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GLYPHOSATE HERBICIDE TOXICITY EFFECTS ON RED HYBRID TILAPIA (*Oreochromis* sp.)

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By

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

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DEDICATION

This thesis is dedicated to my beloved parents.

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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Chairman : Professor Mohd Yunus Shukor, PhD
Faculty : Biotechnology and Biomolecular Sciences

Glyphosate [N-(phosphonomethyl) glycine] is among the most broadly and generally recognised broad-spectrum herbicides used in agriculture due to its low cost and effectiveness in weed management. The pollution of glyphosate in the aquatic environment can be via water run-off from agricultural lands, or by spray drift, aerial spraying or due to industrial discharge, which may be seen as a threat to aquatic biota. Fish is one of the best organisms to study the toxicological aspects of glyphosate. To date, very few studies have been done with high purity glyphosate due to the cost factor, of which this study aims to accomplished. Fish was procured and acclimatized to the laboratory condition within ten days before the commencement of the experiment. A 4 days bioassay was carried out using red hybrid tilapia (*Oreochromis* sp.) as the test organisms to ascertain lethal concentration (LC_{50}) of glyphosate using arithmetic and probit methods. After determining the LC_{50}, fish were exposed to different concentrations (0, 25, 50, 100 and 150 mg/L) of glyphosate herbicide for 4- and 49-days to evaluate its toxicity through changes in fish behaviour, hematological, biochemical, histopathological as well as changes in the growth pattern of the fish. The major physicochemical parameters of the water were ascertained. The water quality parameters during the experiments ranged are temperature (25-29℃), dissolved oxygen (5.3-9.3 mg/L) and pH (6.5-7.4) respectively. The LC_{50} values of glyphosate were 250 mg/L (arithmetic) and 215 mg/L with 162 and 282 mg/L as lower and upper 95% confidence limit (Probit). Fish mortality rate positively correlated with the increase in glyphosate concentration. Glyphosate exerts fewer effects on the behaviour of red hybrid tilapia at low concentrations, but at higher concentrations of 50 mg/L and above various behavioural changes including air gasping, erratic swimming, fin movement, mucus secretions, hemorrhages, and loss of scale were evident. There was significant concentration-dependent decrease between the control and exposed fish in all haematological parameters examined. The total serum protein showed a significant decrease between the control and exposed fish. There was an increase

in the values of total bilirubin, alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase, and gamma-glutamyl transferase in both 4- and 49-days exposure periods. A significant decline was recorded in the cholinesterase enzyme activities in all organs and substrate except the spleen, kidney and gills using PTC substrate during 4 days exposure and gills using BTC substrate during the 49 days glyphosate exposure. BTC and ATC substrates recorded higher activities in the liver and brain both during 4- and 49-days exposure periods. Glyphosate herbicide significantly declined the protein content in all organs during 4- and 49-days toxicity testing with the exception of ovary which shows no significant changes during both toxicity testing. The overall growth performance of the fish was hindered due to exposure to glyphosate herbicide with a reduction in the length (26.3±2.4 to 23.3±1.6 cm), weight (198±5.9 to 175±2.79 g), food conversion ratio (0.21 to 0.02), specific growth rate (4.6 to 4.57), and condition factor (1.28 to 0.95) between the control and glyphosate treated fish. A significant decrease was observed in the hepato-somatic and gonadal-somatic indices of the fish exposed to glyphosate. Glyphosate exposure also resulted in different histopathological changes, with fewer changes in the ovary of red hybrid tilapia. In the liver, hepatopancreas degeneration and its partial detachment from the liver parenchyma, congested blood capillaries, necrosis, hemosiderin deposit, reduced number of mitochondria, pyknosis, degeneration of nucleolus, cytoplasmic vacuolation, and damaged mitochondria result histopathological changes recorded as a result of glyphosate exposure to red hybrid tilapia. Hemosiderin deposit, mitochondria vacuolation, the rapture of the nuclear membrane, margination of chromatin as well as melanin deposit were seen in the spleen of fish. Lifting and congestion of primary and secondary gill lamellae, oedema of interdigitate area, hyperplasia, hypertrophy, mitochondrial degeneration, karyolysis, cellular membrane degeneration were observed in the gills of the fish after glyphosate exposure. Widening of the Bowman's corpuscular space, infiltration of inflammatory cells, tubular degeneration and necrosis, hemosiderin deposit, karyolysis, mitochondria degeneration and vacuolation and dissolution of the cellular membrane were the resulted histopathological alterations seen in the kidney of the fish exposed to glyphosate herbicide. In the brain, partial detachment of the neuronal cells, necrosis of neuronal and glial cells, congestion and dilation of blood vessels, vacuolation of neurophil and glial cells axonal and mitochondrial degeneration and cytoplasmic oedema were the changes observed. Reduced number of previtellogenic follicles and mature yolk granules, increased empty spaces within the mature follicles and degeneration and vacuolation of mitochondria were observed in the ovary of the fish exposed to glyphosate herbicide. According to the findings of this research, exposure of red hybrid tilapia to glyphosate herbicide resulted into overall decline in the health status of the fish. These findings may indicate a health risk for red hybrid tilapia and other aquatic animals exposed to glyphosate in aquatic ecosystems.

