ISOLATION OF SOIL FUNGI FROM MUDON TOWNSHIP, MON STATE AND THEIR ANTIMICROBIAL ACTIVITY

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Abstract

In this research work, soil samples were collected from five different places of Mudon Township including Kwaiwan, Kawkhite, Tarpaton, Tharyargone and Kyauktalone village, during July 2018. Soil fungi were isolated by the serial dilution method from these samples and cultured on 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) and incubated for 3-7 days at room temperature. A total of 41 fungal strains were isolated and the surface color of all strains were white, brown , greenish brown, and their reverse color were cream, black, greenish brown, yellowish brown respectively. In the colony morphology, the isolated fungi were small, medium and large in size. The margins of isolated fungi were entire convex, raised, and the form of isolated fungi circular and irregular. Moreover, physicochemical properties of soil from different locations of Mudon Township were analyzed. All fungal strains were tested by eight test organisms for preliminary study of antimicrobial activity. Among them, six strains showed different level of antimicrobial activity. MP- 7 exhibited the highest antibacterial activity (25.05 mm) against and MP- 25 also showed the moderate activity (23.50 mm) on *Bacillus pumilus* at 5 days. MP- 6 gave the strong antibacterial activity (20.03 mm) against *Bacillus Subtilus* at 6 days. Especially, MP- 41 showed the moderated antimicrobial activity against all test organisms.

Keywords: Soil fungi, antimicrobial activity

Introduction

Microorganisms in soil are important because they affect soil structure and fertility. Soil microorganisms can be classified as bacteria, actinomycetes, fungi, algae and protozoa. Soil is considered one of the most suitable environments for microbial growth, for that the microorganism which have been isolated from the soil. Numerous antibiotics have been isolated from a variety of microorganism; however, studies are still being conducted to identify novel antibiotics effective against pathogenic fungi and bacteria (Cavalcanti, *et. al.*, 2006).

Soil are the foundation of all terrestrial ecosystems and are home to a vast diversity of bacteria, archaea, fungi, insects, annelids and other invertebrates as well as plants and algae. These soil dwellers provide food or nutrients that support organisms that live above and below ground. Soils also play critical roles in buffering and filtering freshwater ecosystems. Consequently, soils are extremely important to human societies (Dominati, 2010). The number and species of microbes in soil vary directly in response to environmental conditions such as nutrient availability, soil texture and type of vegetation cover (Atlas, *et. al*. 1998).

Natural products from microorganisms have been the most successful source that has found many applications in the fields of medicine, pharmacy and agriculture. Most of the antibiotics in current use for the treatment of various infectious diseases are microbial products (Tawiah, *et. al*., 2012).

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Fungi are an important component of the soil microbiotatypically constitution more of the soil biomass than bacteria depending on soil depth and nutrient conditions (Ainsworth & Bisby, 1995). Fungi represent a very important biological resource with an estimated 1.5 million species in the world. The tropics are generally recognized as embracing the greatest variation on earth and in the case of plants about two-thirds (180,000 species) are believed to occur there (Raven, 1988).

Therefore, soil sample is the most effective and popular materials for especially isolating a number of fungi (Ando, 2004). Wide spread efforts have been carried out by many scientists in order to screen for novel antibiotic production microbes (Oskay.M, 2004).

Soil 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 (Nejad, 2013).

Therefore, the aim of the research work was to produce antimicrobial compounds by isolated fungi from five different places soil in Mudon Township. To achieve this aim, the present work has been done according to the following objectives - to collect soil samples from five places of Mudon Township, to isolate soil fungi from these soil samples, to study the cultural characteristics of isolated soil fungi on six different media, to investigate the colony morphology of isolated fungi and to determine the preliminary antimicrobial activity of isolated fungi.

Materials and Methods

Method for collection of soil samples

The soil samples were collected from five different places in various location of Mudon Township, during July 2018. 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 Botany department at Mawlamyine University.

Physicochemical analysis of Soil Samples

The collected soil samples were characterized for its physicochemical properties. Physiochemical parameters include organic nitrogen, phosphorous, potassium oxide, pH, temperature, moisture and texture. Temperature and color of the soil samples was recorded on the spot. The other physicochemical parameters of the soil samples were analyzed at Land Use, Perennial Crops Research & Development center (Mawlamyine).

