# Preliminary Isolation of Soil Fungi at Tha-Yet-Kone Village in Hinthada Township

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## Abstract

The paper presents the result of fungi in the soil obtained at the Tha-Yet-Kone village in Hinthada Township of Ayeyarwady Region. A total of 8 native population of soil fungi was studied from the study soil area. These fungi were isolated by using the dilution method. The main study of this research is focused on a survey of microfungi isolation and colony morphology characters of soil fungi. These soil fungi will be used in the biodegradation of pesticides for agricultural land, in potential bioactive compounds like an enzyme for other industries and as a useful antibiotic for humans, in future studies.

**Keywords:** Soil fungi, dilution method, colony morphological characters

## INTRODUCTION

The soil is a rich habitat for the growth of microorganisms more than any other microbial habitat. Among these microorganisms, fungi are one of the dominant groups present in the soil, which represent the main reservoir of fungi. Fungi belong to the kingdom Fungi (Myceteae). The distinguishing characteristics of this group as a whole are that they are eukaryotic, non-photosynthetic, lack tissue differentiation, have a cell wall of chitin or other polysaccharides and propagate by spores. Soil fungi can be grouped into three general functional groups based on how they get their energy, decomposers, mutualists and pathogens (Toma and Abdulla, 2012). Once the mould is isolated, further culturing may be required before the organism can be identified. Fungal identification is done based on morphological characteristics of the colony, etc. Moulds are characterized by the development of hyphae, which result in the colony characteristics seen in the laboratory (Al-mohanna, 2017).

Soil fungi are also the major source of other industrially important compounds like enzyme inhibitors, antihelminthic, antitumor agents, insecticides, vitamins and immune-suppressants. Soil microbial communities are the most diverse group on earth possessing a plethora of activities useful to mankind (Thamilvanan *et al*., 2018).

In this research, 8 types of soil fungi were isolated at the Tha-Yet-Kone village in Hinthada Township and were studied in the colony morphology characters from isolated fungi.

## MATERIALS AND METHODS

### Area of Study

The soil sample was collected at Tha-Yet-Kone Village of Hinthada Township in Ayeyarwady Region, June 2021. The sample was collected from sampling site for isolation soil fungi as shown in (Figure.1and 2). Tha-Yet-Kone village has located coordinates in degrees, minutes and seconds (DMS) of 17° 37′ 47.772″N and 95° 26′ 24.594″E. The climate of the Tha-Yet-Kone village site is generally characterized by high humidity (89%). The village is located at an elevation of 17m above sea level and the temperature is 26°C in table – 1.

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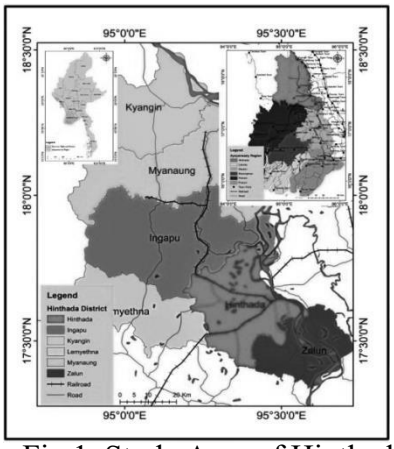



Fig 1. Study Area of Hinthada Township


Fig 2. Tha-Yet-Kone Village Site of Soil Sample

**Collection of Soil sample**

The selected data was collected for the isolation of soil sample at (6 inches) depth and put into a small sterilized polythene bag. The sample was properly labeled and brought to the Microbiology Laboratory, Department of Botany, Hinthada University for further studies.

**Dilution Method (Hayakawa and Kobayashi, 2005)**

The collected soil was air-dried at room temperature and grounded. One gram of soil sample was added into 100mL of sterile water. Soil suspension 0.1mL put 5mL of sterile distilled water and shaken it for 15 minutes. Diluted soil suspension 0.5mL was put into 4.5mL of sterile distilled water and shaken for 10 minutes. One milliliter diluted soil suspension was put into 4mL of sterile distilled water and shaken for 10 minutes. After that took one drop of diluted soil suspension was added to sterile Petri dishes containing Low Carbon Agar (LCA) medium. Each soil fungi was recultured on Petri dishes containing PGA medium and preserved in the PGA slant in (Figure 3).

