UNIVERSITI PUTRA MALAYSIA

# EFFECTS OF MALACHITE GREEN TREATMENT REGIMES ON RED TILAPIA (*Oreochromis* hybrid) AND ITS WITHDRAWAL PERIOD

KWAN PENZ PENZ

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**EFFECTS OF MALACHITE GREEN TREATMENT REGIMES ON RED TILAPIA (*Oreochromis* hybrid) AND ITS WITHDRAWAL PERIOD**



**By**

**KWAN PENZ PENZ**

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

**January 2019**

**DEDICATION**

To my parents,
Eddy Kwan and Karen Lau,
And both my siblings,
Yong Kitt and Tszee Yeung,
Without whom, none of my success would be possible.

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

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**Chair: Dato Mohamed Shariff Mohamed Din, PhD**
**Faculty: Veterinary Medicine**

United Nations reported that the world's average per capita consumption of fish exceeded 20 kg in year 2016. To meet the demand, many farms culture fish intensively which induces stress and increases fish susceptibility to diseases. In order to prevent the occurrence of diseases, the application of chemicals such as malachite green (MG) as a prophylactic treatment has become a common practice in some countries. However, the use of MG is strictly prohibited in fish for human consumption by the European Union and US Food and Drug Administration due to its potential carcinogenic and genotoxic properties. In spite of its health hazard, recent findings indicate the presence of MG and its metabolite, leucomalachite green (LMG) residues in many fish samples from all over the world and are of concern to human health due to the fact that consumption of fish is high in many countries. Therefore, this research examined the toxicity and accumulation of MG residues in red tilapia (*Oreochromis* hybrid) which is one of the highly consumed freshwater fish species in Malaysia. A sensitive liquid chromatography-tandem mass spectrometry (LC-MS/MS) method was used to test the residues of MG and LMG which was validated using red tilapia fish muscle. A simplified method without the use of solid phase extraction for sample preparation was used. The decision limit and detection capability for MG were 0.05 µg/L and 0.09 µg/L and LMG were 0.05 µg/L and 0.08 µg/L. Acute toxicity bioassay was performed, and based on the 96 h $LC_{50}$ result which was 1.06 mg/L, red tilapia were exposed to the acute, sub-acute and sub-lethal concentration of MG. Subsequently, growth parameters, blood chemistry, antioxidant status and oxidative stress were measured. Furthermore, the withdrawal period of MG after exposure to different treatment regimes was determined. Red tilapia exposed to long-term bath showed the highest accumulation of total MG and LMG residues (441.38 µg/kg) immediately after treatment in comparison to dip (once), dips (once for 5 days), short-term bath (once) and short-term bath (once for 5 days). After 30 days, sum residues of MG and LMG were 13.30 µg/L for long-term bath, whereas, for all other treatments it was below 0.26 µg/kg. In addition, MG and LMG residues in muscle tissue of commonly consumed fish such as red tilapia (*Oreochromis* hybrid), African catfish (*Clarias gariepinus*), Asian seabass (*Lates calcarifer*), hybrid grouper (*Epinephelus fuscoguttatus* × *Epinephelus lanceolatus*) and striped catfish (*Pangasius hypophthalmus*) were quantitatively analysed using LC-MS/MS. The fish were purchased from 11 different markets in the state of Selangor, Malaysia. Results showed that sum residues of MG and LMG ranged from 0.53 to 4.10 µg/kg, with the highest residue detected in domestic striped catfish. This study indicates that MG is still being used and aquaculture stakeholders should be made aware of the judicious use of MG for the benefit of public health.