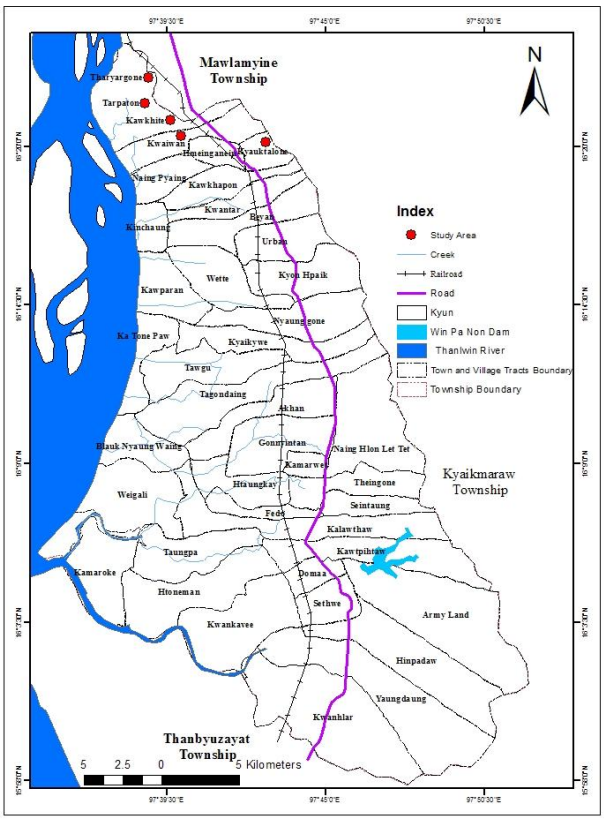
Isolation of fungi from the soil samples

The soil micro fungi were isolated by serial dilution method (Dubey, 2002) on different media such as Blaskeslee's Malt Extract Agar (BMEA Medim), 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 five different places at Mudon Township

Soil sampleNo.	Place	Location
1	Kwaiwan	N 16˚ 19. 353′ E 97˚ 41. 544′
2	Kawkhite	N 16˚ 20.35′ E 97˚ 41. 269′
3	Tarpaton	N 16˚ 20.643′ E 97˚ 40.965′
4	Tharyargone	N 16˚ 20.819′ E 97˚ 40.741 ′
5	Kyauktalone village	N 16˚ 19.732′ E 97˚ 42.082′

Collected Soil Sample Area



Source: Department of Geography, Mawlamyine University

Figure 1 Map of collected soil sample area (Mudon Township)

Serial Dilution Method (Dubey, 2002)

1 g of soil sample was introduced into a conical flask containing 99 ml of distilled water. The flask was than 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 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 45º C and separately poured into each of the petridish containing the respective soil dilutions. The inoculated plates were shaken clock-wise and anti-wise direction for about 5 minutes so as to make uniform

distribution of the fungi inoculums. When the agar was solidified, the inoculated plate were inverted and incubated at 27ºC- 30ºC for 3-7 days. Isolated pure fungi were preserved into slant culture containing BMEA Medium for further experimentations.

Agar Well Method (Collins, 1965)

Isolated strains were tested by agar well method 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 broths (20 µL) were incubated at room temperature for 24- 28 hours. After 24- 48 hours of incubation, the clear zones were measured. Therefore, the diameter of clear zones had seen 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 the experiment were *Escherichia coli* AHU5436, *Bacillus subtilis* IFO 90571, *Bacillus pumilus* IFO 90571, *Candida albicans* NITE 09542, *Pseudomonas fluorescens* IFO94307, *Staphlylococcus aureus* AHU8465, *Agrobacterium tumefaciens* NITE 09678 and *Malassezia furfur*. The organisms were obtained from National Institute of technology and Evaluation (NITE, Japan) and Pharmaceutical Research Department, Yangon, Myanmar.

Results

In present research work, 41 fungal strains were isolated from five different samples collected from Mudon Township. The results of the physicochemical properties of soil samples showed that soil environments of Kwaiwan, Tarpaton and Tharyargone village were sandy loam and the soil sample form Kawkhite and Kyauktalone village were sandy clay loam.

The pH values of the soil samples show that Kwaiwan, Kawkhite, Tarpaton, Tharyargone and Kyauktalone village were moderately acidic with pH of 6.43, 6.41, 6.42, 6.16 and 6.52 respectively. The temperature of soil environments of Mudon Township at the time of this investigation (rainy season) revealed that the soil environment of Mudon Township had temperature range between 20ºC and 28ºC with great variation in present moisture content (1.18-2.99), organic nitrogen (0.09- 0.15), phosphorus (2.40 -11.88), potassium dioxide (4.16 - 12.73). The color of soil samples were red, black and brown. These results were shown in Table 2.