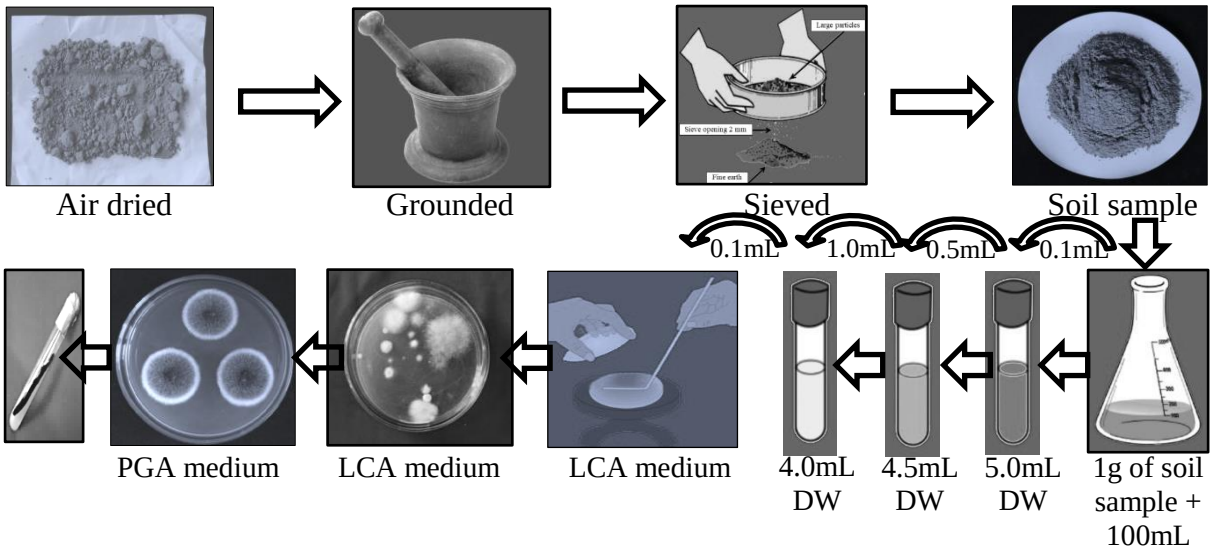



Fig 3. Procedures of Dilution Method (Hayakawa and Kobayashi, 2005)

**Medium used for the isolation (Ando,2004)**

Low Carbon Agar (LCA medium)

| | |
|---|---|
| Glucose | 0.2g |
| Sucrose | 0.2g |
| $K_2HPO_4$ | 0.1g |
| $KNO_3$ | 0.1g |
| KCl | 0.05g |
| Agar | 1.8g |
| Distilled water | 100mL |
| pH | 6.5 |

**Medium used for the transfer (Ando,2004)**

Potato Glucose Agar (PGA) medium

| | |
|---|---|
| Potato | 20g |
| Glucose | 2.0g |
| Agar | 1.8g |
| Distilled water | 100mL |
| pH | 6.5 |

After autoclaving, chloramphenicol (25mg/100mL) was added to the medium for antibacterial activity

## RESULTS

The results revealed the colony morphological characters of fungal from the Tha-Yet-Kone village of soil sample. A total number of 8 fungi was isolated by dilution method as shown in ( Table - 2 and Figure. 4 – 11).

Table 1. Collected of Soil and Isolated of Fungi at Tha-Yet-Kone Village Site

| Soil No. | Collected Site | Location | Collected Date | Temperature | Humidity | Elevation | Isolate Fungi |
|---|---|---|---|---|---|---|---|
| 1 | Tha Yet Kone Village | 17° 37′ 47.772″ N 95° 26′ 24.594″ E | 1.6.2021 | 26°C | 89% | 17m | 8 |

