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Determination of Antimicrobial Activities in Isolated Soil Fungi from Kyonemangay, Whakhema Township, Ayewarwady Region of Myanmar

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Abstract
The soil microorganisms were isolated from five different places of soil samples collected from Kyonemangay, Whakhema Township. For the isolating of fungi, soil samples were collected from 30cm depth after removing the surface layer of soil. Some physicochemical properties of soil samples were determined such as pH range, moisture content, soil temperature and soil texture. Soil fungi were isolated by soil dilution methods and chemical treatment methods. Ten soil fungi were isolated, and their morphological characters were studied. The isolated soil fungi were used to test antimicrobial activities by the paper disc diffusion assay methods against eight test organisms such as *Agrobacterium tumefaciens*, *Bacillus Subtilis, Bacillus pumilus, Candida albicans, Staphylococcus aureus, Escherichia coli, Salmonella typhi* and *Micrococcus luteus*. According to the results of antimicrobial activities, TTSM-01, TTSM-03 and TTSM-07 showed antimicrobial activities against *bacillus subtilis* and *candida albicans*. TTSM-03 was selected for further investigations because it exhibited the highest antifungal activity against *Candida albicans* with 25.9 mm clear zone.

Keywords
Soil microorganism, Antimicrobial activities, Paper disc diffusion assay, Myanmar, Kyonemangay, Whakhema Township

1. Introduction

Soil is the outer layer of the Earth's crust which is loosely arranged and has many forms (Baxter and Wikkiamson, 2001). It generally consists of organic and inorganic matter. The organic fraction of soil is divided into biomass that included living organisms and neuromas which is the dead organisms and their transformation products (Nortcliff et al., 2006).

Soil is a fundamental natural resource with diverse applications across various sectors. Soil is essential for various purposes, including agriculture, construction, environment filtration, habitat for organisms, raw materials and climate regulation. Soil provides habitat vast array of organisms, contributing to biodiversity and the functioning of ecosystems.

Soil microbiology is a branch of microbiology that focuses on the diverse microbial communities present in soil and their interactions with each other, plants and the environments. These microorgansims play a crucial role in maintaining soil health, fertility, and ecosystem functioning.

Soil fungi are essential components of the soil ecosystem, playing crucial roles in nutrient cycling, organic matter decomposition and plant health are diverse, ranging from decomposers to symbiotic partners with plants and pathogens (Tiedje, 1993).

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Soil fungi are microscopic plant-like cells that grow in long threadlike structure or hyphae that make a mass called mycelium. Secondary metabolites can be defined as chemical compounds resulting from specific biosynthetic pathways, whose production is not necessary for normal growth and development of the fungus. Antibiotics derived from fungi have played a crucial role in medicine, particularly in fighting bacterial infection (Fleming, 1929).