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

**KESAN PENGGUNAAN REGIM RAWATAN MALAKIT HIJAU PADA TILAPIA MERAH (*Oreochromis* kacuk) DAN TEMPOH PENGINGKIRANNYA**

Oleh

**KWAN PENZ PENZ**

**Januari 2019**

**Pengerusi : Dato Mohamed Shariff Mohamed Din, PhD**
**Fakulti : Perubatan Veterinar**

Pertubuhan Bangsa-bangsa Bersatu melaporkan jumlah konsumsi ikan per kapita dunia melebihi 20 kg pada tahun 2016. Bagi memenuhi permintaan ini, kebanyakan ladang menternak ikan secara intensif; dimana kultur persekitaran adalah seringkali tertekan (stress) dan ini memudahkan ikan dijangkiti penyakit. Untuk mengelakkan jangkitan penyakit-penyakit ini, penggunaan bahan kimia seperti 'malakit hijau' (MG) sebagai rawatan profilaksis masih sering dipraktikkan di sesetengah negara. Biarpun penggunaan MG untuk rawatan ikan bagi tujuan konsumsi manusia adalah dilarang keras oleh pertubuhan EU dan USFDA disebabkan potensi karsinogenik dan genotoksiknya, sisa MG dan metabolitnya iaitu 'leukomalakit hijau' (LMG) masih ditemui di dalam banyak sampel ikan dari pelbagai negara. Hal ini, menimbulkan kebimbangan dikalangan pengguna terutama kesannya terhadap kesihatan manusia berikutan fakta peningkatan konsumsi ikan di kebanyakan negara. Penyelidikan ini dijalankan bagi mengkaji kesan toksik dan pengumpulan sisa MG pada ikan tilapia merah (*Oreochromis* hibrid), memandangkan ia adalah sejenis ikan air tawar yang paling banyak diternak di Malaysia. Kaedah kromatografi cecair tandem spektrofotometri jisim (LC-MS/MS) telah digunakan untuk mengesan sisa MG dan LMG di dalam isi ikan tilapia merah. Kaedah ringkas penyediaan sampel tanpa menggunakan pengekstrakan fasa pepejal telah digunakan. Had keupayaan pengesanan MG ialah di antara 0.05 µg/L dan 0.09 µg/L manakala LMG adalah 0.05 µg/L dan 0.08 µg/L. Tilapia merah juga didedahkan kepada kepekatan MG akut, sub-akut dan sub-maut berdasarkan keputusan bioassei ketoksikan akut 96 h $LC_{50}$ iaitu pada 1.06mg/L. Seterusnya, parameter pertumbuhan, kimia darah, status antioksidan dan tekanan oksidatif ikan telah diukur. Tempoh penyingkiran MG daripada ikan selepas pendedahan pada regim MG yang berlainan juga dikaji. Tilapia merah yang didedahkan secara mandian jangka panjang mampu mengumpul sisa MG dan LMG yang tertinggi iaitu 441.38 µg/L serta merta selepas rawatan berbanding dengan kaedah celup (sekali), celup (sekali selama 5 hari), mandian jangka pendek (sekali) dan mandian jangka pendek (sekali selama 5 hari). Selepas 30 hari, jumlah sisa MG dan LMG yang terkumpul adalah 13.30 µg/L untuk mandian jangka panjang manakala rawatan-rawatan lain adalah di bawah 0.26 µg/L. Selain itu, sisa MG dan LMG dalam

tisu otot ikan yang lazim dimakan seperti ikan tilapia merah (*Oreochromis* hibrid), keli Afrika (*Clarias gariepinus*), siakap (*Lates calcarifer*), kerapu hibrid (*Epinephelus fuscoguttatus* × *E. lanceolatus*) dan ikan patin (*Pangasius hypophthalmus*) telah dianalisis secara kuantitatif menggunakan LC-MS/MS. Ikan-ikan ini diperolehi dari 11 pasar awam yang berlainan di sekitar negeri Selangor, Malaysia. Hasil kajian menunjukkan sisa MG dan LMG yang dikesan berjumlah di antara 0.53 hingga 4.10 µg/L, dimana kandungan sisa tertinggi dikesan di dalam ikan patin. Kajian ini menunjukkan bahawa penggunaan MG masih dipraktikkan secara meluas dan pihak berkuasa perlu sedar tentang risiko penggunaannya agar tidak menjejaskan kesihatan orang awam.

