

MORPHOLOGICAL CHARACTERISTICS IDENTIFICATION AND  
DIVERSITY OF FUNGSI ISOLATED FROM UNHATCHED (ROTTEN) EGGS OF  
GREEN TURTLE (*Chelonia mydas*) AT CHAGAR HUTANG (*in-situ*)  
AND MA' DAERAH (*ex-situ*)

FAEZAH NOOR BINTI BASIR

FACULTY OF MARITIME STUDIES AND MARINE SCIENCE  
UNIVERSITI MALAYSIA TERENGGANU

2011



16

**MORPHOLOGICAL CHARACTERISTICS IDENTIFICATION AND  
DIVERSITY OF FUNGI ISOLATED FROM UNHATCHED (ROTTEN) EGGS OF  
GREEN TURTLE (*Chelonia mydas*) AT CHAGAR HUTANG (*in-situ*)  
AND MA' DAERAH (*ex-situ*)**

**By**

**Faezah Noor binti Basir**

**Research Report submitted in partial fulfillment of  
the requirements for the degree of  
Bachelor of Science (Marine Biology)**

**Department of Marine Science  
Faculty of Maritime Studies and Marine Science  
UNIVERSITI MALAYSIA TERENGGANU  
2011**

This project report should be cited as:

Faezah Noor, B. 2011. Morphological Characteristics Identification and Diversity of Fungi Isolated from Unhatched (rotten) Eggs of Green Turtle (*Chelonia mydas*) at Chagar Hutang (*in-situ*) and Ma' Daerah (*ex-situ*). Undergraduate thesis, Bachelor of Science in Marine Biology, Faculty of Maritime and Science Marine, Universiti Malaysia Terengganu, Terengganu. 78p.

*No part of this project report may be reproduced by any mechanical, photographic, or electronic process, or in the form of phonographic recording, nor may it be stored in a retrieval system, transmitted, or otherwise copied for public or private use, without written permission from the author and the supervisor(s) of the project.*



DEPARTMENT OF MARINE SCIENCE  
FACULTY OF MARITIME STUDIES AND MARINE SCIENCE  
UNIVERSITI MALAYSIA TERENGGANU

DECLARATION AND VERIFICATION REPORT  
FINAL YEAR RESEARCH PROJECT

It is hereby declared and verified that this research report entitled:  
Morphological Characteristics Identification and Diversity of Fungi Isolated from Unhatched (rotten) Eggs of Green Turtle (*Chelonia mydas*) at Chagar Hutang (*in-situ*) and Ma' Daerah (*ex-situ*) by Faezah Noor Binti Basir, Matric No. UK17018 have been examined and all errors identified have been corrected. This report is submitted to the Department of Marine Science as partial fulfillment towards obtaining the Degree of Bachelor of Science (Marine Biology), Faculty of Maritime Studies and Marine Science, Universiti Malaysia Terengganu.

Verified by:

.....  
Principal Supervisor

Name: Dr. Juanita Joseph

Official stamp:

DR. JUANITA JOSEPH  
KETUA  
Stesen Penyelidikan  
Institut Oseanografi  
Universiti Malaysia Terengganu  
21030 Kuala Terengganu, Terengganu

Date: 28.4.2011

.....  
Second Supervisor

Name: Ms. Siti Nordahliawate Mohamed Sidique

Official stamp:

MS. SITINORDAHLIAWATE MOHAMED SIDIQUE  
Pensyarah  
Unit Agroteknologi  
Fakulti Agroteknologi dan Sains Makanan  
Universiti Malaysia Terengganu  
21030 Mengabang Telipot

Date: 28.4.2011

.....  
Head of Department of Marine Science

Name: Dr. Razak bin Zakariya

Official stamp:

DR. RAZAK ZAKARIYA  
Ketua Jabatan Sains Marin  
Fakulti Pengajian Maritim dan Sains Marin  
Universiti Malaysia Terengganu  
(UMT)

29/4/11  
Date: .....

## TABLE OF CONTENTS

	<b>Page</b>
<b>ACKNOWLEDGEMENTS</b>	ii
<b>LIST OF TABLES</b>	iii
<b>LIST OF FIGURES</b>	iv
<b>LIST OF ABBREVIATIONS</b>	vii
<b>LIST OF APPENDICES</b>	viii
<b>ABSTRACT</b>	ix
<b>ABSTRAK</b>	x
<b>CHAPTER 1: INTRODUCTION</b>	1
1.1 Introduction	1
1.2 Objectives	5
<b>CHAPTER 2: LITERATURE REVIEW</b>	6
2.1 Green Turtle ( <i>Chelonia mydas</i> )	6
2.2 Occurrence of Fungi on Turtle Eggs	9
2.2 Threats and Effects to Turtle's Egg	13
2.2.1 <i>In-situ</i> Nesting	13
2.2.2 <i>Ex-situ</i> Nesting	14
<b>CHAPTER 3: METHODOLOGY</b>	17
3.1 Sampling Sites	17

