# ANTIMICROBIAL ACTIVITY OF NEEM (AZADIRACHTA INDICA A. JUSS) LEAF EXTRACTS AGAINST SNAKEHEAD FISH (CHANNA STRIATA) COMMON PATHOGENS AEROMONAS HYDROPHILA, STREPTOCOCCUS AGALACTIAE, AND STAPHYLOCOCCUS XYLOSUS

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# **Antimicrobial Activity of Neem (*Azadirachta indica* A. Juss) Leaf Extracts against Snakehead Fish (*Channa striata*) Common Pathogens *Aeromonas hydrophila, Streptococcus agalactiae,* and *Staphylococcus xylosus***

**By**

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**requirements for the degree**

**of Master of Sciences**

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# DEDICATION

**To**

**My Mother and My Late Father ….**

**Their words of inspiration and encouragement in pursuit of excellence,**

**still linger on…**

**Thank you**

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**In the name of Allah, Most Gracious, Most Merciful**

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Senan Faik M. Musa

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## LIST OF ABBREVIATIONS

| | |
|---|---|
| **ANOVA** | Analysis Of Variance between groups |
| **ARC** | Aquaculture Research Complex |
| **BLAST** | Basic Local Alignment Search Tool |
| **CFU** | Colony forming unit |
| **DMSO** | Dimethyl sulfoxide |
| **EUS** | Epizootic Ulcerative syndrome |
| **HMDS** | Hexamethyldisilazane |
| **INT** | p-iodonitrotetrazolium chloride |
| **$LC_{50}$** | The concentration of the extracts that kills 50% of the test animals |
| **lux** | Luminous flux |
| **$MgCl_2$** | Magnesium chloride |
| **MH** | Miller Hinton Agar |
| **MIC** | Minimum Inhibitory Concentration |
| **NA** | Nutrient Agar |
| **NCCLS** | National Committee for Clinical Laboratory Standards |
| **nm** | Nanometer |
| **pH** | Hydrogen number |
| **rDNA** | Ribosomal DNA |
| **RNA** | Ribonucleic acid |
| **SPSS** | Statistical package for social science |

**Aktiviti antimikrob ekstrak daun mambu (*Azadirachta indica* A. Juss) terhadap ikan Haruan (*Channa striata*) patogen biasa *Aeromonas hydrophila*, *Streptococcus agalactiae*, dan *Staphylococcus xylosuspada***


## ABSTRAK

Kajian ini menerangkan potensi ekstrak daun mambu (*Azadirachta indica*), untuk mengawal patogen bakteria ikan yang biasa secara *in-vitro* dan *in-vivo*. Eksperimen pertama telah dijalankan untuk menilai aktiviti antimikrob dan kepekatan perencatan minimum tiga ekstrak mentah mambu (asetone, metil alkohol dan air), terhadap tiga patogen ikan biasa *Aeromonas hydrophila*, *Streptococcus agalactiae* dan *Staphylococcus xylosus*. Dengan menggunakan kaedah Difusi Perigi *in vitro*, ekstrak aseton menunjukkan aktiviti terhadap semua bakteria yang diuji dengan zon 13,25 perencatan, 18,75 dan 13,75 mg / ml masing-masing, manakala metil alkohol dan ekstrak air menunjukkan aktiviti sahaja terhadap *A. hydrophila*. Dalam keadaan *in-vitro,* penentuan kepekatan perencatan minimum telah dijalankan dengan menggunakan kaedah pengembangan mikro. Aseton dan ekstrak alkohol metil menunjukkan MIC nilai terendah dengan 0,078 dan 0,156 mg / ml terhadap *S. agalactiae* masing-masing. Eksperimen kedua telah dijalankan untuk menilai aktiviti biologi tiga ekstrak mentah, dengan menggunakan udang air garam *Artemia* nauplii dalam bioesei ketoksikan. Diantara ketiga-tiga ekstrak mentah yang diuji, ekstrak air menunjukkan ketoksikan terendah, dengan LC50 nilai 6.41mg/ml dalam tempoh 24 jam. Dalam eksperimen ketiga, hasil pemeriksaan kualitatif awal fitokimia ekstrak bukan toksik menunjukkan

kehadiran Flavonoids dan Tannins. Sel mikroorganisma yang dirawat dengan ekstrak air daun mambu, dalam eksperimen keempat, menunjukkan sel-sel rosak selepas pemerhatian di bawah Mikroskop Imbasan Elektron (SEM), berbanding mikroorganisma dirawat dengan air suling yang steril. Potensi ekstrak air daun mambu untuk mengawal EUS dalam ikan haruan, telah dijalankan di bawah keadaan *in-vivo*, dalam eksperimen kelima. Dalam tempoh empat hari, kebanyakan ikan dalam Kumpulan 1 dan 3 menunjukkan ulser yang dalam dengan lapisan otot yang menjadi nekrotik di tempat suntikan 61,1 dan 88.8% masing-masing, berbanding dengan Kumpulan 2, yang didapati sedikit bengkak dan melepuh kepada beberapa kes dengan ulser yang dalam. Dalam masa tujuh hari, kebanyakan ikan dalam Kumpulan 1 dan 3 menunjukkan pemulihan daripada ulser yang dalam (93.75 dan 87.5% masing-masing). Kumpulan 2 pula menunjukkan pemulihan 100% dalam tempoh enam hari. Pengenalpastian *A. hydrophila* diasingkan ke tahap spesis telah tertakluk kepada pengekstrakan DNA dan amplifikasi PCR dan penulenan dalam eksperimen keenam. Proses BLAST nukleotida isolat bakteria menunjukkan 100% *Aeromonas hydrophila* strain daripada gen 16S rDNA.