Table 2 Physico-chemical Properties of Soil Samples collected from five different places of Mudon Township

Sample No	Place	Soil color	Texture	pH	T(C^o)	Moisture (%)	Organic N (%)	Nutrients	
								P (ppm)	K_2O (mg)
1	Kwaiwan	Black	SL	6.43	20.7	1.91	0.13	11.88	12.73
2	Kawkhite	Brown	SCL	6.41	28.8	2.41	0.09	5.94	4.16
3	Tarpaton	Brown	SL	6.42	20.75	1.85	0.15	5.94	6.61
4	Tharyar gone	Brown	SL	6.16	20.85	1.18	0.09	5.88	5.09
5	Kyaukta lone	Red	SCL	6.52	20.75	2.99	0.09	2.40	6.19

**SL- Sandy Loam, SCL- sandy clay loam,

N- Nitrogen, P- Phosphorous, K_2O- Potassium oxide

In the present research work, 41 fungal isolates were obtained fifteen strains from Kwaiwan, sixteen strains from Kawkhite, seven strain from Kyauktalone, two strains from Tharyargone and each one strain from Tarpaton. In the present research was used by six culture media. A total of 41 isolated fungi, 17 strains were isolated from BMEA Medium, 13 strains from DRBC Medium, 5 strains from PDA Medium, 4 strains from MEA Medium, each 1 strain from CZA and each 1 strain from GAN Medium. These results were shown in Table 3. The isolated fungi were designated as MP- 1 to MP- 41.

Table 3 Isolation of fungi by using six different media and soil s

Sample No	Places	BMEA Medium	DRBC Medium	PDA Medium	MEA Medium	CZA Medium	GAN Medium	Total
1	Kwaiwan	MP-1, 3,9, 10, 14,	MP- 4, 5, 6, 7, 11, 12, 13, 15	MP- 2	MP- 8	-	-	15
2	Kawkhite	MP- 20, 27, 29, 30, 31	MP- 16, 21, 26,	MP- 17, 18, 19	MP- 23, 24, 25	MP- 22	MP- 28	16
3	Tarpaton	-	MP- 32	-	-	-	-	1
4	Tharyargone	MP- 33	-	MP- 34	-	-	-	2
5	Kyauktalone	MP- 35, 36, 37, 38, 40, 41	MP- 39	-	-	-	-	7
		17	13	5	4	1	1	41