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

KETOKSIKAN HERBISID GLIFOSAT DAN KESANNYA KEPADA TISU IKAN TILAPIA MERAH BAKA KACUKAN (*Oreochromis* sp.)

Oleh

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Ogos 2019

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Glifosat [N- (fosfonometil) glisina] adalah salah satu racun herba berspektrum luas yang paling popular dan lazim digunakan dalam bidang pertanian, disebabkan oleh kosnya yang rendah dan keberkesanannya terhadap pelbagai spesies rumpai. Pencemaran herbisid glifosat di dalam air yang mengancam organisma akuatik boleh berpunca daripada larian air dari kawasan pertanian, tempias semburan, penyemburan pesawat atau pelepasan dari aktiviti perindustrian. Ikan adalah salah satu organisma terbaik untuk mengkaji aspek toksikologi glifosat. Sehingga kini, hanya sedikit kajian yang dilakukan dengan menggunakan glifosat pada kemurnian yang tinggi kerana faktor kos, yang mana kajian ini menjadikan aspek ini sebagai tujuan utama. Ikan terlebih dahulu disesuaikan dengan keadaan makmal selama sepuluh hari sebelum eksperimen dijalankan. Bioasai berdurasi 4 hari menggunakan tilapia merah hibrid (*Oreochromis* sp.) sebagai organisma ujian dijalankan untuk menentukan kepekatan maut (LC_{50}) glifosat melalui kaedah aritmetik dan analisis Probit. Setelah penentuan $LC_{50,}$ ikan didedahkan pula kepada pelbagai kepekatan glifosat (0, 25, 50, 100 dan 150 mg/L) selama 4 dan 49 hari untuk menilai ketoksikan glifosat melalui perubahan pada tingkah laku, hematologi, biokimia, histopatologi serta corak pertumbuhan ikan. Parameter fizikokimia air turut diambil kira dan direkodkan. Parameter kualiti air sepanjang esperimen ialah suhu (25-29℃), oksigen terlarut (5.3-9.3 mg/L) dan pH (6.5-7.4). Nilai LC_{50} glifosat yang diperoleh adalah 250 mg/L (aritmetik) dan 215 mg/L (Probit) dengan 162 dan 282 mg/L sebagai nilai had keyakinan 95% rendah dan tinggi. Kadar kematian ikan didapati berkorelasi positif dengan peningkatan kepekatan glifosat. Herbisid glifosat kurang memberikan kesan terhadap tingkah laku tilapia merah pada kepekatan rendah, namun pada kepekatan 50 mg/L dan ke atas, pelbagai perubahan tingkah laku dapat diperhatikan termasuk ikan tercungap-cungap di permukaan, berenang tidak menentu, pergerakan sirip, rembesan mukus, pendarahan, dan kehilangan sisik. Terdapat penurunan bersandarkan kepekatan yang signifikan di antara ikan kawalan dan ikan yang terdedah pada semua parameter hematologi yang