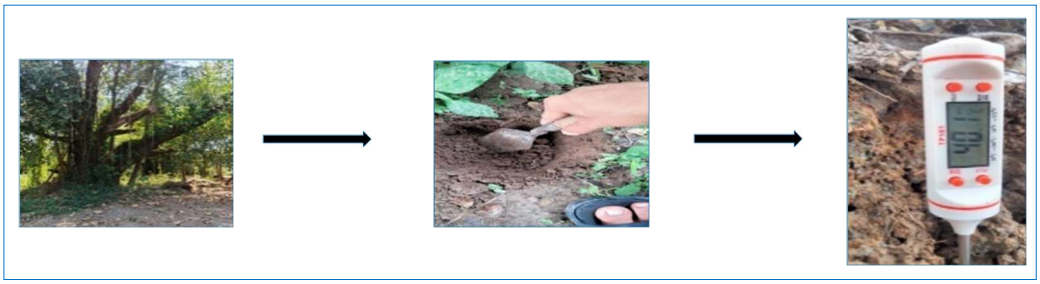
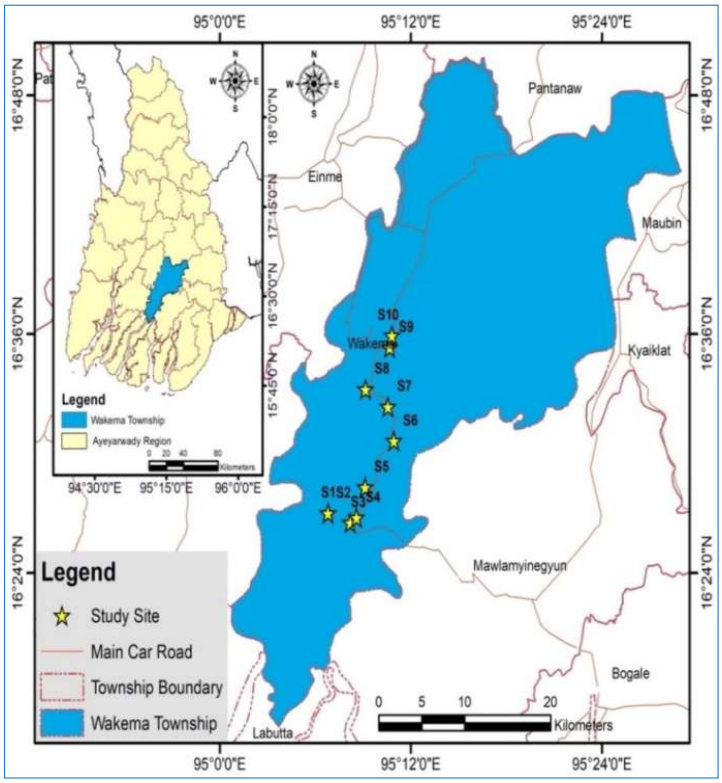


Fig 1. The collection of soil samples

Fig 2. The preparation of soil sample



S-1: Kone Gyi, S-2: Auk Pay Kone, S-3: Kyone Man Gay, S-4: Hnget Kya, S-5: Tone Le Chaung

Fig 3. Map of soil samples collected area

2. Materials and Methods

2.1. Soil Sample Collection and Preparation

For the isolation of soil samples, ten different samples were collected from Kyonemangay, Whakhema Township. Soil sample collection was carried out during July 2024. It was dug 12 inches in depth and tested soil temperature. And then,

these soil samples were placed into sterilized polythene bags. The soil samples were noted and brought to the BRBDC. Soil sample was air dried at room temperature. Then, these soil samples were ground and sieved to remove gravels, roots and other large particles. The soil samples were stored in polythene bag and clearly labeled.

