CULTURE OF DIATOM *Halamphora* sp. USING OIL PALM WASTE AS LIVE FOOD FOR HARPACTICOID COPEPOD AND BLACK TIGER SHRIMP POSTLARVAE

By

NINIE NOOR DIANA BT ENCHE BAHARUDDIN

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Chairman : Professor Natrah Fatin binti Mohd Ikhsan, PhD
Faculty : Agriculture

Malaysia is one of the world's largest producers and exporters of palm oil, accounting for roughly one-third of global production. Palm oil mill effluent (POME) and Palm oil fuel ash (POFA), two types of oil palm waste that are produced in large quantities by palm oil milling activities, pose significant environmental risks and are considered serious environmental polluters in Malaysia. Alternatively, POME and POFA may provide prospects for diatom growth and extraction of high-value products due to their high nutrient and silica levels. The goal of this study was to develop marine benthic diatom *Halamphora* sp. culture exploiting POME and POFA for the enrichment of harpacticoid copepod *Amphiascoides neglectus* and black tiger shrimp *Penaeus monodon* postlarvae culture. This study showed that *Halamphora* sp. can be grown effectively with and without substrate, and PVC substrate is a viable alternative. It was found that the ideal growth parameters for *Halamphora* sp. include the photoperiod (light/dark) of 12:12 h to 18:06 h; light intensities of 80 to 120 μmol m^{-2} s^{-1}; salinities of 30 to 35 ppt; and pH of 8 to 9. The results revealed that higher *Halamphora* sp. growth can be obtained when cultured with 15% POME compared to the commercial medium, f/2 + Si (control). The microalga could also reduce the ammoniacal nitrogen, nitrate, nitrite, and orthophosphate levels in the POME concentrations of 5% and 15%. Meanwhile, there was no significant difference ($P > 0.05$) between the growth of *Halamphora* sp. added with 50%, 75%, and 100% of POFA as the silica sources. In the feeding experiment, the addition of *Halamphora* sp. for harpacticoid copepod enrichment led to higher nutritive values of the zooplankton and maintains the culture water quality between the acceptable ranges with zero water exchange. Furthermore, the addition of *Halamphora* sp. and enriched harpacticoid copepod improved the survival and nutritional content of the *Penaeus monodon* postlarvae compared to the control treatment. The addition of both consortia also protected the postlarvae from Acute Hepatopancreatic Necrosis Disease causing *Vibrio parahaemolyticus*. This study could pave the way for future research on using oil palm waste to culture beneficial microalgal biomass for aquaculture purposes.

Keywords: *Halamphora* sp., *Amphiascoides neglectus*, palm oil mill oil effluent, palm oil fuel ash, *Penaeus monodon* postlarvae

SDG: GOAL 2: Zero hunger; GOAL 14: Life below water

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

PENGKULTURAN DIATOM *Halamphora* sp. MENGGUNAKAN SISA KELAPA SAWIT SEBAGAI MAKANAN HIDUP UNTUK KOPEPOD HARPAKTIKOID DAN POSLARVA UDANG HARIMAU HITAM

Oleh

NINIE NOOR DIANA BT ENCHE BAHARUDDIN

Februari 2023

Pengerusi : Profesor Natrah Fatin binti Mohd Ikhsan, PhD
Fakulti : Pertanian

Malaysia adalah salah satu pengeluar dan pengeksport minyak sawit terbesar di dunia, menyumbang kira-kira satu pertiga daripada pengeluaran global. Efluen kilang kelapa sawit (POME) dan abu minyak sawit (POFA), dua jenis sisa kelapa sawit yang dihasilkan dalam kuantiti yang banyak oleh aktiviti pengilangan minyak sawit, menimbulkan risiko alam sekitar yang ketara dan dianggap sebagai pencemar alam sekitar yang serius di Malaysia. Sebagai alternatif, POME dan POFA boleh menyediakan prospek pertumbuhan diatom dan pengekstrakan produk bernilai tinggi kerana tahap nutrien dan silika yang tinggi. Matlamat kajian ini adalah untuk mengkultur *Halamphora* sp. menggunakan POME dan POFA sebagai makanan hidup kepada *harpacticoid copepod Amphiascoides neglectus* dan poslarva udang harimau hitam *Penaeus monodon*. Berdasarkan penemuan kajian ini, *Halamphora* sp. berjaya dikultur bersama substrat dan juga tanpa substrat, dan substrat PVC adalah alternatif yang paling berkesan. Didapati bahawa parameter