diuji. Jumlah protein serum menunjukkan penurunan yang signifikan antara ikan kawalan dan ikan yang terdedah. Kedua-dua ujian pendedahan 4 dan 49 hari menunjukkan peningkatan pada nilai jumlah bilirubin, alkali fosfatase, aspartate aminotransferase, alanina aminotransferase, dan gamma-glutamyl transferase. Penurunan aktiviti enzim kolinesterase yang signifikan telah dicatatkan dalam semua organ dan substrat yang dikaji kecuali limpa, ginjal dan insang yang menggunakan substrat PTC semasa ujian pendedahan 4 hari, dan insang menggunakan substrat BTC semasa pendedahan glifosat 49 hari. Substrat BTC dan ATC merekodkan aktiviti yang lebih tinggi dalam hati dan otak semasa ujian pendedahan 4 dan 49 hari. Herbisid glifosat turut menyebabkan penurunan kandungan protein yang signifikan dalam semua organ semasa kedua-dua ujian toksisiti 4 dan 49 hari kecuali ovari yang tidak menunjukkan sebarang perubahan yang signifikan. Ikan juga menunjukkan prestasi pertumbuhan keseluruhan yang terbantut setelah pendedahan terhadap glifosat mengakibatkan pengurangan panjang (26.325±2.4 kepada 23.35±1.6 cm), berat (198±5.9 kepada 175±2.79 g), nisbah penukaran makanan (0.21 kepada 0.02), kadar pertumbuhan spesifik (4.677 kepada 4.57), dan faktor keadaan (1.28 kepada 0.95). Ikan yang terdedah kepada glifosat turut menunjukkan penurunan yang signifikan dalam indeks hepato-somatik dan gonado-somatik jika dibandingkan dengan ikan kawalan. Perubahan histopatologi dapat diperhatikan pada tilapia merah hibrid selepas pendedahan kepada glifosat, dengan ovari menunjukkan perubahan yang paling sedikit. Perubahan yang dapat dilihat pada hati misalnya ialah kemerosotan hepatopankreas dan pemisahan separa hepatopankreas daripada parenkima, kongesi kapilari darah, nekrosis, mendapan hemosiderin, penurunan bilangan mitokondria, kemerosotan piknosis nukleolus, pemvakuolan sitoplasma dan kerosakan mitokondria. Limpa ikan pula menunjukkan mendapan hemosiderin, pemvakuolan mitokondria, membran nukleus pecah, marginasi kromatin dan mendapan melanin. Perubahan histopatologi pada insang pula antaranya ialah lamella primer dan sekunder dilihat terangkat dan terdapat kongesi, edema pada kawasan pertemuan, hiperplasia, hipertrofi, kemerosotan mitokondria, kariolisis dan juga kemerosotan membran sel. Peluasan ruang korpuskel Bowman, infiltrasi sel inflamatori, kemerosotan dan nekrosis tubul, mendapan hemosiderin, kariolisis, kemerosotan mitokondria, vakuolasi dan pelarutan membran sel pula adalah perubahan histopatologi yang diperhatikan pada ginjal setelah terdedah pada glifosat. Pemisahan separa sel neuron, nekrosis sel neuron dan glia, kongesi dan pengembangan salur darah, pevakuolan neuropil, kemerosotan akson dan mitokondria pada sel glia, serta edema sitoplasma merupakan perubahan yang dilihat pada otak ikan yang terdedah. Penurunan bilangan folikel previtelogenesis dan granul yolka matang, peningkatan ruang kosong dalam folikel matang, serta kemerosotan dan pemvakuolan mitokondria adalah perubahan yang diperhatikan pada ovari tilapia yang terdedah pada herbisid glifosat. Berdasarkan hasil kajian ini, boleh disimpulkan bahawa pendedahan tilapia merah hibrid kepada herbisid glifosat menjejaskan kesihatan ikan secara keseluruhannya. Penemuan ini menunjukkan glifosat mengakibatkan risiko kesihatan untuk tilapia merah dan juga haiwan lain dalam ekosistem akuatik.

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Umar Abubakar Muhammad.

This thesis was submitted to the Senate of the 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:

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LIST OF ABBREVIATIONS

AChE	Acetylcholinesterase enzyme
ATC	Acetylthiocholine iodide
ALT	Alanine aminotransferase
AP	Alkaline phosphatase
AMPA	Aminomethylphosphonic acid
et al	and friends
AST	Aspartate aminotransferase
BSA	Bovine serum albumin
BTC	Butyrylthiocholine iodide
BuChE	Butyrylcholinesterase enzyme
cm	Centimetre
ºC	Degree Celcius
DNA	Deoxyribonucleic acid
DCA	Dichloroaniline
DO	Dissolved oxygen
dH_2O	Distilled water
DTNB	5, 5-dithio-bis-2-nitrobenzoate
FAO	Food and agriculture organization
GGT	Gamma-glutamyl aminotransferase
GSI	Gonado-somatic index
G	Gram
>	Greater than
HGB	Hemoglobin
HSI	Hepato-somatic index
h	Hour

IFCC	International federation of clinical chemistry
IPA	Isopropylamine
kg	Kilogram
LC	Lethal concentration
<	Less than
L	Liter
MCV	Mean corpuscular volume
MCH	Mean corpuscular hemoglobin
MCHC	Mean corpuscular hemoglobin concentration
µL	Microlitre
µm	Micrometer
mg/L	Miligram per liter
mL	Mililitre
mM	Milimolar
min	Minute
M	Molar
MW	Molecular weight
nm	Nanometre
OP	Organophosphate
PCV	Pack cell volume
PPM	Parts per million
%	Percent
PMSF	Phenylmethylsulfonyl fluoride
PLT	Platelet
POEA	Polyethoxylated tallow amine
PTC	Propionylthiocholine iodide

