EFFECTS OF MARINE MICROALGAE AND CYANOBACTERIA TOWARDS THE MORPHOLOGY AND SURVIVAL RATE OF ZEBRAFISH LARVAE, Danio rerio

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This project is submitted in partial fulfillment of the requirement for the degree of Bachelor of Aquaculture Science

DEPARTMENT OF AQUACULTURE

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This is to certify that I have examined the final project report and all corrections have been made is recommended by the panel examiners. This project complies with the recommended format stipulated in the AKU4999 project guidelines, Department of Aquaculture, Faculty of Agriculture, Universiti Putra Malaysia.

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ABSTRACT

The zebrafish (*Danio rerio*) is becoming internationally recognized as a test model organsims representing animals as well as human. An experiment was conducted to study selected different microalgae and cyanobacteria extracts (*Spirulina platensis*, *Monoraphidium* sp., *Halamphora* sp., *Chaetoceros* sp. and *Chattonella marina*) on the *D. rerio* larvae morphology and survival rate. Samples of microalgae and cyanobacteria at stationary phase were extracted using 95% methanol and were exposed to *D. rerio* eggs at the concentrations of 0 ppm, 10 ppm, 20 ppm, 50 ppm and 100 ppm. The embryonic development was observed daily until hatched. The results showed that no significant differences between egg morphology were detected through the exposure from 0 ppm until 100 ppm. The experiment was then repeated through an increase of concentration up to 200 ppm. It was shown that a toxic *Chattonella marina* induced negative impacts towards the zebrafish larvae morphology causing deformation with low survival rate at (46.67%) compared to control treatment (0ppm, sterile distilled water) which have 100% survival rate. This indicated that the *Chattonella marina* is lethal towards the zebrafish larvae at high concentration.

Keywords: zebrafish, microalgae, cyanobacteria, survival rate

ABSTRAK

Ikan zebra (*Danio rerio*) sudah diiktiraf pada peringkat antarabangsa sebagai model untuk mewakili haiwan dan manusia. Eksperimen dilaksanakan untuk mengkaji mikroalga dan sianobakteria ekstrak yang berbeza (*Spirulina platensis*, *Monoraphidium* sp., *Halamphora* sp., *Chaetoceros* sp. dan *Chattonella marina*) dalam mempengaruhi morfologi larva dan kadar penetasan larva. Sampel mikroalga dan sianobakteria telah diekstrak pada fasa pegun menggunakan 95% metanol dan didedahkan kepada telur *D. rerio* pada kepekatan 0 ppm, 10 ppm, 20 ppm, 50 ppm, and 100 ppm. Pembangunan embrio diperhatikan setiap hari sehingga menetas. Keputusan menunjukkan bahawa tiada perbezaan yang ketara antara morfologi telur dari 0 ppm hingga 100 ppm. Eksperimen kemudian diulang melalui peningkatan kepekatan sehingga 200 ppm. Peningkatan rawatan menunjukkan bahawa kawalan positif, *Chattonella marina* yang beracun telah menyebabkan impak negative terhadap morfologi larva ikan zebra menyebabkan kecacatan bentuk badan dan kadar kelangsungan hidup pada (46.67%) berbanding dengan rawatan kawalan (0ppm, air suling yang steril) yang mempunyai kadar kelangsungan hidup pada 100%. Ini membuktikan bahawa *Chattonella marina* sangat berbahaya kepada larva zebrafish pada kepekatan yang tinggi.

Keywords: ikan zebra, mikroalga, sianobakteria, kelangsungan hidup

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LIST OF ABBREVIATIONS AND SYMBOLS

mL	-	Milliliter
°C	-	Degree Celsius
±	-	Plus minus
<	-	Lower than
%	-	Percent
ppm	-	Part per million
rpm	-	Revolutions per minute
mg/L	-	Milligram/liter
µl	-	Microliter
µmol	-	Micromole
m^2s^{-1}	-	Metre squared per second
>	-	More than
<	-	Less than

CHAPTER 1

INTRODUCTION

1.0 Introduction

Microalgae is a common live feed in aquaculture. Microalgae are very diverse in species, shape and also size which makes it suitable for variety of fish size and stage to feed. They reproduce by vegetative, asexual and sexual mechanisms (Acreman, 1994). Microalgae are mostly autotrophs at which they use light and carbon dioxide to make their own food, although there are some heterotrophs (cannot produce its own food). In general, microalgae contain lipid, protein, fatty acids and amino acids (Kay, 1991). Micronutrients such as various trace metals and the B-complex vitamins such as thiamin (B1), cyanocobalamin (B12) and biotin (B7) were used in energy production and cell division (Kay, 1991). Although microalgae are commonly regard as beneficial organisms, some species are toxic, also known as harmful algal bloom (HAB) species. These toxin productions are correlated with temperature, age of the culture, light intensity, and pH (Kay, 1991).

Meanwhile, cyanobacteria are bacteria that are capable of producing a wide range of potent toxins as secondary metabolites (Van Apeldoorn et al., 2007). There are several similar characteristics of cyanobacteria and microalgae in terms of pigmentation where both of the organisms possessed chlorophyll a (Van Apeldoorn et al., 2007). In tropical countries such as in Malaysia, cyanobacteria multiply rapidly particularly in ponds that are rich in nutrients. Cyanobacteria has the ability to form thick surface with high cell density. Cyanobacterial blooms happen due to the presence of buoyancy-conferring gas vacuoles (Cook et al., 2004; Chorus, 2000). This phenomenon frequently occurs in shallow littoral areas that are shelter to fish larvae and could poison the fish larvae causing chronic effects or even death (Sivonen & Jones, 1999). Some contains cyanotoxins and are responsible for acute and (sub) chronic poisonings of wild/domestic animals and humans (Van Apeldoorn et al., 2007).

Zebrafish can be used as fish model for toxicity investigations that represents both aquatic and human. Zebrafish (*Danio rerio*) is a type of model fish that originates from South Asia which are India, Bangladesh, Nepal, Myanmar and Pakistan (Lawrence, 2007). Their living requirement is highly related towards aquatic vegetation. They can be found in calm water as in rice field, upper reaches of rivers and irrigation ditches (Lawrence, 2007). The zebrafish normally breeds in groups, they are egg laying fish that have no parental care towards the egg (Lawrence, 2007). It can withstand wide range of temperature from 27°C to 34°C, pH 6.0-8.0, and depth range from 16 cm to 57 cm (Engeszer, 2007). In natural environment, the zebrafish have to face predator such as the tire-track eel, swamp eel and dragonfly larvae. Although zebrafish is considered as a hardy species, various harmful activities such as strip mining lead to extensive pollution, agricultural practice increase acidity of the aquatic environment, and poisoning entire stream for livelihood are able to cause zebrafish mortality (Engeszer, 2007). Egan (2009) described that the zebrafish is valid and reliable as a model of stress and affective disorders.

The objectives of this study were to evaluate the toxic effects of microalgae and cyanobacteria towards the survivability of zebrafish larvae based on survival rate and overall morphology and to distinguish the toxic and non-toxic of marine microalgae and cyanobacteria species towards the zebrafish larvae.

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