ISOLATION AND ANTIMICROBIAL ACTIVITY OF ISOLATED SOIL FUNGI FROM HOMALIN TOWNSHIP, SAGAING REGION

Hlaing Myint Thu[1], Zar Zar Yin[2], Ye Myint Aung[3]

Abstract

In the research work, soil samples were collected from six different places of Homalin Township during July 2017. Soil samples were collected from 0-15 cm depth after removing the surface soil for the isolation of fungi. After three days, soil fungi were isolated by the serial dilution method on six different media. A total of 41 fungal strains were isolated and the surface colour of isolated fungi HMT-1 to HMT-41 were white, black, green, pale green, greenish white, dark green, tan, yellow, yellowish green, plum, pale pink, gray and their reverse colour were cream, yellowish cream, greenish cream, buff, brown and purple. In the colony morphology, the isolated fungi were small, medium and large in size. The margin of isolated fungi were entire, undulate, filiform, lobate and the elevation of isolated fungi were raised, convex, flat, umbonate and the form of isolated fungi were circular, irregular, filamentous and rhizoid. Moreover, physicochemical properties of soil from different locations of Homalin Township were analyzed. Some physicochemical properties of soil samples were preliminaries determined and the collected soil samples were found to the rich in fungal strains due to acidic (4.67-5.80), moisture content (0.91 –7.23), organic matter (3.33- 7.98), temperature (25- 27 ˚C) and texture. Soil texture was examined by hydrometer method. Furthermore, all fungal strains were tested by seven test organisms for preliminary study of antimicrobial activity. Among them, twenty strains showed different levels of antimicrobial activity. Especially, HMT-33 showed the moderate antimicrobial activity against all test organisms. Therefore, strain of HMT-33 may be useful by the pharmaceutical industries for the production of antibiotics from local sources.

Keywords: soil fungi, filamentous, physicochemical properties, antimicrobial activity

Introduction

The soil serves as a reservoir for many microbial communities of plants and herbs which can be producing, CO_2 and nitrogen cycle. The microorganisms plays major role in soil ecosystem. Microbial composition and functioning changes the soil quality through decomposition of organic matter, recycling of nutrients and biological control (Stefanis *et al*., 2013). It is very hard to find substrata not isolated any microbes in nature. Therefore, any substrata collected in nature are useful materials for isolating microbes including fungi. Different materials have been reported as the substrata or the samples for fungi. The typical materials are soil, living and fallen leaves, leaf litters, dung, insects, fresh water, marine water and so on. Therefore, soil sample is the most effective and popular materials for especially isolating a number of fungi. The soil sample collected at various places such as paddy field, grain and vegetable fields, conifer or broadleaf wood forests, high mountain area, and etc. For the isolating different fungi, it is more effective to collected surface soil under leaf litter, because the fungal population is densely at the soil surface than the depth (Ando, 2004).

Antimicrobial agents play the most important role in the treatment of bacterial infections (Hacioglu and Dulger, 2011) and wide spread efforts have been carried out by many scientists in

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order to screen for novel antibiotic production microbes (Oskay *et al.*, 2004). The presence of fungi in soil has been well described showing that the environment contains an enormous biodiversity which can be screened for antibiotics production (Gunatilaka, 2006). Through their efforts, many antibiotics have been discovered successfully to combat pathogenic bacteria that cause diseases. Nevertheless, the emergence of new disease and reemergence of multiple-antibiotic resistance pathogens that render the effectiveness of existence clinically used antibiotics have spurred the needs for the discovery of new antibiotics (Roberts, 1998). Several mechanisms of actions of antibiotics have been discovered by scientists. These actions include the inhibition of cell wall, protein and nucleic acids synthesis (Lambert, 1977). However, soil ,which is a naturally occurring loose mixture of mineral and organic particles, still remains the most important target for most researchers in their efforts to discover novel antibiotics which have pharmaceutical values (Najad *et al.*, 2013). According to Dulmage and Rivas(1978), soil microorganisms have continually been screened for their useful biological active metabolites, such as antibiotics since long ago. Therefore, the aim of the research work was to produce antimicrobial compounds by isolated fungi from six different places soil from Homalin Township.

Materials and Methods

Method for Collection of Soil Sample

The soil samples were collected from six different places in various locations of Homalin Township, during July 2017 (Table 1). The soil samples were collected from different places (up to 15 cm depth) into sterilized polythene bags after removing the surface soil for the isolation of fungi and brought to the laboratory of Biological Resources and Biotechnology Development Center at Pathein University.