pertumbuhan yang ideal untuk kultur *Halamphora* sp. termasuk fotoperiod 12:12 h hingga 18:06 h; intensiti cahaya 80 hingga 120 μmol m^{-2} s^{-1}; kemasinan 30 hingga 35 ppt; dan pH 8 hingga 9. Data menunjukkan bahawa pertumbuhan *Halamphora* sp. yang lebih tinggi boleh diperolehi apabila dikulturkan dengan POME 15% berbanding komersial medium, f/2 +Si (medium kawalan). Selain itu, microalga juga boleh mengurangkan tahap nitrogen ammonia, nitrat, nitrit, dan ortofosfat dalam kepekatan POME sebanyak 5% dan 15%. Disamping itu juga, tiada perbezaan ketara ($P > 0.05$) antara pertumbuhan *Halamphora* sp. yang ditambah dengan POFA 50%, 75%, dan 100% sebagai sumber silika. Dalam ujian pemakanan, penambahan *Halamphora* sp. untuk pengayaan *harpacticoid copepod* membawa kepada nilai nutrisi zooplankton yang lebih tinggi dan mengekalkan kualiti air di antara julat yang boleh diterima dengan pertukaran air sifar. Tambahan pula, penambahan *Halamphora* sp. dan *harpacticoid copepod* meningkatkan kelangsungan hidup dan kandungan pemakanan postlarva udang harimau hitam berbanding dengan rawatan kawalan. Penambahan kedua-dua konsortium juga melindungi postlarva daripada *Vibrio parahaemolyticus* yang menyebabkan *'Acute Hepatopancreatic Necrosis Disease'*. Penemuan dalam kajian ini boleh membuka jalan kepada penyelidikan pada masa depan menggunakan sisa kelapa sawit untuk pengkulturan mikroalga bagi tujuan akuakultur.

Kata Kunci: *Halamphora* sp., *Amphiascoides neglectus*, Efluen kilang kelapa sawit, abu minyak sawit, Postlarva *Penaeus monodon*

SDG: MATLAMAT 2: Kelaparan Sifar, MATLAMAT 14: Kehidupan di Bawah Air

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Natrah Fatin binti Mohd Ikhsan, PhD
Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Chairman)

Clement Roy De Cruz, PhD
Senior Lecturer
Faculty of Agriculture
Universiti Putra Malaysia
(Member)

Siti Izera binti Ismail, PhD
Senior Lecturer
Faculty of Agriculture
Universiti Putra Malaysia
(Member)

Norulhuda binti Mohamed Ramli, PhD
Senior Lecturer
Faculty of Engineering
Universiti Putra Malaysia
(Member)

Yosri bin Mohd Siran, PhD
Principal Chemist
Processing Technology
SD Guthrie Research Sdn Bhd
(Member)

ZALILAH MOHD SHARIFF, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 10 October 2024

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

LIST OF SYMBOLS AND ABBREVIATIONS

et al.	et alia
µm	Micrometer
mL	Milliliter
mM	Millimolar
°C	Degree Celsius
v/v	Volume per volume
w/v	Weight per volume
cm	Centimeter
cm^2	Square centimeter
mm	Millimeter
nm	Nanometer
$\mu mol\ m^{-2}\ s^{-1}$	Micromole per square meter per second
$\mu mol\ mL^{-1}$	Micromole per milliliter
$mol\ L^{-1}$	Mol per liter
$g\ L^{-1}$	Gram per liter
h	Hour
rpm	Revolution per minute
s	Second
min	Minute
N	Normality
M	Molar
µL	Microliter
kV	Kilovolt
%	Percentage
sp.	Species

