

UNIVERSITI PUTRA MALAYSIA

PREVALENCE OF CRUSTACEAN PARASITES INFECTING CULTURED FISH IN PENINSULAR MALAYSIA

IBRAHIM MOHAMMED

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By

IBRAHIM MOHAMMED

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

October 2017

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This thesis is dedicated to my late parents and family in recognition of their outstanding contributions to my academic achievements.



Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

PREVALENCE OF CRUSTACEAN PARASITES INFECTING CULTURED FISH IN PENINSULAR MALAYSIA

By

IBRAHIM MOHAMMED

October 2017

Chairman: Murni Marlina Binti Abd Karim, PhD Faculty: Agriculture

Crustacean parasites are among the most harmful pest of fishes infecting cultured fish. The present study aims at determining the prevalence of crustacean parasites and antiparasitic effect of herbs in controlling the parasites currently infecting grow-out fish farms in Peninsular Malaysia. Eleven fish farms were visited to identify fish infested with crustacean parasite. A total of 510 fish consisting of 100 snakehead (Channa striatus), 250 grouper (Epinephelus fuscoguttatus) and 160 goldfish (Carassius *auratus*) were collected randomly from different areas from January to December 2013. Based on the sampling locations, the prevalence of isopods was generally higher in two sampling areas, i.e. in the South of Peninsular Malaysia (97.5%) and the Central (90%) as compared with Argulus sp. that was high in the north of Peninsular Malaysia (92%) but moderate in the Central (74.3%). Our results showed a significant difference in prevalence of these parasites within the three sampling areas. Prevalence of Argulus sp. was found to be higher in snakehead (92%) as compared to goldfish (70%). Our results also showed significant difference in mean intensity values of Argulus infestation between snakehead and goldfish. Three herbs extracts namely neem leaves (Azadirachta indica), wormseed plant (Artemesia cina) and German chamomile (Matricaria chamomilla) were selected to investigate their potential antiparasitic action on Argulus sp. and isopods in in vitro assay. The ectoparasites were exposed to 10, 25, 50, 75 and 100 mg/L of herb concentrations and the mortality was compared with the control groups which used fresh and marine water without addition of any herb extract. In vitro study showed Argulus sp. mortality for all the concentrations of neem leaves, chamomile and wormseed plant extracts. A significantly high antiparasitic effect of these herbs extract solution was observed at concentration of 100 mg/L. In vitro study with isopods also showed mortality of the parasites for all the different treated concentration of neem leaves, chamomile and wormseed plant extracts. The results demonstrated that the three herbs showed high anti-parasitic effect towards crustacean parasites tested. The herbs extracts were also tested against Argulus sp. infested goldfish. The short-term static toxicity test was carried out to determine the median lethal concentration (LC₅₀) of three herbs on goldfish. The immersion experiment was performed using 50, 100 and 150 mg/L of the herbs extracts for 96 hr, along with control in well aerated water in glass aquaria. The concentration of neem



extract, in which 50% of the goldfish were killed in 96 hr was 160 mg/L. In vivo artificial cohabitation Argulus sp. infection test was carried out using naive goldfishes with an average weight of 20-22g. Results showed an average attachment of Argulus sp. ranged from 2-5 parasites per fish during two weeks exposure. In another in vivo assay, goldfish infested with at least three Argulus sp. were treated with bath treatment using two concentrations of herbs (50 and 100 mg/L). Results showed that the three herbs extract were able to reduce the parasites burden and showed anti-parasitic effects on Argulus sp. Three herbs extract at 100 mg/L was tested in tiger grouper infested with one isopod in buccal cavity and the result revealed that neem leaves, chamomile and wormseed plants showed anti-parasitic properties in marine environment and could reduce isopod burden on fish. Analysis of survival using Kaplan-Meier showed that all treatment using the selected herbs in goldfish and tiger grouper showed significant anti-parasitic properties against Argulus sp. and isopod. The efficacies of the three herbs were considered as time-dependent since mortalities increased with longer exposure time. In conclusion, neem leaves, chamomile and wormseed plant extracts showed the ability in controlling the crustacean parasites tested and have potential as anti-parasitic agent in aquaculture.