Physicochemical Analysis of Soil Samples

The collected soil samples were characterized for its physicochemical properties. Physicochemical parameters include organic carbon, organic nitrogen, pH, water content and temperature etc., microbial population density generally decreases with depth as a function of the availability of organic carbon and molecular oxygen, parameters which typically decrease with depth. Temperature and colour of the soil samples was recorded on the spot. The other physicochemical parameters of the soil samples were analyzed at Land Use, Department of Agriculture, Yangon in Myanmar

Isolation of Fungi from the Soil Samples

The soil micro fungi were isolated by serial dilution method (Dubey and Maheshwari, 2002) on different media such as Blaskeslee's Malt Extract Agar (BMEA Medium), Czapek-Dox Agar (CZA Medium), Malt Extract Agar (MEA Medium), Dichloram Rose Bengal–Chloramphenicol Agar (DRBC Medium), Glucose Ammonium Nitrate Agar (GAN Medium), Potato Glucose Agar (PGA Medium) .

Table 1 Collected Soil Samples from Six Different Places at Homalin Township

Soil sample No.	Place	Location
1	Nam Khan Village	N 24º 48' 32.572"
		E 95˚ 09' 23.267"
2	Tone Kham Village	N 24º 50' 05.750"
		E 95˚ 08' 33.089"
3	Naung Taw Village	N 24º 49' 39.211"
		E 95˚ 07' 07.066"
4	Naung Po Aung Village	N 24º 49' 05.805"
		E 95˚ 03' 52.663"
5	Hae Khamm Village	N 24º 51' 46.247"
		E 95˚ 02 12.275"
6	Naung-Pa-Kyit Quarter	N 24º 52' 26.395"
		E 94˚ 54' 43.868"

Serial Dilution Method

One gram of soil sample was introduced into a conical flask containing 99 mL of distilled water. The flask was than shaken for about 30 min in order to make the soil particles free from each other. This solution was then serial diluted from 10^{-3} to10^{-7} dilution in separate test tubes and 0.5 mL each of the above dilution was separately transferred into sterile petridishes under aseptic condition. The sterilized medium in conical flask was cooled down to about 45 ºC and separately poured into each of the petridish containing the respective soil dilutions. The inoculated plates were shaken clock-wise and anti-clock wise direction for about 5 min so as to make uniform distribution of the fungi inoculums. When the agar was solidified, the inoculated plates were inverted and incubated at 27-30 ºC for 3-7 days. Isolated pure fungi were preserved into slant culture (Atlas, 1993) containing BMEA medium for further experiments.

Agar Well Method

Isolated strains were tested by agar well method (Collins, 1965) for the preliminary antimicrobial activities. Cork borer was used to make the wells (8 mm in diameter) in the autoclaved basal antimicrobial test-medium. Wells impregnated with 3-6 days old culture fermented broth (20 µL/ well) were incubated at room temperature for 24-28 h. After 24- 28 h of incubation, the clear zones were measured. Therefore, the diameter of clear zones had been observed as potent activity as shown by respective strain. Clear zones surrounding the wells indicated the presence of antimicrobial activities which inhibit the growth of the test organisms selectively.

Test Organisms

The test organisms used for this experiment were *Agrobacterium tumefaciens* NITE 09678, *Bacillus pumilus*, *Bacillus subtilis* IFO 90571, *Candida albicans* NITE 09542, *Escherichia coli* AHU5436, *Pseudomonas fluorescens* IFO94307, *Staphylococcus aureus* AHU8465. The organisms were obtained from National Institute of Technology and Evaluation (NITE, Japan), and Pharmaceutical Research Department, Yangon, Myanmar.

Results and Discussion

Physicochemical analysis showed that pH of the soil is acidic and is rich with both macro and micro nutrients which is favorable for the growth of fungi. Fungal diversity of any soil depends on a large number of factors of the soil such as pH, organic content, and moisture (Rangaswami and Bagyaraj, 1998).