PchE	Propionylcholinesterase enzyme
RBC	Red blood cell
RTD	Roundup Transcorp
TEM	Transmission electron microscope
U	Unit
WBC	White blood cell
WHO	World Health Organisation

CHAPTER 1

GENERAL INTRODUCTION

1.1 Research background

The exponential increase of human population coupled with development and maximization of agricultural activities led to the increase in chemical herbicides application in agriculture (Gholami-seyedkolaei et al., 2013; Yancheva et al, 2016), which results in a discharge of huge quantity pollutants into aquatic system which is posing a serious threat to aquatic animals including fish (Ayoola, 2008b). The use of pesticides is increasing drastically for the protection of crops against pest and weeds; this may probably harm the non-target aquatic animals like fish (Samanta and Pal, 2016).

Herbicides are generally applied in the control of weeds which may impede the flow of aquatic life and can contribute long-term effects to the environment (Ladipo et al., 2011; Sani and Idris, 2016). Herbicides are considered the biggest proportion of chemical pesticides been used in agriculture globally (Solomon et al., 2013). The global annual pesticide usage is estimated to be 5.6 billion pounds (World ecology Report, 2019), and biggest proportion of about 40% is herbicide (Sihtmäe et al., 2013; US EPA, 2012). Herbicides are mostly used in agriculture and in other areas like highway vegetation control, forestry, industries, and urban bush clearing. Herbicide surface runoff eventually goes into the rivers and streams and accumulated in water bodies to levels that harm planktonic species, the major source of food for many fish (Ladipo et al., 2011).

Glyphosate is among most known and widely recognised broad-spectrum herbicides mostly applied in agriculture due to its low cost and high effectiveness for weed control and management (Eschenburg et al., 2001; Alvarez-moya et al., 2014; Sani and Idris, 2016). The incidence of glyphosate herbicides in an aquatic environment is due to water run-off from agricultural lands, or by spray drift, aerial spray or due to industrial discharge, which may be seen as a hazard to aquatic biota (Samanta et al., 2016a). Glyphosate has been widely applied for the control of weed in the cultivation of wide varieties of plants such as grains and legumes. A huge number of available herbicides that contains glyphosate as the major ingredient are generally accepted by farmers due to their usefulness for weeds control (Zhang et al., 2017b; Jasper et al., 2012). Glyphosate is said to be site-specific, but its use can also reduce the photosynthetic ability of many plants (Ahsan et al., 2008). Monsanto as the first manufacturers of glyphosate, declared it as a harmless herbicide to the environment, which is the main purpose for its wide acceptance with no limitations. Later, some scientists detected its negative constraint following its poisoning report in Latin America after an aerial spray (Watts, 2009).

The continuous movement of glyphosate and other related aquatic pollutants into aquatic habitats can influence aquatic inhabitants in different ways from the death of fish when exposed to high dose or it can at lower doses to slowly poison aquatic inhabitants (Ullah and Zorriehzahra, 2014). Despite its widespread application, the eco-toxicological evidence on glyphosate (technical grade) is inadequate. There is comprehensive information on short-term toxicity of glyphosate on many fish species. However there is little data available on its long-term toxicity (Jiraungkoorskul et al., 2003).

Glyphosate is considered somewhat harmful to birds, fish, and many aquatic animals and it does not bioaccumulate in the food web; however, the surfactants present in glyphosate considered as more harmful than the original glyphosate (Battaglin et al., 2005). Glyphosate pollution due to Roundup application in aquatic environment range from 0.01 to 0.7 mg/L and can reach up to 1.7 mg/L in the aquatic habitats in certain circumstances due to the direct herbicide spray application (Ayumi et al., 2013). Although many researchers reported that glyphosate low toxicity to human, its prevalent and unselective usage as well as its tendency to pollute the aquatic environment are of a major tconcern (Lushchak et al., 2009). Various concentrations of glyphosate in different formulations of herbicide containing up to 480 g/L of glyphosate (48%) and polyoxyethylamine are the real harmful consequence of glyphosate (Gianinni et al., 2013). The dissemination of glyphosate into the ground, water, and sediment renders it challenging to estimate the future environmental destruction triggered by glyphosate long term application (Loro et al., 2015). The time taken for glyphosate to disappear in water is stated to be between 7 and 14 days (Giesy et al., 2000). Fish exposed to glyphosate resulted in the abnormal physical behaviour and other changes. These comprise of unusual movement, air gasping and different other physical anomalies (Kaur and Dua, 2015; Oluwatoyin et al, 2015; Zuberi et al, 2014; Gupta and Dua, 2002; Adedeji et al., 2008).

