SCREENING OF SOIL FUNGI FROM MAGWAY TOWNSHIP AND IDENTIFICATION OF SELECTED SOIL FUNGUS ESPECIALLY AGAINST *STAPHYLOCOCCUS AUREUS*

Phyo Phyo Soe San

Abstract

In the study on the isolation of soil fungi, 43 fungi were isolated from five different soil samples collected at Magway Township, Magway Region. In the investigation of antimicrobial activities of 43 soil fungi, *Agrobacterium tumefaciens, Bacillus pumalis, Candida albicans, Escherichia coli* and *Staphylococcus aureus* were used for the test throughout the research studies. Among them, since PS-13, PS-14, PS-15, PS-16, PS-17 and PS-18 showed the antimicrobial activities against *Staphylococcus aureus and E. coli.* PS-13 showed most highly selective antimicrobial activity against *Staphylococcus aureus* than the other fungi. The characters of selected strain PS-13 are similar to those of *Cephalosporium* sp. (Ando and Inaba (2004), Barnett (1956)). So that strain PS-13 was determined as *Cephalosporium* sp.

Key words: antimicrobial activity, identification

Introduction

Microorganisms live in all parts of the biosphere where there is liquid water, including soil, hot springs, on ocean floor, high in the atmosphere and deep inside rocks within the earth's crust. The typical materials for microbial sources are soil living and fallen leaves, leaf litters, dung, insect, fresh water and marine water. The soil sample is the most effective and popular materials for the isolation of fungi and actinomycetes (Harayama and Isono, 2002). Microorganisms are a virtually unlimited source of novel chemical structures with many potential therapeutic applications (Behal, 2000).

Fungi are well known as prolific producers of biologically active natural products (Hara *et al.*, 2007). Most of the naturally occurring antibiotics have been isolated from soil microorganisms. Isolating microorganisms from the environment is the first step in screening for natural products such as secondary metabolites and enzymes (Hunter-Cevera and Belt, 1999).

Assistant Lecturer, Dr, Department of Botany, Pathein University

Aim and objectives of present work are to know the isolation programs and varieties of fungi from different soil samples, to study the morphology of isolated soil fungi and their antimicrobial potentials.

Materials And Methods

Study area

The fourty-three soil fungi were isolated from five different soil sampls Magway Township, Magway Region (Figure 1). The study site five places(1) Agricultural oil-crops research centre, located between 20˚9'23.29"N and 94˚56'0.24"E, (2)Local Audit Organization, located between 20˚9'19.81" N and 94˚57'10.76" E, (3) Kanther Lake, located between 20˚ 8'43.37" N and 94˚ 56'20.40" E, (4) Magway University, located between 20˚ 7'54.58" N and 94˚56'17.76" E, and (5) Magway Airport, located between 20˚9'29.08"N and 94˚ 58' 22.62" E (Figure 1).

