



APPENDICES

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่

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APPENDIX A

MEDIA

Isolation Media

Water agar

Agar	15 g
Chloramphenicol	0.05 mg
Distilled Water	1000 ml

Dispensed into container and sterilized by autoclaving at 121°C for 15 minutes.

Potato Dextrose Agar

Potato Dextrose Broth	26.5 g
Agar	20.0 g
Distilled Water	1000 ml

Dispensed into container and sterilized by autoclaving at 121°C for 15 minutes.

Liquid media for antimicrobial compound

Potato Dextrose Broth

Potato Dextrose Broth	26.5 g
Distilled Water	1000 ml

SMY Liquid medium* (modified Lee et al., 2005)

Maltose	40 g
Peptone	10 g
Yeast extract	10 g
Distilled water	1 liter

Gelatin semi-solid medium*

Potassium phosphate buffer	0.2% (pH 7.0)
Gelatin	10.0 g
NaCl	3.0 g
MgSO ₄ .7H ₂ O	3.0 g

802C liquid medium*

Yeast extract	2.0 g
MgSO ₄ .7H ₂ O	1.0 g
Peptone	10.0 g
Chitin	1.0 g

F1 medium

Glucose	20.0 g
Soybean meal	5.0 g
CaCl ₂	0.1 g
KH ₂ PO ₄	1.0 g

MgSO ₄ .7H ₂ O	0.5 g
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pH	6.0
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Nutrient agar

Nutrient agar (Bacto)	23.0 g
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Sterilized distilled water	1 liter
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Chitin agar medium for screening chitinase

Colloidal chitin	1%
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Agar	20.0 g
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Distilled water	1000 ml
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Seed culture medium for chitinase (pH 5.0) (Felse and Panda, 2000)

Dextrose	10.1 g
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(NH ₄) ₂ SO ₄	4.2 g
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NaH ₂ PO ₄	6.9 g
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KH ₂ PO ₄	2.0 g
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MgSO ₄ . 7H ₂ O	0.3 g
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Peptone	1.0 g
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Citric acid	10.5 g
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Urea	0.3 g
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Distilled water	1000 ml
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Fermentation medium for chitinase (pH 5.7) (Felse and Panda, 2000)

$(\text{NH}_4)_2\text{SO}_4$	4.2 g
NaH_2PO_4	6.9 g
KH_2PO_4	2.0 g
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	0.3 g
Tween 80	0.2
$\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$	0.005 g
MnSO_4	0.0016 g
ZnSO_4	0.0014 g
$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$	0.002 g
Distilled water	1000 ml

1% skimmed milk agar (pH 5.6) for screening protease

Skimmed milk	10.0 g
Agar	15.0 g
Distilled water	1000 ml

Skimmed milk broth for protease activity (Nimnoi *et al.*, 2010)

Skimmed milk	50.0 g
Casein	10.0 g
Yeast extract	2.5 g
Glucose	1.0 g
Distilled water	1000 ml

Submerged culture for dipicolinic acid cultivation (pH 5.6) (Fargues *et al.*, 1992)

Glucose	30.0 g
Yeast extract	3.0 g
KH_2PO_4	0.39 g
$\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$	1.42 g
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	0.6 g
NH_4NO_3	0.7 g
KCl	1.0 g
Distilled water	1000 ml

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APPENDIX B

PREPARING BUFFER

(A) CTAB extraction buffer

2% CTAB (hexadecyltrimethylammonium bromide)

100 mM TrisHCl (pH=8.0)

20 mM EDTA

1.2 M NaCl

(B) TE buffer (pH 8.0)

10 mM Tris-HCl

1 mM EDTA

(C) Citrate phosphate buffer (pH 5.6) (Dawson, *et al.*, 1986)

Stock solution

A: 0.1M of $C_2H_8O_7 \cdot H_2O$ (21.01 g of citric acid in 1000 ml of distilled water)

B: 0.2M of Na_2HPO_4 (28.40 g of Na_2HPO_4 in 1000ml of distilled water)

Working stock

x ml of 0.1 M of citric acid and y ml of 0.2 M of Na_2HPO_4 mixed.

