ISOLATION OF SOIL FUNGI AND FERMENTATION CONDITIONS OF *FUSARIUM* SP. AGAINST *STAPHYLOCOCCUS AUREUS*

Thet Thet Wai[1], Yu Yu Tin[2] and Swe Swe Myat[3]

Abstract

The present study, soil samples were collected from six different places at Wundwin Township in Mandalay Region. The isolation of fungi were isolated by using physical treatment dilution method (Hayakawa and Kobayashi, 2005) and direct method (Ando,2004).Then, antimicrobial activities of soil fungi were tested by paper disc diffusion assay method. The fungal extract were tested for antimicrobial activity against test organisms *Staphylococcus aureus.* In the fifteen fungi screening, the isolated fungi TW-03 (22.57 mm) and TW-14(34.81mm)exhibited the activity against *Staphylococcus aureus*. In this result, TW-14 showed highest antibacterial activity. *Fusarium* sp. was identified from the selected fungus TW-14. Therefore, this fungus *Fusarium* sp. was selected for further investigation such as age, size, carbon sources and nitrogen sources for suitable fermentation condition. In the study of ages and sizes of inoculum, it was observed that 60hr and 10% seed cultured was best for the fermentation. Then, in this study of carbon and nitrogen sources utilization it was found that soluble starch and soybean gave the best activity on *Staphylococcus aureus*.

Introduction

All micro-organisms require water, sources of energy, carbon, nitrogen, mineral element and vitamin plus oxygen in their growth medium. Specific nutritional requirements of microorganisms used in industrial fermentation processes are as complex and varied as the microorganisms in question. Not only are the types of microorganisms diverse (bacteria, molds and yeast, normally), but the species and strains become very specific as to their respectively. (**Gutcho, Sydney, 1973)**

In the present study, it is an effort to understand the soil fungal diversity in Wundwin Township, Mandalay Region. In many cases the complex or natural media have to be supplemented with mainly inorganic nutrients to satisfy the requirements of the fermenting organism. **(Zabriski, *et al*., 1980)**

Fermentation nutrients are generally classified as: sources of carbon, nitrogen and sulfur, minerals and vitamins. **(Vogel, Henry 1983)**

Many microorganisms can use a single organic compound to supply both carbon and energy needs. Following the carbon source, the nitrogen source is generally the next most plentiful substance in the fermentation media. A few organisms can also use the nitrogen source as the energy source.**(Rhodes and Fletcher, 1966)** Nitrogen can be inorganic such as ammonium salts, or organic such as amino acids, proteins or urea. The carbon substrate has a dual role in biosynthesis and energy generation, with carbohydrates being the usual carbon source for microbial fermentation processes (**Stanbury *et al*. 1995**).

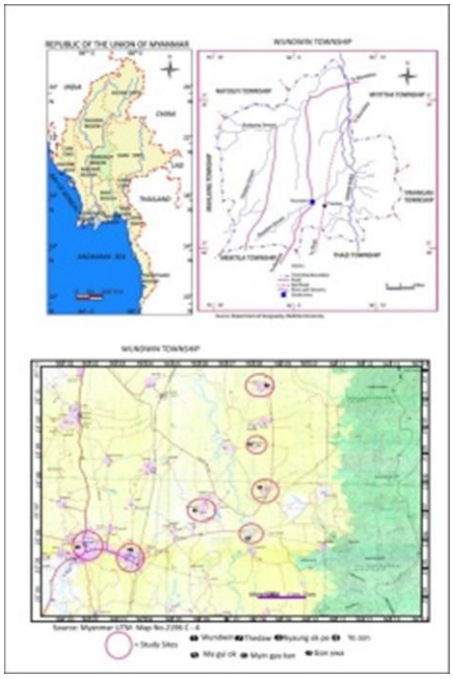
Therefore, the main object of present study is to investigate to find out ages and sizes of inoculum, effects of carbon and nitrogen sources for the production of antibacterial metabolite.

[1] Assistant Lecturer, Department of Botany, Meiktila University
[2] Dr, Associate Professor, Department of Botany, Meiktila University
[3] Dr, Lecturer, Department of Botany, Pathein University

Materials and Methods

Procedure for the effect of ages of inoculum

Isolation and screening soil fungi from different soil samples. Six different soil samples was collected from Wundwin Township, Mandalay Region (Figure 1 and Table.1). The isolation of soil microorganisms were referenced by the following methods. The isolation of fungi were carried out by the physical treatment dilution method (Hayakawa and Kobayashi, 2005) and direct method (Ando, 2005) as shown in Figure 2 and 3.