Eilers *et al.*, (2012) reported that soil microbial abundance and diversity are highest in the top 10 cm and decline with depth. Soil samples were collected from 0-15 cm depth after removing the surface soil for the isolation of fungi. Analysis of six different soils of Homalin Township revealed very optimum moisture and organic contents (Table 2). The colour of soil samples was red, brown and black with variation in pH (4.67–5.80). The temperature of soil environments of Homalin Township at the time of this investigation of July, 2017 (rainy season) revealed that the soil component of Homalin Township had temperature range between 25 °C and 27 °C with great variation in moisture content (0.91-7.23), percent organic carbon (1.93-4.63), organic nitrogen (1.66-3.99), percent humus (3.33-7.98). Total number of colonies obtained in Nam Khan is 14 with pH 4.67 (moisture 3.11%), Tone Kham is 11 strains with pH 5.03 (moisture, 0.91 %), Naung Taw is 11 with pH 5.49 (moisture 5.10 %), Naung Po Aung is one strains with pH 5.80 (moisture 1.91 %), Hae Khamm is 3 strains with pH 5.44 (moisture 7.23 %) and Naung Pa Kyit is 1 strain with pH 4.98 (moisture 4.32 %). This shows that low pH and optimum moisture content favour the growth of Fungi. Distribution of soil fungi depending upon the nature of the organic content, climatic conditions, surface vegetation and soil texture (Marchner *et al*., 2003). Direct relationship is observed between the soil texture and moisture content. Silt and clay soil holds the highest moisture content that is why there is increased population of fungi is observed. The frequency of mycoflora in different fields were found to be regulated by many factors like temperature, humidity, vegetation, organic and inorganic materials, soil type of texture. The fungi were moistly observed in month of June to September due to suitable temperature and humidity (Vinay *et al*., 2015). Brodie *et al.*, (2003) and Pfenning (2006) also reported that the forest areas sampled have higher plant diversity than the other systems of land use (agriculture, pasture and agroforestry). Besides, the results obtained from this study has shown to be similar with the research conducted by Tangjang and Arunachalam (2009) where they found out that there was greater amounts of bacterial and fungal populations in the top soil (0-10 cm) if compared to that of other depths. This might be due to the high organic contents found in the top soil where humus is abundantly presence, especially for the forest floor that is often covered by wilted leaves that tend to decompose.

Table 2 Physico- chemical Properties of the Soil Samples Collected from Six Different Places of Homalin Township

Sample No.	Place	Soil color	Texture	pH	T (˚C)	Moisture (%)	Organic carbon (%)	Organic nitrogen (%)	Humus (%)
1	Nam Khan	Red	CL	4.67	25	3.11	2.48	2.14	4.28
2	Tone Kham	Brown	SCL	5.03	26	0.91	1.93	1.66	3.33
3	Naung Taw	Black	SICL	5.49	26	5.10	2.70	2.33	4.66
4	Naung Po Aung	Red	SCL	5.80	27	1.91	1.94	1.67	3.34
5	Hae Khamm	Black	SIL	5.44	26	7.23	4.63	3.99	7.98
6	Naung Pa Kyit	Red	SICL	4.98	27	4.32	2.12	1.83	3.65

**CL-clay loam, SCL- sandy clay loam, SICL- silty clay loam, SIL- silty loam

In the present investigation, 41 strains fungal were isolated from six different soils samples of Homalin Township by using six different media including BMEA, CZA, MEA, DRBC, GAN and PGA medium and incubated for 3-7 days at room temperature (Table 3). HMT-1 colony diameter was 3.8-4.3 cm, HMT-2 (3.5-4.2 cm), HMT-6 (3.6-4.4 cm), HMT-7 (3.3-4cm), HMT-17 (4.2-4.2 cm), HMT-27 (4.33-6 cm), HMT-28 (3.8-4.5cm), HMT-29 (4.3-5.7 cm) after 5 days incubation on BMEA medium. So, these isolated fungi were large in size and HMT-3 colony diameter was (2.5-2.5 cm), HMT-4 (3.5-3.3 cm) HMT-8 (2.1-2.5 cm), HMT-9 (2.4-2.5 cm), HMT-11 (2-2 cm), HMT-15 (3.8-3.3 cm) HMT-16 (3.7-3.8 cm), HMT-18 (3.0-3.1 cm), HMT-19 (3.1-3.1 cm), HMT-24,30, 31, 33, 39, 40, 41 were (2.5-2.5 cm), HMT-25 (2.3-2.3 cm) after 5 days incubation on BMEA medium. Thus, these isolated fungi were medium in size and other strain colony diameters have less than 2 cm. Therefore, another strains colony diameter were small in size. The surface colour of HMT-1, 4, 6, 15, 20, 23, 26, 27, 28, 29, 34, 40 were white and the surface colour of other strains were greenish white, white cotton, dark green, tan, yellow, yellowish white, yellowish green, plum, black, pale pink, gray, greenish yellow, straw, pale green. The reverse colour of HMT-1, 2, 3, 4, 7, 10, 14, 17, 18, 10, 22, 23, 26, 29, 32, 38, 39 were cream and the other strains were brown, yellow, plum, green, yellowish cream, white cream, greenish cream, dark, buff cream, gray, yellow, purple respectively (Figures 1-5). All fugal strains were cultured, preserved, observed on BMEA medium, due to BMEA medium is best growth and isolated of fungi than other media. Ando (2004) reported that many fungi grow robustly on BMEA medium. After incubating fungi for 5 days, colony morphology of isolated fungi were photographed and measured. The form of colonies were circular, irregular, filamentous and rhizoid. The elevation of colonies were raised, convex, flat, umbonate, and the margin of colonies were entire, undulate, filiform, curled, and lobate.

