ISOLATION OF SOIL FUNGI FROM THREE VILLAGES OF KATHA TOWNSHIP AND THEIR ANTIMICROBIAL ACTIVITIES

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Abstract

In the present research, soil samples were collected from three different places of Katha Township, Sagaing Region, during July 2019 and isolated them by the serial dilution method. The media used for the isolation includes Blakeslee's Malt Extract Agar (BMEA) medium and Potato Dextrose Agar (PDA) medium, incubated for 3-7 days at room temperature. Pure colonies were preserved into slant culture containing PDA medium. Twenty fungal strains were obtained. The surface colours of all isolated fungi are white, black, blue, brown, cream, green, dark green, pale yellow, pink, yellow and greenish yellow and their reserve colours are brown, cream, pale yellow, pink, red and yellow. In the colony morphology, the isolated fungi are medium and large in size. The margin of isolated fungi are entire, undulate, filamentous and the elevation of isolated fungi are flat, umbonate and raised. In the form, isolated fungi are irregular, circular and filamentous. Furthermore, the antimicrobial activity of all fungal strains were tested by agar well diffusion method on eight test organisms. Among them, four fungal strains showed the antimicrobial activity on all test organisms. Especially, TM- 14 and 16 showed the highest antimicrobial activity. These findings suggested that the soil fungi may be utilized for screening of the antimicrobial substances and to treat the diseases caused by pathogenic microorganisms.

Keywords: Soil Fungi, Colony Morphology, Antimicrobial Activity

Introduction

Soil is considered as one of the most suitable environments for microbial growth (Cavalcanti *et al.,* 2006). Fungi are an important component of the soil microbiota typically constituting more of the soil biomass than bacteria, depending on soil depth and nutrient conditions (Anisworth, 1995). Fungi are one of the dominant groups present in soil, which strongly influence ecosystem structure and function. Thus they play a key role in many ecological services (Rajendra, 2016).

Antimicrobial agents play the most important role in the treatment of bacterial infections (Hacioglu. N., 2011) and wide spread efforts have been carried out by many scientists in order to screen for novel antibiotic production microbes (Oskey, 2004).

Several fungal species produces bioactive compounds, secondary metabolites and chemical matels having pharmaceutical importance. There are about 23000 known secondary metabolites, 42% of which are produced by actinobacteria, 42% by fungi (eg. *Penicillium* spp.) and 16% by other bacteria. Antibiotics can be classified according to their made of actions (Lambert, 1977). Antibiotic are classified as broad-spectrum antibiotics when they have the ability to affect a wide range of gram-positive and gram-negative bacteria while antibiotics that only effective towards certain group of bacteria are known as narrow-spectrum antibiotics (Lambert, 1977).

Therefore, the aim of this research work is to produce antimicrobial compounds by isolated fungi from three different places of soil in Katha Township. To achieve this aim, the physicochemical properties of soil from Katha Township were analyzed. Then, fungi were isolated from different soil samples of Katha Township and Secondly, the different forms of colony morphology were studied and recorded them. After that the preliminary antimicrobial activities of isolated fungi were studied through eight test organisms

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Materials and Methods

Collection of soil samples

The soil samples were collected from three different places in various locations of Katha Township, during July, 2019. These samples were taken from different places (up to 15 cm depth) and put into sterilized polyethene bags after removing the surface soil for the isolation of fungi which were brought to the laboratory of Biotechnology and Development Center of Pathein University.

Table 1 Collected soil samples from three different places at Katha Township

No	Place	Location	
1	Kyan Taw	24.194588 N	96.325729 E
2	Between Kyan Taw and Lan Gwa	24.349149 N	96.196676 E
3	Pa Lway Shwe	24.214439 N	96.359594 E

Physicochemical analysis of Soil Samples

The collected soil samples were characterized by its physicochemical properties. Physicochemical parameters include organic carbon, nitrogen, pH, moisture content and temperature etc. The temperature and colour of soil samples were recorded. The physicochemical parameters of the soil samples were analyzed at Department of Agricultural Research, Yezin, Myanmar (Table 3).