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

PREVALENSPARASIT KRUSTASIA MENJANGKITI LADANG PEMBESARAN IKAN DI SEMENANJUNG MALAYSIA DAN KESAN ANTIPARASITIK HERBA

Oleh

IBRAHIM MOHAMMED

Oktober 2017

Pengerusi: Murni Marlina Binti Abd Karim, PhD Fakulti: Pertanian

Parasit krutasia merupakan antara perosak ikan yang berbahaya yang menjangkiti ikan yang diternak. Kajian ini bertujuan untuk menentukan kelaziman parasit krustasia dan kesan antiparasit herba dalam mengawal parasit yang kerap menjangkiti ladang ternakan ikan di Semenanjung Malaysia. Sebelas ladang ikan dilawati untuk mengenalpasti ikan yang dijangkiti parasit krustasia. Sejumlah 510 yang terdiri daripada 100 ekor ikan haruan (Channa striatus), 250 ikan kerapu harimau (Epinephelus fuscoguttatus) dan 160 ikan mas (Carassius auratus) dikutip secara rawak dari beberapa kawasan dari Januari ke Disember 2013. Berdasarkan pada lokasi persampelan, kelaziman isopod adalah pada amnya tinggi di dua kawasan iaitu selatan Semenanjung Malaysia (97.5%) dan tengah (90%) jika dibandingkan dengan Argulus sp. yang mana tinggi di utara Semenanjung Malaysia (92%) tetapi sederhana di kawasan tengah (74.3%). Keputusan kajian menunjukkan terdapat perbezaan yang signifikan dalam kelaziman parasit tersebut dalam ketiga-tiga kawasan persampelan. Kelaziman Argulus sp. didapati lebih tinggi dalam ikan haruan (92%) berbanding dengan ikan mas (70%). Keputusan juga menunjukkan perbezaan yang signifikan dalam nilai purata jangkitan Argulus sp. di antara ikan haruan dan ikan mas.

Tiga ekstrak herba iaitu daun neem (*Azadirachta indica*), pokok 'wormseed' (*Artemesia cina*) dan 'chamomile' Jerman (*Matricaria chamomilla*) telah dipilih untuk dikaji potensi kesan antiparasit mereka ke atas *Argulus* sp. dan isopods di dalan ujian *in vitro*. Parasit didedahkan pada herba dengan kepekatan 10, 25, 50, 75 dan 100 mg/L dan kematian dibandingkan dengan kumpulan kawalan yang menggunakan air tawar dan masin tanpa tambahan sebarang ekstrak herba. Kajian *in vitro* menunjukkan kematian *Argulus* sp. untuk kesemua kepekatan ekstrak daun neem, 'chamomile' dan pokok 'wormseed'. Kesan yang signifikan dapat dilihat untuk ektrak herba-herba tersebut pada kepekatan 100 mg/L. Kajian *in vitro* dengan isopod juga menunjukkan kematian parasit untuk kesemua kepekatan rawatan yang berlainan ekstrak herba daun neem, 'chamomile' dan 'wormseed'. Keputusan menunjukkan kesan antiparasit yang tinggi pada parasit krustasia yang diuji. Ekstrak herba juga diuji pada ikan emas yang dijangkiti *Argulus* sp.. Ujian statik jangka pendek dijalankan untuk menentukan (LC₅₀)

ketiga-tiga herba pada ikan mas. Kajian rendaman yang dijalankan menggunakan 50, 100 dan 150 mg/L ekstrak herba selama 96 jam, bersama kumpulan kawalan dalam akuarium yang mengandungi oksigen. Kepekatan ekstrak daun neem di mana 50% kematian pada ikan mas dalam 69 jam adalah 160 mg/L. Ujian in vivo terhadap jangkitan Argulus sp. secara kohabitasi dijalankan menggunakan ikan mas dengan berat 20-22g. Keputusan menunjukkan perlekatan purata Argulus sp. di antara 2-5 parasit setiap ikan semasa pendedahan selama dua minggu. Pada kajian ujian *in vivo* lain, ikan mas yang dijangkiti sekurang-kurangnya tiga Argulus sp. dirawat menggunakan kaedah rendaman herba dengan kepekatan (50 and 100 mg/L). Keputusan menunjukkan bahawa tiga ekstrak herba boleh mengurangkan parasit dan membuktikan kesan antiparasit terhadap Argulus sp. Tiga ekstrak herba pada kepekatan 100 mg/L diuji pada ikan kerapu harimau yang dijangkiti seekor isopod dalam ruang mulut dan keputusan menunjukkan daun neem, 'chamomile' dan pokok 'wormseed' menunjukkan sifat anti-parasitik dalam persekitaran marin dan boleh merendahkan beban isopod pada ikan. Analisis kemandirian menggunakan Kaplan-Meier menunjukkan kesemua rawatan menggunakan herba terpilih pada ikan emas dan kerapu harimau menunjukkan sifat anti-parasitik terhadap Argulus dan isopod. Keberkesanan ketiga-tiga herba adalah dianggap bersandarkan pada masa kerana kematian meningkat dengan bertambahnya masa pendedahan. Kesimpulannya, ekstrak daun neem, 'chamomile' dan pokok 'wormseed' telah menunjukkan kemampuan dalam mengawal parasit krustasia dan berpotensi sebagai agen anti-parasit dalam akuakultur.

