Identification of *Paecilomyces* and their Fertilizer Effect on *Cucurmis sativus* L.

Tin Tin Hla[1], Myat Myat Moe[2]

ABSTRACT

Genus *Paecilomyces* was isolated from *Sesbania grandiflora* (L.) Poir. belong to the family Fabaceae. These specimens were collected from East Dagon Township, Yangon Region (October to November, 2017). The identification of *Paecilomyces* was undertaken in Microbiology Lab, Dagon University and Chonbuk National University, Jeonju, Korea. This fungus was observed that 72 hrs ages of inoculum optimized for fermentation against *Escherichia coli*. Therefore, *Paecilomyces* used as the fertilizer for *Cucurmis sativus* L. (Thakwa) with various ratios (9:1, 8:2 and 7:3) and compared to the control.

Key words: identification, fertilizer effect

Introduction

Endophytic fungi are micro fungi that colonize or live within the healthy plant tissues without producing any apparent symptoms or obvious negative effects on their hosts (Hirsch and Braun, 1992). They may benefit the host plant by producing bioactive substances to enhance plant growth and competitiveness of the host in nature.

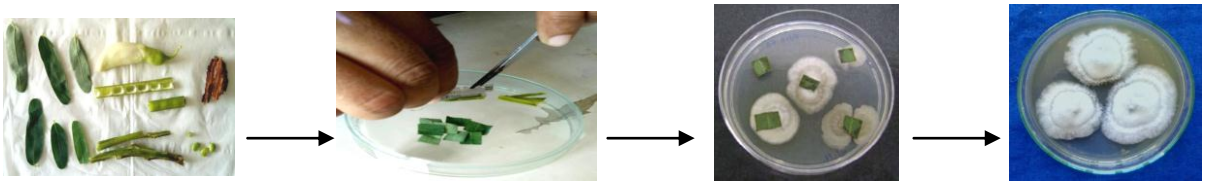
In addition, they produce novel antimicrobial secondary metabolites, which are now recognized as novel bioactive compounds (Carrol, 1988; Strobel *et al.*, 2004). Some endophytic microbes residing within plant tissues have been shown to promote plant growth and endow protection against biotic and abiotic stresses under laboratory conditions (Baltruschat *et al*., 2008; Hubbard et al., 2014; Waller *et al*., 2005).

Sesbania grandiflora (L.) Poir. used for the treatment of headache, in fever, as a tonic, in catarrh, as an astringent etc. The leaves of the plant have been reported to have anxiolytic and anticonvulsant effect, hypolipdemic, antiulcer and anti-inflammatory properties as well (Kirtikar and Basu , 1998). The main objectives of present study are to study the morphological characters, to investigate the effect of fertilizer and to identification of fungus.

MATERIALS AND METHODS

Isolation of endophytic fungi

Surface sterilization method (Ando, 2005)



Dry with filter paper cut in section incubated for 5-7 days pure culture

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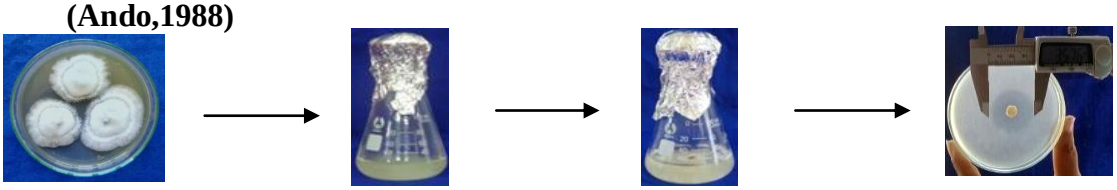
Preliminary study for antimicrobial activities by paper disc diffusion assay (Ando,1988)



7 days old culture seed medium fermentation medium paper disc diffusion assay

Fig 2. Procedure for the antimicrobial activity test

Table 1. Test organisms used in antimicrobial activities

No.	Test organism	Infections
1	*Agrobacterium tumefaciens* NITE 09678	Crown gall tumor
2	*Bacillus subtilis* IFO 90571	Fever and food disease
3	*Escherichia coli* AHU 5436	Diarrhea
4	*Pseudomonas fluroescens* IFO 94307	Leaf blight
5	*Saccharomyces cerevisiae* NITE 52847	Food spoilage
6	*Staphylococcus aureus* AHU8465	Boils and food poisoning

Study on the effects of ages of seed culture on the fermentation

The endophytic fungus was inoculated into the medium, 48 hrs, 60 hrs, 72 hrs, 84 hr, 96 hrs and 108 hrs were employed with 15 % seed culture in 12 hrs intervals for the growth.