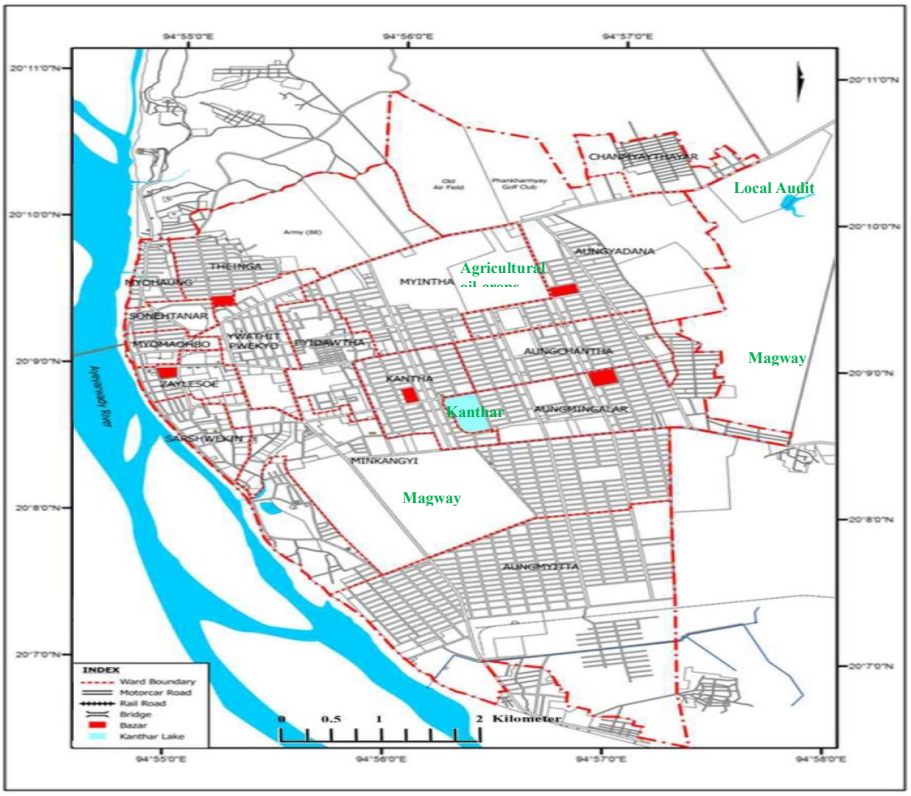


Figure 1. Location map of Magway Township

Study method

1. Physical dilution method (Phay & Amachi,2005)
2. Physical treatment serial dilution method (PBCC, 2004)
3. Physical and chemical treatment dilution method (Phay & Amachi, 2005)
4. Feeding method (PBCC, 2004)

Screening or Preliminary Study for Antimicrobial Activities by Paper Disc Diffusion Assay (Tomita, 1998)

The isolated fungi were grown at 25°C for 7 days on PGA medium. The isolated fungi were inoculated into seed medium and incubated at 25°C for 3 days. 25 ml of seed culture was transferred into the fermentation medium. The fermentation was carried out for 7 days. After the end of fermentation, the fermented broth (20 µl) was used to check the antimicrobial activity against test organisms by paper disc diffusion assay (Figure 4). Paper disc having eight millimeter diameter (Advantec, Toyo Roshi Kaisha Co., Ltd., Japan) was utilized for antimicrobial assays.

The assay medium (Glucose -1%, Polypepton- 0.3%, KNO_3- 0.1 %, Agar-1.8%, Distilled water-100 ml, pH-6.5) was used for the antimicrobial activity test. One percent of test organism was added to assay medium, then poured into plates. After solidification, paper discs impregnated with samples (fermented both) were applied on the agar plates and the plates were incubated for 24-36 hours at 28 to 30°C. Clear zones (inhibitory zones) surrounding the test discs indicate the presence of bioactive metabolites which inhibits the growth of test organisms.

The test organisms used in paper disc diffusion assay were *Agrobacterium tumefaciens, Bacillus pumalis* NITE 47239, *Candida albicans* NITE 83297, *E.coli AHU* 5436, and *Staphylococcus aureus* AHU 8465. The test organisms were supported by NITE (National Institute of Technology and Evaluation, Japan) and Faculty of Agriculture, Hokkaido University, Japan (Table 1).

Table 1. Test organisms used in antimicrobial activities (NITE)

No.	Test organisms	Infections
1.	Agrobacterium tumefaciens (IFO5431)	Crown gall diseases
2.	Bacillus pumalis (NITE47239)	Fever and food poisoning
3.	Candida albicans (NITE09542)	Candidosis
4.	Escherichia coli (AHU5436)	Diarrhoea
5.	Staphylococcus aureus (AHU8465)	Skin disease and Food poisoning

Results

Isolation from Soil Samples

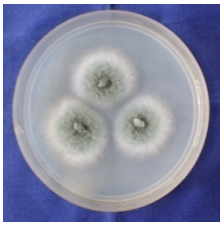
In the course of the isolation for antimicrobial metabolite producing microorganisms, 43 fungi were isolated from five different kinds of soil samples (Table-2 and Figure-2 and 3).