Source: Department of Geography, Meiktila University

Figure 1 Study Area of Wundwin Township

Table 1 Six different soil samples collected at six different places

Soil Samples No.	Collected Area	Texture	pH	Moisture	Location
1	Hitaw Mu Pagoda	Sandy Loam	5.4	9	N 21°04’55.361” E 096°08’48.250”
2	Ma Gyi Oak village	Loamy Sand	4.5	6	N 21°07’22.330” E 096°08’45.861”
3	Myin Kya Kan village	Sandy Loam	6.3	11	N 21°09’21.882” E 096°08’45.641”
4	Gyone Yar village	Sandy Loam	6.8	14	N 21°11’15.357” E 096°08’43.883”
5	Nyaung Oak Phee village	Loamy Sand	6.4	12	N 21°06’58.476” E 096°05’47.607”
6	Quarter(3), Wundwin	Sandy Loam	7.3	12	N 21°05’03.456” E 096°00’38.507”

Isolation By Physical Treatment dilution method (Hayakawa and Kobayashi, 2005)

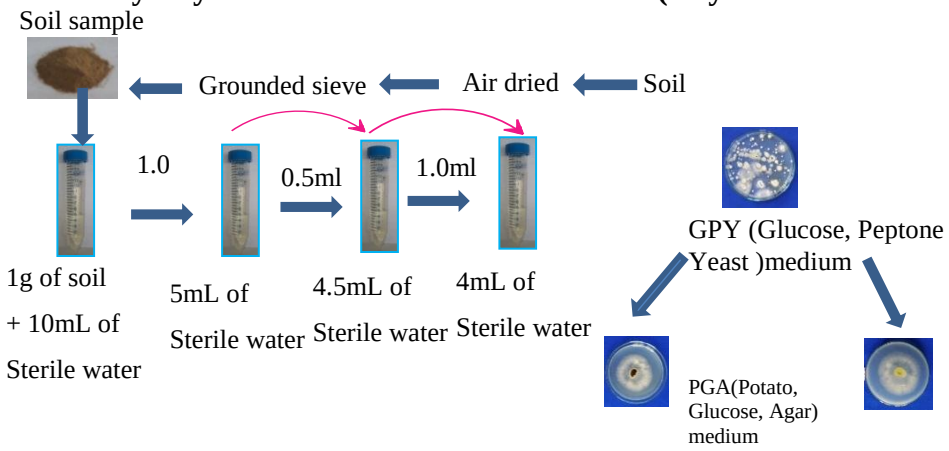


Figure 2 Procedure of physical treatment dilution method

Isolation By Direct Method (Ando, 2004)

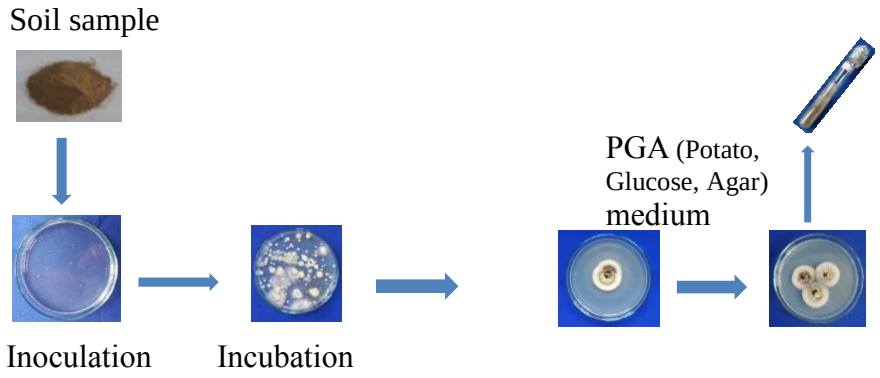


Figure 3 Procedure of direct method

Screening for antimicrobial activities by paper disc diffusion assay (Tomita,1988)