Table 2. Morphological Characters of Isolated Fungal Stains

| | Cultural Character | | | | | |
|---|---|---|---|---|---|---|
| No. | **Isolated Fungi** | **Front color** | **Reverse color** | **Form** | **Elevation** | **Margin** |
| 1 | HTS - 01 | Pale yellow | Dark yellow | Circular | Umbonate | Entire |
| 2 | HTS – 02 | Black in center and pale yellow in edge | Pale yellow | Circular | Flat | Entire |
| 3 | HTS – 03 | Black | White | Circular | Flat | Entire |
| 4 | HTS – 04 | Yellow in the center and white edge | Yellow | Circular | Umbonate | Entire |
| 5 | HTS – 05 | Indigo in the center and ivory edge | Brown | Circular | Umbonate | Enitre |
| 6 | HTS – 06 | Gray | White | Circular | Raised | Filiform |
| 7 | HTS - 07 | White | Pale pink | Circular | Umbonate | Curled |
| 8 | HTS - 08 | White | Orange | Irregular | Umbonate | Undulate |

Fungus HTS – 01(Front view)

Fungus HTS – 01(Reverse view)

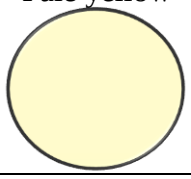
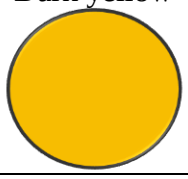
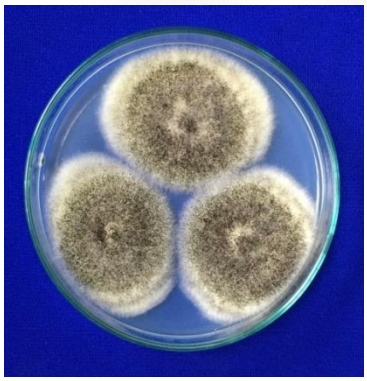
| Cultural Character | | | | | | |
|---|---|---|---|---|---|---|
| **No.** | **Isolated Fungi** | **Front color** | **Reverse color** | **Form** | **Elevation** | **Margin** |
| 1 | HTS - 01 | Pale yellow  | Dark yellow  | Circular  | Umbonate  | Entire  |

Fig 4. Colony Morphology of Isolated Soil Fungus HTS-01(5 days old culture on PGA medium)



Fungus HTS – 02 (Front view)

Fungus HTS – 02 (Reverse view)

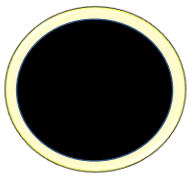
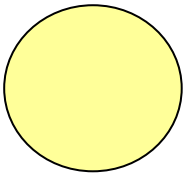
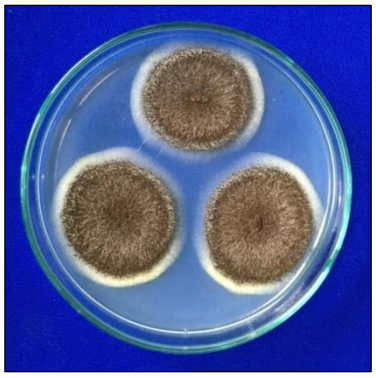
| Cultural Character | | | | | | |
|---|---|---|---|---|---|---|
| **No.** | **Isolated Fungi** | **Front color** | **Reverse color** | **Form** | **Elevation** | **Margin** |
| 2 | HTS - 02 | Black in center and pale yellow in edge  | Pale yellow  | Circular  | Flat  | Entire  |

Fig 5. Colony Morphology of Isolated Soil Fungus HTS-02(5 days old culture on PGA medium)



Fungus HTS – 03 (Front view)

Fungus HTS – 03 (Reverse view)

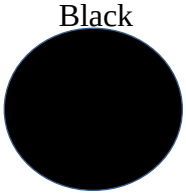
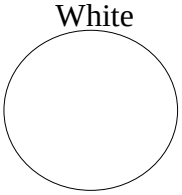
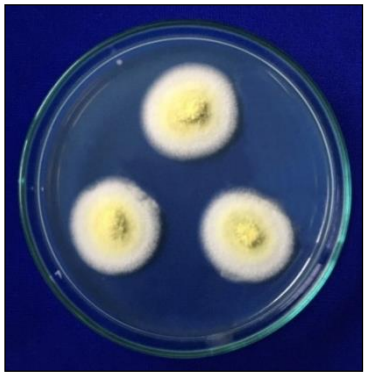
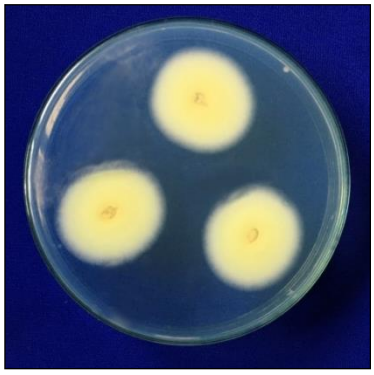
| Cultural Character | | | | | | |
|---|---|---|---|---|---|---|
| **No.** | **Isolated Fungi** | **Front color** | **Reverse color** | **Form** | **Elevation** | **Margin** |
| 3 | HTS - 03 | Black  | White  | Circular  | Flat  | Entire  |