Morphology and Photomicrograph of Isolated Soil Fungi

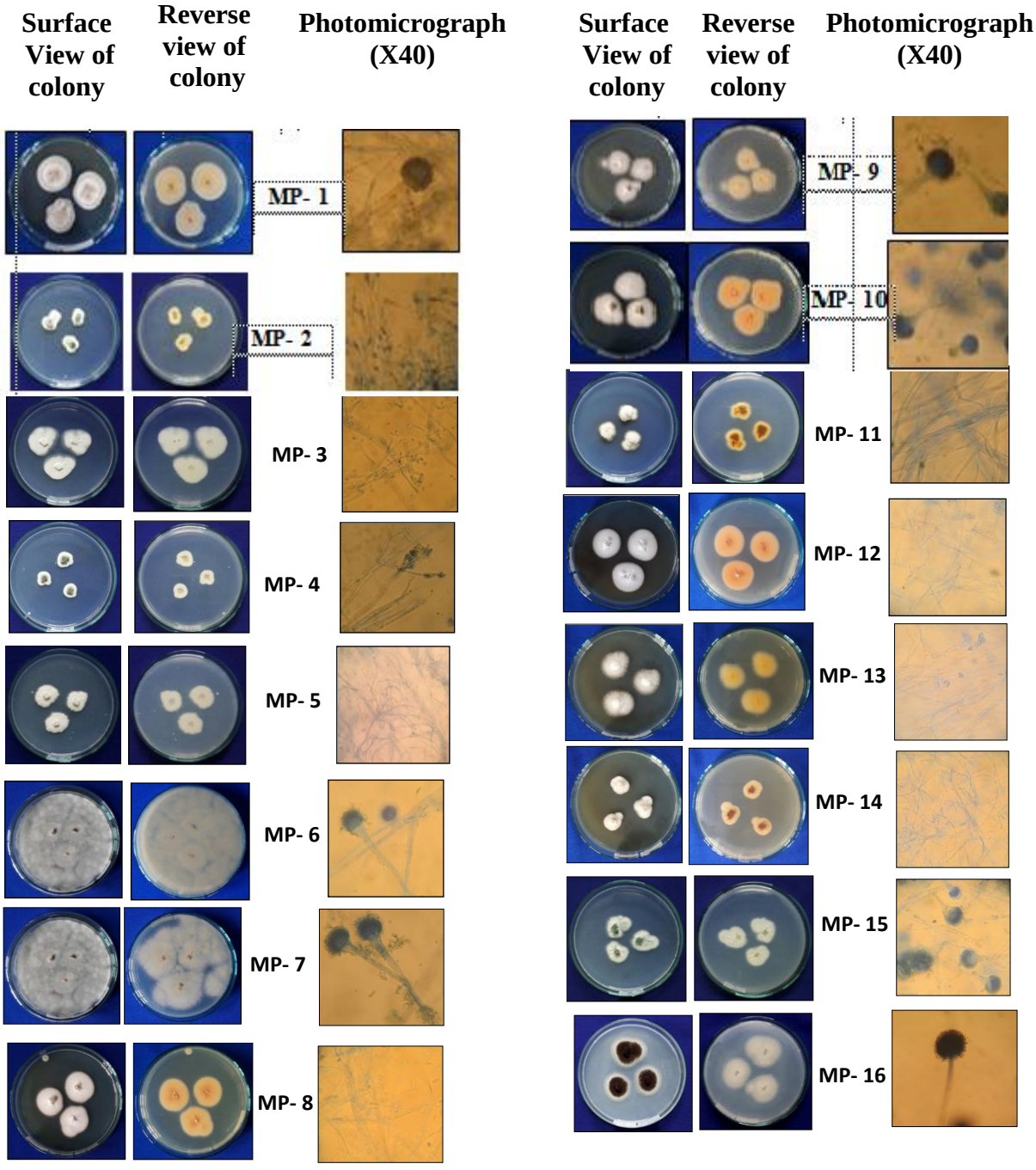


Figure 2 Morphology and microscopical characters of isolated fungi MP-1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 and 16