**Antimicrobial activity of neem (*Azadirachta indica* A. Juss) leaf extracts against snakehead fish (*Channa striata*) common pathogens *Aeromonas hydrophila, Streptococcus agalactiae, and Staphylococcus xylosus***

## ABSTRACT

The present study describes the potential of neem leaf (*Azadirachta indica*) extracts to control common bacterial fish pathogens under *in-vitro* and *in-vivo* conditions. The first experiment was conducted to assess the antimicrobial activity and minimum inhibitory concentrations of three crude extracts of neem (acetone, methyl alcohol and water), against three common fish pathogens *Aeromonas hydrophila*, *Streptococcus agalactiae* and *Staphylococcus xylosus*. Using well-diffusion methods *in-vitro*, the acetone extracts showed activity against all the tested bacteria with zones of inhibition at 13.25, 18.75 and 13.75 mg/ml respectively, while the methyl alcohol and water extracts showed activity only against *A. hydrophila*. Under *in-vitro* conditions, minimum inhibitory concentration determinations were conducted using a micro-dilution method. The acetone and methyl alcohol extracts showed the lowest MIC values, 0.078 and 0.156 mg/ml against *S. agalactiae*, respectively. A second experiment was conducted to evaluate the biological activities of the three crude extracts, using a brine shrimp (*Artemia* nauplii) toxicity bioassay. Among the three tested crude extracts, the water extracts showed the lowest toxicity, with $LC_{50}$ values of 6.41 mg/ml within 24 hours. In the third experiment, the results of qualitative preliminary phytochemical screening of the non-toxic extract showed the presence of Flavonoids and Tannins. In

the fourth experiment, Scanning Electron Microscopy (SEM) of microorganisms treated with the water extract of neem leaf showed the cells were damaged compared to microorganisms treated with sterilized distilled water. The potential of neem leaf water extract to control EUS in snakehead fish was conducted under *in-vivo* conditions in the fifth experiment. Within four days, most of the fish in Groups 1 and 3 developed deep ulcers with underlying necrotic musculature at the injection site (61.1 and 88.8%, respectively), compared to Group 2, which ranged from development of slight swelling and blanching to, in a few cases, deep ulcers. Within seven days, most of the fish in Group 1 and 3 showed recovery from deep ulcers (93.75 and 87.5%, respectively), while Group 2 showed 100% recovery within six days. To identify *A. hydrophila* isolates to the species level, isolates were subjected to DNA extraction and PCR amplification and purification in the sixth experiment. The BLASTed nucleotides from 16S rDNA gene fragments of the bacterial isolates indicated 100% of the isolates were strain *A. hydrophila*.

# CHAPTER ONE

# INTRODUCTION

Aquatic organisms contribute significantly to human nutrition as good sources of proteins, vitamins, trace elements and polyunsaturated fatty acids (Ruxton *et al*., 2005). In Malaysia, aquaculture is a growing industry and the total value of aquaculture production was recently estimated to be RM 1.275 billion (Wei *et al*., 2010). The snakehead fish *Channa Striata*, commonly known as (Haruan) in Malaysia, is an indigenous freshwater carnivorous air breathing fish species (Jais, 2007); which is widely distributed in Malaysia as a popular food source (Ali, 1999), and also as a traditional medicine, especially in hospitals and among post-operative patient to induce wound healing (Zakaria *et al*., 2004).

The growing aquaculture industry and the intensive fish farming system assisted the development of several disease problems which are often caused by opportunistic pathogens, as is evident from general aquaculture (Abraham *et al*., 2008). Fish diseases are widely distributed world-wild and represent serious problems in aquaculture (Lim *et al*., 2001). In the Malaysian aquaculture system, infections that affect cultured fish are a major problem and needs to be dealt with. A study by Wong and Leong (1987) reported that the Malaysian aquaculture sector lost nearly US$ 1.3 million of potential profit due to the spread of disease in its fish stock. Worldwide, bacterial disease represents the major threat to the aquaculture industry, and is reported to cause disease outbreaks and massive economic losses to the farming fish industry (Najiah *et al*., 2011). For instance, in Malaysia in one recent year, the estimated loss of finfish cultured in floating cages in Peninsular Malaysia due to just one type of pathogenic bacteria was reported to be about RM 20 million (Yusuf *et al*., 2007). The pathogenic bacteria *Aeromonas hydrophila, Streptococcus agalactiae* and *Staphylococcus xylosus* are among the common fish bacterial pathogens that infect cultured fish and caused heavy mortalities (Schäperclaus, 1992). In freshwater fish, *A. hydrophila* is the most common pathogen and is known as the aetiological agent for many distinct pathological conditions in the tail, fin rot, haemorrhagic septicaemia and epizootic ulcerative syndrome (Austin and Adams, 1996; Roberts, 1997). Epizootic Ulcerative Syndrome (EUS) is a complex skin disease in both freshwater and brackish water fish, which is identified by the appearance of haemorrhagic ulcerative lesions on the body surface of the fish (FAO, 1986). EUS has spread throughout parts of the Asia-Pacific region, causing heavy mortalities among cultured and wild fish populations in the region (Lilley *et al*., 1992; Roberts *et al*., 1994). Outbreaks of EUS among snakehead fish (*Ophicephalus striatus*) and catfish (*Clarias batrachus*) have been reported to occur annually in both wild and cultured fish in South and Southeast Asia (Lilley *et al*., 1992).

The control of bacterial disease in aquaculture has been based mostly on chemotherapy, which retains a prominent role in fish culture system management (Roberts, 1995). Antimicrobial chemotherapy has been used in aquaculture for more than 50 years (Inglis, 2000). Antibiotics are also used prophylactically in carp culture at times of year when haemorrhagic septicaemia is mostly likely to occur (Inglis *et al*., 1993). Commercial antibiotics such as tetracycline and oxolinic acid have been added to fish feed against different kinds of bacteria pathogens. However, supplementing food with antibiotics releases the antibiotics into the larger environment, which inducing the development of antibiotic resistance among pathogenic bacteria. For example, the earliest isolation of *Aeromonas salmonicida* resistant to a specific antibiotic has often been reported to occur shortly after the introduction of antibiotics into aquaculture system (Wei *et al*., 2010). So it is clear that the use of commercial antibiotics in aquaculture can increase antibiotic resistance among pathogenic bacteria in exposed ecosystems. Moreover, resistances can spread through the environment to different bacterial species, including bacteria that can infect humans (Wei *et al*., 2010). As a result, the occurrence of antibiotic resistant bacteria associated with fish diseases is known to be a worldwide problem in aquaculture, and continues to increase due to the absence of more effective and safer antibiotic use (Bansemir *et al*., 2006). Moreover, problems including solubility, palatability, toxicity, cost delivery and governmental restrictions have played a big part in limiting the use of antibiotics to a selected few, especially in ornamental fish culture (Smith *et al*., 1994).

