

## Investigation the Isolation and Antimicrobial Activities of Soil Fungi Isolated from Some Places in Kangyidaunt Township

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### Abstract

The investigation was conducted to find out the fungal diversity in seven different soil samples at Kangyidaunt Township of Ayeyarwaddy Region. Soil samples were collected randomly from 0-6 cm depth after removing the surface soil. The isolation of fungi was carried out by physical and chemical treatment dilution method and serial dilution method. After the investigation, thirty two soil fungi were isolated and tested for antimicrobial activities by seven test organisms. According to the result, the isolated fungus MTZ-31 exhibited the highest antifungal activity (28.7 mm) against *Candida albicans*. Therefore, this fungus MTZ-31 was selected for further investigation.

**Key words:** isolated soil fungi, antimicrobial activities

### Introduction

The major groups of microorganisms – namely bacteria, archaea, fungi (yeast and molds), algae, protozoa, and viruses. Microbes are important in human culture and health in many ways, saving to ferment foods and treat sewage, and to produce fuel, enzymes, and other bioactive compounds. Microbes are essential tools in biology as model organisms and have been put to use biological warfare and bioterrorism (Michael and Rita., 2022).

Microorganisms are beneficial in increasing the soil fertility and plant growth as they are involved in several biochemical transformations and mineralization activities in soil (Gaddeyya *et al.*, 2012). Microorganisms require a suitable environment include a growth medium that can support their nutritional needs.

Fungi are one of the most important groups of organisms on the planet (Hanson, 2003). Soil fungi play an important role as major decomposers in the soil ecosystem. They also provide mankind with very useful pharmaceutical products, such as antibiotics and other valuable substances, including organic acids, enzymes, pigments and secondary metabolites used in the food industry and fermentation. There are about 75,000 species of soil fungi in the world (Pongsatorn Puangsombat *et al.*, 2010).

Antimicrobial activity can be defined as a collective term for active principles (agents) that inhibit the growth of bacteria, prevent the formation of microbial colonies, and may destroy microorganisms. Antimicrobial assays are important tools to test and screen the inhibitory effects of myriad compounds against microorganisms before establishing their inhibitory spectra (broad vs. narrow) (Bailey, T.W., 2013). The drug used to prevent the pathogenicity of microorganisms is called an antimicrobial agent (Yehia., 2019).

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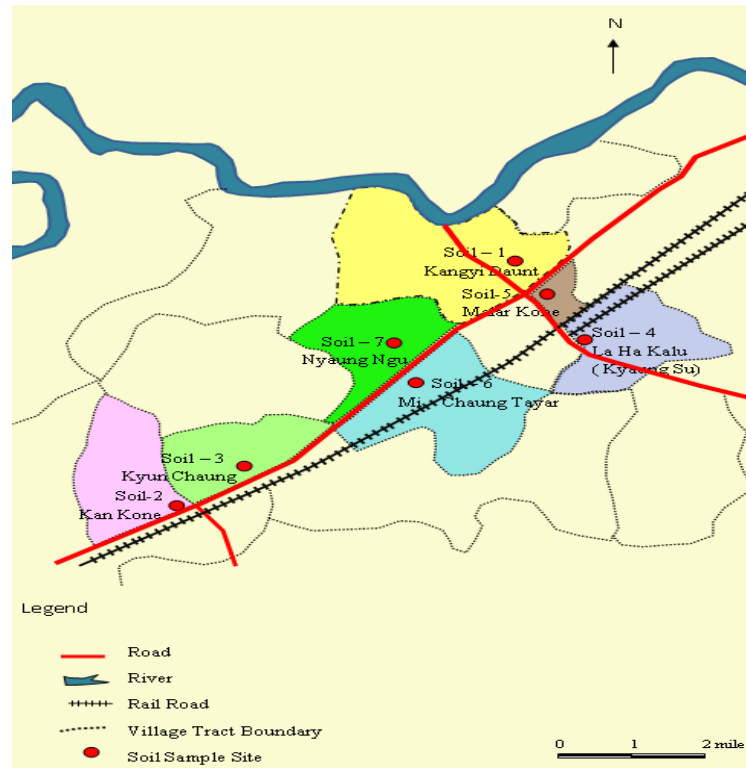
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## Materials and methods

### Collection of soil samples

Soil samples were collected from seven different places in Kangyidaunt Township as shown in (Figure.1.) for isolation of fungi. Soil was analyzed by Department of Agriculture (Land Use), Insein Township in Yangon. The isolation of soil fungi was carried out by the physical and chemical treatment dilution method and serial dilution method (Phay and Yamamura, 2005).