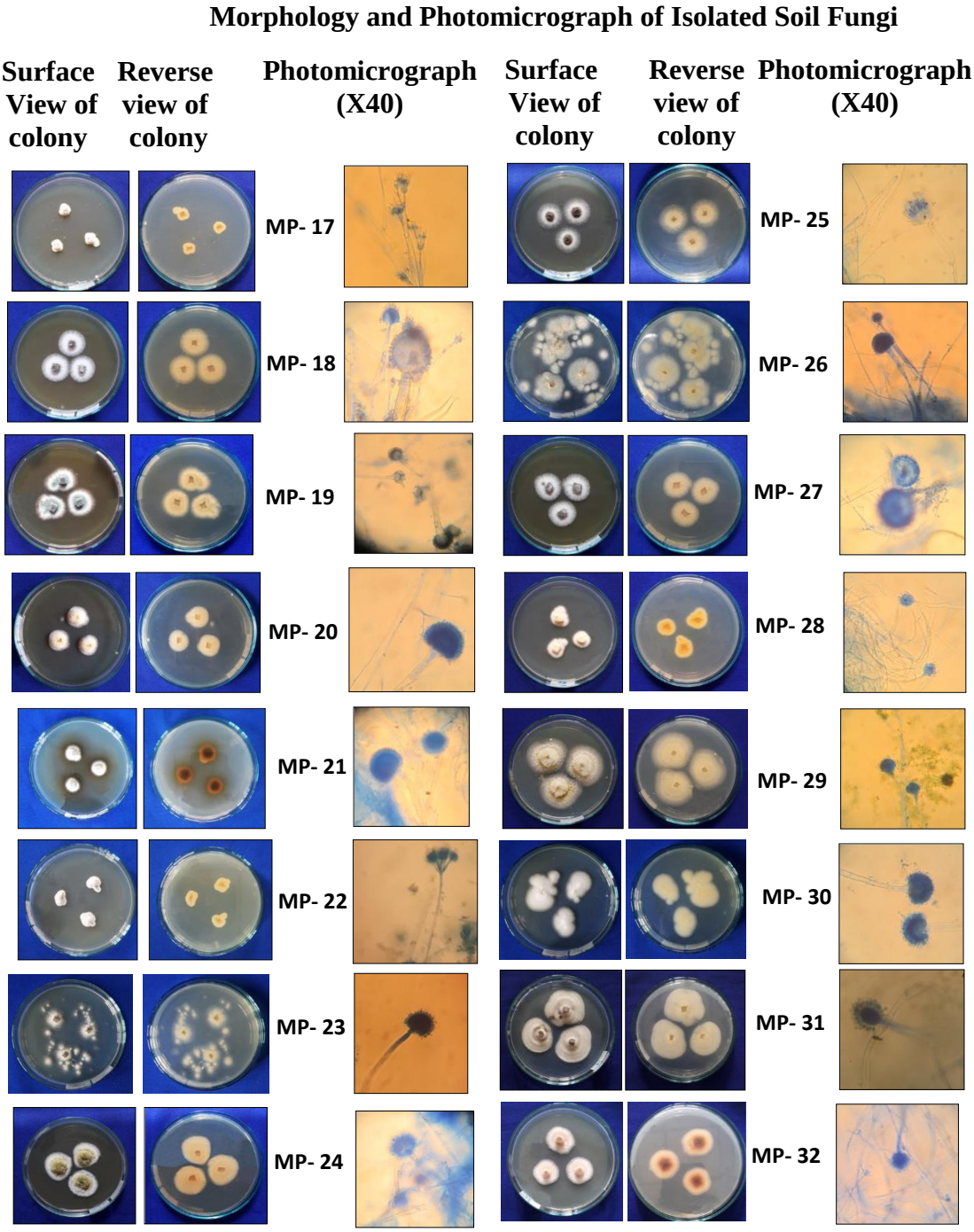


Figure 2 Morphology and microscopical characters of isolated fungi MP-17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31 and 32