## 1.1 Statement of the problem

Due to antibiotic resistance among different kinds of bacterial strains in fish, there is an urgent need for scientists to explore new medicines against these pathogenic bacteria, in an eco-friendly manner. Drugs from natural sources, such as plants, offer one solution to the antibiotic resistance problem among pathogenic fish bacteria (Wei *et al.*, 2010).

For the last two decades, there has been massive interest in the search for various extracts derived from traditional medical plants as a new source of antimicrobial agents (Bonjar and Farrokhi, 2004). Many studies have reported antibacterial activities of different parts of various plant species against different strains of bacteria isolated from fish (Rajendiran *et al*., 2008; Siri *et al*., 2008; Rahman *et al*., 2009; Turker *et al*., 2009; Dhayanithi *et al*., 2010; Najiah *et al*., 2011).

The neem Tree, *Azadirachta indica* of the family Meliaceae, is an evergreen tree with potential medicinal properties that can be observed in most tropical countries (Helmy *et al*., 2007). The use of neem tree as an antibacterial agent has been well known since ancient times (Chaurasia and Jain, 1978; Chawla *et al*., 1995). Many studies reported the antibacterial activities of neem leaves, bark, seeds, seed kernel, twigs, stem bark and fruits (Kraus, 1995).

Generally, the present study is designed to obtain preliminary information on the antibacterial activities of three crude extracts of neem leaf (acetone, methyl alcohol and water) under *in-vitro* conditions against three common fish pathogens: *Aeromonas hydrophila*, *Streptococcus agalactiae* and *Staphylococcus xylosus*, and also evaluate the biological activities of the three crude extracts. Based on the *in-vitro* results with neem leaf extracts and their biological activities, the efficacy of the best crude extract was evaluated under in *in-vivo* conditions in experimentally infected snakehead fish (*C. striata*).

## 1.2 Research objective

This research aimed to achieve the following objectives:

I. To assess the antimicrobial activity and minimum inhibitory concentration of three different kind of neem crude extracts against three snakehead fish (*Channa striata*) common pathogens *in-vitro*.

II. To assess the bio-activities of the neem leaf crude extracts, and perform phytochemical screening and scanning electron microscope (SEM) on the none-biologically active extract.

III. To assess the potential of two pathogenic bacteria (*A. hydrophila* and *S. agalactiae*) to induce epizootic ulcerative syndrome (EUS) in snakehead fish, and the therapeutic efficacies of neem leaf crude extract in snakehead fish experimentally infected with EUS, based on *in-vitro* and in-vivo results.

# CHAPTER TWO

# LITERATURE REVIEW

## 2.1 Aquaculture

### 2.1.1 Introduction

According to the Food and Agriculture Organization (FAO) of the United Nations (FAO, 1997), aquaculture is reported to provide about 16% of the animal protein consumed by the world's population, and is a particularly important source of protein in regions where livestock are few or non-existent. In another report by FAO (2000) fish were reported to provide more than 10% of consumed animal protein in North America and Europe, increasing to 17% in Africa, 26% in Asia and 22% in China. The FAO organization estimates about a billion people worldwide are dependent on fish as their primary source of animal protein, and that by 2030 over half of fish consumed by the world's population will be produced by aquaculture fish farming (FAO, 2000).

There has been a marked increase in aquaculture in terms of total production throughout the last decade or more, especially in certain developing countries (Table 2.1), in contrast to cereals and similar food commodities, which have shown a very little growth. The rapid growth of aquaculture was observed to contribute greatly to world fishery production, which historically has been derived primarily from capture fisheries (New, 1997; 1999).

**Table 2.1:** Aquaculture growth rate and percentage contribution to total fishery production in selected low-incoming food deficit countries (LIFDCs) in Asia (1988 and 1998),

| Country | Total aquaculture production[a] | | Average annual growth (%) | Percentage of aquaculture to total production | |
|---|---|---|---|---|---|
| | 1988 | 1998 | | 1988 | 1998 |
| Bangladesh | 175.261 | 583.877 | 12.99 | 41.72 | 66.02 |
| India | 893.330 | 2.029.619 | 8.69 | 34.27 | 44.81 |
| China[b] | 5.641.232 | 20.739.925 | 14.15 | 52.86 | 59.49 |
| Philippines | 343.059 | 311.933 | -0.82 | 19.43 | 15.74 |
| Thailand | 220.396 | 567.701 | 10.23 | 8.77 | 17.78 |
| Vietnam | 152.617 | 521.870 | 16.12 | 19.67 | 32.98 |
| Indonesia | 414.263 | 694.980 | 5.52 | 17.13 | 17.57 |

**Note:** values in metric tonnes except for percentages, [a]: includes fishes and shellfishes only, [b]: includes China and China Hong Kong SAR. Table adapted from (FAO, 2000a).

A report by FAO (2000b) showed total aquaculture production (excluding seaweed) at about 32.2 million tons in 1999, comprising about 26.18% of the world's total fishery production, compared to 1987, when production was 10.64 million tones and amounted to about 11.13% of total world fishery production.