Table 3 Isolation of Fungi by Using Six Different Media and Soil Samples

No. of Soil Samples	BMEA Medium	CZA Medium	MEA Medium	DRBC Medium	GAN Medium	PGA Medium	No.of isolated fungal strains
1	HMT-1, 2,3,4,5	HMT-6,7,8	HMT-9, 10,11,12,13	-	HMT-14	-	14
2	HMT-15, 16,17	-	HMT-18	HMT-19, 20	HMT-21, 22	HMT-23, 24,25	11
3	HMT-26, 27,28,29	-	-	HMT-30, 31	HMT-32, 33	HMT-34, 35,36	11
4	HMT-37	-	-	-	-	-	1
5	HMT-38, 39,40	-	-	-	-	-	3
6	HMT-41	-	-	-	-	-	1
	17	3	6	4	5	6	41

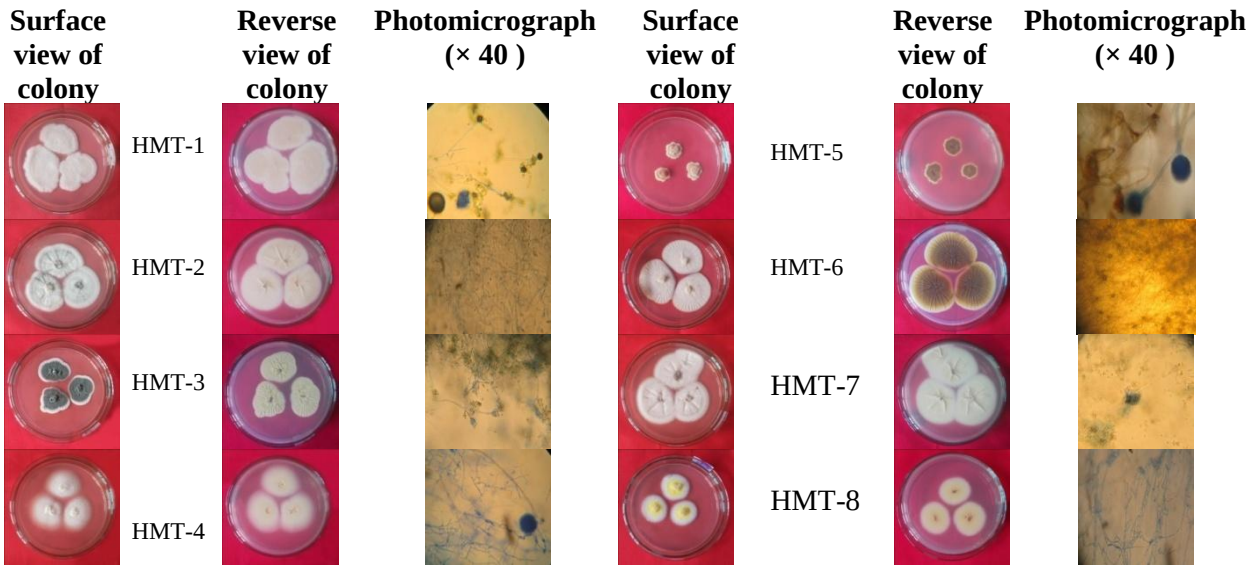

Figure 1 Morphology and photomicrograph characters of isolated fungi HMT-1, 2, 3, 4,5, 6,7,8