Evaluation of Various Dilution Ratio of endophytic fungus on growth and plant height of *Cucumis sativus* L. (Thakwa)

The effect of microbial biofertilizer on the growth of Thakwa seedling was investigated in the plastic bag container of equal sizes holding. The endophytic fungus mixtures 9:1, 8:2 and 7:3 (water : broth) with various doses (0.5mL, 1.0mL and 1.5mL) were applied every weekly interval until two months. The result of growth and plant heights and their yield were recorded.

Identification of endophytic fungus isolated from *S. grandiflora* (L.) Poir.

The identification of endophytic fungus was undertaken in Microbiology Lab, Dagon University and Chonbuk National University, Jeonju, Korea.

Results

Morphlogical characteristics

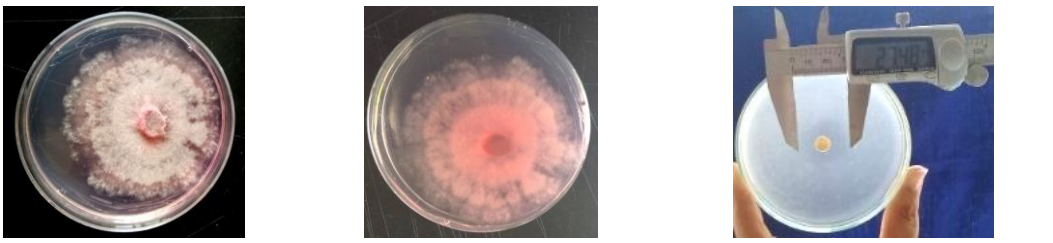
Scientific Name - *Sesbania grandiflora* (L.) Poir.
English Name - Agati
Myanmar Name - Pauk-pan-phyu
Family - Fabaceae
Sub-family - Papilionoideae

Small tree, perennial. Leaves alternate, pinnately compound. Inflorescences axillary raceme. Flower complete, bisexual, irregular, zygomorphic, pentamerous, cyclic and hypogynous. Calyx (5), synsepalous, campanulate, ascending imbricate,

sepaloid, bilabiate (the two upper and three lower united). Corolla 1+2+(2), apopetalous, papilionaceous, consisting of a large posterior petal, standard 1, 2 lateral petals wings and 2 posterior petals fused to form the keel, descending imbricate, petaloid (white). Stamen 5+5, dithecous, introse, dorsifixed, filaments 5 long and 5 short, longitudinal dehiscence. Gynoecium (1), monocapellary, unilocular, style long, stigma globose, ovary superior, marginal placentation. Fruits are legume. Seed endospermic.

Isolation and identification of endophytic fungi from *Sesbania grandiflora* (L.) Poir.
(Ando, 2005 and Barnett, 1956)

Front color pink, reverse pink, mycelium slender, septate, conidiophores long, conidia
1-celled, held together in chain (*Paecilomyces* sp.) as shown in figure (3 and 4).



Front side Reverse side Inhibitory zone (27.48 mm)

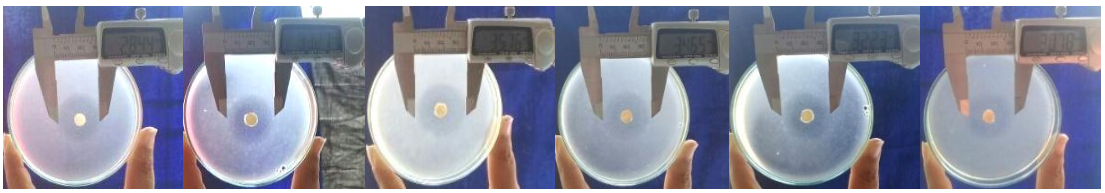
Fig 3.Colony characteristic and antibacterial activity

Starting growth x20 Starting growth x40 center growth x40 conidia x20

Fig 4. Photomicrograph of mycelium and conidia of *Paecilomyces*

Study on the effects of ages of inoculums on the fermentation

It was found that growth phase between 48 hrs and 108 hrs. According to Crueger and Crueger (1989), the ages of inoculums (48, 60, 72, 84, 96 and 108 hrs) with 20 % sizes of inoculum were utilized for the optimization of fermentation. In this investigation, 72 hrs seed culture was the best for fermentation table (2) and figure (5).