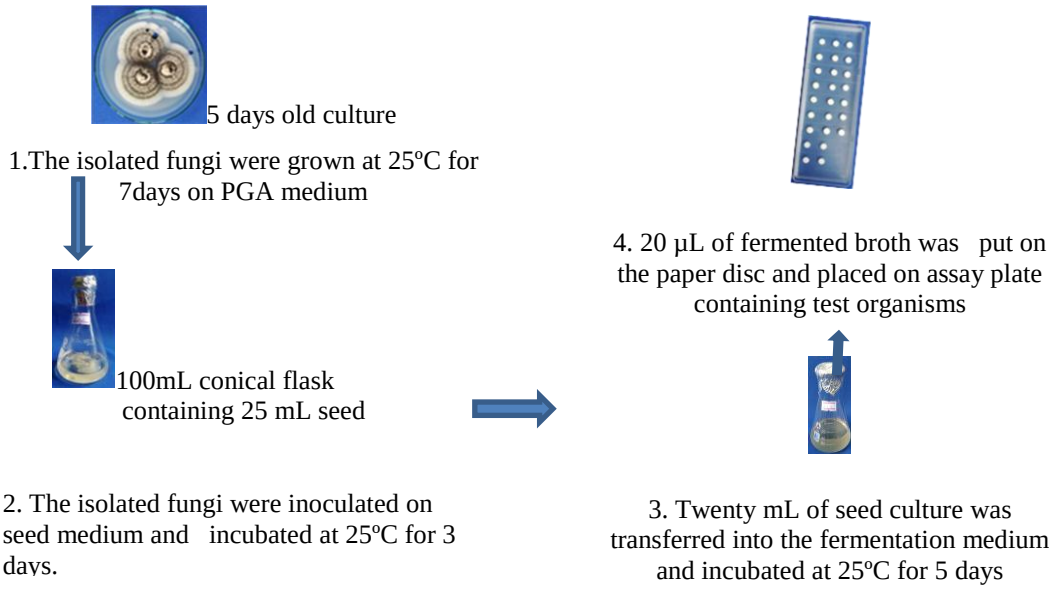


Figure 4 Screening for antimicrobial activities by paper disc diffusion assay

Procedure of fermentation for the effect of ages of inoculum (Omura 1985, Crueger and Crueger 1989)

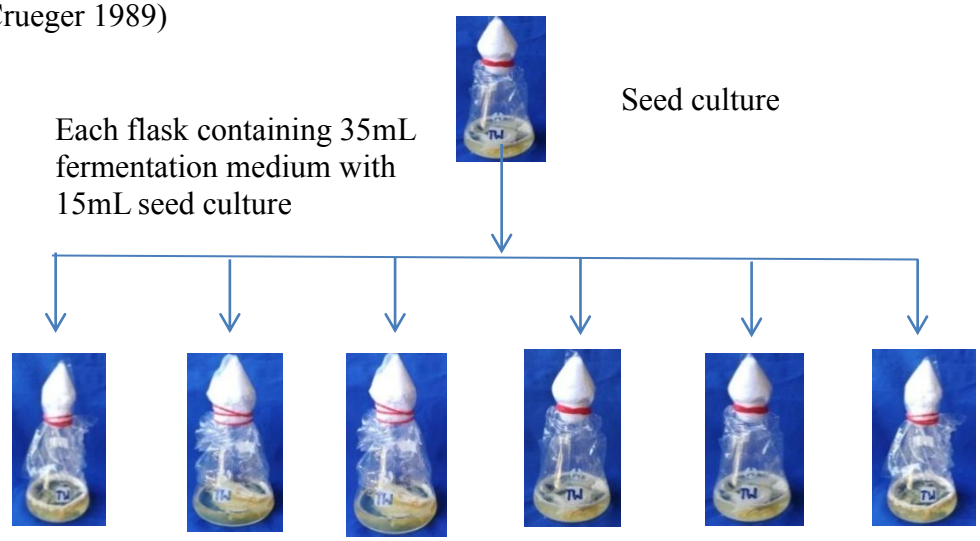


Figure 5 Procedure for the study on the effect of ages of inoculum for fermentation

Procedure of fermentation for the effect of sizes of inoculation (Omura 1985, Crueger and Crueger 1989)