pH	X	Y	pH	X	Y
2.6	89.10	10.90	5.2	46.40	53.60
2.8	84.15	15.85	5.4	44.25	55.75
3.0	79.45	20.55	5.6	42.00	58.00
3.2	75.30	24.70	5.8	39.55	60.45
3.4	71.50	28.50	6.0	36.85	63.15
3.6	67.80	32.20	6.2	33.90	66.10
3.8	64.50	35.50	6.4	30.75	69.25
4.0	61.45	38.55	6.6	27.25	72.75
4.2	58.60	41.40	6.8	22.75	77.25
4.4	55.90	44.10	7.0	17.65	82.35
4.6	53.25	46.75	7.2	13.05	86.95
4.8	50.70	49.30	7.4	9.15	90.85
5.0	48.50	51.50	7.6	6.35	93.65

(D) Tris- HCl buffer

20mM Tris- HCl, pH 7.0

Dissolved 2.422 g of Tris in 1000ml of distilled water and adjusted pH 7.

(E) Colloidal chitin preparation (Mathurot, 2543)

10 g of shrimp cells: 20 ml of acetone: 200 ml of HCl (conc.)

↓
Stirred constantly in ice bath at 4°C for 2 hours

↓
Preserved at 4°C for 24 hours

↓
Filtered through 2-3 layers of fabric filter into 600ml of 50% ethanol which is

constantly stirred

↓
Washed with distilled water for 2-3 times

↓
Adjusted pH 7.0 and precipitate colloidal chitin

↓
Collected the precipitate

(F) Reagent C

Solution A: 1 g of Na₂CO₃ dissolved in 50ml of 0.4% NaOH (2.0g of NaOH in 50 ml of distilled water)

Solution B₁: 1% CuSO₄ · 5H₂O (0.02g of CuSO₄ · 5H₂O in 2ml of distilled water)

Solution B₂: 2% Na₃C₆H₅O₇ · 2H₂O (0.04g of Na₃C₆H₅O₇ · 2H₂O in 2ml of distilled water)

Reagent C = solution A : solution B₁ : solution B₂

= 50 : 0.5 : 0.5

(G) Folin phenol reagent

Dilute 2N folin phenol reagent to 1N before used.

APPENDIX C

(A) Standard curve of N-acetylglucosamine (NAG)

- (1) Prepare the working solution of N-acetylglucosamine (NAG) with a concentration of 1.0 mg/ml of NAG to get 0.1 mg in 100ml of distilled water.

Table . Preparation of standard curve of N-acetylglucosamine (NAG).

No.	N-acetylglucosamine (μg)	Optimal density
1	0	0.000
2	100	0.100
3	200	0.203
4	300	0.318
5	400	0.412
6	500	0.519
7	600	0.626
8	700	0.721
9	800	0.788
10	900	0.881

- (2) Standard curve preparation of reducing sugar was prepared using serial concentration of NAG (N-acetylglucosamine) solution ($1-1000\mu\text{g ml}^{-1}$) in distilled water.
- (3) The reaction mixture was incubated at 37°C in the water bath for 30 minutes.
- (4) Thereafter 0.5 ml of 3,5-dinitrosalicylic acid reagent was added.

- (5) The mixture was placed in a boiling water bath for 15 minutes.
- (6) The reaction was stopped by adding 4 ml of sterilized distilled water.
- (7) N-acetylglucosamine (NAG) was measured spectrophotometrically at 575