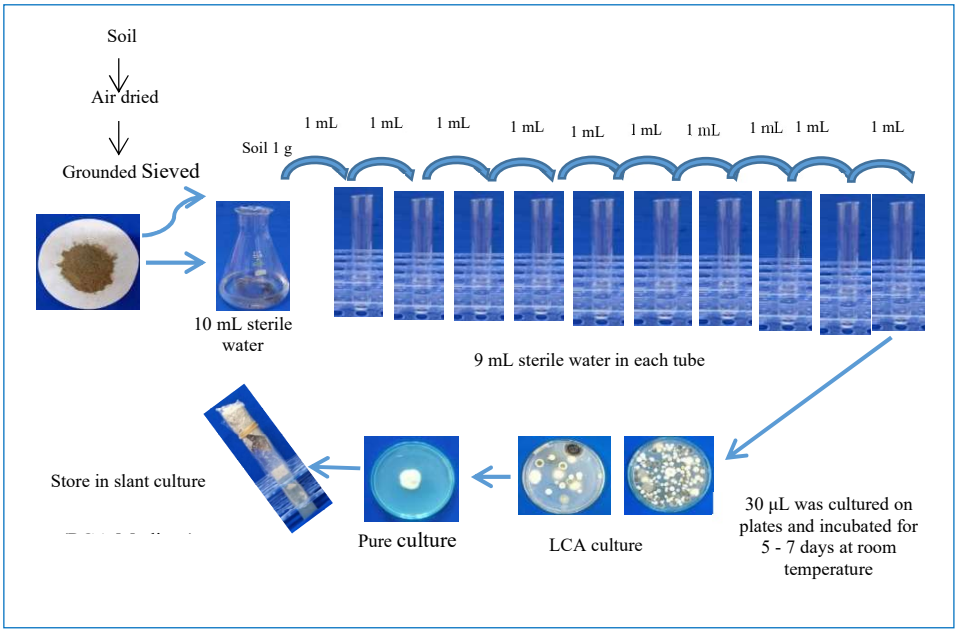


Fig. 4. Soil dilution method (Waksman, 1927)

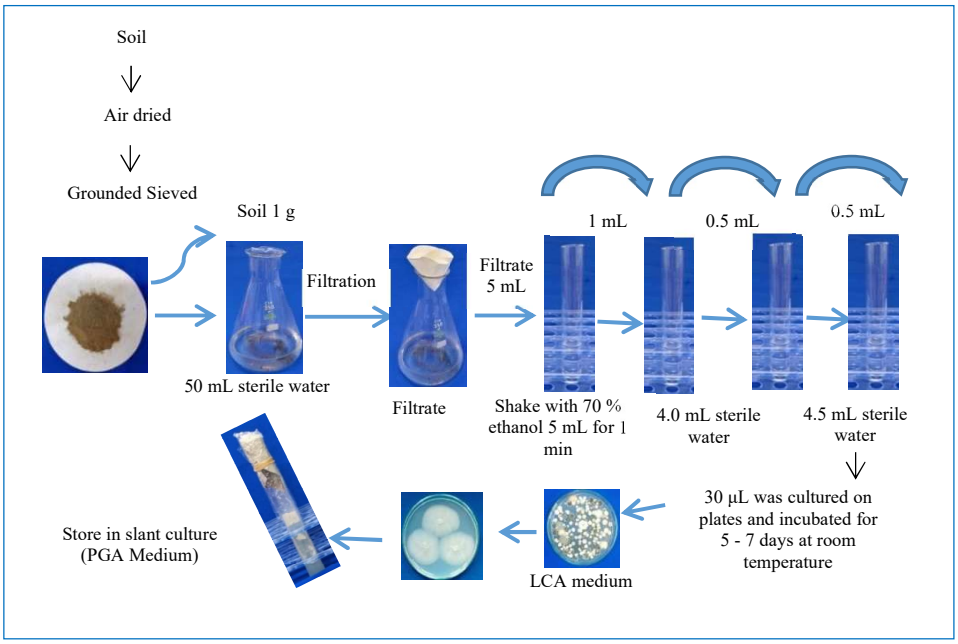


Fig. 5. Chemical treatment dilution method (Hayakawa and Kobayashi, 2005)

Table 1. Medium used for the isolation of fungi (Medium composition g/L)

Potato Glucose Agar Medium		Low Carbon Agar (LCA) Medium		Glucose Soluble Starch Yeast Extract Agar (GSY) Medium	
Potato	200	Glucose	2	Glucose	20
Glucose	20	Sucrose	2	Soluble starch	20
Agar	18	K_2HPO_4	1	Yeast extract	30
		$MgSO_4.7H_2O$	1	K_2HPO_4	0,1
		KNO_3	1	$MgSO_4.7H_2O$	0,1
		KCl	0,5	Agar	18
		Agar	18	pH	6,5

(After autoclaving chloramphenicol was added to the medium)

Table 2. Medium used for Antimicrobial Activity Test (PBCC, 2004)