Serial Dilution Method (Dubey, 2002)

One gram of soil sample was put into a conical flask containing 99 mL of distilled water. The flask was shaken for about 30 minutes in order to make the soil particles free from each other. This solution was then serial diluted from 10^{-3} to 10^{-7} dilution in separated test tubes and 1 mL each of the above dilution was separately transferred into sterile petri dishes under aspetic condition.The sterilized medium in the conical flask was cooled down to about 45°C and separately poured into each of the petri dish containing the respective soil dilutions. The inoculated plates were shaken in a clockwise and anti-clockwise direction for about 5 minutes in order to make uniform distribution of the fungi inoculums. When the agar was solidified, the inoculated plates were inverted and incubated at 27°C-30°C for 3-7 days. The isolated pure fungi had been preserved in slant culture containing PDA medium for further experimentations.

Agar Well Method (Collins, 1965)

Isolated strains were tested by agar well method for the preliminary antimicrobial activities. The wells (8 mm in diameter) were made by Cork borer in the autoclaved basal antimicrobial test medium. Wells impregnated within 3-6 days old culture fermented broth (20 μL) were incubated at room temperature for 24-28 hours. After 24-28 hours of incubation, the clear zones were measured. Therefore, the diameter of clear zones had been observed as potent activity as shown by representative strain. The clear zones which are surrounding the wells indicated the presence of antimicrobial activities which inhabit the growth of the test organism selectively.

Table 2 Eight kinds of Test Organisms used for Antimicrobial Activity (NITE and PRD)

Test No.	Test Organisms	Infection
1	*Escherichia coli* AHU 5436	Diarrhoea, pneumonia, abdominal pain
2	*Bacillus subtilis* IFO 90571	Fever
3	*Bacillus pumilus* IFO 90571	Fever
4	*Candida albicans* NTTE 09542	Candidasis, skin disease
5	*Pseudomonas fluorescens* IFO 94307	Septicemia
6	*Staphylococcus aureus* AHU 8465	Boil and Food poisoning
7	*Agrobacterium tumefaciens* NITE 09678	Crown gall disease
8	*Malassezia furfur* UY	Dandruff, Seborrhoeic dermatitis

NITE = National Institute of Technology Evaluation, Japan
PRD = Pharmaceutical Research Department, Yangon, Myanmar

Results

In the present research work, soil samples were collected and its physicochemical properties were studied. Fungal diversity of any soil depend on a large number of factors of the soil such as pH, organic content, moisture and soil texture. The results of the physicochemical properties of soil samples showed that soil environments between Kyan Taw and Lan Gwa, Pa Lway Shwe were Sandy Loam while the sample from Kyan Taw was Sandy Clay Loam.

The pH values of the soil samples showed that moderately acidic and neutral between 5.1 to 7.18. The temperature of soil environments of Katha Township during this investigation (the rainy season) showed that the soil environment of Katha Township at temperature range between 30°C to 34°C with great variation in present moisture content (4.6-19.3 %), organic carbon (0.26-0.96%), organic nitrogen (41-87 mg/kg) and potassium (50-383 mg/kg). These results were shown in Table 3.

Table 3 Physicochemical Properties of soil samples collected from three different places of Katha Township

Sample No.	Place	Soil Color	Text-ure	pH	T (ºC)	Moisture (%)	Organic Carbon (%)	Organic Nitrogen (mg/kg)	Organic Potassium (mg/kg)
1	Kyan Taw	Brown	SCL	5.1	32	5.7	0.26	41	50
2	Between Kyan Taw & Lan Gwa	Brown	SCL	5.35	30	19.3	052	71	78
3	Pa Lway Shwe	Brown	SL	5.31	33	18.0	0.9	81	79

*CL = clay loam, SCL = sandy clay loam, SL = sandy loam
Soil temperature = between 30 and 35°C

In the isolation of soil fungi, 20 fungal isolates were obtained, 11 strains from Kyan Taw, 4 strains from between Kyan Taw and Lan Gwa, 5 strains from Pa Lway Shwe, These fungi were cultured on potato dextrose agar (PDA) and Blaskeslee's Malt Extract Agar (BMEA) and each ten strains were isolated from these two media.