Source; Department of Geography, Patheingyi University

Figure 1. Map of soil samples collected in Kangyidaunt Township

### Isolation of fungi from soil samples

Physical and chemical treatment dilution method (Phay and Yamamura, 2005)

The collected soil was air dried at room temperature for 5 days. The soil sample was ground and sieved in 2 mm screen. The sample was placed in the hot air oven at

60 °C for 1 hr. The dried soil sample was suspended with 1.5 % phenol and diluted with sterile water. The dilution series were cultured on low carbon agar medium (LCA). Soil suspension (30  $\mu$ L) was cultured on plates containing LCA medium and incubated for 5 - 10 days. Pure colonies were picked up to slant containing in potato glucose agar (PGA) medium.

### Serial dilution method (Phay and Yamamura, 2005)

Soil sample was air dried at room temperature and then grounded and sieved. One gram of the sieved soil was put into 100 mL of sterile distilled water and heated for 10 minutes and then diluted with sterile water. The final test tube in dilution series of microorganisms were placed on LCA medium and incubated for 3-5 days. Single

colonies from the plate were picked and purified for sub culture. The pure fungi were stored in the test tube with PGA medium.

#### Medium used for the isolation of soil fungi

| Low Carbon Agar Medium (LCA)         |      | Potato Glucose Agar Medium (PGA) |      |
|--------------------------------------|------|----------------------------------|------|
| Components of per liter              |      | Components of per liter          |      |
| Glucose                              | 20 g | Potato                           | 20 g |
| Sucrose                              | 20 g | Glucose                          | 20 g |
| K <sub>2</sub> HPO <sub>4</sub>      | 10 g | Agar                             | 18 g |
| MgSO <sub>4</sub> ·7H <sub>2</sub> O | 10 g | pH                               | 6.5  |
| pH                                   | 6.5  |                                  |      |
| KNO <sub>3</sub>                     | 10 g |                                  |      |
| KCl                                  | 5 g  |                                  |      |

After autoclaving, chloramphenicol (25 mg/L) was added to the medium.

#### Preliminary antimicrobial activities test by paper disc diffusion assay method (Tomita, 1988)

The isolated soil fungi was grown on PGA (Potato Glucose Agar) agar medium and were inoculated into seed medium and incubated for 3 days at 25 °C. Seed medium (5 %) was transferred to the fermentation medium. The fermentation was carried out for 7 days. After the end of fermentation, the fermented broth (20 µL) was used to examine the antimicrobial activity against test organisms by paper disc diffusion assay. Paper disc having eight millimeter diameter (Advance, Tokyo Roshi Kiasha Co., Ltd., Japan) were utilized for antimicrobial assays.

The assay medium was used for the antimicrobial activity test. One percent ( $1.6 \times 10^8$  / mL of spore suspension) of test organism was added to assay medium, then poured into plates. After solidification, paper discs impregnated with samples (fermented broth) were applied on the agar plates and the plates were incubated 24-36 hours at room temperature. Clear zones (inhibitory zones) surrounding the test discs indicate the presence of bioactive metabolites which inhibit the growth of test organisms. The test organisms used in paper disc diffusion assay were *Agrobacterium tumefaciens* (plant disease), *Bacillus pumillus* (wound and burn infections), *Candida albicans* (vaginal candidacies infection), *Escherichia coli* (diarrhea), *Micrococcus luteus* (skin disease and chronic cutaneous infection), *Pseudomonas fluorescens* (rice disease), *Staphylococcus aureus* (boils and food poisoning, wound infections).