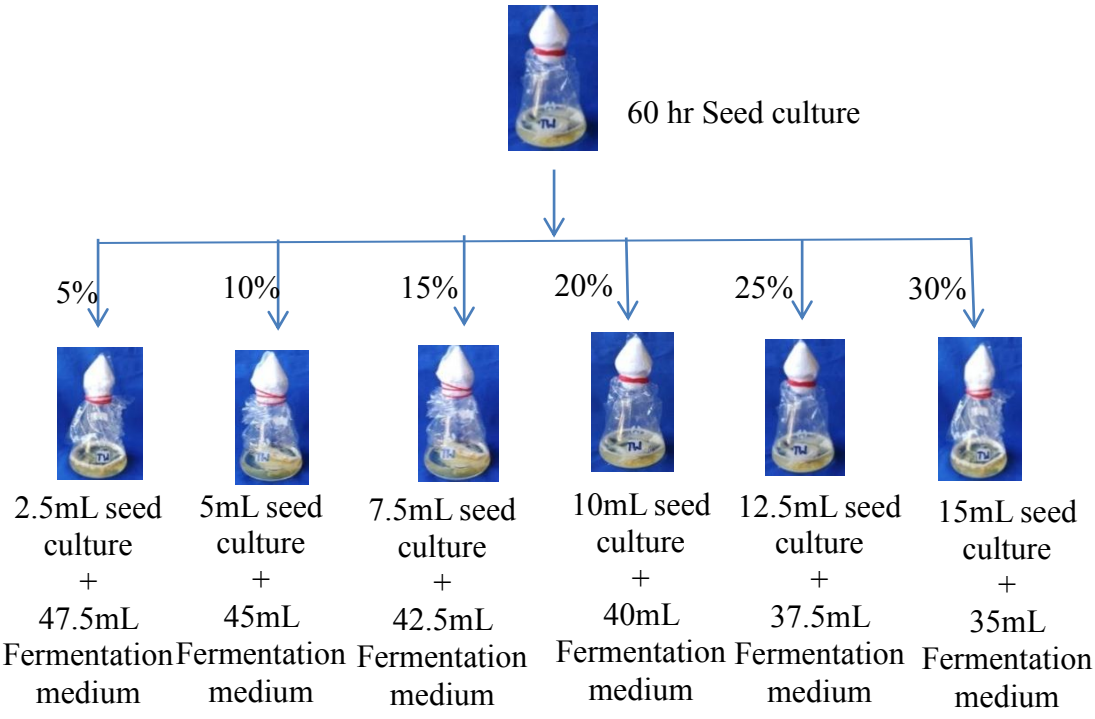


Figure 6 Procedure for the study on the effect of sizes of inoculation for fermentation

Studied on different carbon and nitrogen utilization for the fermentation

Optimal fermentation are very important for maximal productivity metabolites. In this study, carbon and nitrogen sources were employed in the fermentation for the production of antimicrobial metabolites. Carbon sources such as molasses, mannitol, soluble starch, rice powder and corn powder were used. Nitrogen sources such as soybean, Gelatin, NH_4NO_3, $(NH_4)_2SO_3$ and NH_4 CL were also used. In the investigation of carbon and nitrogen sources, each

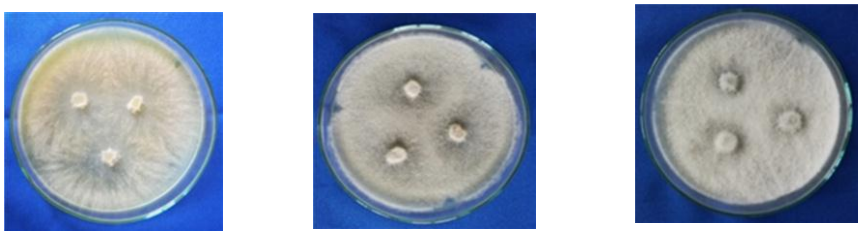
carbon sources 1.0g and nitrogen sources 1.0g were used in the fermentation as antibacterial activity on ***Staphylococcus aureus***.

Results

In the present research work, fungi were isolated from six different soil samples. The total fifteen fungi were isolated which belong to different methods as shown in (Table-2). Two isolated strains were tested for antibacterial activities with *Staphylococcus aureus* . In the present study, TW-3 (22.57 mm) and TW-14 (34.81 mm) were shown in Figure 9.

Table 2 Isolation of Soil Fungi from Six Different Soil Sample

Soil No.	Collected Area	Isolated Soil Fungi		
		Physical treatment dilution method	**Direct method**	**No: of fungi**
1	Hitaw Mu Pagoda	-	TW-01,TW-02 and TW-03	3
2	Ma Gyi Oak village	TW-05	TW-04	2
3	Myin Kya Kan village	TW-06 and TW-09	TW-07 and TW-08	4
4	Gyone Yar village	TW-10	TW-11	2
5	Nyaung Oak Phee village	-	TW-12 and TW-13	2
6	Quarter(3),Wundwin	TW-14	TW-15	2
Total isolated of soil fungi		5	10	15



Fungus TW-01 Fungus TW-02 Fungus TW-03

Figure 7 Morphology of soil fungi (5 days old culture on PGA medium)