mg	Milligram
mg L^{-1}	Milligram per liter
mg mL^{-1}	Milligram per milliliter
cells mL^{-1}	Cells per milliliter
mL min^{-1}	Milliliter per minute
mg m^{-3}	Milligram per cubic meter
S.D	Standard deviation
mg cm^{-2}	Milligram per square centimeter
µg cm^{-2}	Microgram per square centimeter
min	Minute
d^{-1}	Per day
g L^{-1}	Gram per liter
DW	Dry weight
nm	Nanometer
g DW L^{-1}	gram dry weight per liter
mg Kg^{-1} DW^{-1}	Milligram per kilogram dry weight
g	Gram
L	Liter
µg	Microgram
±	Plus, minus
µ	Micron
μ	Specific growth rate
θ	Theta
Ø	Diameter
ANOVA	Analysis of Variance
HSD	Honest Significant Difference

cfu	Colony forming unit
LED	Light Emitting Diode
ppt	Part Per Trillion
OD	Optical Density
b.p	Boiling point
kPa	Kilopascal
kHz	Kilohertz
PL L^{-1}	Postlarvae per liter

CHAPTER 1

INTRODUCTION

1.1 Background of the Study

Aquaculture is a pivotal component of the global food supply and is anticipated to meet the needs of the world's rapidly expanding and growing population (Naylor, 2000; Smith et al., 2010; Oddsson, 2020). The industry's fastest-growing animal production sector has tripled from 34 metric tonnes (MT) in 1997 to 112 MT in 2017 (Naylor et al., 2021). It is forecasted that by 2030, aquatic food production will further increase by 15%, which would mainly come from aquaculture (FAO, 2022).

Among the essential cultured organisms is shrimp, the highest traded animal of the sector with high values. More than 50% of shrimp are produced through farming compared to around 30% that are captured wildly (FAO, 2016). Shrimp production increased from around 3 million MT in 2008 to more than 6 million MT in 2018, accounting for more than 70% of the increment (FAO, 2020). The two most popular farmed species are the whiteleg shrimp (*Penaeus vannamei*) and black tiger shrimp (*Peneaus monodon*).

The intensification of shrimp production has led to disease outbreaks related to water quality management and the proliferation of pathogens (Boyd and McNevin., 2015). Among the established practices are antibiotics, vaccines, and immunostimulants (Marti et al., 2014; Klein et al., 2018; Thornber et al., 2020). However, antibiotics use led to antimicrobial resistance, while the use of vaccines and immunostimulants is minimal. Recently, strategies focussing on ecological approaches involving water recirculation and remediation and the application of probiotic consortium through a microbial management system that includes the use of microalgae, bacteria, and zooplankton are being introduced as more sustainable methods for aquaculture (Schryver and Vadstein, 2014; Xiong et al., 2016). This includes using carbon sources to steer the C/N ratio in the water to stimulate heterotrophic bacteria in the form of flocs or using industrial wastewater as media to culture beneficial microbial biomass (Guldhe et al., 2017; El-Sayed et al., 2021).

Palm oil industry waste such as palm oil mill effluent (POME) and palm oil fuel ash (POFA) was found to support microbial growth due to their high nutrients and silica levels. Both wastes are produced in large amounts by palm oil milling activities and can be used to culture beneficial microorganisms, including the microalgae that are beneficial for aquaculture use. POME, for example, has been used as an alternative media for the culture of various microalgae (Shah et al., 2016), bacteria (Karim et al., 2021), and zooplankton (Isa et al., 2020). It was observed that the media contributed to better nutritive values, growth, and survival of the cultured organisms. POFA, on the other hand, was not discovered in any microbial growth findings but is often used as a supplementary cementitious material in concrete production (Hamada et al., 2022). The effects of microalgae cultivated in oil palm waste medium on aquaculture animals, however, have received remarkably little attention.

This study envisioned exploring further the potential use of oil palm waste as the culture medium for microalga cultivation, particularly microalgae with beneficial properties (e.g., *Halamphora* sp. that was previously reported to show anti-quorum quenching (Aini, 2020) and bioremediation properties (Nurarina, 2022) that can be further be used as enrichment of zooplankton such as the harpacticoid copepod. It is expected that the enhanced microalgal proliferation by the culture medium would lead to a more healthy and better growth of zooplankton that eventually affects shrimp culture performance, particularly in obtaining high-quality vigour shrimp larvae with improved survival.