Fig 6. Colony Morphology of Isolated Soil Fungus HTS-03 (5 days old culture on PGA medium)



Fungus HTS – 04 (Front view)



Fungus HTS – 04 (Reverse view)

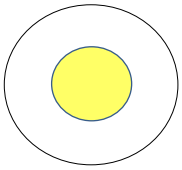
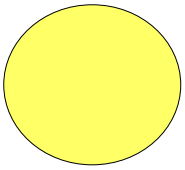
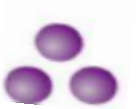
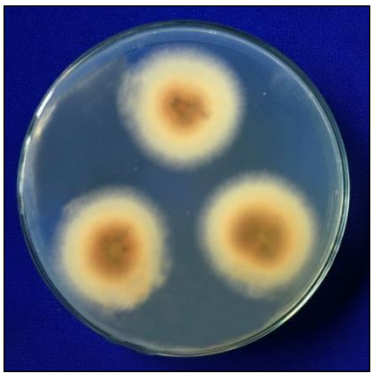
| Cultural Character | | | | | | |
|---|---|---|---|---|---|---|
| **No.** | **Isolated Fungi** | **Front color** | **Reverse color** | **Form** | **Elevation** | **Margin** |
| 4 | HTS - 04 | Yellow in center and white in edge  | Yellow  | Circular  | Umbonate  | Entire  |

Fig 7. Colony Morphology of Isolated Soil Fungus HTS-04(5 days old culture on PGA medium)

Fungus HTS – 05 (Front view)



Fungus HTS – 05 (Reverse view)

**Cultural Character**

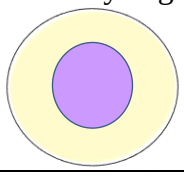
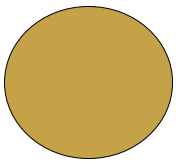
| No. | Isolated Fungi | Front color | Reverse color | Form | Elevation | Margin |
|---|---|---|---|---|---|---|
| 5 | HTS - 05 | Indigo in the center and ivory edge  | Brown  | Circular  | Umbonate  | Entire  |

Fig 8. Colony Morphology of Isolated Soil Fungus HTS-05(5 days old culture on PGA medium)

Fungus HTS – 06 (Front view)

Fungus HTS – 06 (Reverse view)

**Cultural Character**

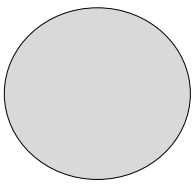
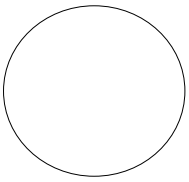
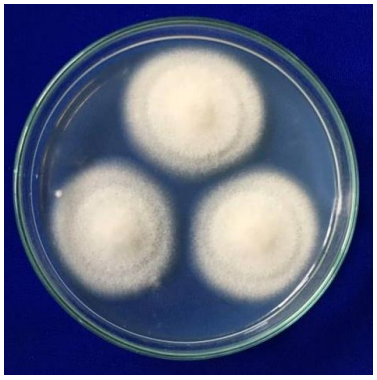
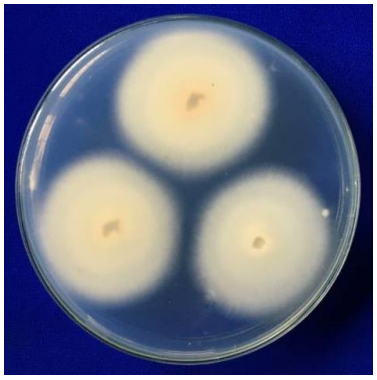
| No. | Isolated Fungi | Front color | Reverse color | Form | Elevation | Margin |
|---|---|---|---|---|---|---|
| 6 | HTS - 06 | Gray  | White  | Circular  | Raised  | Filiform  |