Seed medium		Fermentation medium		Assay medium	
Glucose	7	Glucose	15	Glucose	5
Yeast extract	5	Yeast extract	7	Peptone	7
KNO_3	0,2	Peptone	8	KNO_3	3
K_2HPO_4	0,01	K_2HPO_4	0,1	Agar	18
pH	6,5	$MgSO_4.7H_2O$	0,1		
		pH	6,5		

Table 3. Test organisms used in antimicrobial activities

No	Test Organism	Diseases
1	*Agrobacterium tumefaciens*	Tumor cell in plants
2	*Bacillus subtilis*	Fever
3	*Bacillus pumilus*	Sepsis, endocarditis, skin infection and food poison
4	*Candida albicans*	Candidosis
5	*Staphylococcus aureus*	Skin disease, Food poison
6	*Escherichia coli*	Diarrhoea
7	*Salmonella typhi*	Typhoid
8	*Micrococcus luteus*	Skin disease

2.2. Some Physicochemical Properties of Soil Samples (pH, Moisture and Soil Texture)

Soil pH is a measure of the acidity or alkalinity of soil, expressed on a scale from 0 to 14. A pH of 7 is neutral, while values below 7 indicate acidic soil and values above 7 indicate alkaline soil. Soil moisture refers to the amount of water held in the soil pores, which is crucial for plant growth, microbial activity and various soil processes. Soil texture refers to the relative proportion of sand, silt, clay particles in the soil. It plays a crucial role in determining soil properties such as water retention, drainage, aeration and nutrient availability (Brady and Weil, 2016).

Table 4. Soil sample collected from different places in whakhema township

Soil No	Collected Places	Location		Collected Date
S-1	Kone Gyi	N 16° 27′ 0.60″	E 95° 6′ 48.48″	1.7.2024
S-2	Auk Pay Kone	N 16° 27′ 0.39″	E 95° 6′ 47.35″	1.7.2024
S-3	Kyone Ma Ngay	N 16° 26′ 31.68″	E 95° 8′ 11.50″	1.7.2024
S-4	Hnget Kya	N 16° 26′ 46.96″	E 95° 8′ 34.95″	1.7.2024
S-5	Tone Le Chaung	N 16° 28′ 18.79″	E 95° 9′ 7.60″	1.7.2024

Table 5. Analytical data of ten different soil samples

Soil No	Collected Places	pH	Moisture (%)	Temperature (°C)	Soil Texture
S-1	Kone Gyi	6.2	3.01	27.2 - 40.1	Sandy Clay Loam
S-2	Auk Pay Kone	6.6	4.87	27 - 40.1	Sandy Clay Loam
S-3	Kyone Ma Ngay	6.5	3.33	27.9 - 40.1	Sandy Clay Loam
S-4	Hnget Kya	6.5	3.34	27.1 - 40.1	Sandy Loam
S-5	Tone Le Chaung	6.3	2.44	27.5 - 40.1	Sandy Loam

Table 6. Isolated Soil Fungi from Different Soil Samples

Soil sample no	Isolated soil fungi		No. of fungi
	Soil dilution method	Chemical treatment dilution method	
1.	TTSM-01, TTSM-02	TTSM-03	3
2.	TTSM-04, TTSM-05,	-	2
3.	TTSM-06,	-	1
4.	TTSM-07	TTSM-08	2
5.	TTSM-09	TTSM-10	2
	Total		10

In this study, different places of soil samples in Whakhema Township were determined the pH, moisture and soil texture by Land Utilization Division, Department of Agriculture, Pathein Township, Ayeyarwady Region.

2.3. Isolation of Soil Fungi from Soil Samples

Isolation of soil fungi from soil samples was carried out by Waskman (1927) and Hayakawa and Kobayashi (2005). Low carbon agar medium (LCA) and glucose soluble starch agar (GSY) medium were used to isolate soil fungi. Potato glucose agar medium was used to transfer visible colony from LCA and GSY media.