## Medium used for antimicrobial activities test

| Seed medium                     |        | Assay medium            |      |
|---------------------------------|--------|-------------------------|------|
| Components of per liter         |        | Components of per liter |      |
| Glucose                         | 20 g   | Glucose                 | 10 g |
| Soluble starch                  | 3 g    | Yeast extract           | 3 g  |
| Yeast extract                   | 3 g    | Polypeptone             | 3 g  |
| KNO <sub>3</sub>                | 1 g    | KNO <sub>3</sub>        | 1 g  |
| K <sub>2</sub> HPO <sub>4</sub> | 0.01 g | Agar                    | 18 g |
| pH                              | 6.5    | pH                      | 6.5  |

## Fermentation medium

| Components of per liter         |        |
|---------------------------------|--------|
| Glucose                         | 10 g   |
| Soluble starch                  | 5 g    |
| Yeast extract                   | 5 g    |
| KNO <sub>3</sub>                | 0.01 g |
| K <sub>2</sub> HPO <sub>4</sub> | 0.01 g |
| MgSO <sub>4</sub>               | 0.01 g |
| C-CC                            | 1 -    |

**Results and Discussion**

## Collection of soil samples

Soil samples were collected from seven different places in Kangyidaunt Township as shown in (Table 1) for isolation of fungi. Soil was analyzed by Department of Agriculture (Land Use), Insein Township in Yangon as shown in (Figure 2). Different soil types are clay, clay loam, loam and sandy loam.

Table 1. Texture, Moisture and pH of soil samples

| Sr.No | Collected Place                | Texture    | Moisture % | pH   | Location                   |
|-------|--------------------------------|------------|------------|------|----------------------------|
| S.1   | Ball lone kwin (Kan Gyi Daunt) | Loam       | 1.98       | 5.72 | 16° 55'35" N, 94° 53'47" E |
| S.2   | Kan Kone                       | Clay loam  | 2.73       | 5.73 | 16° 51'12" N, 94° 50'43" E |
| S.3   | Kyun Chaung                    | Sandy loam | 0.83       | 4.84 | 16° 52'9" N, 94° 51'12" E  |
| S.4   | La Har Ka Lu                   | Loam       | 2.33       | 5.68 | 16° 53'42" N, 94° 55'20" E |
| S.5   | Mar Lar Kone                   | Sandy loam | 1.15       | 5.31 | 16° 54'52" N, 94° 54'42" E |
| S.6   | Mi chaung Tayar                | Clay       | 5.35       | 4.31 | 16° 53'57" N, 94° 53'36" E |
| S.7   | Nyaung Ngu                     | Clay       | 4.16       | 4.65 | 16° 54'42" N, 94° 53'32" E |