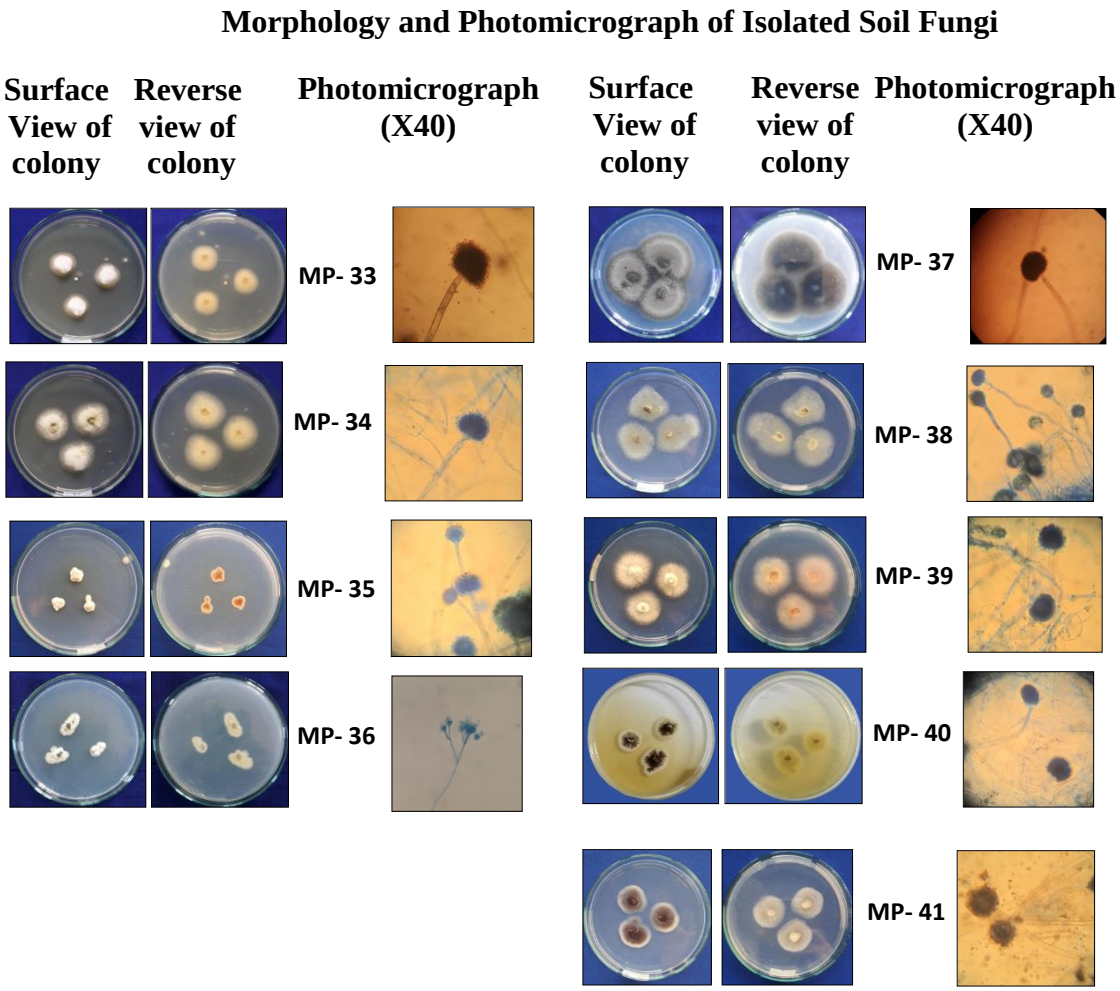


Figure 3 Morphology and microscopical characters of isolated fungi MP-33, 34, 35, 36, 37, 38, 39 40 and 41

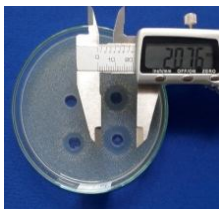
All fungal strains were tested by eight test organisms for preliminary study of antimicrobial activity. Among them, six strains showed different level of antimicrobial activities. In 4 days old culture, MP- 7 showed activities are (20.76 mm) and MP- 26 showed (23.50 mm) activities against *Escherichia coli* at 5 days. In 4 days, MP- 6 (20.03 mm) and MP- 7 showed (16.99 mm) activities are against *Bacillus subtilus* at 6 days. MP- 7 showed activity (25.05 mm) at 5 days and MP- 26 (20.53 mm) against *Bacillus pumilus* at 4 days fermentation period. In 6 days, MP- 6 showed (14. 76 mm) and MP- 26 (15.29 mm) against *Candida albican* at 5 days. MP- 26 (14.75 mm) and MP- 41 showed (13.33 mm) against *Pseudomonas fluorescens* at 6 days. In 5 days, MP- 25 (13.20 mm) and MP- 41 showed (16. 30 mm) against *Straphylococcus aureus.* MP- 7 (15. 34 mm) and MP- 41 (16.07 mm) showed against *Agrobacterium tumefaciens.* In 5 days, MP-7 (15.77 mm) and MP-26 showed against *Malassezia furfur.*

Among them, MP - 7 exhibited the highest antibacterial activity (25.05 mm) against *Bacillus pumilus* at 5 days and MP- 25 also showed the moderate activity (23.50 mm) on *Bacillus pumilus* at 5 days. MP- 6 gave the strong antifungal activity (20.03 mm) against *Bacillus subtilus* at 4 days. Especially, MP- 41 showed the moderated antimicrobial activity against all test organisms.

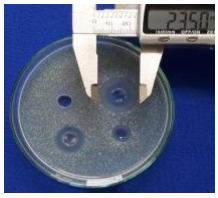
Table 4 Antibacterial activity of six fungal strains against *Escherichia coli*

No	Isolated Fungi	Fermentation Period (days) and Inhibitory zone (mm)			
		3 days	4 days	5 days	6 days
1	MP- 6	15.04	15.71	21.99	20.39
2	MP- 7	20.23	20.76	23.35	24.08
3	MP- 25	+	13.56	18.28	16.70
4	MP- 26	-	20.12	**23.50**	20.72
5	MP- 33	-	11.31	11.87	14.32
6	MP- 41	16.47	14.73	16.99	20.15

(+)present (-) absent well size=8mm



MP- 7 against *E coli* at 4 days of fermentation period



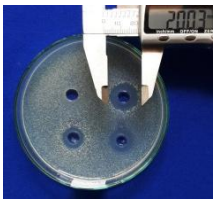
MP-26 against *E coli* at 5 days of fermentation period

Figure 4 Antibacterial activity of selected fungal strains against *Escherichia coli*

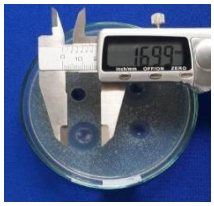
Table 5 Antibacterial activity of six fungal strains against *Bacillus subtilis*

No	Isolated Fungi	Fermentation Period (days) and Inhibitory zone (mm)			
		3 days	4 days	5 days	6 days
1	MP- 6	-	**20.03**	13.81	15.94
2	MP- 7	15.44	16.75	13.43	16.99
3	MP- 25	+	14.71	14.16	13.52
4	MP- 26	-	19.77	15.96	15.13
5	MP- 33	-	19.09	11.25	12.64
6	MP- 41	17.60	12.95	18.05	19.12