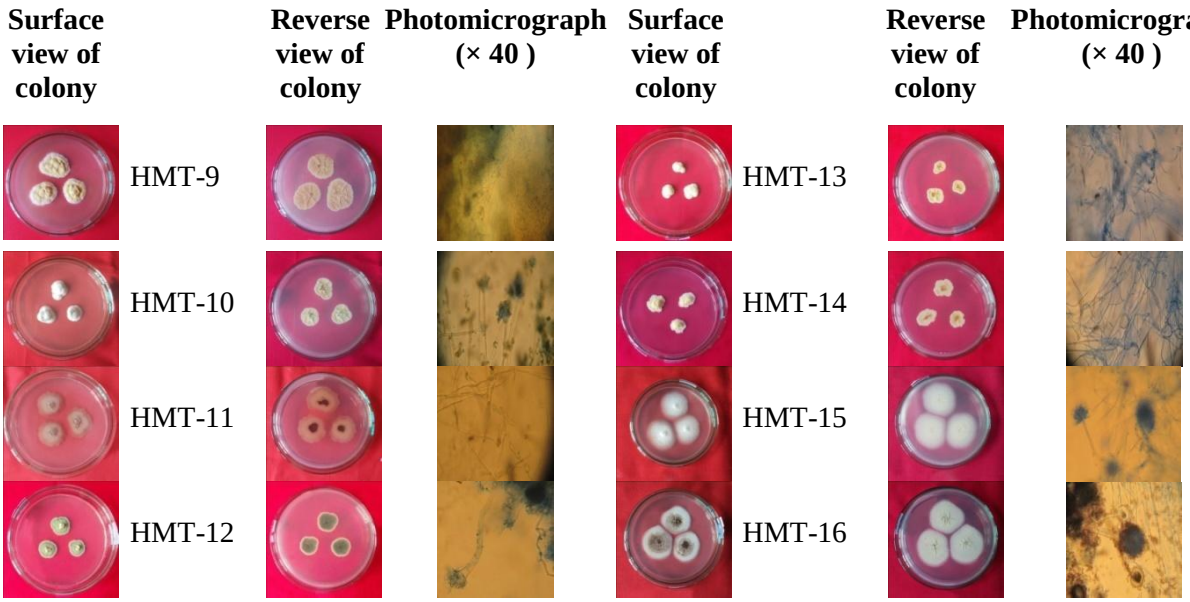

Figure 2 Morphology and photomicrograph characters of isolated fungi HMT-9, 10,11,12,13, 14, 15, 16

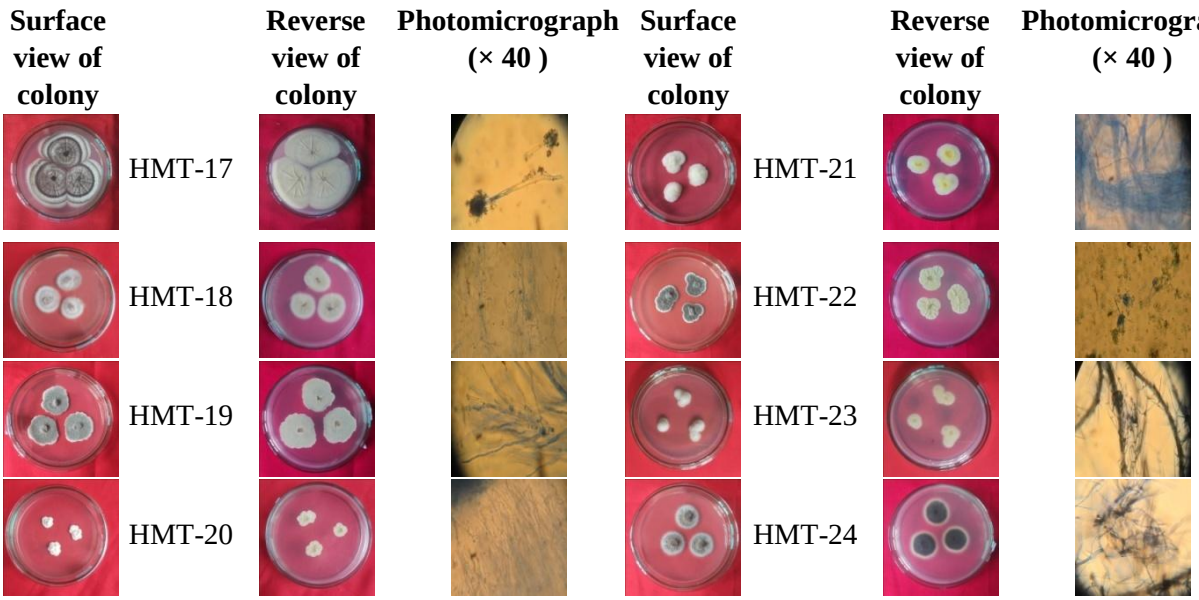

Figure 3 Morphology and photomicrograph characters of isolated fungi HMT-17, 18, 19, 20, 21, 22, 23, 24