Fig 9. Colony Morphology of Isolated Soil Fungus HTS-06(5 days old culture on PGA medium)



Fungus HTS – 07 (Front view)

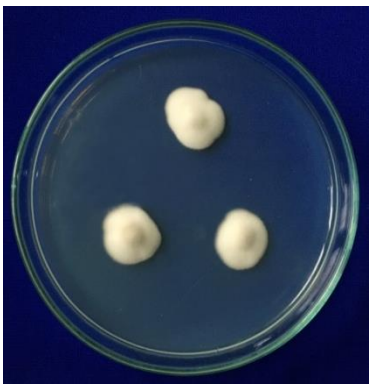


Fungus HTS – 07 (Reverse view)

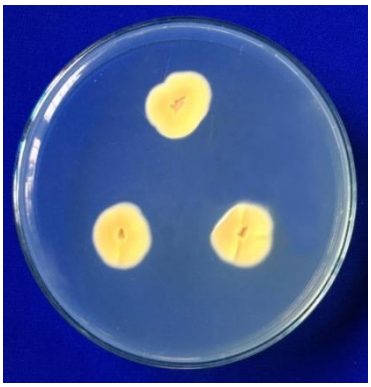
**Cultural Character**

| No. | Isolated Fungi | Front color | Reverse color | Form | Elevation | Margin |
|---|---|---|---|---|---|---|
| 7 | HTS - 07 | White | Pale pink | Circular | Umbonate | Curled |

Fig 10. Colony Morphology of Isolated Soil Fungus HTS-07 (5 days old culture on PGA medium)



Fungus HTS – 08 (Front view)



Fungus HTS – 08 (Reverse view)

**Cultural Character**

| No. | Isolated Fungi | Front color | Reverse color | Form | Elevation | Margin |
|---|---|---|---|---|---|---|
| 8 | HTS - 08 | White | Orange | Irregular | Umbonate | Undulate |

Fig 11. Colony Morphology of Isolated Soil Fungus HTS-08(5 days old culture on PGA medium)

## DISCUSSION AND CONCLUSION

In the Preliminary isolation of soil fungi, 8 different fungi were collected from the Tha-Yet-Kone village in Hinthada Township. According to Hayakawa and Kobayashi (2005) these fungi were isolated by the dilution method. One drop of diluted soil suspension was added to sterile Petri dishes containing Low Carbon Agar (LCA) medium. Each soil fungi was recultured on Petri dishes containing a Potato Glucose Agar (PGA) medium and preserved in the PGA slant. Pure cultures of isolated soil fungi were inoculated on the Petri plates containing Potato Glucose Agar (PGA) medium with chloramphenicol and incubated for 3 to 5 days. The macroscopic features of the isolated fungi were described including the colony morphological color (front and reverse views) and the growth characteristics such as form, elevation and margin of the whole Nguyen *et al*., (2019). According to Table 2, fungi HTS-01, 02, 03, 04, 05, 06, 07 and 08 were isolated from a soil sample collected at the Tha-Yet-Kone village. In the study of colony morphology characters, it was found that these isolated soil fungi were of different colors on front and reverse views. All isolate exhibited a circular colony form except fungus HTS-08 which was irregular shaped. In elevation, fungi HTS–01, 04, 05, 07 and HTS–08 were exhibited umbonate. The isolated fungi HTS–02 and HTS–03 were exhibited flat and only fungus HTS–6 was exhibited raised. The isolated fungi HTS–01, 02, 03, 04 and HTS–05 were found entire margin and fungus HTS-06 was found filiform margin and fungus HTS–07 was found curled margin and fungus HTS–08 was found undulate margin, respectively. After preliminary isolation, antimicrobial tests will be performed and identified on isolated 8 soil fungi. The soil fungi will be tested in future studies for the biodegradation of pesticides for agricultural land, in potential bioactive compounds like an enzyme for other industrially and as a useful antibiotic for humans.

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