(+)present (-) absent well size=8mm



MP- 6 against *Bacillus subtilis* at 4 days of fermentation period



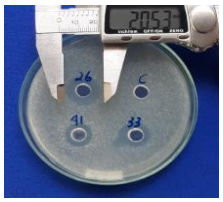
MP- 7 against *Bacillus subtilis* at 6 days of fermentation period

Figure 5 Antibacterial activity of selected fungal strains against *Bacillus subtilis*

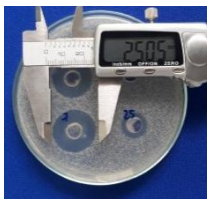
Table 6 Antibacterial activity of six fungal strains against *Bacillus pumilus*

No	Isolated Fungi	Fermentation Period (days) and Inhibitory zone (mm)			
		3 days	4 days	5 days	6 days
1	MP- 6	17.74	19.77	23.26	21.78
2	MP- 7	20.47	19.69	**25.05**	23.86
3	MP- 25	13.62	15.40	15.49	14.89
4	MP- 26	-	20.53	20.51	21.11
5	MP- 33	-	18.30	+	13.11
6	MP- 41	18.30	16.27	14.46	20.36

(+)present (-) absent well size=8mm



MP- 7 against *Bacillus pumilus* at 5 days of fermentation period



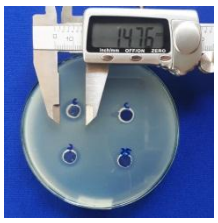
MP- 26 against *Bacillus pumilus* at 4 days of fermentation period

Figure 6 Antimicrobial activity of selected fungal strains against *Bacillus pumilus*

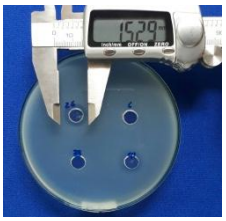
Table 7 Antifungal activity of six fungal strains against *Candida albicans*

No	Isolated Fungi	Fermentation Period (days) and Inhibitory zone (mm)			
		3 days	4 days	5 days	6 days
1	MP- 6	-	+	11.48	14.76
2	MP- 7	13.62	+	13.27	14.17
3	MP- 25	12.60	+	13.79	11.65
4	MP- 26	-	+	**15.29**	+
5	MP- 33	-	+	+	12.23
6	MP- 41	12.44	+	14.46	15.60

(+)present (-) absent well size=8mm



MP- 6 against *Candida albicans* at 6 days of fermentation period



MP- 26 against *Candida albicans* at 5 days of fermentation period

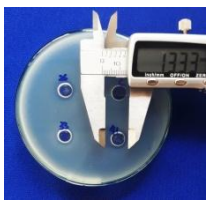
Figure 7 Antifungal activity of selected fungal strains against *Candida albicans*

Table 8 Antibacterial activity of six fungal strains against *Pseudomonas fluorescens*

No	Isolated Fungi	Fermentation Period (days) and Inhibitory zone (mm)			
		3 days	4 days	5 days	6 days
1	MP- 6	-	+	+	+
2	MP- 7	+	+	11.92	13.47
3	MP- 25	+	+	+	12.52
4	MP- 26	-	+	**14.75**	12.05
5	MP- 33	-	+	+	+
6	MP- 41	-	12.01	13.18	13.33

(+)present (-) absent well size=8mm

MP- 26 against *Pseudomonas fluorescens* at 5 days of fermentation period



MP- 41 against *Pseudomonas fluorescens* at 6 days of fermentation period

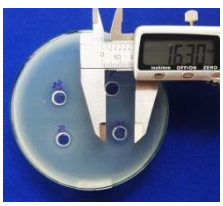
Figure 8 Antibacterial activity of selected fungal strains against *Pseudomonas fluroescens*

Table 9 Antibacterial activity of six fungal strains against *Straphylococus aureus*

No	Isolated Fungi	Fermentation Period (days) and Inhibitory zone (mm)			
		3 days	4 days	5 days	6 days
1	MP- 6	-	11.82	+	+
2	MP- 7	+	12.18	11.96	+
3	MP- 25	-	12.64	13.20	+
4	MP- 26	-	11.84	13.93	12.59
5	MP- 33	-	+	+	+
6	MP- 41	+	12.16	16.07	**16.30**

(+)present (-) absent well size=8mm

MP- 25 against *Straphylococus aureus* at 5 days of fermentation period



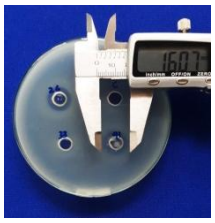
MP- 41 against *Straphylococus aureus* at 6 days of fermentation period