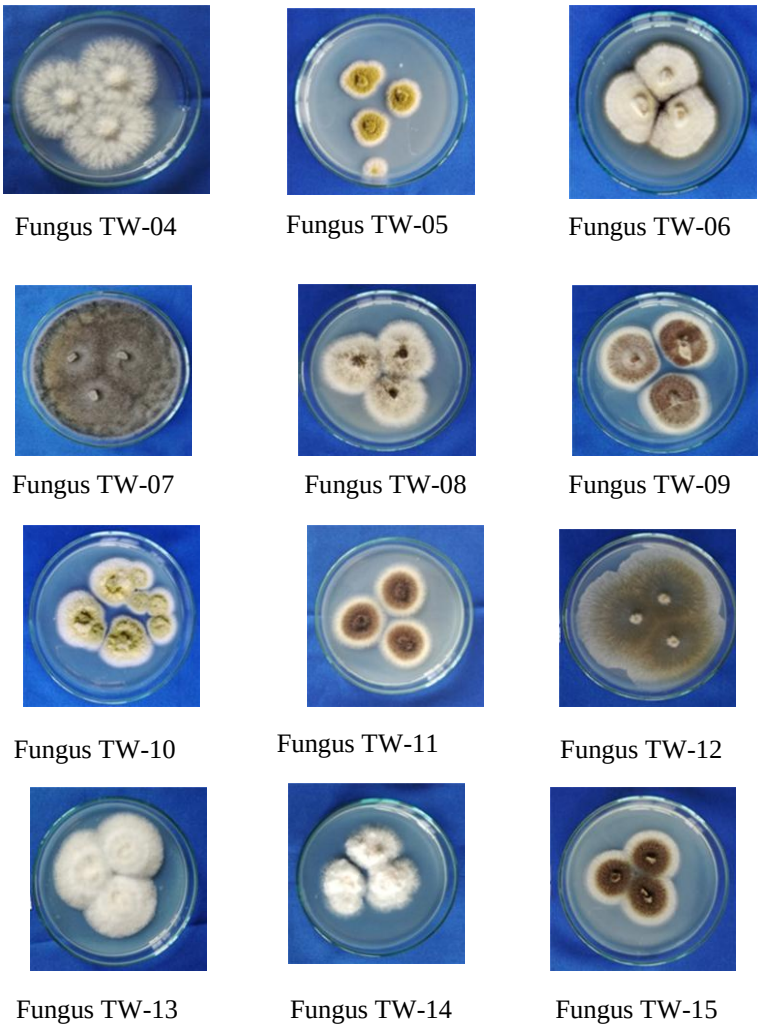


Figure 8 Morphology of soil fungi (5 days old culture on PGA medium)

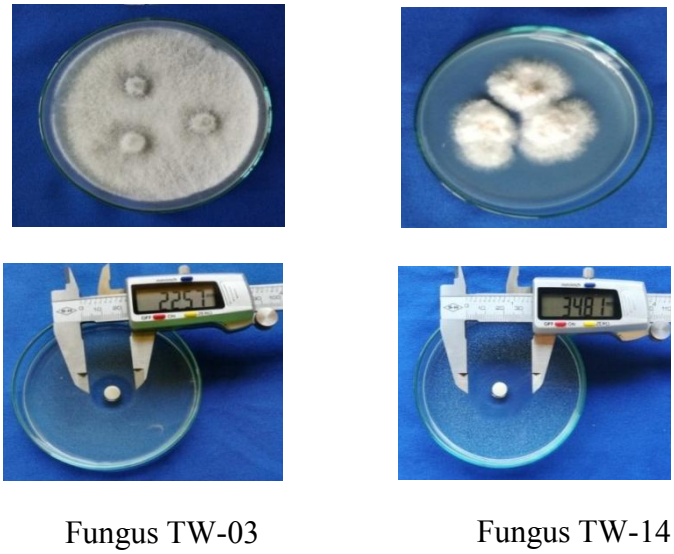
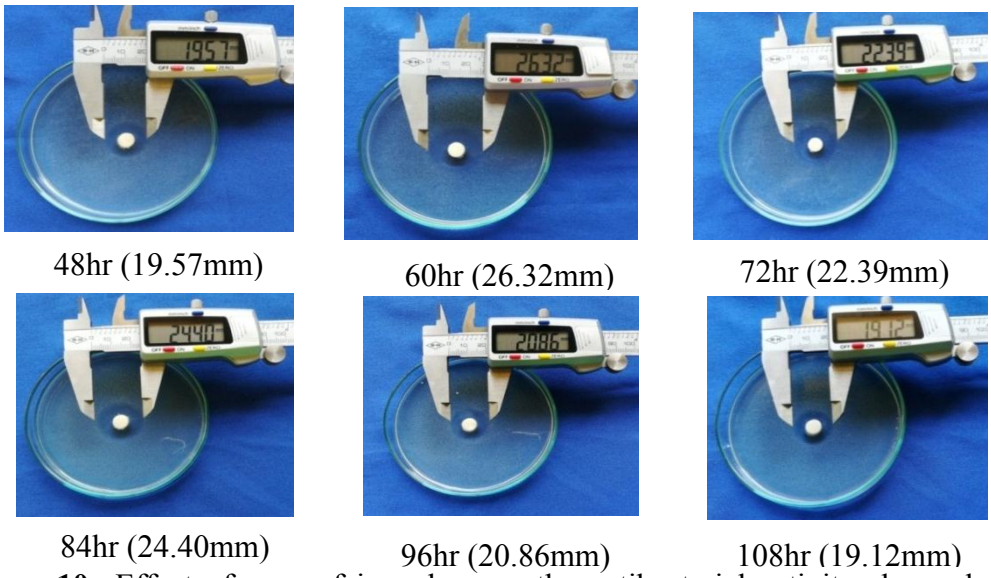


