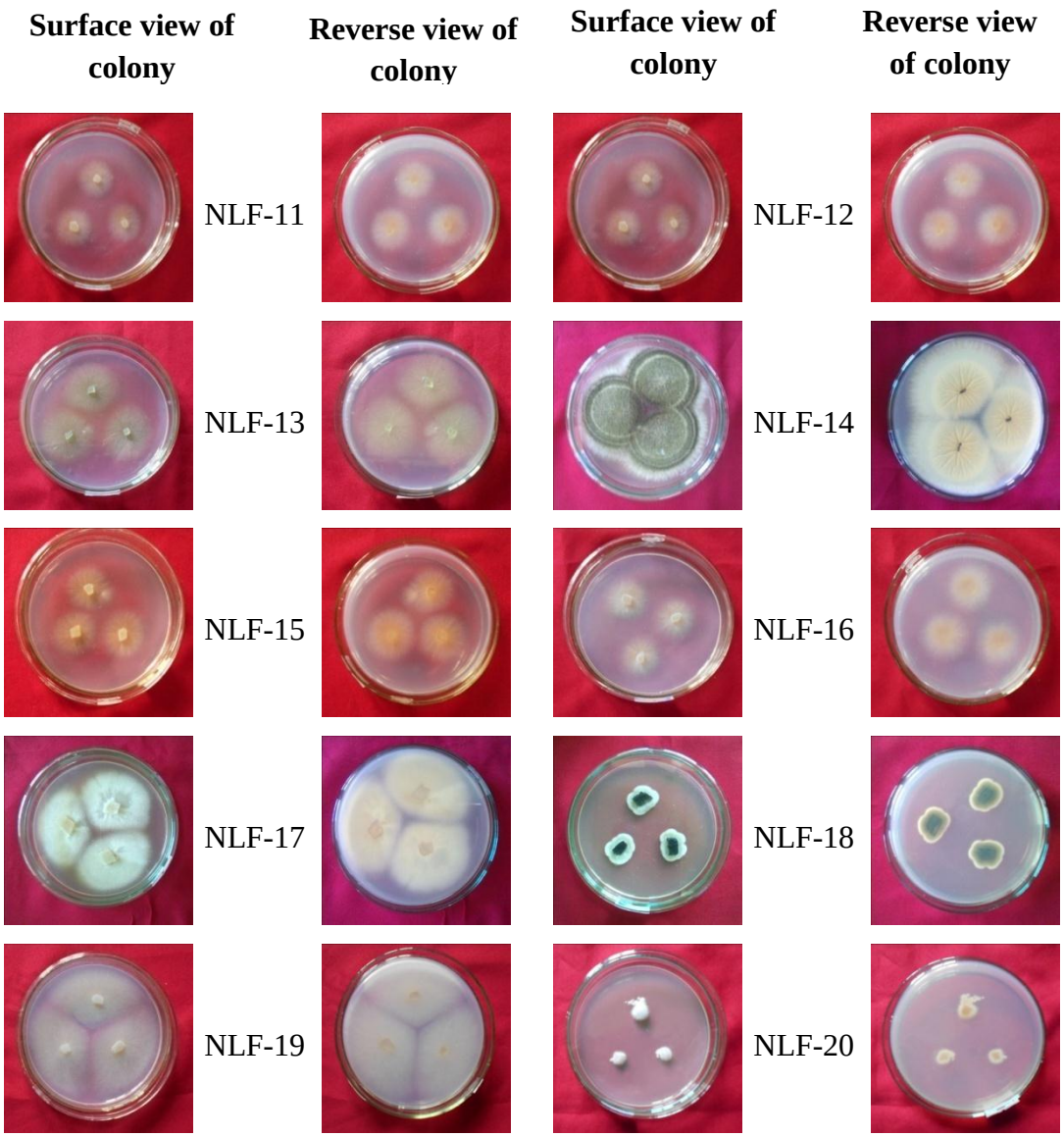

Figure 4 Morphological characters of isolated fungi NLF-11 to NLF-20 (5-days)

Figure 5 Pure culture of isolated fungi from soil sample 4 (NLF 1-20)

Antimicrobial activities of isolated fungal strains

Nine isolated fungi (NLF-6, 7, 9, 10, 12, 14, 15, 16 and 18) had antimicrobial activity and remaining eleven isolates (NLF-1, 2, 3, 4, 5, 8, 11, 13, 17, 19 and 20) could not produce antimicrobial metabolites. NLF-14 showed the highest antimicrobial activity (32.53mm and

32.43mm) against *Agrobacterium tumefaciens* and *Aspergillus paraciticus* in 6 days fermentation period respectively. NLF-12 showed the best inhibition zone (34.85mm) on *Micrococcus luteus* and NLF-6 showed the antibacterial activity (30.26mm) against *Pseudomonas fluorescence* in 5 days fermentation period. These results were displayed in figure (6, 7, 8). Among them, antibacterial activity of isolated fungus NLF-12 showed the maximum inhibitory zone against *Micrococcus luteus*.