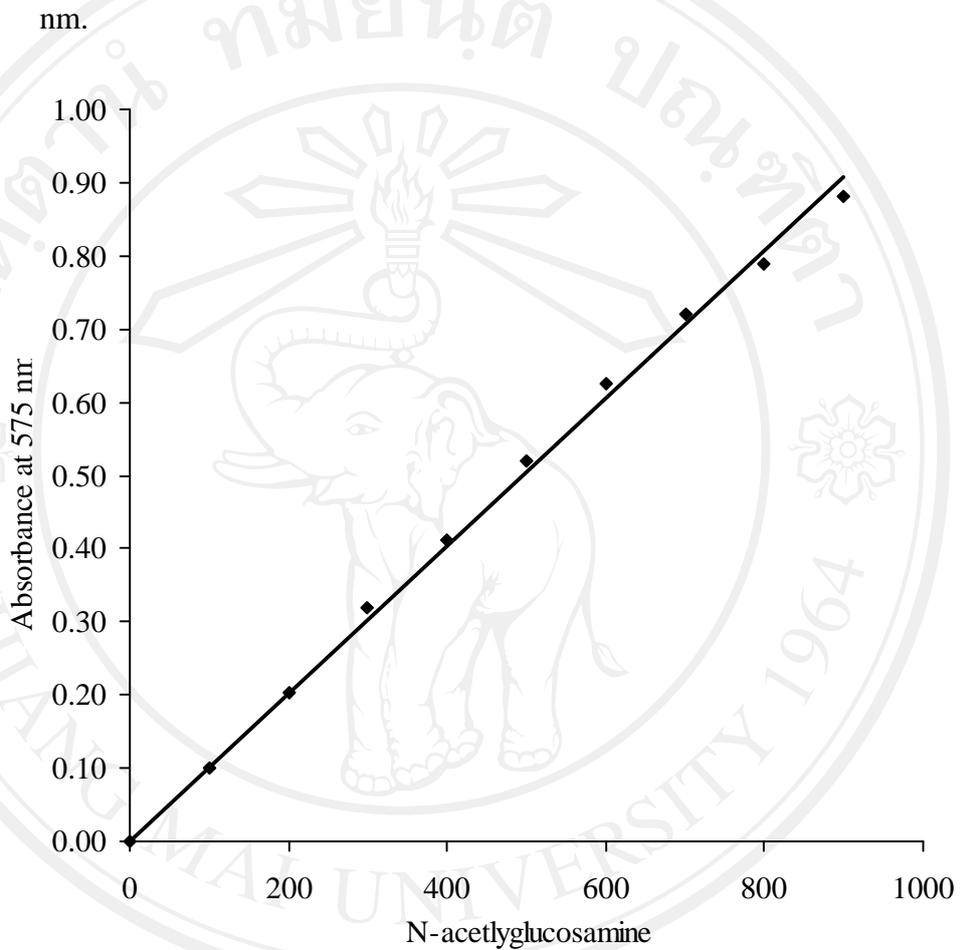


Figure1 Standard curve of N-acetylglucosamine at different concentrations.

(B) Standard curve of Bovine Serum Albumin (BSA)

- (1) Prepare a solution of the bovine serum albumin or BSA (stock solution) concentration of 1,000 $\mu\text{g/ml}$ (0.1 g of BSA in 100ml of distilled water) and then prepare a standard solution of BSA concentrations from 0-1,000 $\mu\text{g/ml}$ as follows;

Table 2. Preparation of standard solution of the protein bovine serum albumin (BSA) at different concentrations.

Concentration ($\mu\text{g/ml}$)	Solution (μl)	Stock solution (μl)
0	300	0
100	270	30
200	240	60
300	210	90
400	180	120
500	150	150
600	120	180
700	90	210
800	60	240
900	30	270
1000	0	300

- (2) Added the reagent C solution and incubated at room temperature for 20 minutes.
- (3) Mixed with 0.3ml of folin phenol reagent and left for 30 minutes at room temperature.
- (4) Measured the absorbance at 750 nm.

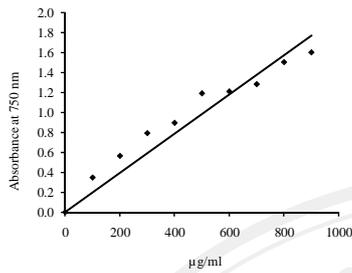


Figure 2 Standard curve of the protein bovine serum albumin at different concentrations

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(C) Calculating the amount of reducing sugar (chitinase activity)

$$1. \text{ Amount of reducing sugar} = \text{OD (ES-EC-SC)}$$

Where:

ES = enzyme substrate

EC = Enzyme control

SC = Substrate control

2. Change the OD value to the –in milligram (mg)

$$\text{Equation from slope } y = mX$$

Where:

M = slope of standard graph

Y = value OD (ES-EC-SC)

X = reducing sugar amount (mg)

$$X = \frac{y}{m} \text{ mg/ml}$$

$$3. \text{ Chitinase activity} = \frac{X \times \text{crude enzyme dilution}}{221.21 \times 0.25 \times 30} \text{ unit/ml}$$