Figure 9 Effect of inoculation on the antibacterial activity shown by *Staphylococcus aureus*

The effect of ages of inoculation on the fermentation

In the effect of age of inoculum, TW-14(*Fusarium* sp.*)* was investigated by using 48, 60, 72, 84, 96, 108hr old culture age of inoculums. The results showed that 60hr age of inoculum gave the highest activities (26.16 mm) followed (24.40 mm) at 84hr and (22.39 mm) at 72hr age of inoculum. The results were shown in Table 3 and Figure 10.



48hr (19.57mm) 60hr (26.32mm) 72hr (22.39mm)

84hr (24.40mm) 96hr (20.86mm) 108hr (19.12mm)

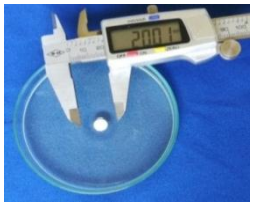
Figure 10 Effect of ages of inoculum on the antibacterial activity shown by *Staphylococcus aureus*

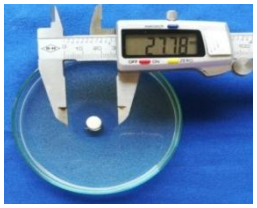
Table 3 The effect of ages of inoculums on the fermentation for TW-14 against *Staphylococcus aureus*

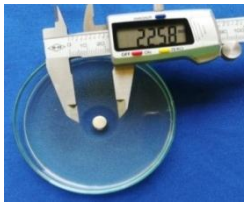
Sr. No	Age of inoculum	Activity (clear zones, mm)
1	48 hr	19.57 mm
2	60 hr	26.16 mm
3	72 hr	22.39 mm
4	84 hr	24.40 mm
5	96 hr	20.86 mm
6	108 hr	19.12 mm

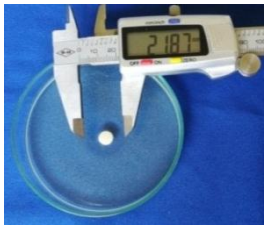
The effect of sizes of inoculums on the fermentation

In this research work, the effect of size of inoculums was studied using 5%, 10%, 15%, 20%, 25% and 30% inoculums. Using 10% showed significantly higher (27.78mm) than others, followed by 25% and 30% (24.04mm and 26.12mm) respectively in Table 4 and Figure 11.


5% (20.02mm)


10% (27.78mm)


15% (22.58mm)


20% (21.78mm)


25% (24.04mm)

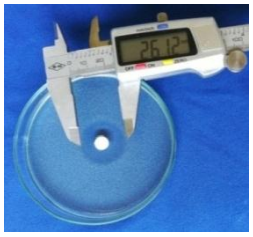

30% (26.12mm)

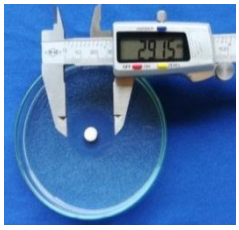
Figure 11 Effect of sizes of inoculum on the antibacterial activity shown by *Staphylococcus aureus*

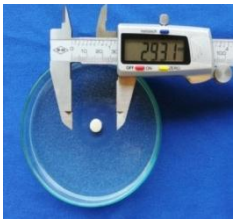
Table 4 The effect of sizes of inoculation on the fermentation for TW-14 against *Staphylococcus aureus*