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This thesis was submitted to the Senate of Universiti 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:

**Mohamed Shariff Mohamed Din, PhD**
Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Chairman)

**Fatimah Md. Yusoff, PhD**
Professor
Institute of Bioscience
Universiti Putra Malaysia
(Member)

**Sanjoy Banerjee, PhD**
Research Fellow
Institute of Bioscience
Universiti Putra Malaysia
(Member)

**ROBIAH BINTI YUNUS, PhD**
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

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Name of Chairman of Supervisory Committee: Dato' Dr. Mohamed Shariff Mohamed Din

Signature: ______________________

Name of Member of Supervisory Committee: Prof Dr. Fatimah Md. Yusoff

Signature: ______________________

Name of Member of Supervisory Committee: Dr Sanjoy Banerjee

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# LIST OF ABBREVIATIONS

| | |
|---|---|
| 2D | Two dimension |
| A | Absorbance |
| ABC | ATP binding cassette |
| ATP | Adenosine triphosphate |
| C | Safe concentration |
| CAS | Chemical abstracts service |
| CAT | Catalase |
| CCα | Decision limit |
| CCβ | Detection capability |
| CE | Collision energy |
| CID | PubChem compound identification |
| $d_6$-LMG | Leucomalachite green internal standard |
| DNA | Deoxyribonucleic acid |
| DO | Dissolved oxygen |
| EINECS | European Inventory of Existing Commercial Substances |
| EPA | Eicosapentaenoic acid |
| EU | European Union |
| FCR | Feed conversion ratio |
| FDA | Food and Drug Administration |
| FMC | A mixture of formalin, malachite green and methylene blue |
| GIFT | Genetically improved farmed tilapia |
| GPx | Glutathione peroxidase |
| GSH | Reduced glutathione |
| H-ESI | Heated electrospray ionization |
| HPLC | High-performance liquid chromatography |
| H-SRM | Highly selective reaction monitoring |
| IgM | Immunoglobulin M |
| ISO | International Organization for Standardization |
| IUPAC | International Union of Pure Applied Chemist |
| LC-ACPI-MS | Liquid chromatography with atmospheric pressure chemical ionization mass spectrometry |
| LC-ITMS | Liquid chromatography-ion trap mass spectrometry |
| LC-MS/MS | Liquid chromatography-tandem mass spectrometry |
| LC-VIS | Liquid chromatography with visible detection |
| LC-VIS/FLD | Liquid chromatography with visible and fluorescence detection |
| LMG | Leucomalachite green |
| LOD | Limit of detection |
| LOQ | Limit of quantitation |
| MDA | Malondialdehyde |
| MDL | Method detection limit |
| MG | Malachite green |
| MMCs | Melanomacrophage centres |
| MQL | Method quantitation limit |
| MRLs | Maximum residue limits |
| MRPLs | Minimum required performance limits |
| ms2 | Mass selection stage 2 |
| MXR | Multixenobiotic resistance |

| | |
|---|---|
| NBT | Nitroblue tetrazolium activity |
| NL | Neutral loss constituents |
| PBS | Phosphate buffer saline |
| P-gp | P-glycoprotein |
| pH | Potential of hydrogen |
| RASFF | Rapid Alert System for Food and Feed |
| RM | Ringgit Malaysia |
| ROS | Reactive oxygen species |
| RSD | Relative standard deviation |
| SGR | Specific growth rate |
| SOD | Superoxide dismutase |
| SPE | Solid phase extraction |
| SRM | Selected reaction monitoring |
| TBA | Thiobarbituric acid |
| TCA | Trichloroacetic acid |
| TI | Total immunoglobulin levels |
| TIC F | Total ion chromatogram fragment |
| TPP | Total plasma protein |
| TSQ | Triple stage quadrupole |
| US FDA | US Food and Drug Administration |
| USD | US dollars |

## LIST OF NOTATIONS

| | |
|---|---|
| % | percent |
| ×*g* | times gravity |
| °C | degree Celsius |
| µ | micro |
| ‰ | parts per thousand |
| g | gram |
| kg | kilogram |
| L | litre |
| $LC_{50}$ | lethal concentration 50 |
| M | molar |
| $M^+$ | molecular ion |
| mg | milligram |
| $MH^+$ | molecular ion |
| mL | millilitre |
| mM | millimolar |
| mTorr | millitorr |
| n | number of replicate observations |
| $n_b$ | number of blank observations |
| nm | nanometer |
| ppm | parts per million |
| ppt | parts per thousand |
| $R^2$ | correlation coefficient |
| rpm | revolutions per minute |
| $s_0$ | standard deviation |
| $s'_0$ | standard deviation for calculating MDL and MQL |
| $t_2 - t_1$ | time/duration of exposure |
| V | voltage |
| $W_1$ | initial weight |
| $W_2$ | final weight |
| α | alpha |
| β | beta |