(D) Abbotts' formula (Abbott, 1925)

$$\text{Corrected \%} = \left(1 - \frac{n \text{ in T after treatment}}{n \text{ in Co after treatment}} \right) \times 100$$

Where:

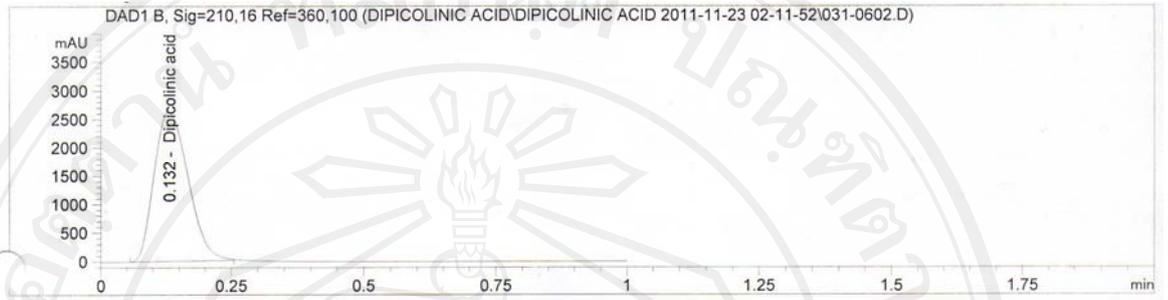
n = Insect population

T = Treated

Co = Control

APPENDIX D

HPLC Result of Dipicolinic acid



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Education background

2001 - 2004 Master of Agricultural Science (M.Agr.Sc- Entomology) in Yezin Agricultural University, Yezin, Pyinmanar Township, Mandalay Division, Myanmar.

1993 - 1998 Bachelor of Agricultural Science (B.Agr.Sc) in Yezin Agricultural University, Yezin, Pyinmanar Township, Mandalay Division, Myanmar.

Work experiences

July, 2007 to October, 2008 Technical Advisor, Manage the plantation Myanmar Singapore Plantation Limited

6-9-2000 to July.2007 Research Works and Admin. Management for Plantation Crops: Deputy Manager, Research and Factory Division (MPCE), Ministry of Agriculture and Irrigation (MOAI), The Republic of Union of Myanmar.

16-10-99 to 6-9-2000 Arava International Center for Vegetable Production, Israel

30-11-98 to 15-10-99 Nursery and Bud-wood nursery Manager: Assistant Supervisor, Zayat Queen Rubber Estate (Hlegu), MPCE,