3.1.1	Chagar Hutang, Redang Island	19
3.1.2	Ma' Daerah Turtle Sanctuary	20
3.2	Samples Collection	21
3.2.1	Observation and Isolation of Fungi on Turtle Eggs Shell and Soil.	21
3.3	Laboratory Analysis	22
3.3.1	Isolation of Fungi from Soil	22
3.4	Fungi Identification	24
3.4.1	<i>Fusarium</i> spp. Identification	24
3.4.2	<i>Aspergillus</i> spp. Identification	25
3.4.3	Other Fungi Identification	26
3.5	Diversity Analysis	26

## **CHAPTER 4: RESULTS**

4.1	<i>In-situ</i> and <i>Ex-situ</i> Nesting Sites Excavation Process	27
4.2	Identification of Fungi	30
4.2.1	<i>Fusarium solani</i>	30
4.2.2	<i>Fusarium</i> sp.	35
4.2.3	<i>Aspergillus niger</i>	37
4.2.4	<i>Trichoderma</i> sp.	43
4.2.5	<i>Pestalotiopsis</i> sp.	44
4.2.6	Unknown fungi	45
4.3	Fungi Isolated from The Unhatched Turtle Eggs	48

4.4	Fungi Isolated from Soil and Debris	49
4.3.1	Dilution Plate Technique	49
4.3.2	Direct Plating Technique	51
4.3.3	Debris Plating Technique	52
4.5	Fungi isolated from Chagar Hutang and Ma' Daerah.	53
4.6	Diversity of Fungi in Chagar Hutang Beach and Ma' Daerah Turtle Sanctuary	54
	<b>CHAPTER 5: DISCUSSION</b>	<b>55</b>
5.1	Fungi Infection and Habitat Comparison	55
5.2	Fungi Distribution and Diversity	58
5.3	Possible Mycotoxin Effects	61
	<b>CHAPTER 6: CONCLUSION RECOMMENDATION</b>	<b>64</b>
	<b>REFERENCES</b>	<b>66</b>
	<b>APPENDICES</b>	<b>72</b>
	<b>CURICULUM VITAE</b>	<b>78</b>



## ACKNOWLEDGEMENTS

In the name of Allah, the most Gracious and Merciful, I would like to express my deepest gratitude to Allah S.W.T. the Almighty for His blessing in completing this thesis. I would also like to thank my supervisors, Dr. Juanita Joseph and Ms. Siti Nordahliawate Mohamed Sidique for their advise, teaching, support and assistance during the project research.

My sincere appreciation goes to the Sea Turtle Research Unit (SEATRU), Institute of Oceanography, Universiti Malaysia Terengganu (UMT) and the Department of Fisheries Malaysia for allowing the sampling to be done at Chagar Hutang Turtle Sanctuary and Ma' Daerah Turtle Sanctuary. Thanks are also extended to both staff from SEATRU and Ma' Daerah Turtle Sanctuary for their help and cooperation. I am also appreciating the facilities provided by SEATRU and transportation sponsored by Laguna Redang Resort during the sampling field at Chagar Hutang Turtle Sanctuary.

I would also like to thank laboratory staff from Post Harvest Laboratory (Faculty of Agrotechnology and Food Science) and Instrumentation Laboratory (Faculty of Maritime Studies and Marine Science) for assistance during laboratory work. Special thanks are dedicated to my family members and classmates for their love, patience, support and encouragement. Finally, I am also thankful to all of my friends that had helped during the field trips, laboratory work and as well as for the moral support.

## LIST OF TABLES

<b>Table</b>		<b>Page</b>
2.1	Fungi isolated from failed eggs surface and soil/nest	11
4.1	Data recorded for 11 nests at Chagar Hutang beach.	28
4.2	Data recorded for six nests at Ma' Daerah Turtle Sanctuary.	29
4.3	Colony Formation Unit (CFU)/g soil of $10^{-3}$ dilution	52
4.4	Fungi isolated from soil and debris.	54
4.5	Number and percentage of fungi isolates from Chagar Hutang and Ma' Daerah	55
4.6	Species diversity (H) of fungi at Chagar Hutang and Ma' Daerah	56