Table 5 Antibacterial Activity of Nine Selected Fungi against *Agrobacterium tumefaciens*

Sr No.	Selected fungi	Fermentation Period (Days) and Inhibition Zone (mm)							
		3	4days	5days	6days	7days	8days	9days	10days
1	NLF-6	17.8	19.56	21.84	28.49	26.50	24.32	23.66	20.71
2	NLF-7	19.3	25.99	29.40	28.70	25.43	19.80	17.23	15.63
3	NLF-9	18.4	20.56	23.73	18.43	15.66	14.73	13.56	12.00
4	NLF-10	13.6	16.54	17.06	23.43	18.95	15.12	14.00	13.21
5	NLF-12	16.3	16.66	18.95	24.38	25.41	22.80	21.32	17.00
6	NLF-14	23.1	25.04	29.00	32.53	30.05	28.74	25.00	20.00
7	NLF-15	14.5	18.00	21.52	24.00	27.00	25.12	23.00	19.00
8	NLF-16	12.5	13.73	17.60	29.56	18.74	14.20	13.18	-
9	NLF-18	19.7	20.93	25.11	27.43	22.00	23.48	21.73	18.56

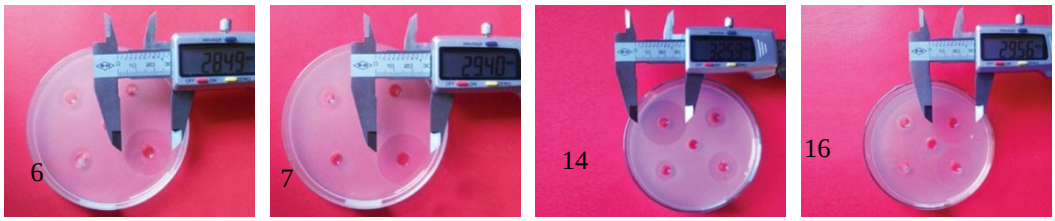

Figure 6 Antibacterial activity of isolated fungi against *Agrobacterium tumefaciens*

Table 6 Antifungal Activity of Nine Selected Fungi against *Aspergillus paraciticus*

Sr No.	Selected fungi	Fermentation Period (Days) and Inhibition Zone (mm)							
		3	4days	5days	6days	7days	8days	9days	10days
1	NLF-6	20.	27.34	28.93	30.73	30.10	29.40	28.50	23.00
2	NLF-7	15.	18.16	22.96	29.96	27.14	25.56	23.12	19.00
3	NLF-9	22.	27.89	29.02	31.09	28.50	27.73	26.18	18.00
4	NLF-10	16.	19.38	20.32	25.25	17.78	16.13	14.50	-
5	NLF-12	18.	25.76	30.12	28.34	24.70	20.82	17.12	15.56
6	NLF-14	16.	17.19	24.45	32.43	19.67	19.46	17.64	16.59
7	NLF-15	15.50	18.23	20.12	25.43	17.56	16.12	15.04	-
8	NLF-16	17.13	19.28	23.43	25.76	20.84	22.76	19.43	17.21
9	NLF-18	20.44	24.01	26.05	25.22	24.78	22.02	19.82	16.00

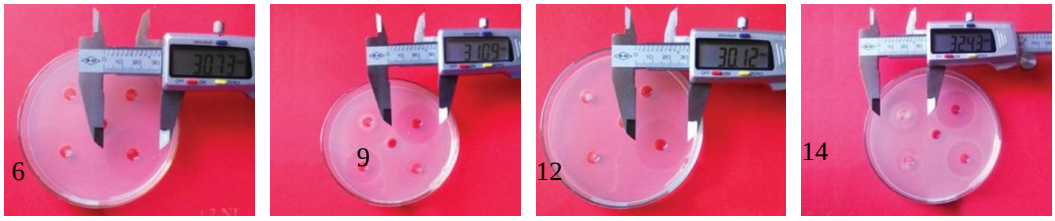

Figure 7 Antifungal activity of isolated fungi against *Aspergillus paraciticus*

Table 7 Antibacterial Activity of Nine Selected Fungi against *Micrococcus luteus*

Sr No.	Selected fungi	Fermentation Period (Days) and Inhibition Zone (mm)							
		3	4days	5days	6days	7days	8days	9days	10days
1	NLF-6	24.35	16.89	27.12	28.43	26.43	23.12	20.42	18.00
2	NLF-7	14.12	20.95	28.54	26.42	23.78	20.43	19.12	17.13
3	NLF-9	20.12	24.56	28.43	30.09	27.12	25.43	24.56	19.84
4	NLF-10	18.12	19.00	23.43	25.73	20.45	18.84	12.11	11.56
5	NLF-12	22.54	28.76	34.85	29.74	28.70	25.43	20.12	18.64
6	NLF-14	17.84	19.21	23.42	27.12	18.43	17.18	15.43	13.21
7	NLF-15	15.10	17.64	19.81	20.43	18.12	16.00	14.21	12.55
8	NLF-16	11.51	16.54	19.21	23.78	25.64	24.12	22.56	13.96
9	NLF-18	14.56	17.12	19.96	25.43	18.53	16.12	15.41	14.12