### 2.1.2 Aquaculture in Asia and Malaysia

In Asia, aquaculture is known to be an ancient tradition; for example, the culturing of Chinese carp for food is thought to be at least 2500 years old, while in the case of Indian carp the practice was known for at least 10 centuries. In Indonesia and the Philippines, the farming of Milkfish in brackish water ponds was practiced since the 16th century (Hora and Pillay, 1962). The aquaculture industries in Malaysia have relatively recent antecedents. According to a study by Tan (1998) the culture of

Chinese carp in mining pools in the early part of the century was the first trial recorded in Malaysia. In the middle of the 1990s, the aquaculture industry in Malaysia started to build a strong economic profile in food and ornamental fish production. In 1994, total aquaculture production in Malaysia reached 114.144 million tons of food fish valued at US$145.8 million and 227.8 million aquarium fish valued at US$17.5 million (Department of Fisheries, 1994), The growth of the aquaculture industry in Malaysia was spurred by government support in the form of research and development, extension and support services, as well as financial incentives. Under these conditions, it is likely that production will continue to increase in the future (Mohamed *et al*., 2000).

Due to the rapid expansion of aquaculture to meet ever-increasing demands for fish, it is now considered the fastest growing food-producing industry in the world. Many researchers have given a high profile to the importance of fish culture as a food source to meet the needs of the growing world population. Moreover, many countries are interested in encouraging fish culture as part of a global policy to improve the efficiency of their populations though improvement in their nutritional content and livelihoods (Al-Dohail *et al*., 2009). World fisheries witnessed rapid changes during the last decades including, for example, new aquaculture technologies, the implementation of Exclusive Economic Zones for fishing, the UN meeting on the law of the sea in 1982 and other development activities, all of which played major roles in enhancing fisheries management and production (Ahmed *et al*., 1999).

## 2.2 Snakehead fish

### 2.2.1 Taxonomy and Synonymy of Snakehead fish

According to Nelson (2006), a group of teleostean fish known as snakehead fish are classified as follows:

Class Actinopterygii

Subclass Neopterygii

Order Perciformes

Suborder Channoidei

Family Channidae

Recently, two genera were recognized as part of the Channidae family (Courtenay and Williams, 2004): *Channa* (Scopoli, 1777) (snakeheads of Asia, Malaysia and Indonesia) and Parachanna (Teugels and Daget, 1984) (African snakeheads). Generic synonyms of *Channa* include *Channa* (Gronow, 1763), a nomen nudum; Ophicephalus (Bloch, 1793) and its misspelled variant *Ophiocephalus*; Bostrychoides (Lacépéde, 1801); and philypnoides (Bleeker, 1849). A decision by (Myers and Shapovalov, 1931) to distinguish the two genera of Ophicephalus and Channa according to the presence (Ophicephalus) or absence (Channa) of pelvic fins was invalid. Currently, there are 29 species of snakehead fish recognized in the family Channidae, as shown in Table 2.2.

**Table 2.2:** Recognized species of the family Channidae after (Vierke, 1991b), (Musikasinthorn, 2000), (Musikasinthorn and Taki, 2001), and (Zhang *et al*., 2002).

| Species | References | Name |
|---|---|---|
| *Channa amphibeus* | (McClelland, 1845) | Chel snakehead1 |
| *Channa argus* | (Cantor, 1842) | northern snakehead1 |
| *Channa asiatica* | (Linnaeus, 1758) | Chines snakehead |
| *Channa aurantimaculata* | (Musikasinthorn, 2000) | Orangespotted snakehead1 |
| *Channa bananensis* | (Bleeker, 1852) | Bangka snakehead[1] |
| *Channa baramensis* | (Steindachner, 1901) | Baram snakehead[1] |
| *Channa barca* | (Hamilton, 1822) | Barca snakehead |
| *Channa bleheri* | (Vierke, 1991) | Rainbow snakehead |
| *Channa Burmanica* | (Chaudhuri, 1919) | Burmese snakehead[1] |
| *Channa cyanospilos* | (Bleeker, 1853) | Bluespotted snakehead[1] |
| *Channa gachua* | (Hamilton, 1822) | Dwarf snakehead[3] |
| *Channa harcourtbutleri* | (Annandale, 1918) | Inle snakehead[1] |
| *Channa Lucius* | (Cuvier, 1831) | Splendid snakehead |
| *Channa maculate* | (Lacepède, 1802) | Blotched snakehead[1] |
| *Channa marulius* | (Hamilton, 1822) | Bullseye snakehead[1,3] |
| *Channa marulioides* | (Bleecker and Miller, 1851) | Emperor snakehead |
| *Channa melanoptera* | (Bleeker, 1855) | Blackfinned snakehead[1] |
| *Channa melasoma* | (Bleecker and Miller, 1851) | Black snakehead |
| *Channa micropeltes* | (Cuvier, 1831) | Giant snakehead[3] |
| *Channa nox Zhang,* | (Zhang *et al*., 2002) | Night Snakehead[1] |
| *Channa orientalis* | (Schneider, 1801) | Ceylon snakehead[2] |
| *Channa panaw* | (Musikasinthorn, 1998) | Panaw snakehead[1] |
| *Channa pleurophthalma* | (Bleecker and Miller, 1851) | Ocellated snakehead[1] |
| *Channa punctuate* | (Bloch, 1793) | Spotted snakehead[3] |
| *Channa stewartii* | (Playfair, 1867 ) | Golden snakehead |
| *Channa striata* | (Bloch, 1797) | Chevron snakehead[3] |
| *Parachanna africana* | (Steindachner, 1879) | Niger snakehead[1] |
| *Parachanna insignis* | (Sauvage, 1884) | Congo snakehead[1] |
| *Parachanna obscura* | (Günther, 1861) | African snakehead |

**Note:** [1]: Proposed common name, [2]: Common name tentative, [3]: Species complex. Table adopted from (Courtenay and Williams, 2004).

### 2.2.2 Snakehead fish (*Channa striata*)

#### 2.2.2. (a) Taxonomy and distribution

The snakehead fish (*Channa striata*: Channidae), known locally as Haruan or snakehead mureel, is a tropical, fresh water carnivorous, air breathing fish species, widely distributed within Malaysia, Plate 2.1. However, other members of the same family Channidae are found also in neighbouring countries in the region (Jais, 2007).