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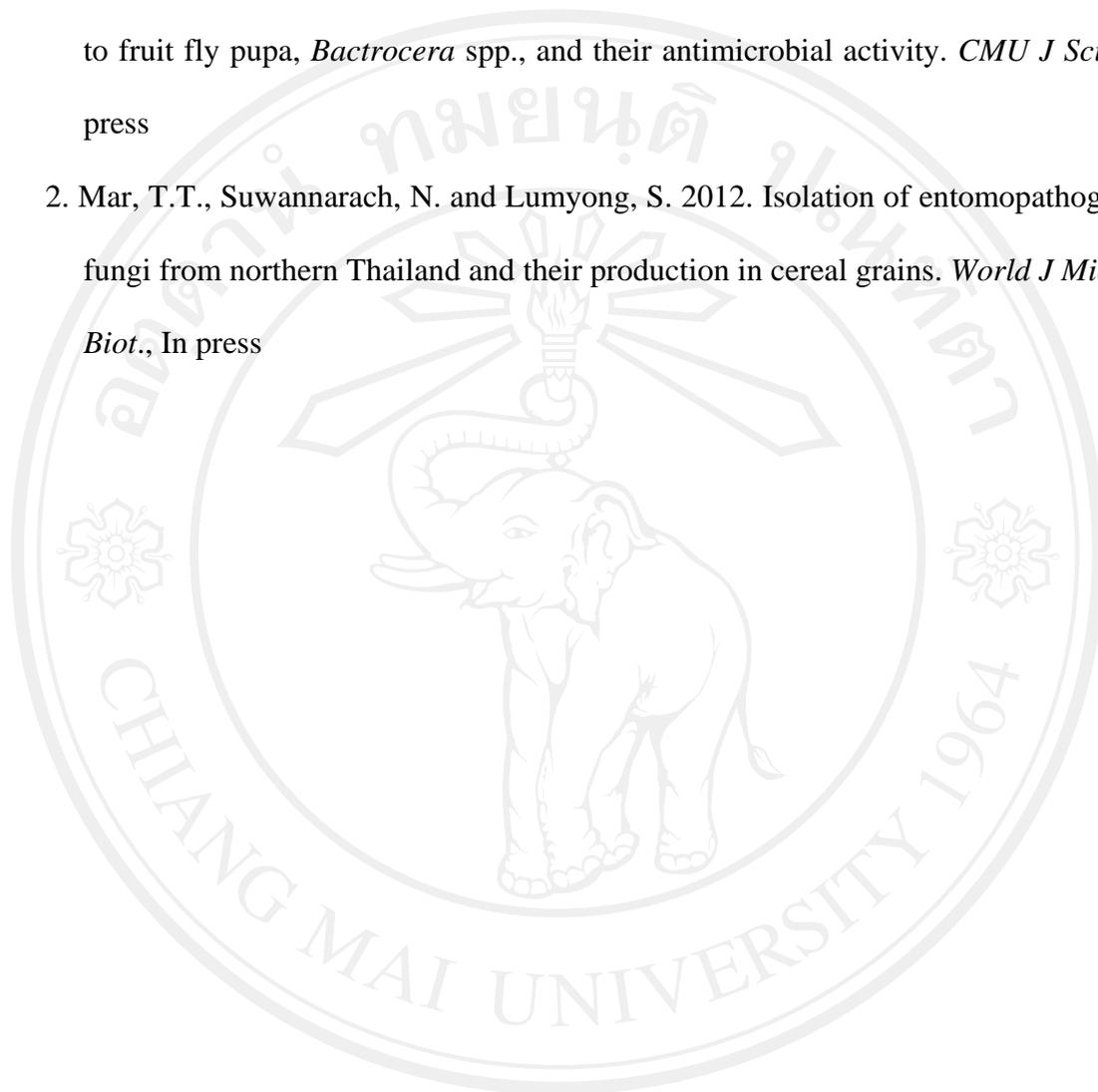
16-9-98 to 30-11-98 Rubber nursery Manager: Deputy Assistant Supervisor, MayanGone Rubber Estate (Kyaikhto), MPCE, Ministry of Agriculture and Irrigation (MOAI), The Republic of Union of Myanmar.

Presentations

1. Thet Thet Mar, Saisamorn Lumyong. 2010. Efficacy of secondary metabolic compounds in entomopathogenic fungi and their pathogenicity. Seminar I. 13 January, 2010. Microbiology Division, Department of Biology, Faculty of Science, Chiang Mai University.
2. Thet Thet Mar, Saisamorn Lumyong. 2011. Production of entomopathogenic fungal inoculums by using cereal grains. Seminar II. 19 January, 2011. Microbiology Division, Department of Biology, Faculty of Science, Chiang Mai University.
3. Thet Thet Mar, Saisamorn Lumyong. 2011. Conidial production of entomopathogenic fungi in solid state fermentation. 31 August, 2011. The 4th International on Fermentation Technology for Value Added Agricultural Products with Joint Sessions from Asian Core Program. Khon Kaen. Thailand. Abstract see online: http://fervaap.kku.ac.th/inter/images/Fervaap2011/CD_LINK/CD_Fervaap2011/System.file/Fer4_O.asp.htm. Fer4 O10.
4. Thet Thet Mar, Saisamorn Lumyong. 2011. Evaluation of effective entomopathogenic fungi to fruit fly pupa, *Bactrocera* sp.. Seminar III. 21 September, 2011. Microbiology Division, Department of Biology, Faculty of Science, Chiang Mai University.

Published papers

1. Mar, T.T. and Lumyong, S. 2012. Evaluation of effective entomopathogenic fungi to fruit fly pupa, *Bactrocera* spp., and their antimicrobial activity. *CMU J Sci.*, In press
2. Mar, T.T., Suwannarach, N. and Lumyong, S. 2012. Isolation of entomopathogenic fungi from northern Thailand and their production in cereal grains. *World J Microb Biot.*, In press



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