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I certify that a Thesis Examination Committee has met on (date on viva voce) to conduct the final examination of Name on his thesis entitled "Prevalence of Crustacean Parasites Infecting Cultured Fish in Peninsular Malaysia" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U. (A) 106] 15 March 1998. The committee recommends that the student be awarded the Doctor of Philosophy.

Members of the Thesis Examination Committee were as follows:

Annie Christianus, PhD

Senior Lecturer Faculty of Agriculture Universiti Putra Malaysia (Chairman)

Aziz bin Ar<mark>shad, PhD</mark>

Professor Faculty of Agriculture Universiti Putra Malaysia (Internal Examiner)

Md Sabri bin Mohd Yusoff, PhD

Associate Professor Faculty of Veterinary Medicine Universiti Putra Malaysia (Internal Examiner)

Mohamed Sayed Mohamed Marzouk, PhD

Professor Cairo University Egypt (External Examiner)

NOR AINI AB. SHUKOR, PhD Professor and Deputy Dean

School of Graduate Studies Universiti Putra Malaysia

Date:

This thesis was submitted to the Senate of University Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

Murni Marlina Abd Karim, PhD

Senior Lecturer Faculty of Agriculture Universiti Putra Malaysia (Chairman)

Mohd Salleh Kamarudin, PhD Professor Faculty of Agriculture Universiti Putra Malaysia (Member)

Hassan M. Daud, PhD

Associate Professor Faculty of Veterinary Medicine Universiti Putra Malaysia (Member)

ROBIAH BINTI YUNUS, PHD

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UPM

Signature: Name of Member of Supervisory Committee:

Mohd Salleh Kamarudin

Signature: Name of Member of Supervisory Committee:

Hassan M. Daud

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LIST OF ABBREVIATIONS

°C	Degree Celsius
5	Percentage
BW	Body weight
BL	Body length
Conc.	Concentration
(C)	Central part
CI	Confidence interval
Cham	Chamomile
hr	Hour
Gm	Gram
Kg	Kilogram
L	Liter
Mg	Milligram
ml	Milliter
mg	Milligram
min	Minutes
(N)	Northern parts
pН	Potential of hydrogen
ppt	Part per million
SE	Standard error
(S)	Southern parts
WSP	Worm seed plant

CHAPTER 1

INTRODUCTION

Aquaculture is among the rapid growing food production sectors of the world with a yearly average increase approximately 10% since 1984, and a comparable trend is being tracked for ornamental farming which is also a sector of aquaculture (FAO, 2011). Infectious diseases are common and huge economic losses are encountered due to intensification of aquaculture (Kumar *et al.*, 2012). The major causative agents are parasites, bacteria and virus. Crustacean parasites such as *Argulus* sp., copepod and isopods cause serious problems in commercial fish farming including small-scale coastline fish farms in the Mediterranean Sea, Northern Hemisphere and South East Asia (Fast and Lester, 2013).

Crustacean is in the phylum Arthropoda which includes among others Branchiura (Fish lice), parasitic copepods (Entomostraca) and parasitic isopods (Eumalacostraca). Sea lice belong to family Caligidae which comprises of over 400 species but only a few of these species have been reported as pests in the fish farming facilities infesting the external surface of marine and brackish water (Fast and Lester, 2013). The earliest occurrence infection of unknown marine parasitic copepods in Malaysia was reported in *Lutjanus johni* (golden snapper), *Lates calcarifer* (sea bass) and *Epinephelus malabaricus* (grouper) (Leong and Wong, 1988).

Low infection of *Argulus* and isopod usually are not harmful to the hosts nevertheless heavily infected hosts could have serious damage to gill tissues, muscle and skin which eventually will lead to secondary infections, deficiency of hemoglobin, abnormally thin, severe epizootics and mortality (Richard, 2003). Among the common symptoms of this type of parasite infections are loss of appetite, slower growth rate and high mortalities (Woo *et al.*, 2002).