1.2 Problem Statement

Disease is one of the most critical challenges affecting the shrimp industry. Shrimp are susceptible to a variety of viral, bacterial, and fungal infections, which can lead to high mortality rates and severe economic losses for producers (Lee et al., 2022). Furthermore, environmental factors such as temperature, salinity, and water quality can all have an impact on shrimp postlarvae survival and growth (Boyd and McNevin., 2015). Shrimp postlarvae also require a special diet, and the availability and quality of feed can all impact survival and growth (Rasdi and Qin, 2014).

The benefits of live food include the presence of viable and motile zooplankton and phytoplankton, which increases the bioavailability of nutrients and improves predatory reactions (Radhakrishnan et al., 2019). The plankton acts as a potential exogenous nutrient for marine larvae high in nutritive values with bioremediation properties (Conceição et al., 2010; Nielsen et al., 2017; Radhakrishnan et al., 2019). The culture conditions and the quality of live food used during cultivation could also determine the nutritional value of the cultured animals (Radhakrishnan et al., 2019). The nutrition of zooplankton is usually derived from microalgae, which may require a massive amount of microalgae culture. However, the high cost of culture media is a substantial barrier to the mass culture of microalgae. The nutrients produced using synthetic chemicals are expensive and scarce in the local market (Michael et al., 2018). According to Xia and Murphy (2016), the cost of culture media accounts for approximately 50% of the total biomass production cost. Rawat et al. (2013) stated that high prices could be overcome using less expensive elements.

POME and POFA are waste products generated in large quantities by the palm oil industry that are both cost-effective and environmentally sustainable. The use of microalgae in phycoremediation applications such as POME and biomass production has gained much attention because of their ability to support metabolic activities by absorbing contaminants in a short period (Nur et al., 2017). The high nutritional values of POME such as nitrogen, phosphorus and carbon can promote microalgae biomass productivity (Nur et al., 2017). Silica, on the other hand, is essential for diatoms to grow and form new cells. The high silica content of POFA has several benefits that make it a useful by-product of the palm oil industry, with potential applications in construction, materials science such as zeolites and silica gels, and other fields (Nurdin et al., 2018). POFA, due to its special characteristics, may boost the growth of diatoms. Hence, the use of POME and POFA as a culture medium can also benefit in reducing the environmental impact of the palm oil industry. However, the effects of POME and POFA on microalgal growth, nutrient content, and biochemical composition present in the zooplankton and shrimp postlarvae have not been thoroughly investigated.

1.3 Research Objectives

This study explores the potential use of waste from the palm oil industry to cultivate marine benthic diatom *Halamphora* sp. for harpacticoid copepod *Amphiascoides neglectus* and black tiger shrimp *Peneaus monodon* postlarvae. The objectives of this research are as follows:

1. To determine the optimal culture conditions for *Halamphora* sp. growth enhancement
2. To develop palm oil mill effluent (POME) and palm oil fuel ash (POFA) as a modified medium for *Halamphora* sp. culture
3. To determine the growth and biochemical properties of *Halamphora* sp. grown in oil palm waste modified medium
4. To assess the growth and biochemical composition of harpacticoid copepod *Amphiascoides neglectus* and *Penaeus monodon* postlarvae fed with *Halamphora* sp. and to evaluate the protective effect of *Halamphora* sp. against Acute Hepatopancreatic Necrosis Disease - positive *Vibrio parahaemolyticus*.

1.4 Thesis Outline

This thesis is structured into seven chapters. Chapter 1 introduces aquaculture and the present challenges facing the shrimp and palm oil industries. In Chapter 2, a review of the state of utilising palm oil waste to cultivate microalgae and marine benthic diatom *Halamphora* sp. is highlighted, which has its novelty in preventing disease outbreaks and improving water quality in the aquaculture industry (Figure 1.1). In Chapter 3, culture conditions such as substrate type, light intensity, light duration, salinity, and pH are evaluated in order to cultivate *Halamphora* sp. under optimal conditions. In Chapter 4, oil palm waste (POME and POFA) is investigated as a modified culture medium for *Halamphora* sp. growth, as well as the nutrient removal efficiency. In Chapter 5, *Halamphora* sp. is mass cultured in commercial media and palm oil waste modified medium to compare its growth performance. The biochemical compositions of *Halamphora* sp. cultivated in different media are analysed. Chapter 6 determines the growth performance, water quality, and biochemical compositions of harpacticoid copepod and *P. monodon* postlarvae fed with *Halamphora* sp. cultivated in various media. Finally, Chapter 7 detailed the outcomes of all studies. The overall results of the study and future recommendations are also highlighted.