Table 4 Isolation of Soil Fungi on Two Different Media

Sample No.	Place	PDA	BMEA	Total
1	Kyan Taw	TM-1, 2, 3, 4, 5	TM-6, 7, 8, 9, 10, 11	11
2	Between Kyan Taw and Lan Gwa	TM-12, 13	TM-14, 15	4
3	Pa Lway Shwe	TM-16, 17, 18	TM-19, 20	5
Total		10	10	20

In the colony morphology, isolated strains were medium and large in size, entire in margin, raised, flat, convex in elevation and form in circular and irregular. Their colony morphology, microphotograph and their antimicrobial activities were also performed.

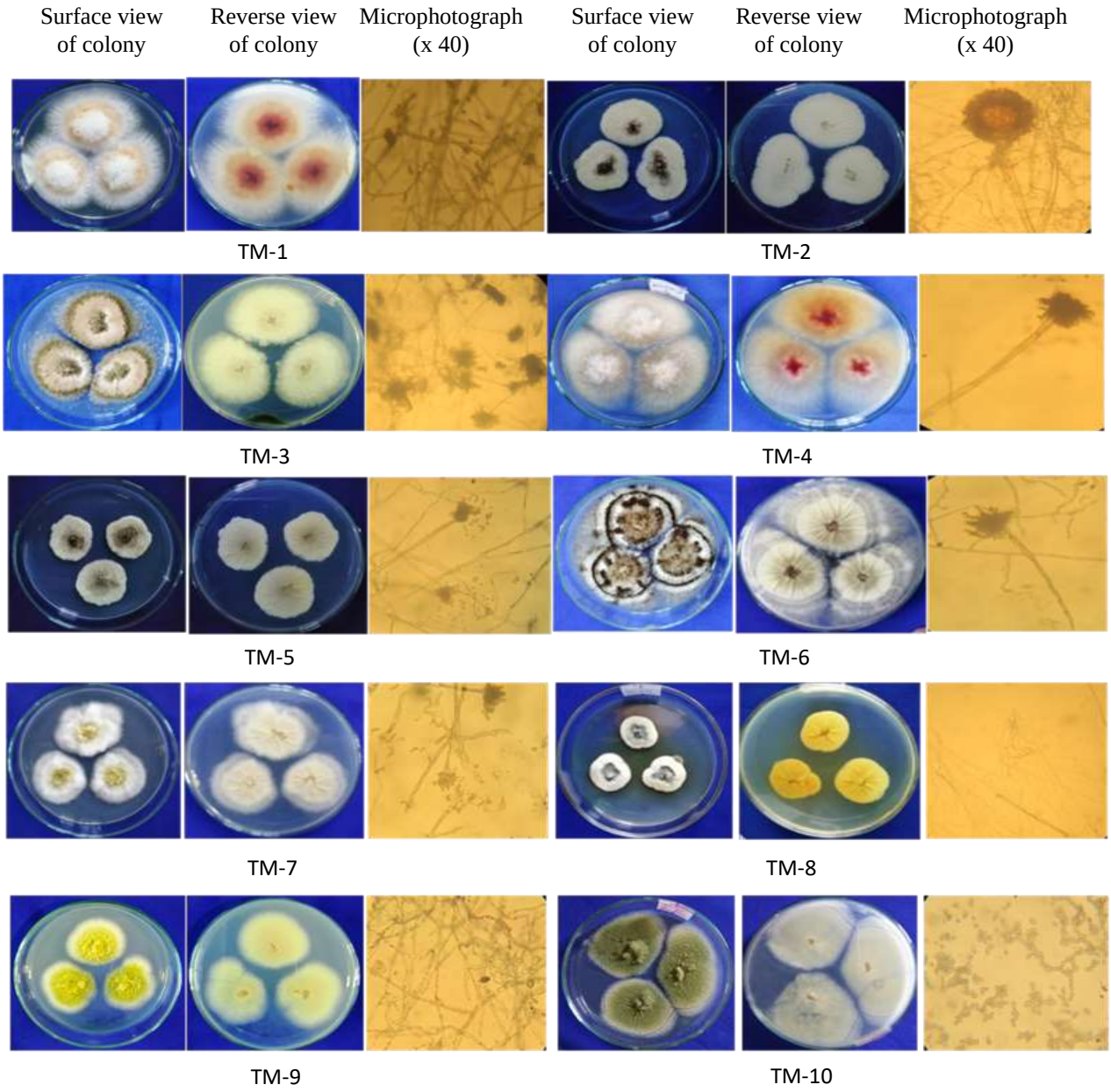


Figure 1 Morphology and their microscopical characters of isolated fungi TM-1 to TM-10