Table 2. Isolated soil fungi from two different soil samples by using four methods (PS-01 to PS-18)

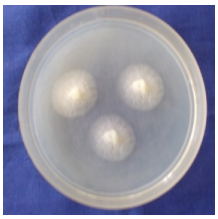
Soil No.	**Isolation method**	**Isolated fungi**
S-1	Physical dilution method	PS-01,02,03
	Physical Treatment serial dilution method	PS-04,05
	Physical and chemical treatment dilution method	PS-06,07
	Feeding method	PS-08,09
S-2	Physical dilution method	PS-10,11,12
	Physical treatment serial dilution method	PS-13,14
	Physical and chemical treatment dilution method	PS-15,16
	Feeding method	PS-17,18

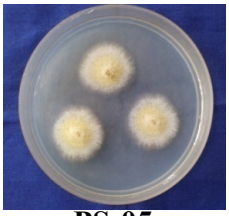
Soil No.	Isolation method	Isolated fungi
S-3	Physical dilution method	PS-19,20
	Physical treatment serial dilution method	PS-21,22
	Physical and chemical treatment dilution method	PS-23,24
	Feeding method	PS-25,26
S-4	Physical dilution method	PS-27,28
	Physical treatment serial dilution method	PS-29,30,31
	Physical and chemical treatment dilution method	PS-32,33
	Feeding method	PS-34,35
S-5	Physical dilution method	PS-36,37
	Physical treatment serial dilution method	PS-38,39
	Physical and chemical treatment dilution method	PS-40,41
	Feeding method	PS-42,43