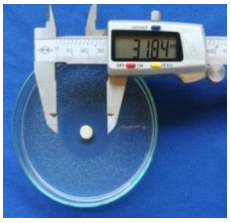
Sr. No.	Sizes of inoculum	Activity (clear zones, mm)
1	5%	12.58 mm
2	10%	27.78 mm
3	15%	20.01 mm
4	20%	21.87 mm
5	25%	24.04 mm
6	30%	26.12 mm

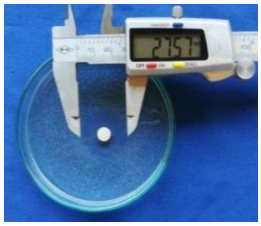
Effects of Carbon Utilization on Fermentation

There were variations in the level of antimicrobial activity when the different carbon sources were tested in the fermentation medium. The addition of different carbon sources displayed the highest antibacterial activities on soluble starch (31.84mm) followed by mannitol (29.31mm), molasses (29.15 mm), rice powder(27.57 mm) and corn powder(16.96 mm) were found as activity (Table 5 and Figure 12)


Molasses


Mannitol


Soluble starch


Rice powder


Corn powder

Figure 12 Effect on the carbon sources of selected fungi TW–14 against *Staphylococcus aureus*

Table 5 Effect on the carbon sources of selected fungi TW–14 against *Staphylococcus aureus*

Sr. No	Carbon Sources	Activity (Clear zones, mm) TW-14
1	Molasses	29.15mm
2	Mannitol	29.31mm
3	Soluble starch	31.84mm
4	Rice powder	27.57mm
5	Corn powder	16.96mm

Effects of Nitrogen Utilization on Fermentation

There were variations in the level of antimicrobial activity when the different nitrogen sources were tested in the fermentation medium. When the addition of various nitrogen sources, the significant inhibition zones on soybean (31.68mm) followed by NH_4NO_3(30.60mm), KNO_3 (24.34mm), gelatin (24.12mm) and $(NH_4)_2SO_4$(23.98mm), were found on activity (Table 6 and Figure 13)

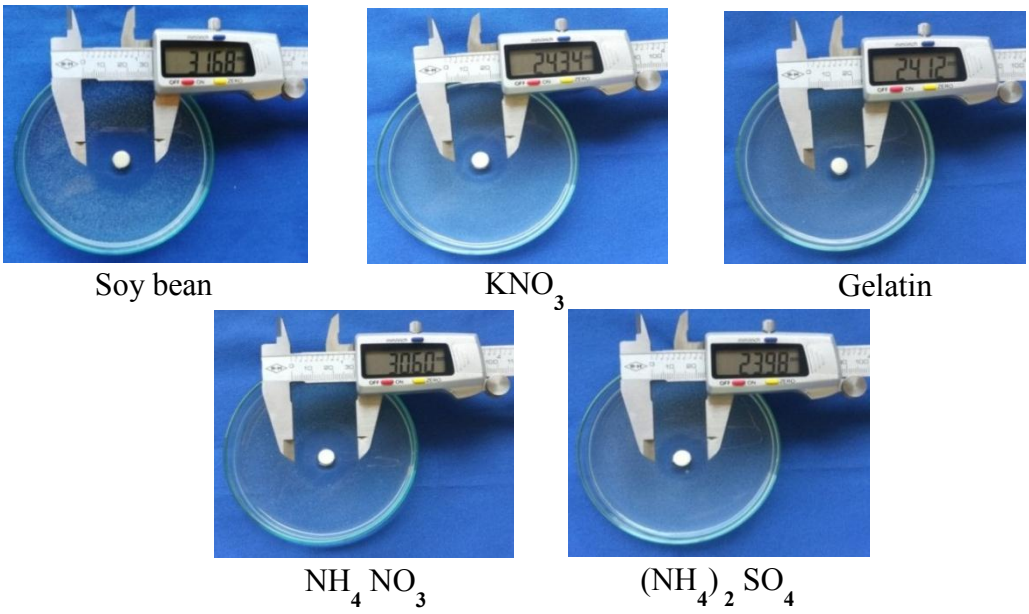


Figure 13 Effect of the nitrogen on the antibacterial activity of TW–14 against *Staphylococcus aureus*

Table 6 Nitrogen Utilization for the Fermentation on *Staphylococcus aureus*

Sr. No	Nitrogen Sources	Activity (Clear zones, mm) TW-14
1	Soybean	31.68mm
2	KNO_3	24.34mm
3	Gelatin	24.12mm
4	NH_4NO_3	30.60mm
5	$(NH_4)_2SO_4$	23.98mm

Discussion and Conclusion

The environmental factors such as pH, temperature, moisture, organic carbon and nitrogen play an important role in distribution of mycophora.(Adams *et al*., 1999).