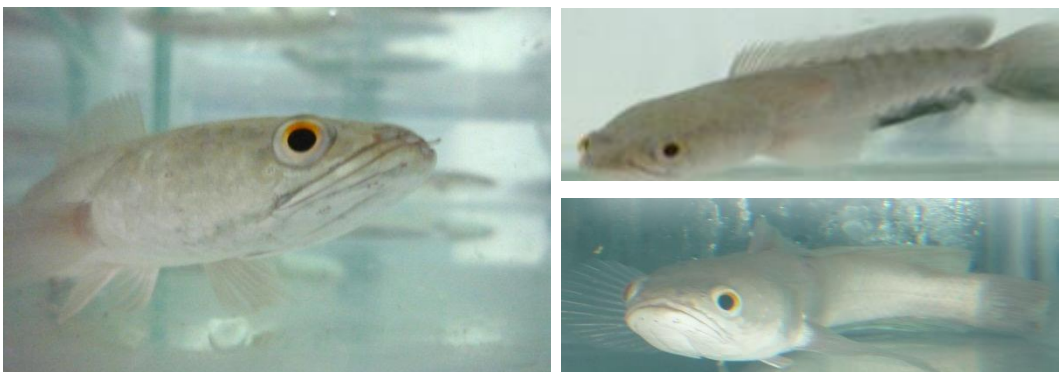

**Plate 2.1:** Picture of snakehead fish (*Channa striata*)

According to several studies by Kajima *et al*. (1994) and Kumar (1995) on the genetic variability in snakehead mitochondrial DNA, *C. striata* has been present in Malaysia for more than 600,000 years, proving that this fish is truly an indigenous Malaysian species. *C. striata*, which belongs to the Channidae family, is also found in rivers and lakes across tropical and subtropical Asian countries, from Pakistan and India to Southeast Asia and Southern China (Mohammad and Ambak, 1983; Hossain *et al*., 2008). About 30 species of this family have been reported worldwide and seven are found in Malaysia, including: *Channa bankanensis*, *Channa gachua*, *Channa Lucius*, *Channa marulioides*, *Channa melasoma*, *Channa micropeltes* and *Channa striata* (Lee and Ng, 1994). The species *C. striata* is the most widely introduced species of snakehead fish; many studies by (Cobb, 1905; Jordan *et al*., 1905; Smith, 1907; Tinker, 1944; Brock, 1952; 1960) have shown that this species was released in Hawaii prior to 1900, and in Madagascar in 1978 (Raminosoa, 1987; Reinthal and Stiassny, 1991; Stiassny and Raminosoa, 1994; Lévêque, 1998). While the exact date is unknown in the Philippines (Seale, 1908; Herre, 1924; 1934; Conlu, 1986), the consensus is it was probably first introduced during the 1970s or 1980s, as well as in the Vogelkop Peninsula, Papua and Indonesia (Allen, 1991).

### 2.2.2. (b) Habitat preference of *C. striata*

Snakehead *C. striata* can be found in freshwater ponds, streams, and most commonly in stagnant muddy waters; in India it can primarily be found on the plains (Talwar and Jhingran, 1992), and is also reported in reservoirs in Sri Lanka (Fernando and Indrasena, 1969). In Malaysia, the species can be found in rivers, swamps, rice paddies, mining pools, lakes and roadside ditches (Mohammad and Ambak, 1983; Lee and Ng, 1991). Despite the fact that *C. striata* is known to be carnivorous, this species is not a good swimmer, but a rapid and fast flipper action assists them in catch prey. Moreover, this species is also known as an air breather, whereby the fish need to surface for air, and for that reason *C. striata* prefers slow running or stagnant, shallow water not more than 2 meter deep, with aquatic plants and some dead logs to hide or hunt in. On the other hand, *C. striata* have also been reported in waters up to 12 meters deep and 4 to 80 meters wide (Jais, 2007).

### 2.2.2. (c) Morphology of *C. Striata*

Snakehead *C.striata* possesses an angular head without patches of scales, with a large mouth. Between 4 and 7 canines lie inside the lower jaw behind a single row of villiform teeth, which widen to 6 rows at the jaw symphysis; there are villiform teeth on the prevomer and palatines. The pectoral fin length is about half the length of the head. The dorsal fin contains 37–46 fin rays; the anal fin has 23–29 fin rays; the pectoral fins bear about 15–17 rays and the pelvic fins contain 6 rays, with a rounded caudal fin. On top of the head the scales are large with rosette of head scales between the orbits, while the scales of the frontal head create a central plate of rosettes; there are 9 rows of scales between the preopercular angles and the posterior border of the orbit; 18–20 predorsal scales and 50–57 scales in lateral series. The colours of the snakehead fish can be highly variable or complex in *C. striata*, but most often the dorsum appears to be dark brown to black; commonly, a dark stripe, extending from the maxillary poster ventrally toward the opercular curvature, is known to be a distinctive mark of this species (Talwar and Jhingran, 1992; Courtenay and Williams, 2004).

### 2.2.2. (d) Medicinal properties of *C. striata*

The medicinal properties of *C. striata* are attributed to two major components: amino acids and fatty acids.

### A. Amino acids

The earliest study on the amino acid profile of *C. striata* was performed on filleted *C. striata* meat in 1994, which was found to be rich in glycine, a non-essential amino acid (Jais *et al*., 1994). The result of this study was confirmed later by many studies such as (Dahlan, 2011). Moreover, non-essential amino acids were found in abundance in *C. striata* extracts, including; glutamic acid (Gam *et al*., 2006; Zuraini *et al*., 2006), arginine (Witte and Barbul, 2002) and aspartic acid (Zuraini *et al*., 2006). In another study, the presence of protein in *C. striata* mucus was reported and found to be variable and dependent on the type of crude extraction. Aqueous extracts were shown to contain high levels of protein, 0.589 mg/ml and 0.291 mg/ml, respectively, while 0.291 mg/ml were found in an acidic extract (Wei *et al*., 2010).