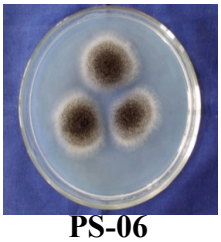

PS-01

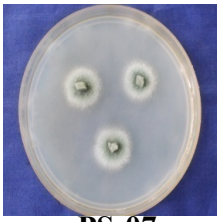

PS-02


PS-03


PS-04


PS-05


PS-06


PS-07


PS-08

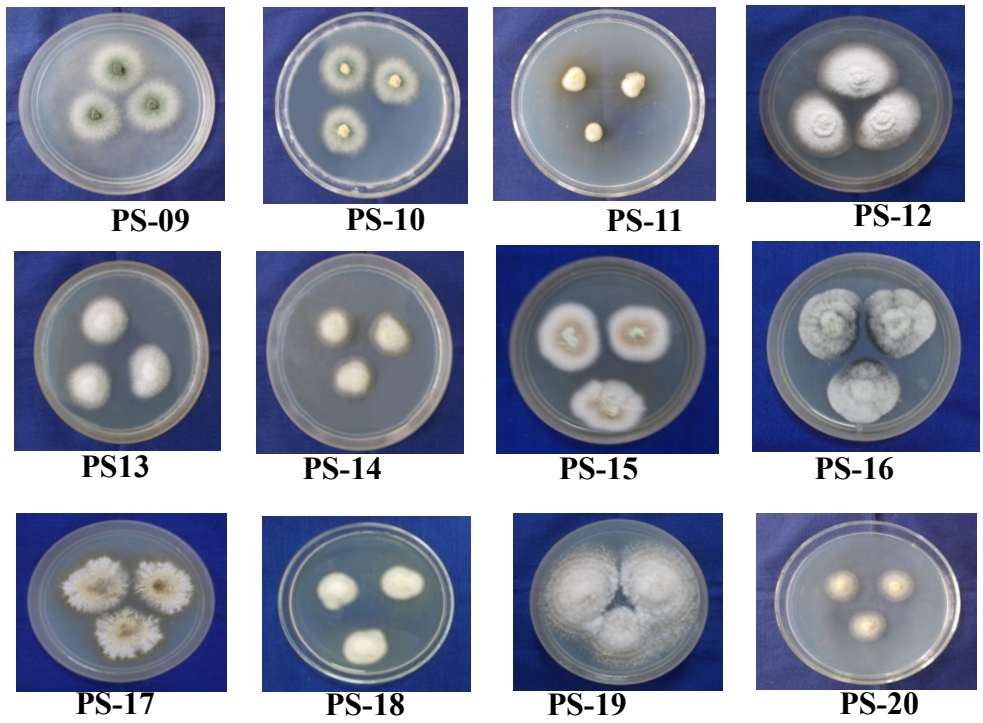
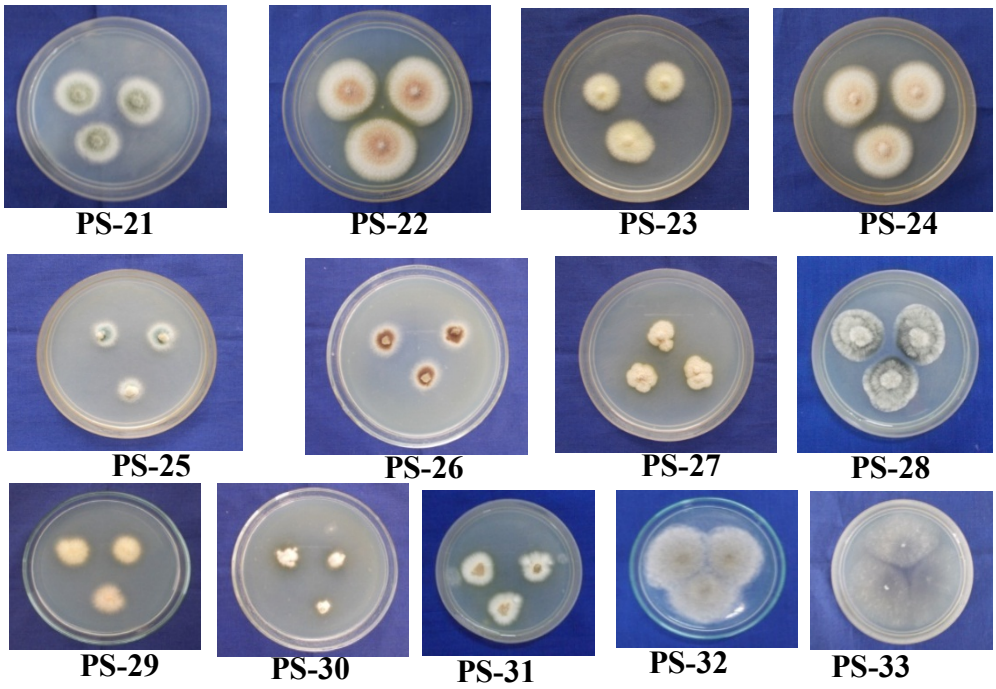


Figure 2. Morphology of isolated fungi on PGA medium (5 days old culture)



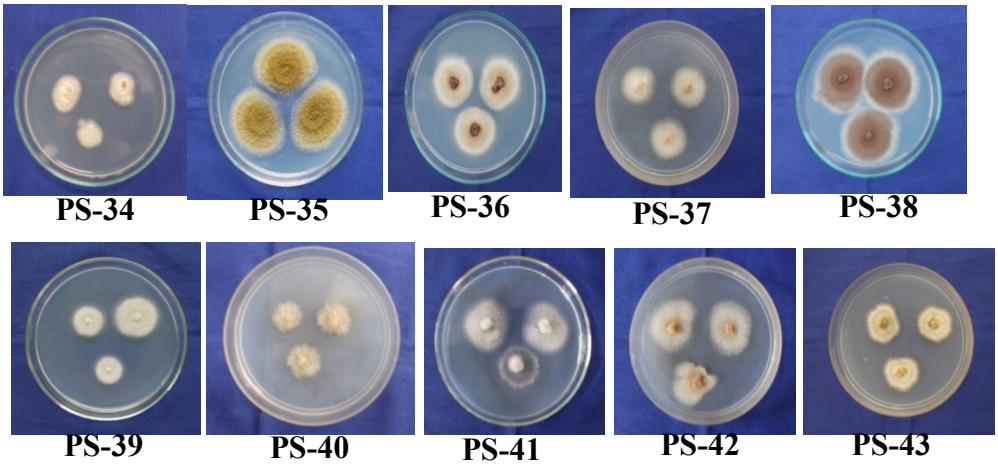


Figure 3. Morphology of isolated fungi on PGA medium (5 days old culture)