## LIST OF FIGURES

Figure	Page
2.1 Female green turtle	8
2.2 Debris from soil at Chagar Hutang beach soils.	14
3.1 Map of Chagar Hutang, Redang Island (Chan and Liew, 1999)	18
3.2 Map of Ma' Daerah Turtle Sanctuary (taken from: <a href="http://www.google earth.com">http://www.google earth.com</a> ).	18
3.3 <i>In-situ</i> nest under incubation at Chagar Hutang. Wooden sticks are used to mark the nest and a net are used to cover the nest from predators.	19
3.4 Infected and predated turtle eggs.	22
3.5 Culture of <i>Aspergillus niger</i> on Czapek Yeast Extract Agar (CYA25, CYA37 and CYA20S) and MEA (Malt Extract Agar).	25
4.1 A) Healthy eggs. Symptoms of fungi infection on turtle egg's surface (arrow) at B) Chagar Hutang Turtle Sanctuary; C) and D) from Ma' Daerah Turtle Sanctuary.	30
4.2 Macroscopic characteristic of <i>Fusarium solani</i> on PDA medium. Pigmentation is various with cream, yellow and orange pigmentation and mycelium growth were robust.	31
4.3 Cream and green sporodochia on mycelium of <i>Fusarium solani</i> (arrow).	31
4.4 Blunt end of macroconidia(circle) with 3 to 4 septa. (Scale: 10µm).	32
4.5 Microconidia with 0 to 1 septa, oval and reniform shape. (Scale: 10µm).	33
4.6 Single chlamyospores (arrow).	33
4.7 Long monophialades (arrow) with microconidia (circle). (Scale: 50µm).	34

4.8	A) Abundance of uniform macroconidia (scale: 100µm) from B) sporodochia (circle).	34
4.9	White mycelium and produce orange to brown pigmentation	35
4.10	Macroconidia (circle) and abundance of chlamydospore (arrow).	36
4.11	Chlamydospore with short branched (arrow).	36
4.12	A) Macroconidia and B) microconidia. (Scale: 10µm).	37
4.13	Macroscopic characteristics of <i>Aspergillus niger</i> on A) CYA37, B) CZ, C) CYA20S, D) CYA25 and E) MEA.	38
4.14	Conidial head with phialdes and conidia (circle). (100x magnification).	39
4.15	Macroscopic characteristics of <i>Aspergillus niger</i> on A) CYA37, B) CZ, C) CYA20S, and D) MEA.	40
4.16	A) Mycelium and conidial head (40x magnification) B) Smooth conidia (40x magnification).	40
4.17	Conidial head with biseriate foot cell (40x magnification).	41
4.18	A) Conidial head of <i>Aspergillus niger</i> (100x magnification) B) Finely rough conidia (100x magnification).	42
4.19	Macroscopic characteristics of <i>Trichoderma</i> sp.	43
4.20	Abundance of green, smooth and spherical spores.	44
4.21	<i>Pestalotiopsis</i> sp. culture from debris on soil from Ma' Daerah Turtle Sanctuary.	45
4.22	A) Mycelium and B) Conidia of <i>Pestalotiopsis</i> sp. (40x magnification).	45
4.23	Macroscopic of unknown fungi.	46
4.24	Mycelium and spores of unknown fungi. (Scale: 50 µm).	46
4.25	Unknown fungi from nest A) 105, B) and C) 115; and D) 129.	47
4.26	Number of fungi isolated from the unhatched turtle eggs at Chagar Hutang beach	48

4.27	Number of fungi isolated from the unhatched turtle eggs at Ma' Daerah Turtle Sanctuary.	49
4.28	Colonies of fungi growth for CFU counting.	50
4.29	Colonies of fungi from spread plating technique on PDA medium (in circle).	51
4.30	Fungi from debris growth on PDA medium.	52
5.1	A) Bluish symptoms and B) Dead embryo infected by <i>Fusarium solani</i> . (Source: Sarmiento-Ramirez <i>et al.</i> , 2010).	56

## **LIST OF ABBREVIATIONS**

<b>C</b>	-	<b>Celcius</b>
<b>CFU</b>	-	<b>Colony Formation Unit</b>
<b>CLA</b>	-	<b>Carnation Leaves Agar</b>
<b>cm</b>	-	<b>centimeter</b>
<b>CYA</b>	-	<b>Czapex Yeast</b>
<b>CZ</b>	-	<b>Czapex Dox Agar Agar</b>
<b>E</b>	-	<b>East</b>
<b>g</b>	-	<b>gram</b>
<b>H</b>	-	<b>Index of species Diversity</b>
<b>m</b>	-	<b>meter</b>
<b>MEA</b>	-	<b>Malt Extract Agar</b>
<b>mm</b>	-	<b>milimiter</b>
<b>N</b>	-	<b>North</b>
<b>PDA</b>	-	<b>Potato Dextrose Agar</b>
<b>ppb</b>	-	<b>Part per billion</b>
<b>µm</b>	-	<b>micrometer</b>