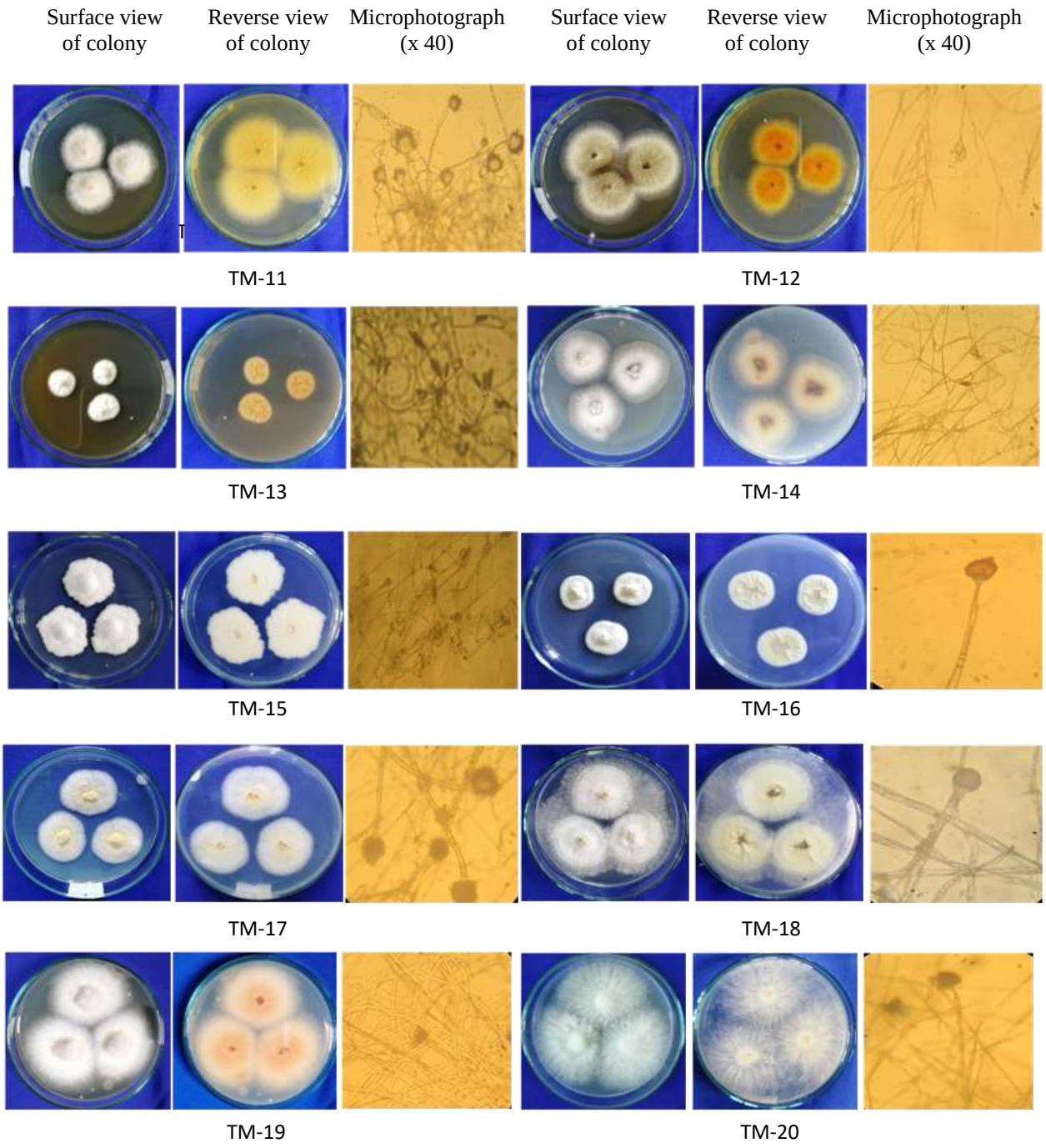


Figure 2 Morphology and their microscopical characters of isolated fungi TM-11 to TM-20

In the screening of antimicrobial activity, all strains were tested on eight test organisms. Among them, four strains showed different levels of antimicrobial activities and were selected for further study.