# CHAPTER 1

# INTRODUCTION

The world's population is projected to reach 9.7 billion by 2050 (United Nations 2015). Food demand is expected to increase by 59% to 98% (Elferink and Schierhorn 2016). This pose a threat to food security, considering that the limited natural resources have shown declining signs of sustainability. Hence, farmers worldwide will have to increase their agricultural crop production. Fish is an important source of food as it provides more than 4.5 billion people with at least 15% of their average per capita intake of animal protein (Béné *et al.* 2015). However, capture fisheries have been relatively static since the 1980s (FAO 2016). This is shown by the slow growth of global total capture fisheries production which was 87.7 million tonnes in 1996 (FAO 2011), 90.0 million tonnes in 2006 (FAO 2012) and 93.4 million tonnes in 2014 (FAO 2016). On the other hand, an increasing trend was seen in aquaculture production (Figure 1.1), which is one of the fastest growing sectors of global food industry which amounted to 47.3 million tonnes in 2006, 59.9 million tonnes in 2010 (FAO 2012) and 73.8 million tonnes in 2014 (FAO 2016). Asian countries are the leading producers of aquaculture species in the world (Table 1.1). Thus, the production of fish should meet the food safety standards for the safety of consumers worldwide.

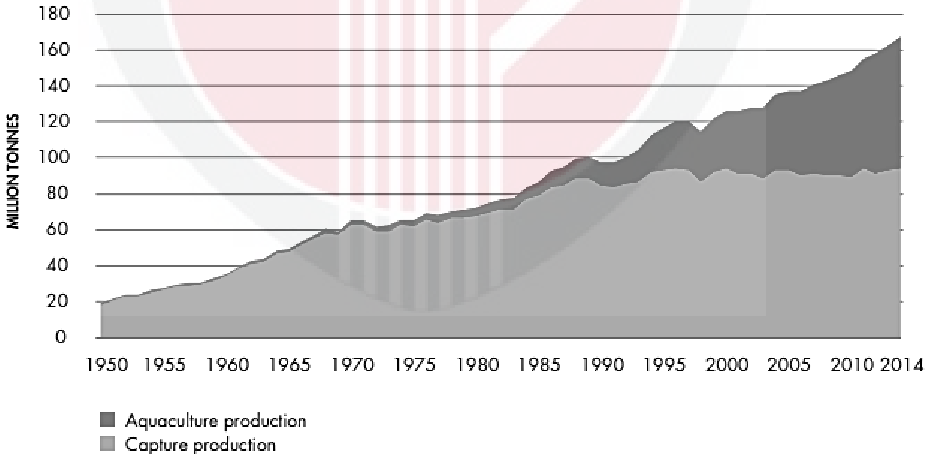


**Figure 1.1: World capture fisheries and aquaculture production** (Source: FAO, 2016).

**Table 1.1: The top 15 producers of farmed aquatic species in 2014** (FAO, 2016).

| | Country | Total aquatic animals | Total aquatic animals & plants |
|---|---|---|---|
| | | (Thousand tonnes) | |
| 1 | China | 45, 469.0 | 58, 795.3 |
| 2 | Indonesia | 4, 253.9 | 14, 330.9 |
| 3 | India | 4, 881.0 | 4, 884.0 |
| 4 | Vietnam | 3, 397.1 | 3, 411.4 |
| 5 | Philippines | 788.0 | 2, 337.6 |
| 6 | Bangladesh | 1, 956.9 | 1, 956.9 |
| 7 | Republic of Korea | 480.4 | 1, 567.4 |
| 8 | Norway | 1, 332.5 | 1, 332.5 |
| 9 | Chile | 1, 214.5 | 1, 227.4 |
| 10 | Egypt | 1, 137.1 | 1, 137.1 |
| 11 | Japan | 657.0 | 1, 020.4 |
| 12 | Myanmar | 962.2 | 964.3 |
| 13 | Thailand | 934.8 | 934.8 |
| 14 | Brazil | 561.8 | 562.5 |
| 15 | Malaysia | 275.7 | 521.0 |

Aquaculture is an alternative sustainable solution and is one of the most resource-efficient methods to produce a continuous and consistent supply of protein for human consumption as compared to other domestic livestock in terms of food conversion ratio. Figure 1.2 illustrates that farmed fish is the most efficient means in producing protein when compared to cattle, hogs and broiler chicken. However, shortage of agricultural land due to urbanization and freshwater scarcity urges fish farmers to move towards intensive aquaculture to meet the rising demand for inexpensive food for the people. Although high-value fish gets the lion's share of attention, the low-value inland finfish is the bulk of world's aquaculture production (Subasinghe *et al.* 2000).