Soil samples were collected from six different places at Wundwin Township in Mandalay Region. The isolation of fungi were isolated by using physical treatment dilution method (Hayakawa and Kobayashi, 2005) and direct method (Ando, 2004). According to the result, three fungi TW-01, TW-02 and TW-03 were isolated from the soil no-1.Two fungi TW-04 and TW-05 were isolated from the soil no-2.Four fungi TW-06, TW-07, TW-08 and TW-09 were isolated from the soil no-3.Two fungi TW-10 and TW-11 were isolated from the soil no-4. Two fungi TW-12 and TW-13 were isolated from the soil no-5. Two fungi TW-14 and TW-15 were isolated from the soil no.6.

Then, antimicrobial activities of soil fungi were tested by paper disc diffusion assay method. The fungal extracts were tested for antimicrobial activity against test organisms *Staphylococcus aureus.* Among them TW-14(*Fusarium* sp.*)* showed highest antibacterial against *Staphylococcus aureus*. In this investigation six different hours of 48hr, 60hr, 72hr, 84hr, 96hr and 108hr were consumed. For the sizes of inoculation 5%, 10%, 15%, 20%, 25% and 30% were used respectively. According to the results from this study, it is considered that the optimum ages of inoculum is 60hr (26.16 mm) and optimization size is 10% (27.78 mm).

In carbon and nitrogen sources utilization, carbon sources such as glucose, sucrose, lactose, glycerol, and soluble starch were used. Nitrogen sources such as yeast extract, $NaNO_3$, urea, soybean and peanut cake were also used. The results obtained in carbon sources study indicated that soluble starch (31.84 mm) is highest activity and corn powder(16.96 mm) is lower activity(Table 4 and Figure 12). In nitrogen sources study, soybean (31.68mm) is highest activity and $(NH_4)_2 SO_4$ (23.98 mm) is lower activity (Table 5 and Figure 13).

In the present study, the isolated fungus (*Fusarium* sp.) was then screened for the production of antimicrobial compound and it is an effort to understand the soil fungal diversity in Wundwin Township, Mandalay Region.

References

Adams, C.P., Bamford, K. M. and Early, M. P. (1999). **Principles of Horticulture** (3rd Ed.), utterworth Heineman, p. 25.

Aina VO, Adewuni AAJ, Hauwa Haruna and Amina Zaraki. Isolation and identification of fungi associated with the deterioration of painted wall surfaces within Kaduna polytechnic. Asian Journal of Medical Sciences,(2011); 3 (6): 250-253.

Ando, K. (2004). **Isolation and identification of fungi**. Workshop at Pathein University.

B.I. Duerden, T.M.S. Reid, J.M. Jewsbury, **Microbial and parasitic infection**, 7th ed., Great Britain: Edward Arnold, (1993).

Gutcho, Sydney,(1973). **Proteins from Hydrocarbons**, Noyes Data Corporation, Park Ridge, NJ

Hayakawa , A. and M. Kobayashi. (2005). **Screening for rare actinomycetes from soil.** J Microbial., 76, 240-242

Omalu, I.C.J., Ayanwale, V.A., Ajalaruru, A.B., Mohammed, A..Z., Bala, J. and Chukwuemeka V. (2011) Isolation Of Fungi And Bacteria From Housefly (Musca Domestica L.) Larvae. The Internet Journal of Microbiology. 9(1).

Smith, Biotechnology, Cambridge, New York, USA, (1996).

Stanbury, P.F., Whitaker, A. and Hall, S.J. (1995). **Media for industrial fermentations**. In Principles of Fermentation Technology pp. 93–121. Oxford: Pergamon Press

Vogel, Henry C. (ed.), (1983). **Fermentation and Biochemical Engineering Handbook**, Noyes Publications, Park Ridge, NJ

Y. Kulkarni and Y. Aynihojri, **Isolation and characterization of actinomycetes and optimization of its production**, M.Sc thesis, Department of Microbiology, University of Pune, (1995).

Zabriski, D. W., *et al*.,(1980). **Trader's Guide to Fermentation Media Formulation**, Trader's Oil Mill Co., Ft. Worth, TX