Table 5 Antibacterial Activity of Isolated Fungal strains against *Escherichia coli*

No.	Isolated Fungal	Fermentation Period (Days) and Inhibitory Zone (mm)			
		3rd day	4th day	5th day	6th day
1	TM-4	-	-	19.09	+
2	TM-14	18.88	22.32	**31.59**	20.22
3	TM-16	-	-	**24.60**	+
4	TM-20	+	+	+	18.21

Table 6 Antibacterial Activity of Isolated Fungal strains against *Bacillus subtilis*

No.	Isolated Fungal	Fermentation Period (Days) and Inhibitory Zone (mm)			
		3rd day	4th day	5th day	6th day
1	TM-4	17.38	20.76	**26.49**	22. 98
2	TM-14	20.85	21.58	22.94	19.44
3	TM-16	+	18.95	20.34	19.53
4	TM-20	+	+	**25.10**	21.2

Table 7 Antibacterial Activity of Isolated Fungal strains against *Bacillus pumilus*

No.	Isolated Fungal	Fermentation Period (Days) and Inhibitory Zone (mm)			
		3rd day	4th day	5th day	6th day
1	TM-4	16.59	**26.22**	26.62	15.22
2	TM-14	18.23	20.44	24.30	17.18
3	TM-16	-	21.26	22.19	18.21
4	TM-20	+	**25.24**	17.10	+

Table 8 Antifungal Activity of Isolated Fungal strains against *Candida albicans*

No.	Isolated Fungal	Fermentation Period (Days) and Inhibitory Zone (mm)			
		3rd day	4th day	5th day	6th day
1	TM-4	+	+	22.54	+
2	TM-14	+	22.64	23.66	18.15
3	TM-16	21.32	**33.68**	20.12	+
4	TM-20	-	28.00	**24.38**	+

Table 9 Antibacterial Activity of Isolated Fungal strains against *Pseudomonas fluorescence*

No.	Isolated Fungal	Fermentation Period (Days) and Inhibitory Zone (mm)			
		3rd day	4th day	5th day	6th day
1	TM-4	-	+	+	18.29
2	TM-14	-	+	17.45	**29.74**
3	TM-16	-	29.35	**32.59**	19.19
4	TM-20	-	+	29.35	17.27

Table 10 Antibacterial Activity of Isolated Fungal strains against *Staphylococcus aureus*

No.	Isolated Fungal	Fermentation Period (Days) and Inhibitory Zone (mm)			
		3rd day	4th day	5th day	6th day
1	TM-4	-	+	21.36	-
2	TM-14	-	-	18.45	+
3	TM-16	18.00	**22.70**	19.33	+
4	TM-20	-	+	**20.25**	19.52

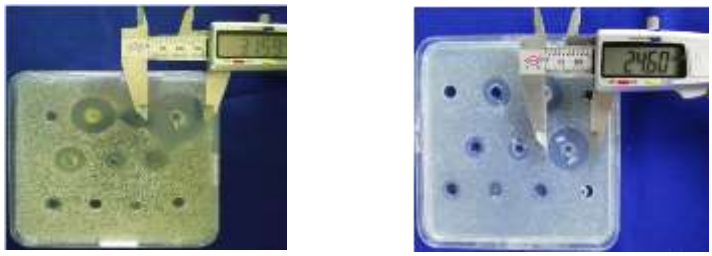
Table 11 Antibacterial Activity of Isolated Fungal strains against *Agrobacterium tumefaciens*