Table 3. Antimicrobial activities and starch hdrolyzing activities of isolated soil fungi

Stain No.	*Bacillus pumalis*	*Candida albicans*	*E. coil*	*S. aureus*	*Agro. tumefaciens*
PS-01	-	-	-	-	-
PS-02	-	-	-	-	-
PS-03	-	-	-	-	-
PS-04	-	-	-	-	-
PS-05	-	-	-	-	-
PS-06	-	-	-	-	-
PS-07	-	-	-	-	-
PS-08	-	-	-	-	-
PS-10	-	-	-	-	-
PS-11	-	-	-	-	-
PS-12	-	-	-	-	-
PS-13	-	-	27mm	37mm	-
PS-14	-	-	26mm	32mm	-
PS-15	-	-	23mm	29mm	-
PS-16	-	-	25mm	30mm	-

Stain No.	*Bacillus pumalis*	*Candida albicans*	*E. coil*	*S. aureus*	*Agro. tumefaciens*
PS-17	+	-	27mm	28mm	-
PS-18	-	-	29mm	33mm	-
PS-19	-	-	-	-	-
PS-20	-	-	+	-	-
PS-21	-	-	-	-	-
PS-22	-	-	-	-	-
PS-23	-	-	-	-	-
PS-24	-	-	-	-	-
PS-25	-	-	-	-	-
PS-26	-	-	-	-	-
PS-27	-	-	-	-	-
PS-28	-	-	-	-	-
PS-29	+	-	-	-	-
PS-30	-	+	-	-	-
PS-31	-	-	-	-	-
PS-32	-	-	-	-	-
PS-33	-	-	-	-	-
PS-34	-	-	-	-	-
PS-35	-	-	-	-	-
PS-36	-	+	-	-	+
PS-37	-	-	-	-	-
PS-38	-	-	-	-	-
PS-39	-	-	-	-	-
PS-40	-	-	-	-	-
PS-41	-	-	-	+	-
PS-42	-	-	-	-	-
PS-43	-	-	-	-	-

Screening of Effective Microorganisms Isolated from Soil by Paper Disc Diffusion Assay