2.4. Screening for Antimicrobial Activities by Paper Disc Diffusion Assay Method

The isolated fungi were grown at room temperature for 5 days

on PGA. The isolated fungi were inoculated on seed medium and inoculated at room temperature for 2 days. Twenty mL of seed culture was transferred into the fermentation medium (30 mL) and was incubated at room temperature for 10 days. Twenty µL of fermented broth was put on paper disc and placed on assay plate containing test organisms.

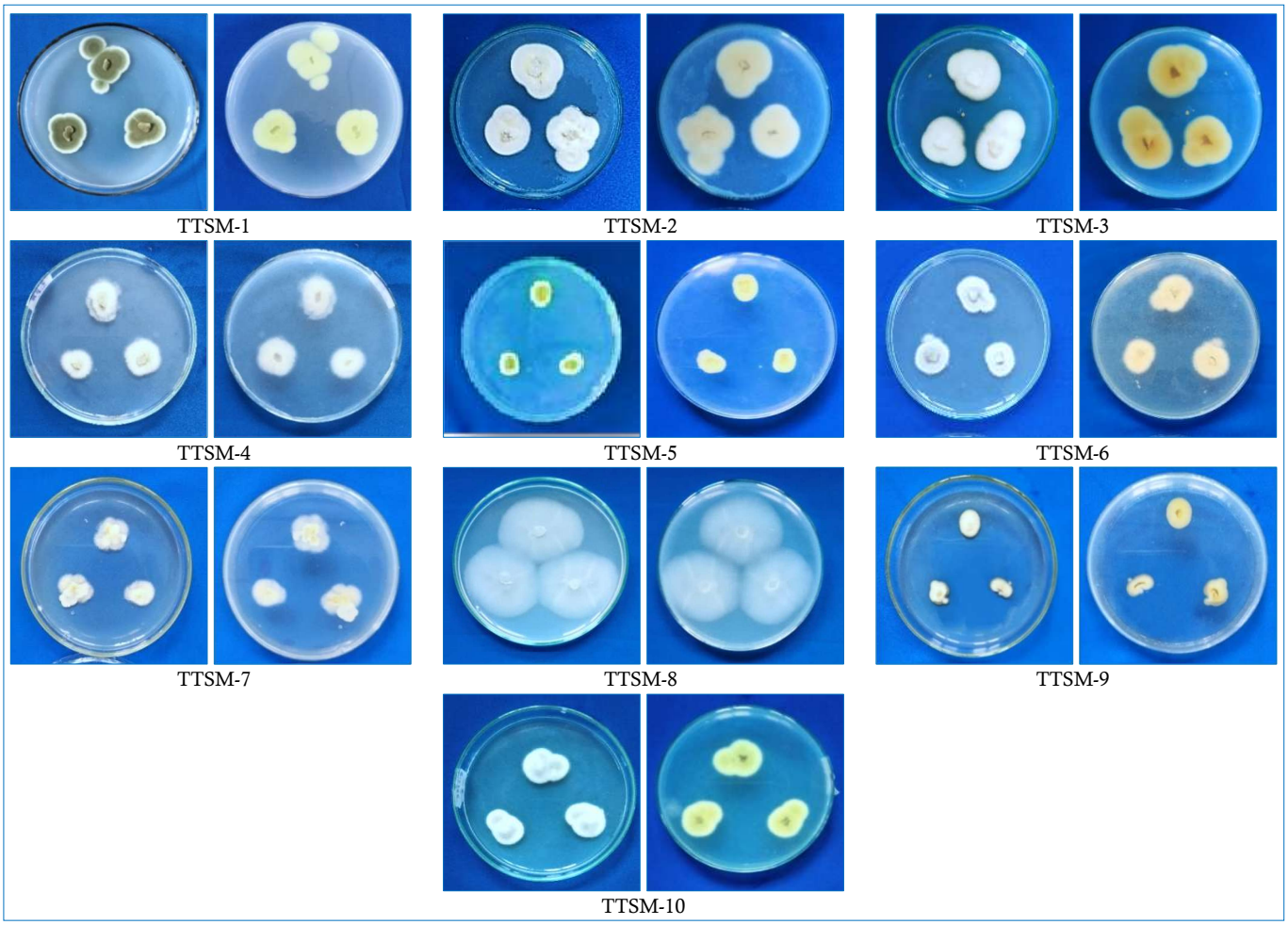


Fig. 6. Morphological characters of soil fungi front views and reverse views of TTSM-01 to TTSM-10 of 5 days old culture on PGA medium

Table 5. Morphological characters of isolated soil fungi (TTSM-1 to TTSM10)

Isolated fungi	Front view	Reverse view
TTSM-01	Dark green	Green
TTSM-02	White	Cream
TTSM-03	White	Pale yellow
TTSM-04	White	White
TTSM-05	Green	Yellow
TTSM-06	White	Cream
TTSM-07	White	Yellow
TTSM-08	White	White
TTSM-09	White	Pale yellow
TTSM-10	Pale yellow	Green

3. Results

3.1. Soil Sample Collection

Ten soil samples were collected from different places of Kyonemangay, Whakhema Township, Ayeyarwady Region, Myanmar.

3.2. Some Physicochemical Properties of Soil Samples (pH, Moisture and Soil Texture)

The different places of soil samples in Whakhema Township were determined the pH, moisture and soil texture by Land Utilization Division, Department of Agriculture, Pathein Township, Ayeyarwady Region.

3.3. Isolation of Soil Fungi from Soil Samples

A total of sixteen soil fungi were isolated from different places of soil samples in Whakhema Township.