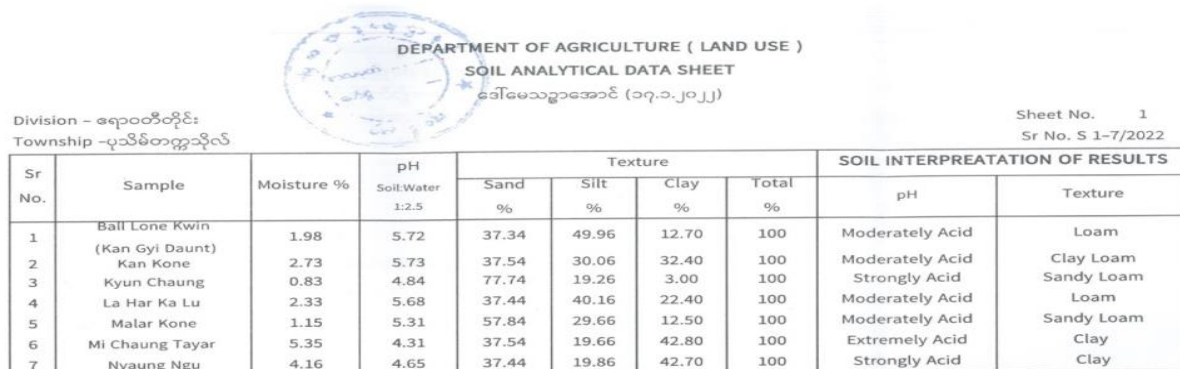


Figure 2. The interpretation results of seven soil samples

Thirty two soil fungi (Table 2. and Figure 3.) were isolated in seven different soil samples by the physical and chemical treatment dilution method and serial dilution method (Phay and Yamamura, 2005). Among them, fifteen fungi isolated from physical and chemical treatment method and seventeen fungi also isolated from serial dilution method.

Table 2. Used of isolated method and their isolated fungi

| <b>Sr. No</b> | <b>Isolation Method</b>         | <b>Isolated fungi</b>     |
|---------------|---------------------------------|---------------------------|
| S.1           | Physical and Chemical Treatment | MTZ-01, MTZ-02            |
|               | Serial Dilution                 | MTZ-03, MTZ-04            |
| S. 2          | Physical and Chemical Treatment | MTZ-05, MTZ-06            |
|               | Serial Dilution                 | MTZ-07,MTZ-08             |
| S. 3          | Physical and Chemical Treatment | MTZ-09, MTZ-10,<br>MTZ-11 |
|               | Serial Dilution                 | MTZ-12, MTZ-13,<br>MTZ-14 |
| S. 4          | Physical and Chemical Treatment | MTZ-15,MTZ-16             |
|               | Serial Dilution                 | MTZ-17,MTZ-18             |
| S. 5          | Physical and Chemical Treatment | MTZ-19, MTZ-20,<br>MTZ-21 |
|               | Serial Dilution                 | MTZ-22, MTZ-23,<br>MTZ-24 |
| S.6           | Physical and Chemical Treatment | MTZ-25                    |
|               | Serial Dilution                 | MTZ-26, MTZ-27,<br>MTZ-28 |
| S.7           | Physical and Chemical Treatment | MTZ-29, MTZ-30            |
|               | Serial Dilution                 | MTZ-31, MTZ-32            |