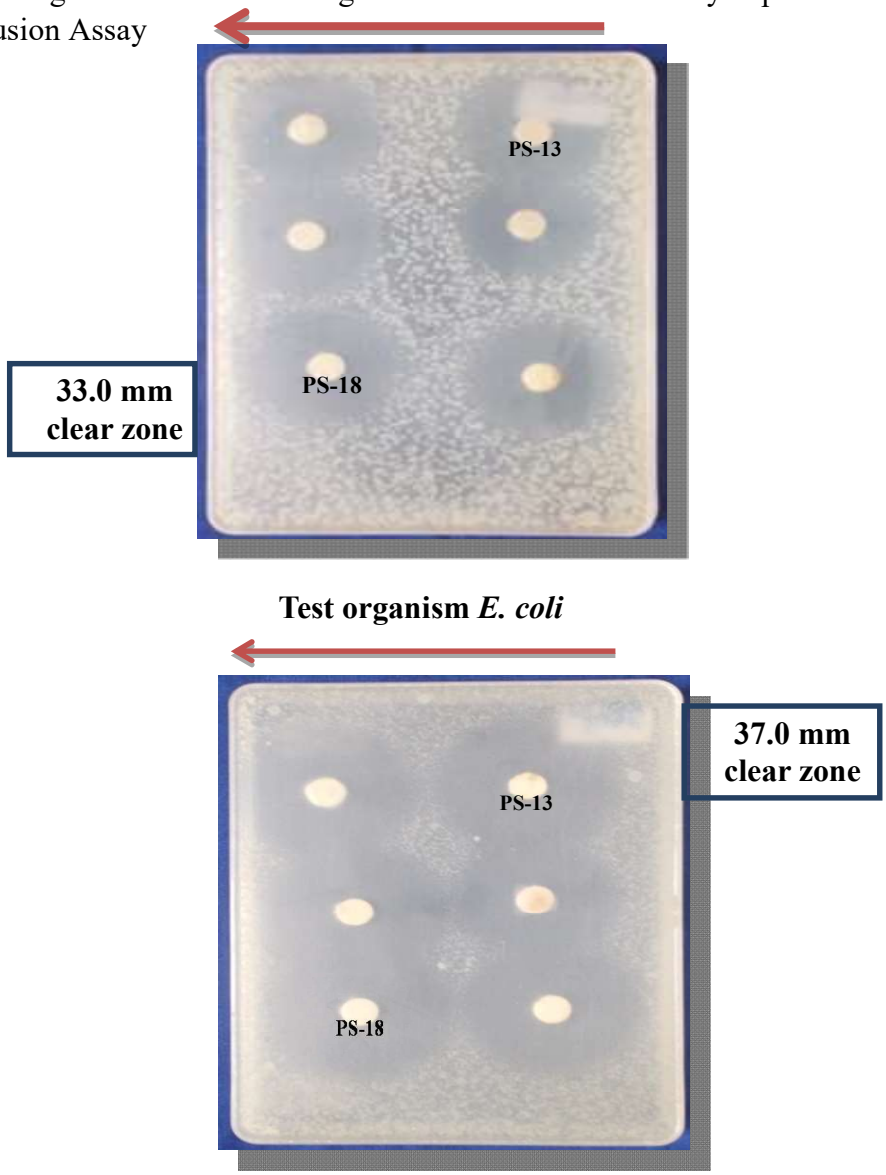
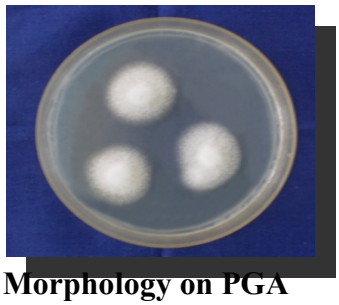
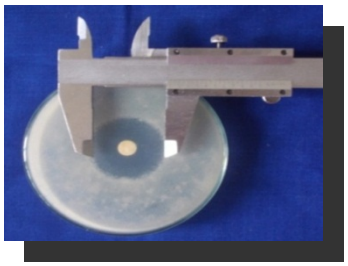


Figure 4. Antibacterial activity of isolated soil fungi



Morphology on PGA medium (5 days old culture)

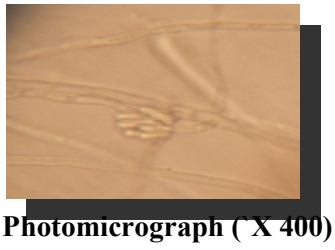


This fungus showed the antibacterial activity against *Staphylococcus aureus*(37.0 mm, clear zone)

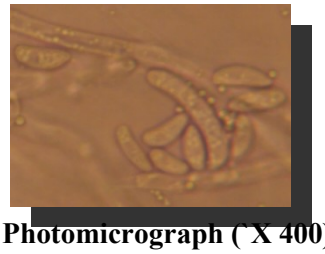
Figure 5. Morphology and antimicrobial activity of selected soil fungus PS-13.

Morphology of soil fungus PS-13

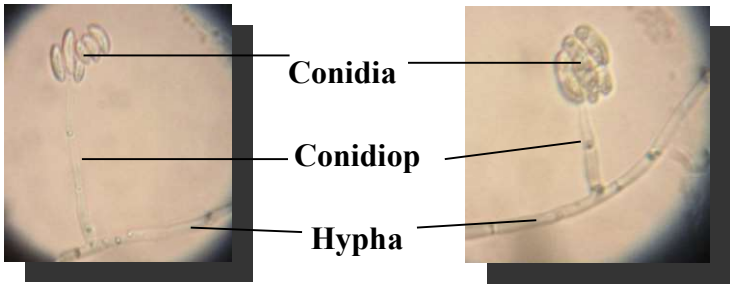
- Characteristically present hyphae with septa regularly
- Conidiophore upright, hyaline, unbranch, elongated with conidial production
- Conidia hyaline, lacking septum, amerospore, elliptical to elliptic fusiform, produce successively at the tip and collecting in a slime drop.