## LIST OF APPENDICES

		<b>Page</b>
1	Form for macromorphology characteristic of <i>Aspergillus</i> spp.	72
2	Fungi isolation from Chagar Hutang Turtle Sactuary.	73
3	Fungi isolation from Ma' Daerah Turtle Sactuary.	74
4	Shannon-Wiener Index Diversity of Fungi Isolated from Chagar Hutang Turtle Sactuary.	76
5	Shannon-Wiener Index Diversity of Fungi Isolated from Ma' Daerah Turtle Sactuary.	77

## ABSTRACT

This study was conducted at Chagar Hutang Turtle Sactuary, Redang Island and Ma' Daerah Turtle Sactuary, Kemaman. The samples of green turtle eggs with fungi infection symptoms at the sampling sites and the soil from nest were brought back to the laboratory for further analysis and identification. All fungi isolated were regularly culture in the PDA, CLA, CZ, CYA or CLA medium at the laboratory for further identification through macroscopic and microscopic morphological characteristics. Five species of fungi was identified from both sampling sites which were *Fusarium solani*, *Fusarium* spp., *Trichoderma* sp., *Aspergillus niger* and *Pestalotiopsis* sp.. There were also unknown fungi isolated from the surface of unhatched turtle eggs and from the nest. The *Fusarium solani* was more dominant compared to other fungi in both sampling sites and have consistence occurrence on egg shells. Number of colonies was also isolated from soil at Chagar Hutang with the range of 0 to 1690 CFU/g soil and 0 to 250 CFU/g at Ma' Daerah Turtle Sanctuary. Based from the Shannon-Weiner index, diversity of fungi species was higher at Ma' Daerah Turtle Sanctuary ( $H= 1.05$ ) than in Chagar Hutang ( $H= 1.01$ ). The fungi colonization on the surface of egg shells may risk the survival of embryonic development of green turtle. The study will be valuable knowledge on the occurrence of fungi isolated from the unhatched turtle eggs and will be a platform to be a better understanding of fungi colonization that may risk the survival of embryonic development of green turtle.



# IDENTIFIKASI CIRI-CIRI MORFOLOGI DAN DIVERSITI FUNGUS PADA PERMUKAAN TELUR PENYU AGAR (*Chelonia mydas*) YANG TIDAK MENETAS DI CHAGAR HUTANG(*in-situ*) DAN MA' DAERAH(*ex-situ*)

## ABSTRAK

Kajian ini telah dijalankan di Santuari Penyu Chagar Hutang, Redang Island dan Santuari Penyu Ma' Daerah, Kemaman. Fungus yang didapati daripada permukaan telur penyu agar telah di ambil dan pasir daripada kawasan sarang penyu tersebut juga telah di bawa pulang ke makmal untuk analisis seterusnya untuk menentukan spesiesnya. Kesemua fungus yang telah didapati telah dikultur menggunakan medium PDA, CLA, CZ, CYA atau CLA di makmal untuk menentukan spesiesnya melalui ciri-ciri morfologi makroskopik and mikroskopiknya. Lima spesies fungus telah dikenalpasti daripada kedua-dua tempat iaitu *Fusarium solani*, *Fusarium* sp., *Trichoderma* sp., *Aspergillus niger* dan *Pestalotia* sp.. Terdapat juga spesies fungus yang tidak diketahui yang didapati daripada permukaan telur penyu dan kawasan persarangannya. *Fusarium solani* adalah fungus yang paling dominan berbanding fungus lain di kedua-dua kawasan persampelan dan mempunyai kehadiran yang konsisten di setiap permukaan telur penyu. Bilangan koloni yang didapati daripada Chagar Hutang mempunyai julat dari 0 hingga 1690 CFU/g soil dan 0 hingga 250 CFU/g di Santuari Penyu Ma' Daerah. Berdasarkan Shannon-Weiner index, diversiti fungus adalah tinggi di Santuari Penyu Ma' Daerah ( $H= 1.05$ ) berbanding di Chagar Hutang ( $H= 1.01$ ). Koloni fungus yang terdapat pada permukaan telur penyu mungkin mengurangkan risiko kelangsungan hidup embrio penyu agar di dalam telur. Kajian ini akan memberi pengetahuan yang bermanfaat bagi kehadiran fungus dari telur

penyu yang tidak menetas dan bakal menjadi platform untuk lebih memahami kolonisasi fungus yang mungkin berisiko untuk mengganggu perkembangan embrio penyu hijau.