### B. Fatty acids

The earliest study on the fatty acid profile of *C. striata* was in 1993. The study reported a high level of fat in fish wet weight (11–17%), a high level of unsaturated fat (1.2–2.3%) and a low level of omega-3 (Tan, 1993). Overall, the fat or lipid content of snakehead fish *C. striata* has remained controversial due to several studies that found a high amount of total fat, ranging between 5.7 and 11.9 % in one study by Zuraini *et al*. (2006), and up to 35.93 % in another study by Zakaria *et al*. (2007). On the other hand, other studies found low levels of total fat, ranging from 3.25 % (Rahman *et al*., 1995) to 0.99% (Karapanagiotidis *et al*., 2010) and 1.47 % (Chedoloh *et al*., 2011) of total body weight.

## 2.3 Fish diseases

### 2.3.1 Introduction

Fish diseases are wide-spread globally and are also known to cause serious problems in aquaculture industries (Lim and Webster, 2001). The most important financial losses in aquaculture have been attributed to fish disease (Grisez and Ollevier, 1995). In Malaysia, aquaculture sites infected with fish diseases are considered a major problem and need to be dealt with immediately. During 1989-1993, the Malaysian aquaculture sectors lost US$ 1.3 million in potential profits, due to fish diseases spread in their stocks (Wong and Leong, 1987). Moreover, when there is a disease outbreak, the costs of aquaculture production increase due to the cost of the treatment applied loss of fish stocks and reductions in fish growth during the recuperation period. Meanwhile, disease problems facing wild fish occur less frequently than in aquaculture systems, due to removal of infected fish from the environment by predation and also to less crowding of fish in natural systems compared to aquaculture systems (Francis-Floyd, 2005). Bacteria, parasites and viruses that might not cause a serious problem in the natural environment may result in severe problems for fish in aquaculture systems due to their high density and stress.

Diseases are the outcome of the complex interaction between the host, the pathogen and the environment (Snieszko, 1974), as shown in Figure 2.1.

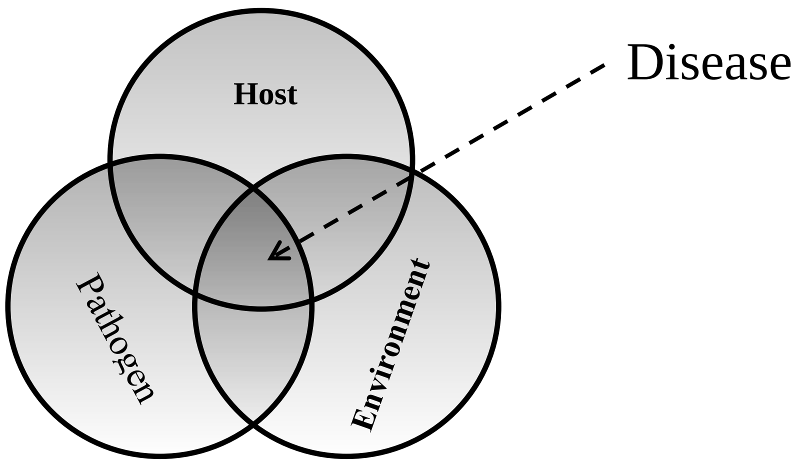


**Figure 2.1:** The relationship between pathogen, host and environment, adopted from (Snieszko, 1974; Francis-Floyd, 2005)

Based on Olivier (2002), in order for a disease to break out or spread from either cultured fish to wild fish or vice-versa, several conditions must be met:

a. Existence of a pathogen in both fish and water sources;

b. Existence of a susceptible host;

c. Viability regarding number and longevity of pathogen in the environment;

d. Applicable infection route.

However, once the pathogen or disease factor has already been introduced into the natural environment, the chances for either treatment or eradication will be minimal.

Environmental problems, including poor quality of water provided or other stressors, effectively contribute to disease outbreaks. Based on that fact, limiting environmental stress to cultured fish can play an important role in preventing disease outbreaks. Moreover, continuous observation of fish behaviour and feeding activity will also aid in discovering problems early before the majority of the population becomes infected. In such cases, applying treatments in the early stages of the disease can lead to successful treatment (Snieszko and Axelrod, 1971; Francis-Floyd, 2005).

Good and successful management of healthy fish is also important in order to avoid and prevent disease outbreaks, which can often be achieved in the course of maintaining good water quality, nutrition and sanitation. The most common fish pathogens that can cause serious problems for their health include bacteria, parasites, virus and fungi. Because of this, sterilization technologies in use in aquaculture, such as ultraviolet and ozonation, might be able to eliminate some, but not all, potential pathogens from the environment. In addition, allowing poor water quality and poor nutrition for fish stocks may also weaken their immune systems by creating a stressful condition for the fish, and finally enable a pathogen to cause disease in the fish (Francis-Floyd, 2005). Outbreaks of fish diseases are usually a complex that involves both infectious and non-infectious factors.

In aquaculture systems, the combination of faecal waste and dead plant materials can result in a high organic load in the water, due to decomposing organic material. In addition, by removing $O_2$ and replacing it with $CO_2$ and other chemicals produced by decomposition, including ammonia, nitrites and other fish metabolites, these will finally cause the fish environment to degrade, either killing the fish or rendering them more susceptible to infectious disease due to the stress (Shariff *et al*. 1992), as shown in Figure 2.2.