Photomicrograph (`X 400)



Photomicrograph (`X 400)



Photomicrograph (`X 400)** **Photomicrograph (`X 400)

Figure 6. Photomicrograph of *Cephalosporium*

Kingdom - Fungi
Division - Ascomycota
Class - Ascomycetes
Order - Moniliales
Family - Moniliaceae
Genus – *Cephalosporium*

Discussion And Conclusion

In the course of the screening of antimicrobial metabolite producing soil fungi from five different places collected at Magway township were utilized in order to find a new and effective antimicrobial activities with a specific target of fungi.In the precent study, 43 soil fungi were isolated from five different soil samples. During the studies of antimicrobial activities of 43 soil fungi, *Agrobacterium tumefaciens, Bacillus pumalis, Candida albicans, Escherichia coli* and *Staphylococcus aureus* were used for the test throughout the research studies. Among them, PS-13, PS-14, PS-15, PS-16, PS-17 and PS-18 showed the antimicrobial activities against *Staphylococcus aureus* and *Escherichia coli.* Fungus PS-13(37.0 mm clear zone) showed the highest activity against *Staphylococcus aureus* than the other fungi. Selected fungus PS-13 was isolated from the soil collected at Local Audit Organization. In the investigation of identification, fungus PS-13 possessing antibacterial activity was identified as *Cephalosporium* sp. on the basis of morphological-microscopial characters and reference keys (Ando and Inaba (2004), Barnett (1956)). In conclusion these selected soil fungus *Cephalosporium* sp. obtained from Local Audit Organization, Magway township, Magway region was observed that inhibit harmful diseases causing agents *Staphylococcus aureus.*So, these selected soil fungus can be regarded as a good source of antibiotic for human and animals.

Acknowledgements

I wish to express my sincere gratitude to Dr Nyunt Phay, Rector, Pathein University for his permission to do this research. I am greatefully indebted to Dr Wai Wai Nyunt and Dr Mi Mi Gyi, Pro-Rectors, Pathein University, for their encouragement. My special thanks go to Professor Dr Kay Thi Mya, Head of Botany Department Pathein University, for her invaluable support and suggestions. I extend my thanks to Dr Wah Wah Lwin, Professor, Department of Botany Pathein University, for her advice and kind help. I would like to express my heartfelt gratitude to my supervisor Dr Mya Htet Htet Aung, Lecturer, Department of Botany, Pathein University, for her invaluable overall guidance, suggestions and co-operation during my study.

References

Ando, K and S Inaba (2004). Isolation and identification of fungi, Workshop, BDBRC-Pathein University.

Barnett,H.L.(1956).Illustrated Genera of imperfect fungi.

Behal, v. (2000). Bioactive products from Streptomyces. Malaysian Journal of Microbiology 1, 1-6.

Hara-kishore, K., S. Misra, D. R. Chandra, K. V. V. R. Prakash, U. S. Murty. (2007) Antimicrobial efficacy of secondary metabolites from Glomerella cingulata. Braz. J. Microbiol., 38, 150-155.

Harayama, T. and K. Isono. (2002). Sources of Microorganisms, J. Microbiology, 48: 46-50.

Hunter-Cevera J. C., and A. Belt. (1999). Isolation of cultures. In: Manual of Industrial Microbiology and Biotechnology(eds AL Demain, JE Davies) ASM Press. Washington, DC.

Phay and Amachi, (2005). Approach method for rare microorganisms from soil sources, J. Microbiology, 76, 237-239.

Tomita, F.(1988). Laboratory Method, Hokkaido University, Japan.

NITE (National Institute of Technology and Evaluation) (2004). Isolation method for microorganisms, Kisarazu, Japan.

PBCC (Pathein Biotechnology Culture Collection media), (2004). Pathein University.