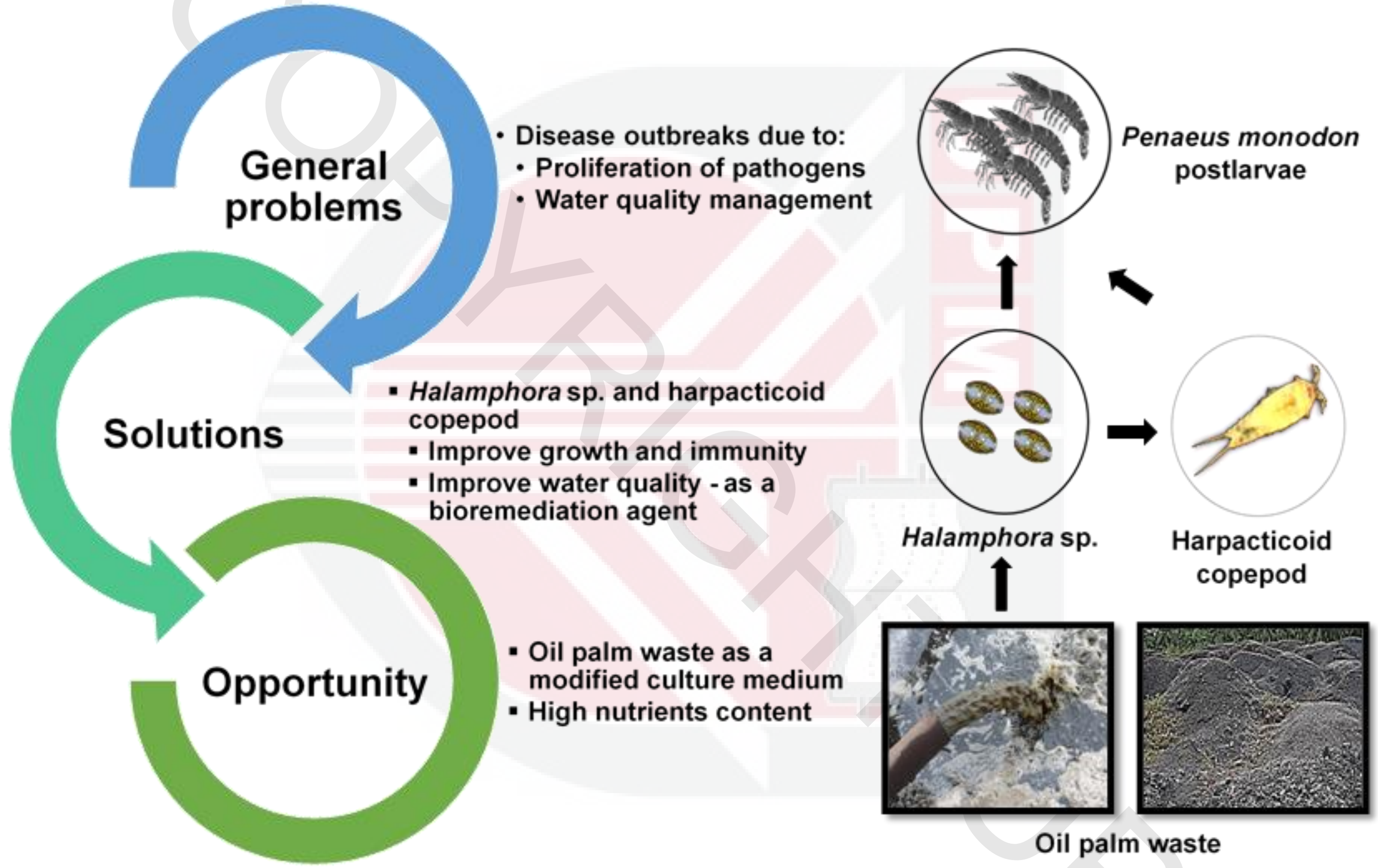

Figure 1.1 : Research Overview

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