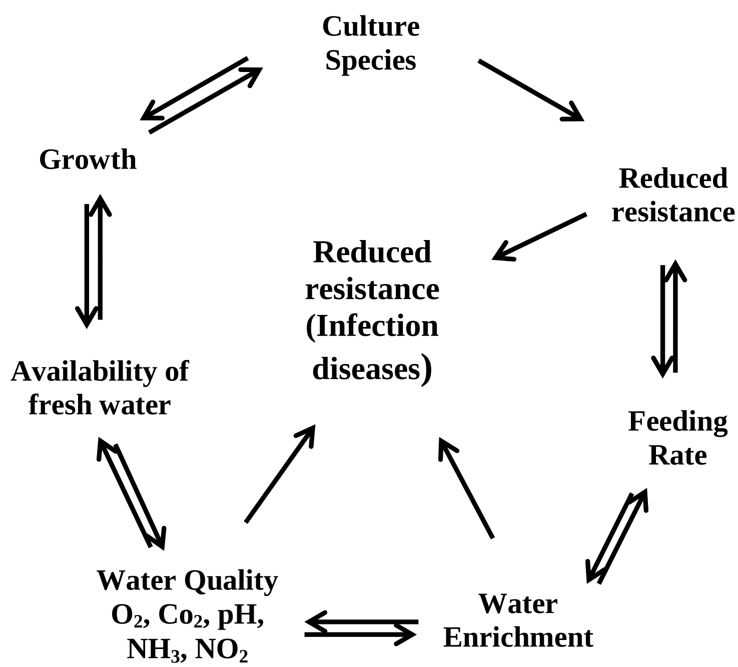


**Figure 2.2:** The relationship of the environmental condition in the aquaculture pond to fish infectious disease, adopted from (Snieszko, 1974; Shariff *et al*., 1992)

### 2.3.2 Types of fish diseases

#### 2.3.2.(a) Infectious Diseases

Infectious disease is usually caused by the presence of a pathogenic factor in the environment or carried by the fish. There are four type of infectious disease that negatively impact fish health: bacterial, parasitic, viral and fungal diseases. Therefore, as stated earlier, infectious diseases require some type of treatment to avoid disease outbreaks (Francis-Floyd, 2005).

#### 2.3.2.(a).1 Bacterial disease

Bacteria are considered a major group of pathogens, which pose one of the most significant threats to successful fish production throughout the world. In both aquaculture and wild fish, bacteria diseases are responsible for heavy mortality throughout the world (Roberts, 1989). Moreover, most of the causative microorganisms are naturally occurring opportunist pathogens, which usually invade hosts that are susceptible to infection. In aquaculture, bacterial diseases are most commonly caused by bacteria such as *Aeromonas hydrophila*, *Staphylococcus xylosus*, and *Streptococcus agalactiae*. By contrast, in nature, the major bacterial fish pathogens include Aeromonad, Pseudomonad and *Edwardsiella tarda* (Banu, 1996; Islam, 1996). Most bacterial disease symptoms include haemorrhagic spots (bleeding where the blood loss escapes from the circulatory system), skin erosion (wearing away from the surface tissue), or ulcers along the body wall and around the eyes and mouth (also known as areas of tissue erosion), as well as diseases of kidney and liver. Bacterial disease can also cause an enlarged, fluid-filled abdomen and protruding eyes. The mortality and acuteness of bacterial diseases depends on the temperature, bacteria strain, fish species and stress (Ministry of Agriculture, 1990).

The pathogen *A. hydrophila*, a member of the Vibrionaceae family, Gram-negative, motile and rod-shaped bacteria, has the ability to infected cold-blooded vertebrates and mammals which exist freely in water (Ho *et al*., 1990), and is a primary and secondary pathogen of a number of aquatic and terrestrial animals, including humans (Howard and Buckley, 1985). Moreover, *A. hydrophila* is considered an essential cause of bacterial haemorrhagic septicaemia in freshwater fish (Frerichs, 1989), and is also reported to be associated with different ulcerative conditions / syndromes including Epizootic Ulcerative Syndrome (EUS) in Thailand and the Philippines (Llobrera and Gacutan, 1987; Lio-Po *et al*., 1992). Based on reports by Mastan and Qureshi (2003), varying degree of degeneration been observed in the epidermis, dermis, hypodermis and underlying musculature of infected *C. striata*. Outbreaks of EUS among snakehead fish (*Ophicephalus striatus*) and catfish (*Clarias batrachus*) have occurred annually in both wild and cultured fish in South and Southeast Asia (Lilley *et al.,* 1992). *Aeromonas hydrophila* was found to be consistently associated with EUS-affected fish in these countries and its pathogenicity has been confirmed by Boonyaratpalin (1989) and Lio-Po *et al*. (1992). Streptococcal infection, induced by the pathogen *Streptococcus* sp., has become one of the most important fish diseases since it was first, reported in the middle of the last century. In many studies, the pathogen *A. hydrophila* was reported to associate with tail and fin rot, haemorrhagic septicaemia and EUS (Austin and Adams, 1996; Roberts, 1997). More recently, numerous aquatic animals, including humans, have been reported to be infected by *Streptococcus* sp. (Evans *et al*., 2002; Hernández *et al*., 2009). Streptococcal infection can cause severe economic losses in numerous species of cultured and wild fish (Shariff *et al*., 1992); similarly, *Streptococcus iniae* emerged as significant pathogens in barramundi, *Lates calcarifer* (Bromage and Owens, 2000). Moreover, *Streptococcus agalactiae*, which is known as group B *Streptococcus,* has been reported to cause neonatal pneumonia and meningitis in humans (Brimil *et al*., 2006), mastitis in cows and horses (Yildirim *et al*., 2002; Brochet *et al*., 2006) and streptococcal infection in fish (Toranzo, *et al*., 2005).

Bacterial diseases can be treated effectively using antibiotics such as potentiated sulphonamide, chloramphenicol, neomycin, nitrofurantoin and oxytetracycline (Wiklund and Dalsgaard, 1998). The use of antibiotics in human medicines applied experimentally to treat bacterial infections in fish is not without problems, such as determination of appropriate solubility, palatability, toxicity, cost, delivery and governmental restrictions. In the case of government restrictions, only limited types of antibiotics are allowed to be used, especially in ornamental fish cultures (Smith *et a*l., 1994). However, even with all these restrictions being implemented, the development of antibiotic resistance continues to be observed in different strains of pathogens. A study by Aoki *et al*. (1971) reported that *A. salmonicida* exhibited resistance factors against antibiotics. It was also reported that multiple drug resistance cases were detected from atypical *A. salmonicida* as well as a variety of marine bacteria (Sandaa and Enger, 1996).