No.	Isolated Fungal	Fermentation Period (Days) and Inhibitory Zone (mm)			
		3rd day	4th day	5th day	6th day
1	TM-4		+	24.14	**26.19**
2	TM-14	-	17.59	18.33	+
3	TM-16	-	+	16.57	+
4	TM-20	-	**25.23**	22.59	19.19

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

Table 12. Antifungal Activity of Isolated Fungal strains against *Malassezia furfur*

No.	Isolated Fungal	Fermentation Period (Days) and Inhibitory Zone (mm)			
		3rd day	4th day	5th day	6th day
1	TM-4	+	18.21	19.55	+
2	TM-14	+	17.08	16.45	+
3	TM-16	14.87	**24.00**	**27.67**	18.04
4	TM-20	+	20.33	25.57	18.23.

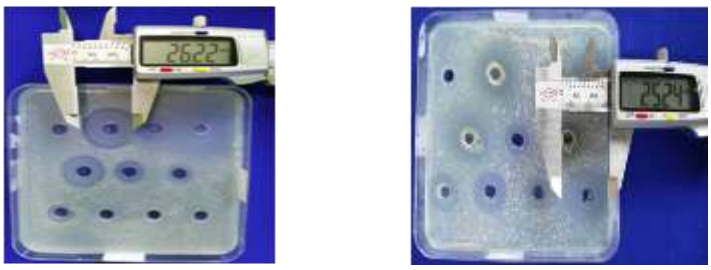


TM-14 TM-16

Figure 3 Antibacterial Activity of Isolated Fungal strains against *Escherichia coli*

TM-4 TM-20

Figure 4 Antibacterial Activity of Isolated Fungal strains against *Bacillus subtilis*

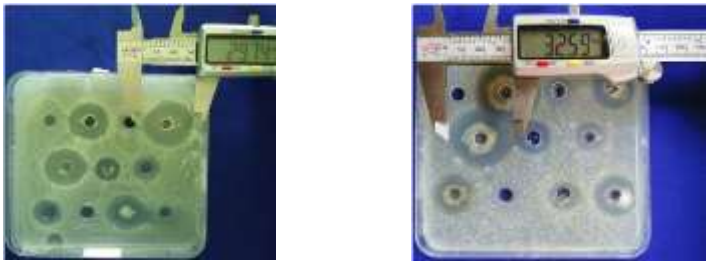


TM-4 TM-20

Figure 5. Antibacterial Activity of Isolated Fungal strains against *Bacillus pumilus*

TM-16 TM-20

Figure 6 Antifungal Activity of Isolated Fungal strains against *Candida albicans*

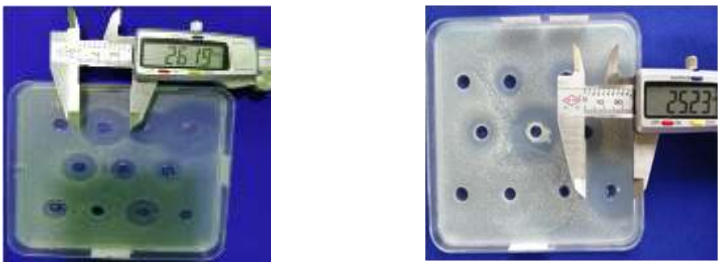


TM-14 TM-16

Figure 7 Antibacterial Activity of Isolated Fungal strains against *Pseudomonas fluorescence*

TM-16 TM-20

Figure 8 Antibacterial Activity of Isolated Fungal strains against *Staphylococcus aureus*



TM-4 TM-20

Figure 9 Antibacterial Activity of Isolated Fungal strains against *Agrobacterium tumefaciens*

TM-16 TM-16

Figure 10 Antifungal Activity of Isolated Fungal strains against *Malassezia furfur*

Discussion and Conclusion

In the present study, the colour of soil samples is brown with variation in pH (5.11-7.18). During the investigation (rainy season) showed that the soil environment of Katha Township at temperature ranging between 30°C to 35°C with great variation in present moisture content (4.6-19.3 %), organic carbon (0.26-0.96 %), organic nitrogen (41-87 mg/kg) and potassium (50-79 mg/kg). Total number of colonies obtained in Kyan Taw is eleven with pH 5.11 (moisture 5.7 %). The results showed that low pH and optimum moisture content favour for the growth of fungi. Normal soil contains a large number of microbes and substantial quantities of microbial biomass.