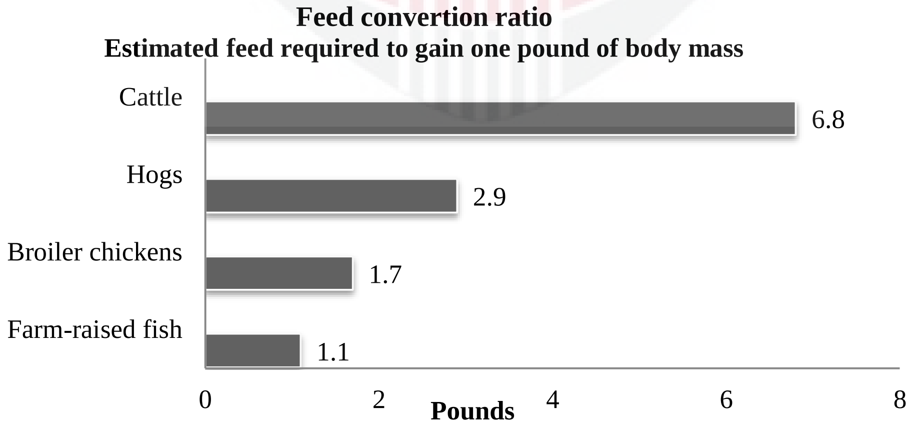


**Figure 1.2: A comparison of feed conversion ratio for the production of beef, poultry and farmed fish** (Global Aquaculture Alliance, 2016).

Through intensive farming, operating cost is more economical and farmers are able to easily monitor their fish and get higher productivity in a limited amount of land. However, culturing fish beyond its carrying capacity stresses the environment as well as the cultured animals and usually results in disease outbreak (Mehana *et al.* 2015). Survey on the annual losses due to diseases in 16 Asian countries was reported to be equivalent to USD 16 billion (OIE 2016), indicating that disease is a primary constraint in aquaculture. Farmers, however, often take risk by practicing high-density cultures and frequently use chemicals as prophylaxis to minimise the occurrence of diseases by opportunistic infectious agents (Subasinghe 2009).

The success of fish farming is highly dependent on external inputs, and one of them is chemicals (Subasinghe *et al.* 2000). Chemicals have been used in aquaculture for centuries not only in health management but also for soil and water management, aquatic productivity enhancement (probiotic), live fish transportation, feed formulation, growth promotion and as a value addition to the final product (Chowdhury *et al.* 2012). In addition, chemicals (vitamin and immunostimulant) are also used to manipulate and enhance reproduction (Arthur *et al.* 1996), improve the survival rate of fingerlings, and build stronger immune system against pathogenic bacteria. However, the type of chemicals depends on the purpose, culture system, and species. Apart from antibiotics, there are pesticides, herbicides, biocides, algaecides, and biosecurity disinfectants to control the aquatic pest, treat disease, and protect farm infrastructure. For instance, anti-fouling paints such as copper oxide (Abdel-Khalek *et al.* 2015) are used to coat net and cages to prevent attachment of marine organisms which could reduce the water flow rate, decrease dissolved oxygen and affect the durability of nets. In aquaculture, formalin, florfenicol, tricaine methanesulfonate, oxytetracyline, hydrogen peroxide, sulfamerazine and sulfadimethoxine are among the approved drugs by Food and Drug Administration (FDA) (Table 1.2).