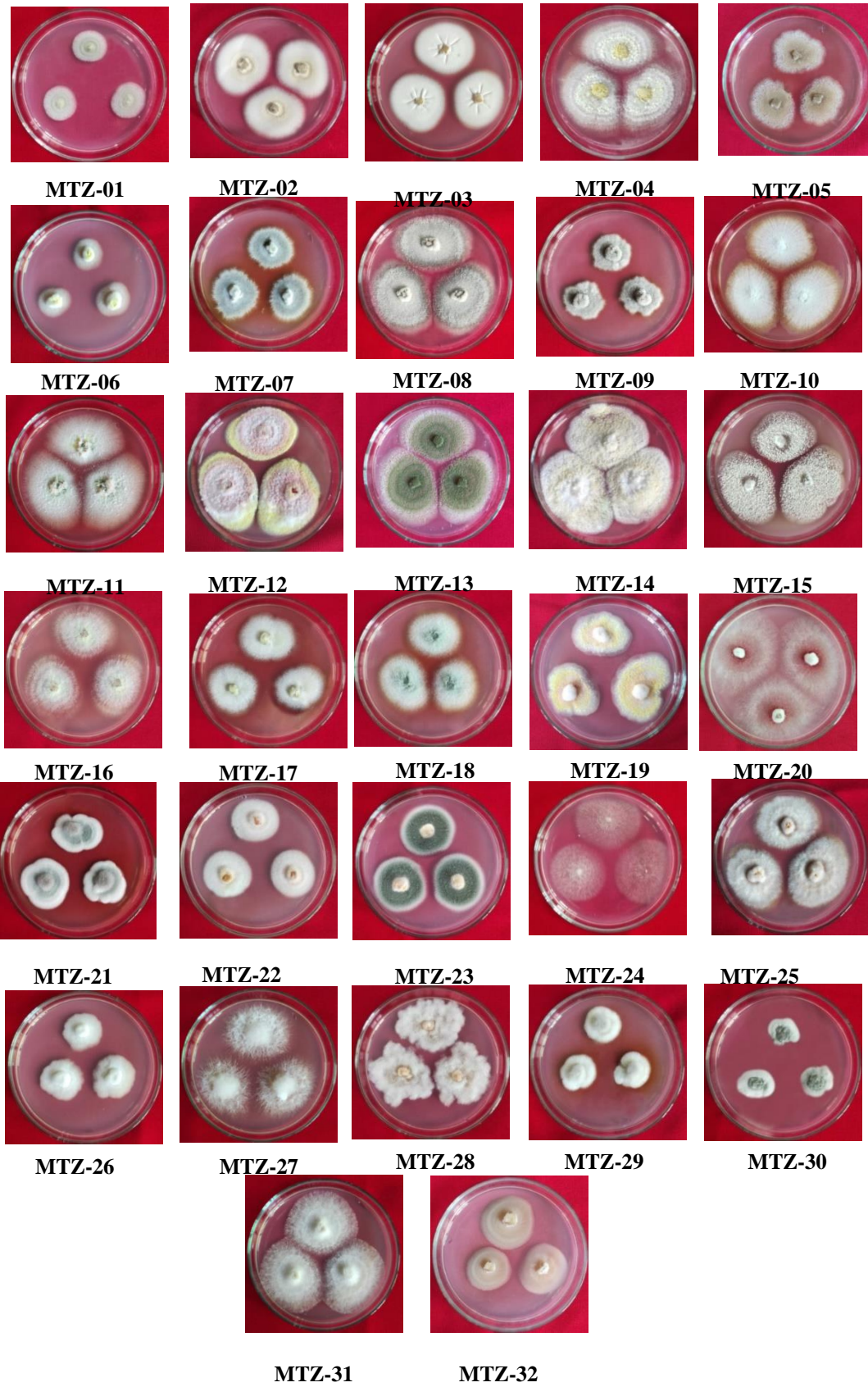


Figure 3. Morphology of isolated soil fungi MTZ-01 to MTZ-32

## Preliminary antimicrobial activities test by paper disc diffusion assay method

According to the preliminary study of antimicrobial activities result, MTZ-31 showed the highest antifungal activity against *Candida albicans* (28.7 mm) as shown in Table 3. and Figure 4. (Paper disc = 8 mm in diameter)

Table 3. Preliminary antimicrobial activities test by paper disc diffusion assay method