It is also known that the bacteria thrive well in natural and alkaline soils, whereas fungi show the best activity under acidic conditions. A total of 20 fungi were isolated from three different soil samples and cultured on BMEA and PDA medium. The isolated fungi were designated as TM-1 to TM-20. The surface colours of all isolated fungi are white, black, blue, brown, cream, green, dark green, pale yellow, pink, yellow and greenish yellow and their reserve colour are brown, cream, pale yellow, pink, red and yellow.

Among all of the strains, the surface color of TM-1 has changed from red to orange and the reverse colour of TM-6 has changed from gray to cream on slant culture after six days. The reverse

color of TM-9 has occured yellow pigment in slant culture after seven days on PDA medium. A wide range of media is used for growing fungi, as a result media have affected on colony morphology and their colour. All fungal strains were tested by eight test organisms for preliminary study of antimicrobial activities among them, 4 strains showed that the different levels of antimicrobial activities.

TM-14 exhibited the antibacterial activity (31.59 mm) on *Escherichia coli* at 5th day, (22.94 mm) on *Bacillus subtilis* at 5th day, (24.30 mm) on *Bacillus pumilus* at 5th day and (23.66 mm) on *Candida albicans* at 6th day. TM-16 showed the highest antimicrobial activities (33.68 mm) on *Candidas albicans* at 4th day, (27.67 mm) *on Malassezia furfur* at 5th day and then (32.59 mm) on *Pseudomonas fluorescens* at 5th day and (22.70 mm) on *Staphylococcus aureus* at 4th day. Especially TM-16 showed moderate antimicrobial activity against most of the test organism.

It can be concluded that the present research is to isolate the fungi from different soil samples and to study the antimicrobial activities of isolated fungi on eight test organisms. This study will be focused on the fermentation conditions of selected fungus and extraction of antimicrobial compounds.

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References

Ainsworth G.C, Bisby G.R.(1995). **Dictionary of the Fungi,** eight edition**,** common wealth Mycological institute. Kew, Survey. P-44.

Ando K.M, Suto and Inada S. (2004). **Sampling and isolation methods of fungi**, workshop at University of Pathein.

Cavalcanti MA, Oliveira LG, Fernandes MJ and Lima DM (2006). **Filamentous fungi isolated from soil in districts of the Xingo region,** *Braz. Acta Bot. Bras.* 20, 831-837

Collin, C.H. (1965), **Microbiological Methods.** Butfer worth and Co., Publishers Ltd., Landon.

Dubey.R.C and Maheshwari D.K. (2002) **Practical Microbiology.5.**chand and company Ltd. Ram Nagar, New Dehli. 110-155 ELBS and E. And S. Living stone Ltd.

Dulmage. H. T and Rivas. R. (1978). **A survey to soil microganisms, with particular reference to the actinomycetes as sources of substances toxic to Heliothis viresuns.** Journal of invertebrate pathology, Vol.31, pp. 118-122.

Hacioglu, N. B. Dulger, (2011). **European Journal of Experimental Biology,** 1(4), 158-163.

Lambert. A. (1977). **Pharmaceutical microbiology.** Five Edition, Blackwell scientific publications, Oxford.

Oskay. M, Tamer. A. U. Azeri. C. (2004). **African Journal of Biotechnology .3(9),** 441-446.-

Ramann. E, Schzllhorn. R. C, Krausse. (1899). **Amzhal and Bedeutung derneidernpffanz lichen orgonismen in wald and moobodien.** 31: 575-608.

Rangaswai . G, Bagyaraj D. J. (1998). **Agriculture Microbiology,** Second Edition published by prentice Hall of India Pvt. Ltd. N, Delhi.

Rajendra Kumar Seth* *et al*, **"Isolation and identification of Soil Fungi from Wheat Cultivated Area of Uttar Pradesh"** *Journal of Plant Pathology and Microbiology,* 2016, : 2157-7471.