On the other hand, chloramphenicol, nitrofurans, fluoroquinolones, quinolones, malachite green (MG) and steroid hormones are not approved to be used in the production of fish for consumption (FDA 2011) due to the deleterious effects they pose to human health. Malachite green is a very effective antiparasitic and antifungal chemical in aquaculture. Although it is not permitted in aquatic food animal by the US Food and Drug Administration (US FDA), European Union (EU) and Canadian Government (López-Gutiérrez *et al.* 2013) the residues of MG and its metabolite are still found in fishes in recent years (RASFF 2017) and were non-compliant with the food standards. The metabolite of MG, also known as leucomalachite green (LMG) can persist in the fish muscles up to 12 months (Máchová *et al.* 1996). However, the presence of this residue is influenced by the fish species, climate and culture system. Malachite green affects the immune system, reproductive system and is carcinogenic and can cause genotoxicity (Srivastava *et al.* 2004). However, because it is cheap, highly effective and readily available (Wong and Cheung 2009), farmers are still using MG in certain countries.

**Table 1.2: The list of FDA-approved aquaculture drugs** (FDA, 2011).

| Drugs | Compounds/ Active chemicals |
|---|---|
| Chorulon | Chorionic gonadotropin |
| Paracide-F, Formacide-B, Formalin-F | Formalin solution |
| Aquaflor-Type A, Aquaflor-CA1 | Florfenicol |
| Finquel and Tricaine-S | Tricaine methanesulfonate (MS-222) |
| Terramycin-200 for fish Type A, OxyMarine | Oxytetracycline |
| 35% PEROX-AID | Hydrogen peroxide |
| Sulfamerazine | Sulfamerazine |
| Romet-30 | Sulfadimethoxine or ormetoprim combination |
| Others | Acetic acid, calcium chloride, calcium oxide, carbon dioxide gas, fuller's earth, garlic (whole form), ice, magnesium sulphate, onion (whole form), papain, potassium chloride, povidone iodine, sodium bicarbonate, sodium chloride, sodium sulphide, thiamine hydrochloride, urea & tannic acid |

Most countries, including Asia, do not have reliable data on the number of chemicals used in aquaculture (Rico *et al.* 2012). The use of chemicals is an important issue and should be of great concern to policymakers and consumers because fish consumption is on the rise and is high in Asian countries (Table 1.3). Thus, the study on the toxicity of MG and the withdrawal period of MG and LMG is important to serve as baseline information for the authorities that are responsible in monitoring the usage of MG, as well as for researchers to conduct further studies with the intention to protect human health. The present study was conducted using the local red tilapia (*Oreochromis* hybrid) due to its abundance and high market demand. There are FDA guidelines regarding the toxicity of MG but it may only be suitable for temperate countries since the data for the guidelines are based on studies (Bilandžić *et al*. 2012; Yonar and Yonar 2010; Silveira-Coffigny *et al*. 2004) in the temperate region. However, records for tropical conditions are scarce. In addition, MG toxicity differs with fish species and can be easily influenced by factors such as temperature, pH, hardness and dissolved oxygen of test water (Srivastava *et al.* 2004). Thus, this study was conducted using locally cultured species under tropical conditions.

**Table 1.3: Per capita fish consumption in Asian countries.**

| Year | Country | Per capita (kg/year) | Reference |
|---|---|---|---|
| 2013 | Malaysia | 52.4 | (DOFM 2014) |
| 2012 | Hong Kong | 70.4 | (Helgi Library 2015) |
| | Macao | 55.7 | |
| | Myanmar | 54.9 | |
| | Taiwan | 35.5 | |

| | | | |
|---|---|---|---|
| 2011 | Maldives | 166.0 | (Helgi Library 2015) |
| | South Korea | 58.1 | |
| | Japan | 53.7 | |
| | Vietnam | 33.0 | |

The results of this study targets to provide information and knowledge regarding the toxicity of MG in red tilapia. The results of this study could be used as a guideline to establish and recommend the proper usage of MG in fish farming practices based on the withdrawal period of MG and LMG. Hence, this research aimed for the following objectives:

1. To validate a modified method for determination of malachite green (MG) and leucomalachite green (LMG) in fish muscle using liquid chromatography-tandem mass spectrometry (LC-MS/MS)

2. To investigate the uptake of MG residues in fish exposed to acute, sub-acute and sub-lethal concentrations of MG under tropical conditions

3. To determine the effects of MG residues on growth, immunological parameters, oxidative stress and antioxidant status in fish exposed to acute, sub-acute and sub-lethal concentrations of MG
4. To assess the withdrawal period of MG and LMG in fish exposed to different treatment regimes of MG
5. To screen residues of MG and LMG in commonly consumed fish from the domestic market

The present study can be hypothesised as follows:

$H_0$: There is no effect on the growth, immunological parameters, oxidative stress and antioxidant status in red tilapia when exposed to MG.