| Sr.No                | <i>Agrobacterium tunefaciens</i> | <i>Bacillus pumillus</i> | <i>Candida albicans</i> | <i>Escherichia coli</i> | <i>Micrococcus luteus</i> | <i>Pseudomonas fluorescens</i> | <i>Staphylococcus aureus</i> |
|----------------------|----------------------------------|--------------------------|-------------------------|-------------------------|---------------------------|--------------------------------|------------------------------|
| Inhibitory Zone (mm) |                                  |                          |                         |                         |                           |                                |                              |
| MTZ-01               | 22.6                             | 20.8                     | -                       | 20.4                    | 16.4                      | 16.9                           | 22.2                         |
| MTZ-02               | -                                | -                        | -                       | 12.2                    | -                         | -                              | -                            |
| MTZ-03               | 23.8                             | 22.3                     | -                       | 22.8                    | 19.0                      | 13.2                           | 22.3                         |
| MTZ-04               | 13.9                             | 14.9                     | 13.0                    | 15.4                    | -                         | -                              | 13.7                         |
| MTZ-05               | 16.7                             | 16.6                     | 17.9                    | 16.1                    | -                         | -                              | 18.2                         |
| MTZ-06               | 20.6                             | 21.3                     | 17.7                    | 22.1                    | 15.5                      | 17.1                           | 22.4                         |
| MTZ-07               | 16.5                             | 14.4                     | 16.8                    | 11.8                    | -                         | -                              | 16.3                         |
| MTZ-08               | 16.9                             | 11.7                     | 12.3                    | 16.0                    | -                         | -                              | 16.6                         |
| MTZ-09               | -                                | -                        | -                       | -                       | -                         | -                              | -                            |
| MTZ-10               | 22.8                             | 22.3                     | 17.7                    | 21.6                    | 14.8                      | 17.1                           | 23.4                         |
| MTZ-11               | 18.2                             | 16.7                     | 18.2                    | 16.1                    | -                         | -                              | -                            |
| MTZ-12               | -                                | -                        | -                       | 11.3                    | -                         | -                              | -                            |
| MTZ-13               | 16.0                             | 16.7                     | 11.8                    | 18.8                    | -                         | 12.8                           | 18.0                         |
| MTZ-14               | 20.7                             | 16.8                     | 21.1                    | 20.5                    | 12.8                      | 12.5                           | 16.5                         |
| MTZ-15               | -                                | -                        | 12.5                    | -                       | -                         | -                              | -                            |
| MTZ-16               | 16.5                             | 13.8                     | 16.3                    | -                       | 15.1                      | -                              | 15.0                         |
| MTZ-17               | -                                | -                        | -                       | -                       | -                         | -                              | -                            |
| MTZ-18               | 19.7                             | -                        | 17.6                    | 19.3                    | -                         | 12.0                           | 18.5                         |
| MTZ-19               | 22.6                             | 22.6                     | 15.9                    | 20.9                    | 14.7                      | 17.3                           | 21.8                         |
| MTZ-20               | 18.0                             | 15.4                     | 17.9                    | 14.5                    | -                         | -                              | 16.5                         |
| MTZ-21               | 14.0                             | -                        | -                       | -                       | -                         | -                              | -                            |
| MTZ-22               | 20.4                             | 17.5                     | 19.7                    | 18.6                    | -                         | -                              | 15.4                         |
| MTZ-23               | 21.1                             | 19.5                     | 19.2                    | 19.4                    | -                         | -                              | 18.2                         |
| MTZ-24               | 24.6                             | 22.5                     | 16.4                    | 20.4                    | 15.3                      | 17.5                           | 22.7                         |
| MTZ-25               | 20.7                             | 18.6                     | 20.3                    | 18.4                    | -                         | 13.2                           | 18.2                         |
| MTZ-26               | 21.0                             | 17.0                     | 21.1                    | 15.8                    | -                         | 12.5                           | 18.7                         |
| MTZ-27               | -                                | -                        | 13.5                    | -                       | -                         | -                              | -                            |
| MTZ-28               | 20.1                             | 22.5                     | 20.2                    | 19.8                    | 15.9                      | 16.9                           | 22.0                         |
| MTZ-29               | 20.6                             | 18.7                     | -                       | 18.8                    | -                         | 11.4                           | 19.5                         |
| MTZ-30               | 19.7                             | 19.1                     | 14.2                    | 19.1                    | -                         | 13.1                           | 19.4                         |
| MTZ-31               | 27.9                             | 22.6                     | 28.7                    | 25.2                    | 16.1                      | 18.6                           | 24.7                         |
| MTZ-32               | 20.8                             | 17.6                     | 21.0                    | 19.2                    | -                         | 12.4                           | 19.2                         |