**2.3.2.(a).2 Parasitic diseases**

Parasitic diseases of aquaculture are also widely caused by protozoan organisms that live in the aquatic environment. These protozoans (*Ichthyophthirius multifiliis*, *Chilodonella* sp., *Trichodina* sp., *Ichthyobodo necator*, *Henneguya* sp.) are known to infect gills, skin, muscle, and the internal organs of both freshwater and aquarium fish, eventually causing irritation, weight loss and finally death. Infection by protozoans in fish is usually controlled by using standard chemicals, for example potassium permanganate, formalin and copper sulfate (Klinger and Francis-Floyd, 2002).

Investigations of snakehead fish parasites have been concentrated on those species of importance in aquaculture (Hoffman and Schubert, 1984). In addition, most of the fish were shown to be able to host more than one parasite at a time, and snakeheads were no exception (Courtenay and Williams, 2004). In China, research done by Jinhui (1991) listed parasitic crustaceans of *C. argus*, *C. asiatica*, and *C. punctata* from Chinese waters. Another list of known parasites of *C. gachua*, *C. marulius*, C. punctata and *C. striata* was provided from Bangladesh (Arthur and Ahmed, 2002). They also found that all parasites but *C. gachua* were equalled or far outnumbered by the parasites reported by Bykhovskaya-Pavlovskaya (1964) for *C. argus*, as shown in Table 2.3. The parasitic disease gnathostomiasis, which is known also to affect humans and is caused by a helminthic parasite, *Gnathostoma spinigerum*, which is recognized as a highly important disease, with about 800 suspected cases per year in two hospitals in Bangkok and Thailand between 1985 and 1988 (Setasuban, 1990). *Channa striata*, was identified as an intermediate host for this parasite, which was mostly found in muscle tissues and occurred in 100% of fish examined over 41 cm in length (Setasuban *et al*., 1991).

**Table 2.3:** Parasites of snakehead (*Channa argus*)

| Parasite | Group | Host Tissues | Other Fish Affected |
|---|---|---|---|
| *Azygia hwangtsiüi* | Trematoda | Intestine | |
| *Clinostomum complanatum* | Trematoda | Body cavity | Perches |
| *Cysticercus gryporhynchus cheilancristrotus* | Cestoidea | Gallbladder, intestine | Cyprinids, perches |
| *Gyrodactylus ophiocephali* | Myxosporidia | Fins | |
| *Henneguya ophiocephalia* | Myxosporidia | Gill arches, supra-branchial chambers | |
| *Henneguya vovki* | Myxosporidia | Body cavity | |

**Table 2.3:** Continued

| Parasite | Group | Host Tissues | Other Fish Affected |
|---|---|---|---|
| *Henneguya zschokkei?* | Myxosporidia | Gills, subcutaneous and musculature | Salmonids (tubercle disease of salmoinds) |
| *Lamproglena chinensis* | Copepoda | Gills | |
| *Mysosoma acuta* | Myxosporidia | Gill filaments | Crucian carp |
| *Myxidium ophiocephali* | Myxosporidia | Gallbladder, liver ducts | |
| *Myxobolus cheisini* | Myxosporidia | Gill filaments | |
| *Neomyxobolus ophiocephalus* | Myxosporidia | Gill filaments | |
| *Paracanthocephalus curtus* | Acanthocephala | Intestine | Cyprinids, esocids, sleepers, bagrid, catfishes |
| *Paracanthocephalus tenuirostris* | Acanthocephala | Intestine | |
| *Pingis sinensis* | Nematoda | Intestine | |
| *Polyonchobothrium ophiocephalina* | Cestoidea | Intestine | |
| *Thelohanellus catlae* | Myxosporidia | Kidneys | |
| *Zschokkella ophiocephalli* | Myxosporidia | Kidney tubules | |

**Note:** Table adopted from (Bykhovskaya-Pavlovskaya,1964)

### 2.3.2.(a).3 Viral diseases

Viral diseases can lead to serious problems in every aspect of aquaculture, especially when precaution fails to prevent the introduction of a viral agent, which can also lead to severe economic losses. Regardless of the species cultured in aquaculture, viral diseases can only be treated by quarantine, and eliminating the infected stock. Cleaning, disinfection of all equipment, facilities, and water resources must be followed before attempting to reintroduce animals and access virus-free stocks (Wolf, 1988). Viral diseases are not easy to differentiate from bacterial diseases unless they are tested in the laboratory. Moreover, viral diseases are difficult to diagnose and no certain medications are available for treating viral infections. Viral diseases are known to cause epizootic hematopoietic necrosis in red fin perch (*Perca fluviatilis*) and rainbow trout (*O. mykiss*) (Langdon *et al*., 2006; Langdon and Humphrey, 1987; Whittington *et al*., 1994). Infectious hematopoietic necrosis, infectious pancreatic necrosis and viral haemorrhagic septicaemia are the major viral diseases of salmon and trout (Post, 1983; Wolf, 1988).

Throughout the South Central and South Eastern areas of the United States, the virus to the American channel catfish (*Ictalurus punctatus*), seems to be endemic and to present in most cultured stocks of catfish; outbreaks of viral disease seem to be related to the stressful environmental conditions of aquaculture together with the presence of a bacterial co-pathogen. When epizootic outbreaks occur, losses among fingerlings and fray are reported to be very much higher than adults, which are intermittent and do not affect the commercial production of the catfish (Plumb and Gaines, 1975; Post, 1983; Wolf, 1988; Anonymous, 1990b). In shellfish cultures, viral disease is reported to be a major cause of losses, but a number of viruses have been associated with Epizootic disease such as: herpes-like viral disease and reo-like viral disease in shrimp and crabs, a baculovirus disease of shrimp; and a herpes-type virus disease of oysters (Sindermann and Center, 1974; Sindermann and Lightner, 1988).

#### 2.3.2.(a).4 Fungal diseases

Fungal diseases are usually considered an opportunistic infection that results from acute or chronic exposure to stress, which then compromises the immune system.