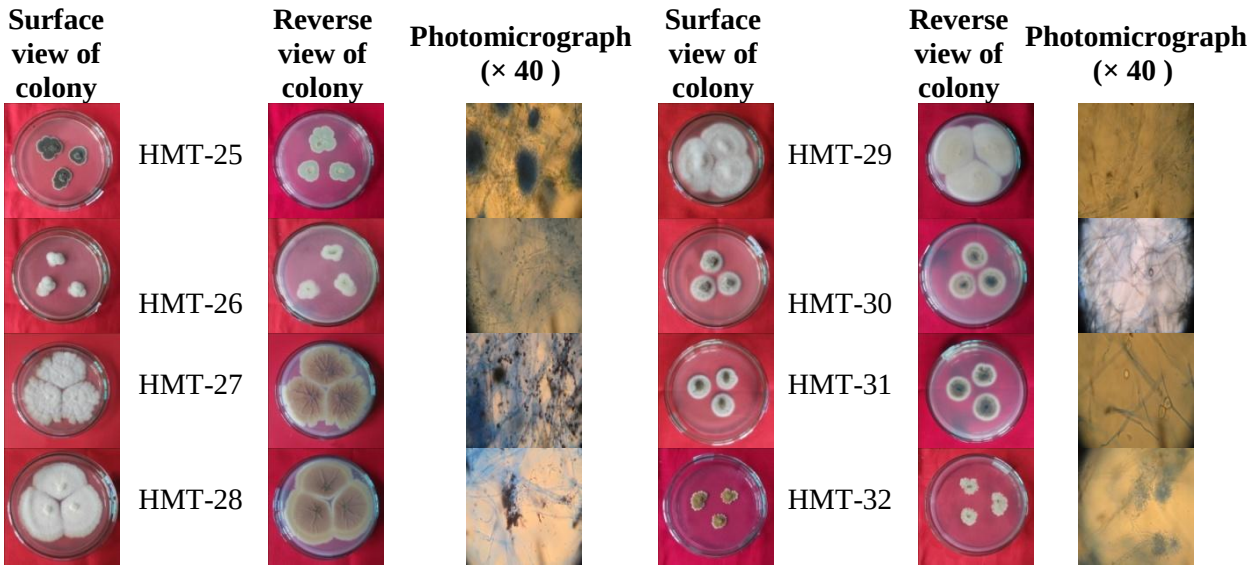

Figure 4 Morphology and photomicrograph characters of isolated fungi HMT-25, 26, 27, 28, 29, 30, 31, 32

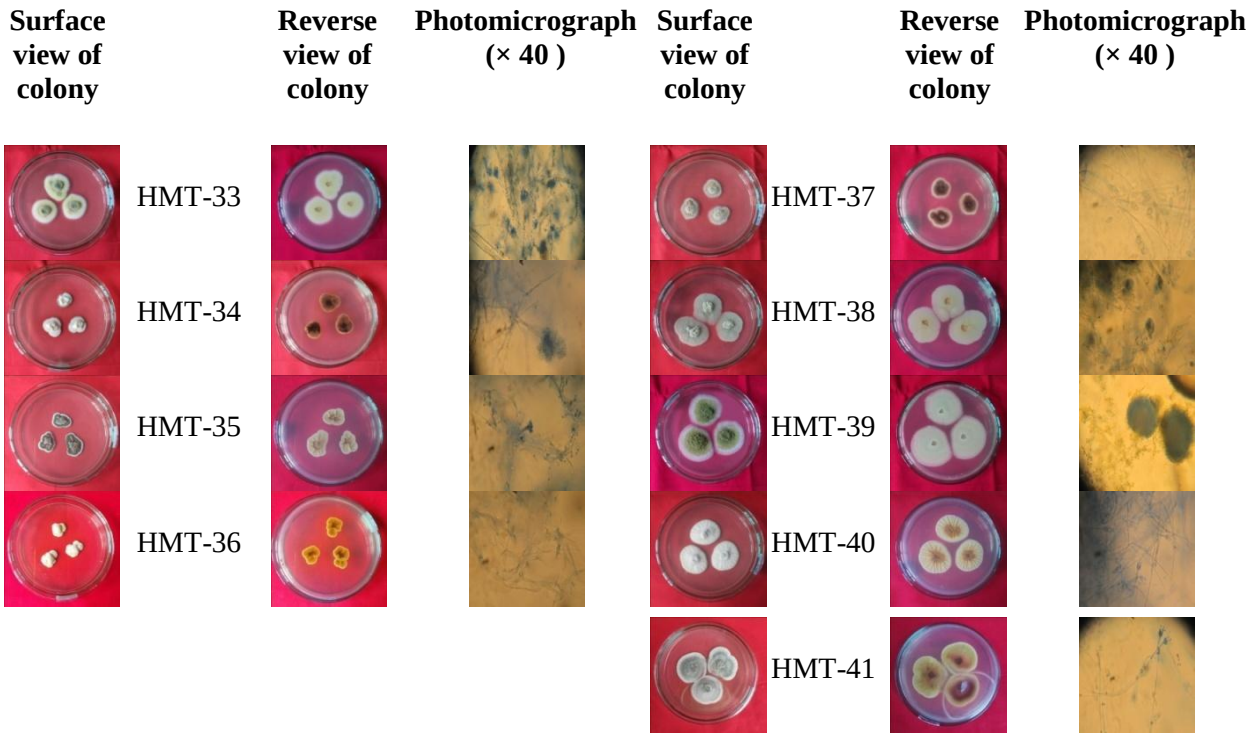

Figure 5 Morphology and photomicrograph characters of isolated fungi HMT-33, 34, 35, 36, 37, 38, 39, 40, 41