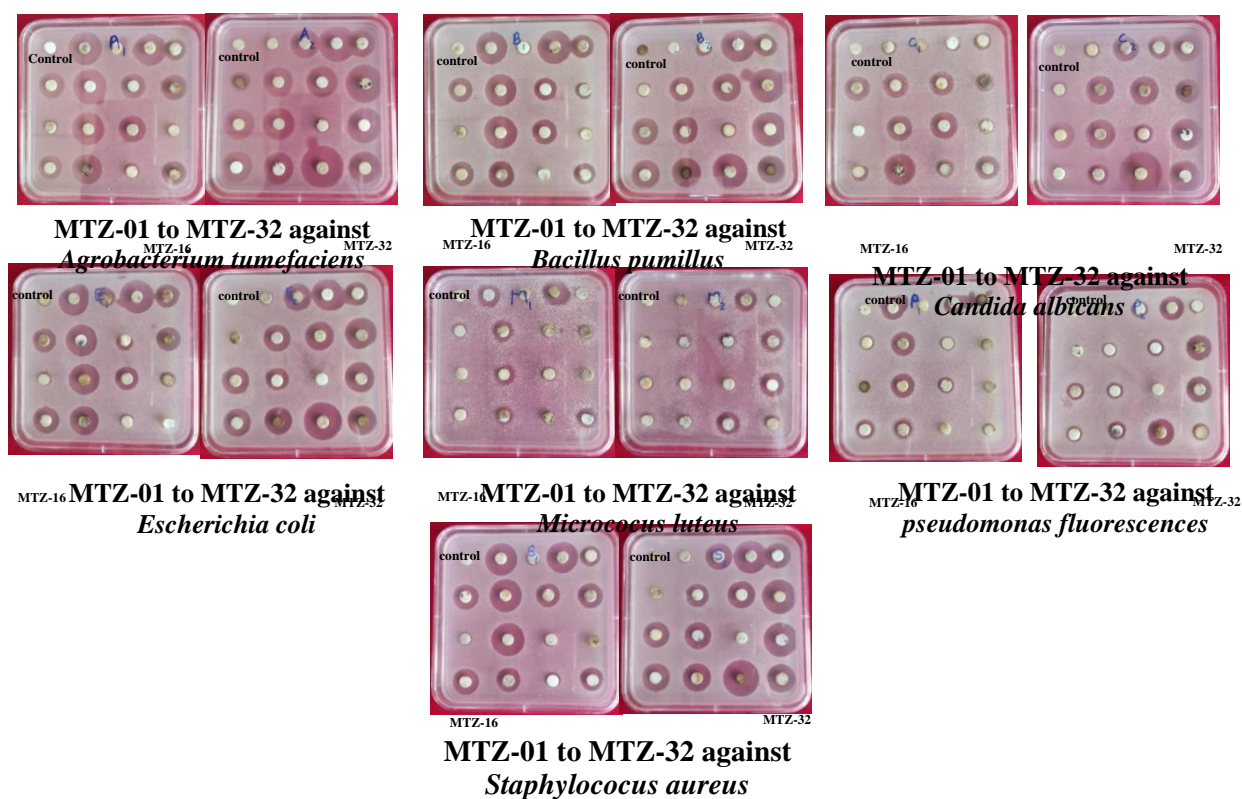


Figure 4. Antimicrobial activities of isolated soil fungi MTZ-01 to MTZ-32 against seven test organisms

### Conclusion

The soil is considered a habitat of several organisms includes fungi that differ in their phenotype and genotype, more to their various economic and ecological importance according to the Latifa *et al.*,2021. M. Raja *et al.*, 2017 mentioned that fungi are not only beautiful but play a significant role in the daily life of human beings besides their utilization in industry, agriculture, medicine, food industry, textiles, bioremediation, natural cycling, as biofertilizers and many other ways. Fungal biotechnology has become an integral part of the human welfare. Therefore, fungi isolated from the seven different soil samples places in Kangyidaunt Township.

These soil was analyzed in Department of Agriculture (Land Use), Insein Township in Yangon. In order to the isolation of soil fungi was studied by physical and chemical treatment dilution method, and serial dilution method. According to the result, thirty two soil fungi were isolated. The isolated fungi was given as temporary name MTZ-01 to MTZ- 32.

According to Yehia., 2019 antimicrobial products kill or slow the spread of microorganisms. In the preliminary study of antimicrobial activity, all isolated soil fungi (MTZ-01-32) were evaluated by paper disc diffusion assay method according to Tomita., 1998. Based on the result, MTZ -31 showed the highest antifungal activity (28.7 mm) against *Candida albicans* which causes vaginal candidacies infection. The front colour and reverse colour of isolated soil fungi (MTZ-31) was white. These fungus was isolated from (soil 7) Nyaung Ngu village. The soil character is clay, pH-4.65 and moisture 4.16. And then, further experiment will be investigated.

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