Ten soil fungi were obtained using the soil serial dilution method and while six soil fungi were isolated through chemical treatment dilution method.

3.4. Screening for Antimicrobial Activities by Paper Disc Diffusion Assay Method

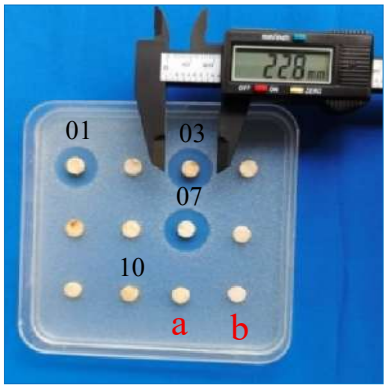
The isolated soil fungi were used for antimicrobial activities by using the paper disc assay methods. Initially, the isolated soil fungi (TTSM-01 – TTSM-10) were inoculated into a seed medium and incubated for 2 days. Subsequently, the cultures were transferred to a fermentation medium. Antimicrobial activity was evaluated starting from the third day of fermentation. The presented result here shows the antimicrobial activity observed on the fourth day of fermentation, which exhibited the highest activity. In this study, three fungi (TTSM-01, TTSM-03 and TTSM-07) showed antimicrobial activities against *Bacillus subtilis* and *Candida albicans*. In this time, the isolated fungus TTSM-03 (25.9 mm) showed antifungal activity on *Candida albicans*.

3.4.1. Antimicrobial Activities of Selected Fungi (2nd times)

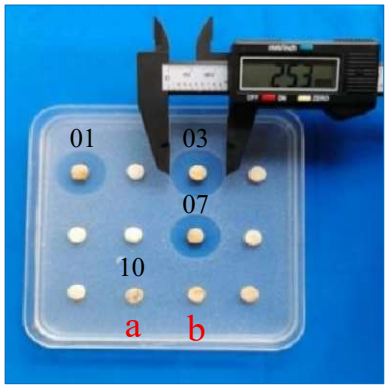
The isolated soil fungi were used for antimicrobial activities by using the paper disc assay methods. In this study, three fungi were shown antimicrobial activities against *Bacillus subtilis* and *Candida albicans*. So, these fungi were selected for the second time on test activities. This time, the fungus TTSM-03 (25.9 mm) showed more active on *Candida albicans.* The results discussed above are depicted in Figs. 8 and 9 and summarized in Table 7. Therefore, this strain TTSM-03 fungus will be used for further more investigation on the fermentation.