Figure 8 Antibacterial activity of isolated fungi against *Micrococcus luteus*

Table 8 Antibacterial Activity of Nine Selected Fungi against *Pseudomonas fluorescens*

SrNo.	Selected fungi	Fermentation Period (Days) and Inhibition Zone (mm)							
		3 days	4days	5days	6days	7days	8days	9days	10days
1	NLF-6	20.41	25.64	30.26	28.12	26.43	24.12	22.76	19.48
2	NLF-7	15.53	17.12	18.94	20.42	23.73	20.65	18.56	16.72
3	NLF-9	17.34	19.42	23.43	29.99	22.56	20.43	18.76	17.00
4	NLF-10	18.74	20.60	26.60	24.12	22.76	20.12	18.42	16.00
5	NLF-12	15.64	18.67	25.27	23.00	21.56	19.56	17.43	16.72
6	NLF-14	19.93	21.43	23.56	26.00	24.12	20.56	19.00	17.76
7	NLF-15	13.97	15.82	16.36	17.84	18.03	19.67	16.43	15.00
8	NLF-16	14.54	16.76	18.43	20.76	17.12	15.43	14.12	-
9	NLF-18	21.43	25.64	26.00	27.77	23.43	20.56	18.80	17.00

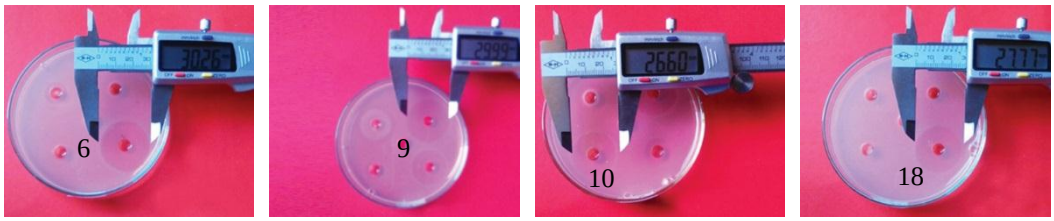

Figure 9 Antibacterial activity of isolated fungi against *Pseudomonas fluorescens*

Discussion and Conclusion

Fungi grow on diverse habitats in nature and are cosmopolitan in distribution requiring several specific elements for growth and reproduction. In laboratory, these are isolated on specific culture medium for cultivation, preservation, microscopical examination and biochemical and physioloigcal characterization. A wide range of media are used for isolation of different groups of fungi that influence the vegetative growth and colony morphology, pigmentation and sporulation depending upon the composition of specific culture medium, pH, temperature, light, water availability and surrounding atmospheric gas mixture (Northolt and Bullerman, 1982).

A total of twenty fungi were isolated from four soil samples collected at Beikthano Ancient City, Taungdwingyi Township, Magway Region. The fungi NLF-1, 2, 3, 4, & 5 were isolated from Gokkone village (Loam), NLF-6, 7, 8, 9 & 10 from Shwe Young Taw (sandy loam), NLF-11, 12, 13, 14 & 15 from Nan Twin Taw Ya monastery, (sandy loam) NLF-16, 17, 18, 19 & 20 from Beikthano station (sandy loam). All these fungi were different according to their morphological characters. NLF-1, 2, 6, 11, 12, 16 & 17 were white, greenish yellow, pale orange, pale brown, gray and white in the surface and reverse color was white, cream, brown, gray and pale yellow on the LCA medium.

Sharma (2010) reported that white to orange with green spores at centre and bright orange on reverse side in Potato Dextrose Agar (PDA) and colourless on both side Low Carbon Agar (LCA) medium. NLF-3, 4, 5, 7, 8, 9, 10, 13, 14, 15, 18, 19 & 20 were white, green, black, pale orange, greenish white and brownish yellow in the surface and reverse color were pink, white, gray, pale yellow, greenish yellow, cream, black green on the MEA medium. Ilhan (2006) reported that colonies on MEA were soluble pigment was lemon yellow, reverse was luteus to lemon yellow in colour.

In the investigation of antimicrobial activities, twelve fungi were tested with four test organisms by using Agar Well Method. Among them, NLF-6, 7, 9, 10, 12, 14, 15, 16 & 18 showed the antimicrobial activity on *Agrobacterium tumefaciens, Aspergillus paraciticus, Micrococcus luteus* and *Pseudomonas fluorescens.*

All of them, NLF-14 showed the highest antimicrobial activity (32.53 mm and 32.43 mm) against *A. tumefaciens* and *A. paraciticus* respectively. Moreover, NLF-12 showed the best inhibition zone (34.85 mm) on *Micrococcus luteus* and NLF-6 showed the antibacterial activity (30.26 mm) against *P. fluorescens.* According to the results, NLF-12 gave the highest antibacterial activity.

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