$H_0$: There is no difference on the persistence of MG and LMG residues in red tilapia muscle upon exposure to different treatment regimes of MG.

$H_1$: There are effects on the growth, immunological parameters, oxidative stress and antioxidant status in red tilapia when exposed to MG.

$H_1$: There are differences on the persistence of MG and LMG residues in red tilapia muscle upon exposure to different treatment regimes of MG.

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**BIODATA OF STUDENT**

Kwan Penz Penz was born in Muar, Johor on the 18th of May 1990 and she is the eldest of three siblings. She received her secondary education at MARA Junior Science Collage (MJSC) Kuala Klawang, Negeri Sembilan and MJSC Batu Pahat, Johor. In year 2005 and 2007, she was awarded the best co-curriculum student award for actively representing the state of Johor in Chess competitions. In 2008, she enrolled in Universiti Malaysia Terengganu to pursue her diploma in Fisheries and Bachelor's degree in Science in Agrotechnology (Aquaculture) and she graduated with first-class honours in 2014. She also received gold awards from Novel Research and Innovation Competition (2014) and International Invention, Innovation and Technology Exhibition (2015) for her final year project entitled "*Tetraselmis chuii* beads for home aquarium". Apart from that, she also got 1st place in the Malaysian Undergraduate Competition and 2nd place in the Asia Pacific Undergraduate Competition in the Alltech Young Scientist Program (2014-2015). During her graduation, she was awarded the Vice Chancellor's award and was the valedictorian during the graduation ceremony in 2014. Due to her passion for research work, she furthered her studies in the degree of Doctor of Philosophy in Universiti Putra Malaysia (UPM) with a scholarship from MyBrain15 by Kementerian Pelajaran Malaysia (KPM). She also got 1st place in the 3 minutes thesis (3MT) competition at faculty level and received consolation prize at UPM final competition in 2016.

## LIST OF PUBLICATIONS

### Journal publications

Kwan, P.P., Banerjee, S., Shariff, M., Ishak, N.A.S., Yusoff, F.M. (2018). Quantitative analysis of malachite green and leucomalachite green residues in fish purchased from the markets in Malaysia. Food Control. 92(1): 101-106. https://doi.org/10.1016/j.foodcont.2018.04.031

Kwan, P.P., Banerjee, S., Shariff, M., Yusoff, F.M. (2019). Residual quantification and oxidative stress induced by malachite green after subacute and sublethal exposure in red tilapia. Veterinary World. 12(9): 1416-1421.

Kwan, P.P., Banerjee, S., Shariff, M., Yusoff, F.M. (2019). Persistence of malachite green and leucomalachite green in red tilapia (*Oreochromis* hybrid) exposed to different treatment regimens. Food Control. In Press. https://doi.org/10.1016/j.foodcont.2019.106866

Kwan, P.P., Banerjee, S., Shariff, M., Yusoff, F.M. (2018). An Update on the Use of Malachite Green in Aquaculture: A Review. Reviews in Fisheries Science and Aquaculture. (Submitted 5 Nov 2018)

### Conferences

Kwan, P.P., Ishak, N.A.S., Shariff, M., Banerjee, S. (2017). Malachite green and leucomalachite green residues in local and imported striped catfish, *Pangasius hypophthalmus*. World Aquaculture Society: Aqua Pacific Aquaculture (*APA 2017*). 24-27 July 2017, Kuala Lumpur, Malaysia.

Kwan, P.P., Banerjee, S., Shariff, M., Yusoff, F.M. (2018). Effects of sub-lethal exposure to malachite green on red tilapia (*Oreochromis* hybrid). 3rd International Congress & Expo on Biotechnology and Bioengineering (*ICEBB 2018*). 25-26 June 2018, Kuala Lumpur, Malaysia.

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