All fungal strains were tested by seven test organisms for preliminary study of antimicrobial activities. Among them, twenty strains showed different level of antimicrobial activities. HMT-38 exhibited the highest antibacterial activity (27.23 mm) against *Agrobacterium tumefaciens* at 5 days (Table 4 and Figure 6) and HMT-21 showed the high activity (23.26 mm) on *Bacillus pumilus* at 4 days (Table 5 and Figure 7). HMT-20 gave the moderate antimicrobial activity (24.54 mm, and 24.23 mm) against both *Bacillus subtilis* at 4 days(Table 6 and Figure 8) and *Candida albicans* at 5 days(Table 7 and Figure 9). And then, HMT-12 showed the antibacterial activity (24.67 mm) on *Esherichia coli* (Table 8 and Figure 10) and HMT-29

(21.69 mm) against *Pseudomonas fluorescens* (Table 9 and Figure 11) respectively. Especially, HMT-33 showed the moderated antimicrobial activity against all test organisms (Table 10 and Figure 12). Brooks (2001) reported that antibiotics are classified as broad-spectrum antibiotics when they have the ability to affect a wide range of gram- positive and gram-negative bacteria as well as fungi while antibiotic that only effective towards certain group of bacteria are known as narrow spectrum antibiotics. Therefore, HMT-33 strain was broad-spectrum antibiotics.

Table 4 Antibacterial Activity of the Isolated Fungal Strains against *A. tumefaciens*

No.	Isolated Fungi	Fermentation Period (days) and Inhibitory zone (mm)			
		3	4	5	6
1	HMT-1	+	24.84	27.21	20.99
2	HMT-5	-	22.56	26.45	19.75
3	HMT-7	+	22.38	22.37	21.32
4	HMT-29	15.55	13.57	23.04	-
5	HMT-33	+	13.93	20.75	17.16
6	HMT-37	+	+	26.80	+
7	HMT-38	+	+	27.23	-
8	HMT-39	-	-	25.17	+
9	HMT-40	+	+	26.80	+

(+) present (-) no activity Agar well =8mm

Figure 6 HMT-38 against *A. tumefaciens* at 5 days of fermentation period

Table 5 Antibacterial Activity of the Isolated Fungal Strains against *Bacillus pumilus*

No.	Isolated Fungi	Fermentation Period (days) and Inhibitory zone (mm)			
		3	4	5	6
1	HMT-1	-	+	-	-
2	HMT-5	-	+	-	-
3	HMT-12	12.99	-	-	18.36
4	HMT-18	+	+	+	-
5	HMT-20	+	21.61		-
6	HMT-21	+	23.26	--	-
7	HMT-29	-	15.87	--	-
8	HMT-30	+	15.60		-
9	HMT-33	19.61	19.74	19.67	15.24

(+) present (-) no activity Agar well =8mm

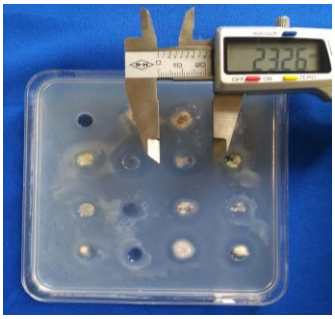

Figure 7 HMT-21 against *B.pumilus* at 4 days of fermentation period

Table 6 Antibacterial Activity of the Isolated Fungal Strains against *Bacillus subtilis*

No.	Isolated Fungi	Fermentation Period (days) and Inhibitory zone (mm)			
		3	4	5	6
1	HMT-12	+	-	19.20	20.16
2	HMT-16	-	22.65	+	-
3	HMT-18	-	+	17.00	-
4	HMT-20	-	24.54	21.61	-
9	HMT-21	-	23.29	-	-
6	HMT-27	-	+	13.43	13.43
7	HMT-29	-	19.57	18.63	18.63
8	HMT-30	-	16.92	-	-
9	HMT-33	18.61	19.06	17.35	17.54

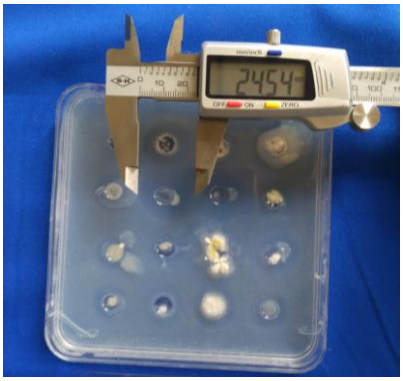

Figure 8 HMT-20 against *B.subtilis* at 4 days of fermentation period

(+)present (-) no activity

(+) present (-) no activity Agar well =8mm

Table 7 Antifungal Activity of the Isolated Fungal Strains against *Candida albicans*

No.	**Isolated Fungi**	**Fermentation Period (days) and Inhibitory zone (mm)**			
		3	**4**	**5**	**6**
1	HMT-12	12.99	-	18.24	17.98
2	HMT-16	-	-	18.65	-
3	HMT-17	-	+	14.85	-
4	HMT-18	-	+	17.98	-
9	HMT-20	-	+	24.23	-
6	HMT-27	-	+	13.42	-
7	HMT-28	-	+	+	-
8	HMT-29	-	+	15.45	-
9	HMT-33	18.59	22.97	18.47	17.54