Table 6 Antimicrobial activities of isolated fungi TTSM-01 to TTSM10 (1st times)

Isolated No	*Agrobacterium Tumefaciens*	*Bacillus subtilis*	*Bacillus pumilus*	*Candida albican*	*Staphylococcus aureus*	*E. coli*	*Salmonella typhi*	*Micrococcus luteus*
TTSM-01	-	21.7mm	-	22.6mm	-	-	-	-
TTSM-02	-	-	-	-	-	-	-	-
TTSM-03	-	**22.8mm**	-	**25.3mm**	-	-	-	-
TTSM0-4	-	-	-	-	-	-	-	-
TTSM-05	-	-	-	-	-	-	-	-
TTSM-06	-	-	-	-	-	-	-	-
TTSM-07	-	22.6 mm	-	23.7 mm	-	-	-	-
TTSM-08	-	-	-	-	-	-	-	-
TTSM-09	-	-	-	-	-	-	-	-
TTSM-10	-	-	-	-	-	-	-	-



Antimicrobial activity on *Bacillus subtilis*
(a: ethanol control, b: chloramphenicol control)



Antimicrobial activity on *Candida albicans*
(a: ethanol control, b: chloramphenicol control)

Fig. 7. Antimicrobial activities of isolated soil fungi TTSM-01 to TTSM-10

Table 7 Antimicrobial activities of selected fungi (2nd times)

Isolated No	*Bacillus Subtilis* ± SD	*Candida albicans* ± SD
TTSM-01	23.1 mm ± 0.01	23.4 mm ± 0.02
TTSM-03	**24.7 mm** ± 0.08	**25.9 mm** ± 0.01
TTSM-07	23.3 mm ± 0.03	23.9 mm ± 0.01

4. Discussion

In the present study, a total of ten soil fungi isolates were obtained from five different soil types, suggesting that soil heterogeneity plays a significant role in determining fungal diversity and distribution. Variations in fungal isolates among soil samples may be attributed to differences in physicochemical properties such as pH, moisture content, organic matter, and nutrient availability, all of which are known to influence microbial growth and survival. Soils rich in organic matter tend to support a higher diversity of fungi, whereas nutrient-poor soils may limit fungal proliferation (Gadd, 2007 and Hawksworth, 2001).

The antimicrobial activity exhibited by the isolated fungi indicates their potential to produce bioactive secondary metabolites. Fungi are well known for their ability to synthesize a wide range of antimicrobial compounds, including antibiotics, as a defense mechanism to compete

with other microorganisms in their natural habitat (Demain and Sanchez, 2009). This competitive interaction in soil ecosystems is a major factor driving the production of such metabolites, which may explain the inhibitory effects observed in this study.

Furthermore, the variation in antimicrobial activity among the isolates suggests differences in their metabolic capabilities and genetic potential to produce secondary metabolites. Some isolates may produce higher concentrations of bioactive compounds, resulting in stronger antimicrobial activity, while others may exhibit weaker or no activity.

Similar findings have been reported in previous studies, highlighting the diversity in antimicrobial potential among soil fungi (Bills and Gloer, 2016).