48hr 60hr 72hr 84hr 96hr 108hr

Fig 5. The effects of ages of inoculums for fermentation of *Paecilomyces* against *E. coli*

Table 2. The effects of ages of culture on fermentation

Culture time (Ages of culture, hrs)	Activity (clear zone, mm)
48	28.44
60	33.27
72	**35.76**
84	34.65
96	32.27
108	30.78

Evaluation of Various Dilution Ratio of strain SD-4 on growth and plant height of *C. sativus* L. (Thakwa)

In this study, the selected endophytic fungus SD-4 mixtures 9:1, 8:2 and 7:3 (water : broth) with various doses (0.5mL, 1.0mL and 1.5mL) were applied every weekly interval until two months. The best growth and plant height were found in (8:2) dose (1.0mL) observed and ratio (9:1) dose (1.5mL) found the best yield of these various ratios. The result of growth and plant heights and their yield as shown in table (3) and figure (6).

Table 3.The effects of Various Ratio of selected strain SD-4 on growth and plant height of *C. sativus* L.(Thakwa)

Time (cm)	Control	Ratio (0.5mL)			Ratio (1.0mL)			Ratio(1.5mL)		
		9:1	8:2	7:3	9:1	**8:2**	7:3	**9:1**	8:2	7:3
1 week	23.6	24.5	26	38	29.2	**43.2**	26.5	**38**	35	27
2 week	30.5	31.6	31	47	37.1	**67**	36	**63.4**	55.2	32.3
4 week	79.4	113.2	42	131.6	112.3	**224.3**	109.7	**212.5**	176.2	57.9
8 week	265	219.3	42.4	265	212.7	**304.2**	280.1	**296.8**	270.5	139.2

A B C D E F G H

Fig 6. Four weeks of plant height and yield of *C. sativus* L. (Thakwa)
A. Control B. 1.5mL C. 1.0mL D. 0.5mL
E. Control F. Ratio (9:1) 1.5mL G. ratio (8:2) 1.5mL H. Ratio (7:3) 1mL

Discussion and Conclusion

The samples of *Sesbania grandiflora* (L.) Poir. belonging to the family Fabaceae, collected from East Dagon Township, Yangon Region. The isolation of *Paecilomyces* by using surface sterilization method and cultured on potato glucose agar (PGA) medium. The identification of endophytic fungus was undertaken in Microbiology Lab, Dagon University and Chonbuk National University, Jeonju, Korea by using the method of Ando, 2005 and Barnett, 1956. The endophytic fungus *Paecilomyces* isolated from *Sesbania grandiflora* leaf. The isolated fungus was studied morphological characteristics. According to Pannapa Powthong (2013), based on their morphological characteristics, isolated endophytic fungi were *Acremonium* spp., *Fusarium* spp., *Phaeoacremonium* spp., *Phomopsis* spp., *Paecilomyces* spp., and *Cladosporium* spp. and different isolates exhibited different strengths of antibacterial and antioxidant activities.

In the study of antimicrobial test with six kinds of test organisms such as *A. tumefaciens, B. subtilis, E. coli, P. fluroescens, S. cerevisiae* and *S. aureus.* Among them, *Paecilomyces* was found the highest activity (30.29 mm) against on *E. coli. E. coli* appeared less susceptible to the extracts compared to the other high profile resistance of this organism against several antimicrobials. The activity of *S. grandiflora* leaf was shown zone of inhibition on both *E.* coli, *B. subtilis*, *S. aureus* and *P. aeruginosa* agreement by Lakshmi *et al.* (2011). *Pacieliomyces* sp. isolated from *Sesbania grandiflora* leaf, and thus was selected used for biofertilizer effect on *Cucumis sativus* L. (Thakwa).

Crueger and Crueger (1989) reported that, the ages of inoculums 72 hrs seed culture was the best for fermentation. In the evaluation of dilution of various ratios, it was found that ratio (8:2) dose (1.0mL) observed the best growth and plant height and ratio (9:1) dose (1.5mL) were found that the best yield of these various ratios. Endophytic microorganisms affect plant growth directly or indirectly and can provide the hosts with compounds that are produced by the fungi for facilitating the uptake of nutrients from the environment. Endophytes can also act by decreasing or preventing the deleterious effect of pathogens. Varma *et al*. (1999) agreed that the fungus increases the growth of various hosts suggesting that it may be useful for the promotion of plant growth.

S. grandiflora may represent new sources of antimicrobial with stable, biologically active components that can establish a scientific base for the use of plants in modern medicine and bioactive compounds. Therefore, it was found that the potential bioactive compounds produced by endophytic fungi isolated from *S. grandiflora* (L.) Poir. It was concluded that the endophytic fungi are one of the potential sources of new natural bioactive products from their secondary metabolites. However, many endophytic fungi have shown using fertilizers on plant growth and height as well as yielding.

Acknowledgements

My special thanks to Professor and Head Dr Myat Myat Moe, Department of Botany, Dagon University and Professor Beng, Project manager (CBNU) for guidance and providing infrastructural support to carry out the research work.

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