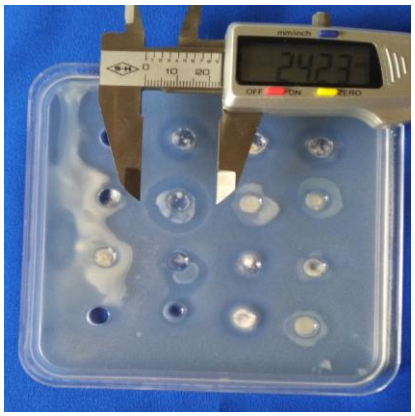

Figure 9 HMT-20 against *C. albicans* at 5 days of fermentation period

(+) present (-) no activity Agar well =8mm

Table 8 Antibacterial Activity of the Isolated Fungal Strains against *Escherichia coli*

No.	**Isolated Fungi**	**Fermentation Period (days) and Inhibitory zone (mm)**			
		3	**4**	**5**	**6**
1	HMT-1	-	24.27	-	-
2	HMT-5	-	24.27	-	-
3	HMT-12	13.58	24.67	-	17.63
4	HMT-16	-	17.52	22.12	-
9	HMT-17	-	17.14	21.40	-
6	HMT-18	-	15.82	19.17	-
7	HMT-20	-	19.30	+	-
8	HMT-21	-	16.86	-	-
9	HMT-33	19.48	20.75	18.60	16.44

Figure 10 HMT-12 against *E. coli* at 4 days of fermentation period

(+) present (-) no activity Agar well =8mm

Table 9 Antibacterial Activity of the Isolated Fungal Strains against *P. fluorescens*

No.	Isolated Fungi	Fermentation Period (days) and Inhibitory zone (mm)			
		3	4	5	6
1	HMT-16	-	-	+	-
2	HMT-17	-	-	+	-
3	HMT-18	-	-	+	-
4	HMT-20	-	-	-	-
9	HMT-21	-	-	-	-
6	HMT-27	-	-	-	-
7	HMT-28	-	-	-	-
8	HMT-29	-	-	20.52	21.69
9	HMT-33	18.06	18.79	20.13	18.70

(+) present (-) no activity Agar well =8mm

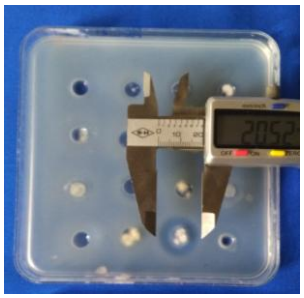

Figure 11 HMT-29 against *P. fluorescens* at 5 days of fermentation period

Table 10 Antibacterial Activity of the Isolated Fungal Strains against *S. aureus*

No.	Isolated Fungi	Fermentation Period (days) and Inhibitory zone (mm)			
		3	4	5	6
1	HMT-12	-	-	18.90	17.63
2	HMT-16	17.53	-	+	-
3	HMT-17	17.53	-	+	-
4	HMT-18	16.07	-	+	-
9	HMT-21	16.08	-	-	-
6	HMT-29	16.69	-	-	-
7	HMT-33	18.37	19.50	23.26	20.12
8	HMT-34	-	-	-	-
9	HMT-35	-	-	-	-

(+) present (-) no activity Agar well =8mm

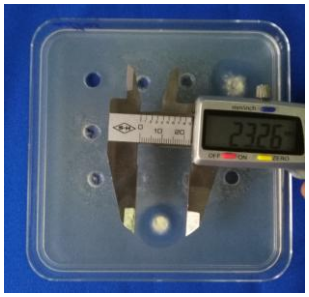

Figure 12 HMT-33 against *S. aureus* at 5 days of fermentation period

Conclusion

Results revealed that acidic pH and optimum moisture content and rich mineral content is most favourable condition for the growth of fungi. The soil which as large amount of organic matter due to acidic soil pH and slit and clay soil texture holds good amount of moisture content harboured a good quantitative isolated fungi in the soil for the purpose of recycling of dead organic matter thus making them available to the maintenance of global carbon cycle and ecological balance in the environment with dominant and sporulating genera. This research was done by the isolation of fungi occurring in the soil and their colony morphology and preliminary study of antimicrobial activity was performed by using seven test organisms. This investigation revealed that soil fungus HMT-33 isolated from soil of Homalin Township showed the moderated antimicrobial activity against (plant and animal pathogen) seven test organisms. It can be concluded that there is potential to discover useful antibiotic producing fungi in Homalin Township and produce some form of antimicrobial drug development programs.

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