Figure 9 Antimicrobial activity of selected fungal strainag ainst *Straphylococus aureus*

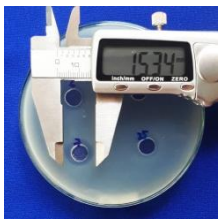
Table 10 Antibacterial activity of six fungal strains against *Agrobacterium tumefaciens*

No	Isolated Fungi	Fermentation Period (days) and Inhibitory zone (mm)			
		3 days	4 days	5 days	6 days
1	MP- 6	-	+	11.28	14.45
2	MP- 7	+	+	14.75	15.34
3	MP- 25	-	12.45	+	13.22
4	MP- 26	-	13.05	15.54	13.84
5	MP- 33	-	+	+	13.04
6	MP- 41	+	13.63	16.07	15.51

(+)present (-) absent well size=8mm



MP-41 against *Agrobacterium tumefaciens* at 5days of fermentation period



MP-7 against *Agrobacterium tumefaciens* at 6 days of fermentation period

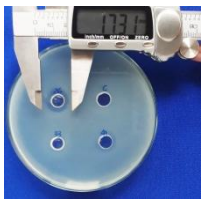
Figure10 Antimicrobial activity of selected fungal strains *Agrobacterium tumefaciens*

Table 11 Antifungal activity of six fungal strains against *Malassezia furfur*

No	**Isolated Fungi**	**Fermentation Period (days) and Inhibitory zone (mm)**			
		3 days	4 days	5 days	6 days
1	MP- 6	+	+	12.89	14.41
2	MP- 7	15.86	+	15.77	14.12
3	MP- 25	+	13.63	15.41	+
4	MP- 26	-	+	**17.31**	13.41
5	MP- 33	-	+	+	+
6	MP- 41	+	14.46	12.88	17.12

(+)present (-) absent well size=8mm

MP- 7 against *Malassezia furfur* at 5 days of fermentation period



MP- 26 against *Malassezia furfur* at 5 days of fermentation period

Figure11 Antimicrobial activity of selected fungal strains against *Malassezia furfur*

Discussion and Conclusion

Soil 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 (Nejad *et. al.*, 2013). Scientist are continuously searching for novel antibiotic producing microbes because drug resistant strains of pathogen emerge more quickly than the rate of discovery of new drugs and antibiotics (Kumar et al., 2010).

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 , 1998).

In the present study, physicochemical properties of soil from Mudon Township were analyzed. The color of soil samples was red, black and brown.The temperature of soil environment of Mudon Township at the time of this investigation of July, 2018 (rainy season) revealed that these places had temperature range between 20 ° C and 28 ° C with great variation

in present moisture content (1.18- 2. 99 %) organic nitrogen (0.09- 0.15 %), phosphorous nutrient (5.88- 11.88 ppm) and potassium oxide nutrient (4.16- 12. 73 mg).Physicochemical analysis of five different soils from Mudon Township.

Ramann *et al*., 1899 also reported that due to the accumulation of more litter in scrub and deciduous forest more percentage of fungi are present in the soil for the purpose of recycling of dead organic matter. It is known that the bacteria thrive well in neutral and alkaline soils, whereas fungi show the best activity under acidic conditions.

The surface color of other strains were brown, greenish brown, and their reverse color were black, greenish brown, yellowish brown respectively. In the colony morphology, the isolated fungi were small, medium and large in size. The margins of isolated fungi were entire and the elevation of isolated fungi were flat, convex, raised, and the form of isolated fungi circular and irregular.

All fungal strains were tested by eight test organisms for preliminary study of antimicrobial activity. Among them, six strains showed different level of antimicrobial activity. MP- 7 exhibited the highest antibacterial activity (25.05 mm) against *Bacillus pumilus* at 5 days and MP- 25 also showed the moderate activity (23.50 mm) on *Bacillus pumilus* at 5 days. MP- 6 gave the strong antibacterial activity (20.03 mm) against *Bacillus subtilus* at 4 days. Especially, MP- 41 showed the moderated antimicrobial activity against all test organisms.

For the human health and nutrition fungi are well known to produce both beneficial and deleterious natural agents and continue to be explored as useful sources of natural antimicrobial agents. In comparison to plants, microorganisms are highly diverse but narrowly explored (Chioma et al., 2016). It was concluded that the present research was to isolate the fungi from different soil samples and to study the antimicrobial activity of isolated fungi on eight test organisms. Further study will be focused on the fermentation conditions of selected fungus and extraction of antimicrobial compounds.

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