Argulus is a crustacean parasite that causes injury to major carps predominantly cyprinids during culture. The manner of this parasite feeding embroils secretion and ejection of huge amounts of digestive fluids with host tissue tearing using the buccal apparatus and attachment of appendages, which cause severe fleshly injury and hemorrhages to the host. The extremely toxic secretion of the buccal gland can cause a high inflammatory reaction and loss of weight (Jackson *et al.*, 2001).

Cymothoidae which is a family of isopod is considered as one of the biggest fish parasites (Brusca, 1981). Juvenile isopods are free-living organism whereas adult isopods are usually ectoparasitic and the parasites are often found on gills, oral cavity or skin of the hosts (Bunkley-Williams and Williams, 1998). Isopods lifespan seems to differ from species to species, with maximum living of one to two year (Brusca, 1981), even though others may live above nine year on their host (Maxwell, 1982). In



several studies, fish infected with isopods showed a reduction in condition index, shorter lifespan and eventually decreased production (Adlard and Lester, 1994).

However, numerous other studies found no injurious effects on the fish host (Chang *et al.*, 1999; Maxwell, 1982). Furthermore, such unobvious absence of effect on the host condition index due to the isopod infection can be clarified by the host reimbursing higher rate of energy loss through increase rate of feeding as compare to non-parasitized individuals (Östlund-Nilsson *et al.*, 2005). In order to manage the parasitism and harmful outcome, several effective parasiticides have been developed earlier for example hydrogen peroxide, parathion, cypermethrin and dichlorvos (Toovey and Lynon, 2000). Other chemicals, for example, formalin and trichlorofon are far from being acceptable (Glover *et al.*, 2004).

Recent research efforts have aimed at developing alternative drug formulations which include medicinal plants (Kumar *et al.*, 2012). Recently organic based medicines (herbs) are widely used to treat diseases which have been proved to be effective throughout the world (Kumar *et al.*, 2012). The importance of studying the prevalence and mean intensity of crustacean parasites and their control with herbs cannot be over emphasized, as it can provide information on the situation of the disease within the communities.

1.1 Problem Statements and justification

Fish parasites are frequently reported to be a main constraint in the emerging industry of finfish marine culture in Malaysia, due to severe parasitic disease outbreaks (Rueckert *et al.*, 2008). The ectoparasites *Argulus* spp. is typically attached to the host, which directly decreases the growth and is also an effective vector of several viral and bacterial pathogens. Most isopods feed mainly on blood; and they ingest the mucus, subcutaneous and epithelium tissues of their host. The use of chemotherapeutics and antibiotics for treatment and control of diseases in aquaculture has tremendous adverse effects as these can lead to resistance and accumulation of toxins.

1.2 Hypothesis

Prevalence of crustacean parasites (*Argulus* and isopods) is expected to be high in some farms and water bodies across Peninsular Malaysia. Plant extracts such as Neem (*Azadirachta indica*) leaves, German chamomile (*Matricaria chamomilla*) and worm seed plant (*Artemesia cina L*, WSP) with either anticancer, antibacterial, antifungal, repellent, and pesticidal properties were tested on different organism for both *in vitro* and *in vivo* toxicity test. Present study expected that these herbs possess anti parasitic activity with high comparable in *in vitro* and *in vivo* toxicity on respective crustaceans parasites.

1.3 Scope and objectives

In Malaysia studies on the prevalence of crustacean parasites in goldfish, grouper and snakehead in grow out farm is limited, also the use of plant extract (Neem leaves, Wormseed plant and chamomile) against crustacean parasites in grow out farm is lacking. Hence, the present study was undertaken to provide this vital information on the prevalence of crustacean parasites in goldfish, grouper and snakehead in selected grow out farms in Peninsular Malaysia and also to assess the effect of plant extracts of Neem leaves, WSP and chamomile for *in vitro* and *in vivo* test against *Argulus* and isopods, with the following specific objective.

1.4 Specific objectives

- 1. To determine the prevalence and mean intensity of crustacean parasites (*Argulus* and isopods) in cultured marine and freshwater fishes in selected farms of Peninsular Malaysia.
- 2. To evaluate the efficacy of neem leaves, chamomile and worm seed plant extracts in *in vitro* study against crustacean parasites (*Argulus* and isopods).
- 3. To investigate the efficacy of neem leaves, chamomile and worm seed plant extracts in controlling freshwater parasite (*Argulus*) in infested goldfish under *in vivo* condition.
- 4. To examine the efficacy of neem leaves, chamomile and worm seed plant extracts in controlling marine water parasite (isopods) in tiger grouper *in vivo* condition.

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