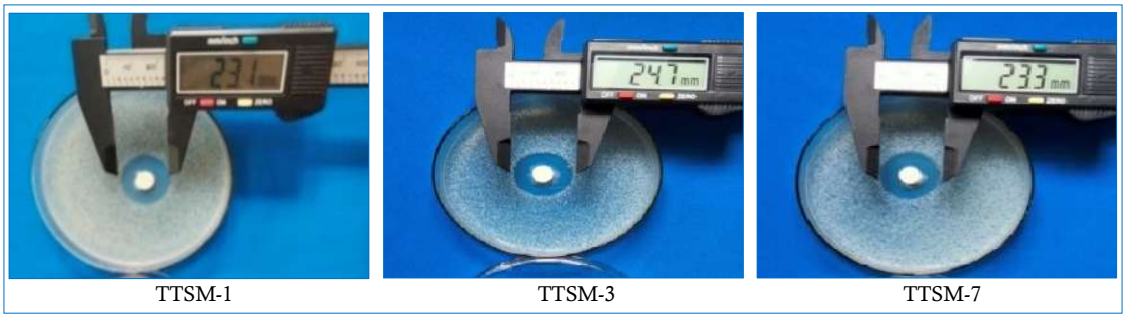


Fig. 8. Antibacterial activities of selected soil fungi against *Bacillus subtilis*

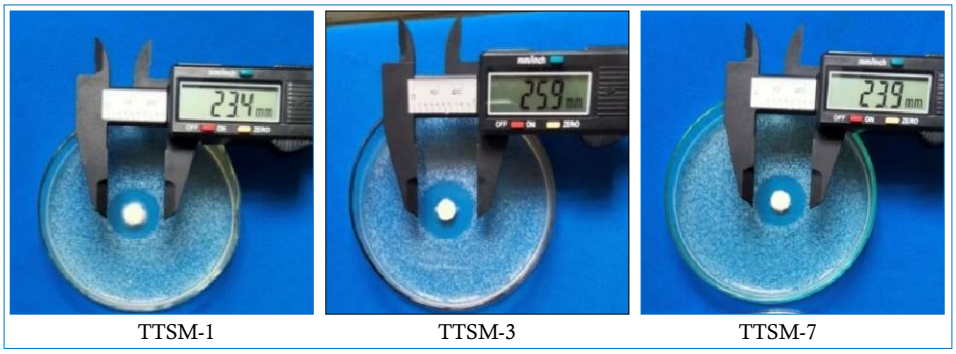


Fig. 9. Antifungal activities of selected soil fungi against *Candida albicans*

These findings are in agreement with earlier reports indicating that soil fungi are a prolific source of antimicrobial agents and have contributed significantly to the discovery of novel drugs (Bills and Gloer, 2016). Overall, the results suggest that fungi isolated from diverse soil environments possess promising antimicrobial potential and could serve as valuable sources for the development of new antimicrobial agents. Further studies will be focused on identification, purification, and characterization of active compounds.

5. Conclusion

Soil samples were collected from Whakhema Township, Ayeyarwady Region. Ten different soil samples were collected, and a total of ten fungi were isolated. In order to isolate soil fungi, Soil Dilution Method and Chemical Treatment Dilution Method (CTDM) were used. In this study, in total seven fungi were isolated from the soil dilution method and three fungi by chemical treatment dilution method. Morphological characters of isolated fungi were also studies according to their front views and reverse views.

The pH value of soil samples was reported in the range from (4.9–6.6). The moisture content of soil samples (S-1 3.01 %, S-2 4.87 %, S-3 3.33 %, S-4 3.34 % and S-5 2.44 %) were recorded. The temperature of ten soil samples was located between (27°C and 40.1°C). The texture of soil sample was observed that soil samples (S-1, S-2, S-3 were sandy clay loam and (S-4 and S-5) were sandy loam.

The isolated soil fungi were used for antimicrobial activities by using paper disc diffusion methods. According to the results of antimicrobial activities, TTSM-03 isolated from the soil samples (Soil-1) showed the antimicrobial activities against *Bacillus subtilis* (24.7 mm clear zone at 4-day fermentation) and *Candida albicans* (25.9 mm clear zone at 4-day fermentation). The fungus TTSM-03 showed more active on *Candida albicans*. Therefore, this fungus TTSM-03 was selected for further more investigation on fermentation.

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