In current times, different xenobiotic materials drained into the water environment, these including pesticides, heavy metals and other pollutants that have been speedily increased due to agricultural, industrial as well as domestic discharges (Sekabira et al., 2010). Water pollution poses a danger to aquatic biota, which attracted many scientists the world over (Yancheva et al., 2016). The non-ionic constituent surfactants in herbicide preparations is designed to aid the effectiveness of active constituents and to facilitate the passage of chemicals into the plant's cuticle and also present a potential toxic (Brausch and Smith, 2007).

Fish is considered as the primary and cheapest source of protein for many people. In Malaysia, there is a rise in fish production as well as its consumption from 49 kg to 53 kg per capita between the year 2000 to 2005 (Abdi et al., 2012). Tilapia is considered among the most consumed warm water fishes (He et al., 2016). Their capability to tolerate a wide variety of environment and different cultivation systems yielded a fast expansion of its farming and its introduction to subtropical and sometimes even the temperate regions of the globe (Vander et al., 2013). It is regarded as a globally important fish as a result of its fast growth rate, easy handling, palatability and easy reproduction in a controlled environment (He et al., 2016). *Oreochromis* spp (tilapia) is regarded as a potential bioindicator in aquatic toxicology studies due to its worldwide distribution and acceptance (Iva et al., 2007; Muttappa et al., 2015). Red hybrid tilapia is cultivated in aquatic habitats such as reservoirs, lakes, paddy fields, former mining areas and irrigation canals (Hamzah et al., 2008). Red hybrid tilapia was chosen because it is readily available and it is used just as representative experimental species for study.

1.2 Problem statement

Globally, about 800 million people suffer from malnutrition and the world population might reach 9.6 billion in 2050 with a high concentration in coastal regions which mostly relied on fish as a source of protein (FAO, 2018). This issue poses too much pressure on fish and fisheries resources of the world. Fisheries and aquaculture provide not only protein for the improvement of the health status of human beings, but can also provide employment for wealth generation as fish are considered most commercially important commodities the world over (FAO, 2018). Fish is considered a high-quality food due to its high protein content, a rich source of vitamins and has different quantities of fats as well as calcium for improving human health condition (Ali and Abubakar, 2015). Inland fisheries are regarded as the cheapest source of protein, especially to the rural populace (Abubakar et al., 2006; Sandun et al., 2015). There is an increase in demand in reducing the impacts of anthropogenic activities on the water bodies. The use of fish as bioindicator for glyphosate pollution can provide information necessary in realizing this demand (Karami et al., 2012).

Experts recommend fish over many sources of animal protein as it is cheaper compared to other protein sources. Fish can live only in water, which is not free from various sources of pollution mostly from anthropogenic activities which involve the use of chemicals in agriculture, industries, municipal wastes that pass through run-off into the aquatic environment and render it unfit for the survival of many aquatic organisms including fish. Identifying the effect of a particular chemical in a natural water body is difficult as many chemicals dissolved in water and deposited into streams and rivers through run-off (Anitha and Rathnamma, 2016; Narra, 2016).

Malaysia is one of the producers and users of many herbicides including glyphosate and therefore this compound is expected to be found in natural water bodies of Malaysia. Glyphosate is used as an ingredient in many herbicides which make it the most broadly used herbicide. Subsequently, after its initial introduction in the early seventies its application increased 100-times, more than 600,000 tons of glyphosate is approximately used globally (Zhang et al., 2017b). Investigation on biochemical and physiological constraints of glyphosate against fish can give important ecotoxicological data and also assist in choosing more biomarkers of glyphosate pollution and its constraints to fish species.

Red hybrid tilapia contributed approximately 90% of total tilapia production in Malaysia; its production expanded from 8,214 tonnes in 1998 to 20,061 tonnes in 2003 (Hamzah et al., 2008). There is scanty or no information about the toxicity of technical grade glyphosate on red hybrid tilapia, which is one of the commercial fish species consumed in Malaysia. The choice of red hybrid tilapia for this research is due to its availability and just as a representative species and not because of its exposure to glyphosate pollution as most Malaysian fish farms are far from agricultural farmlands. Jayasumana et al, (2014) reported that Sri Lanka and El Salvador banned the use of glyphosate due to its link to fatal chronic kidney diseases. In Malaysia, about 2.5 million people were diagnosed with kidney-related problems which might be attributed to the uncontrolled usage of glyphosate (Idris, 2016). In this present study, tissues of red hybrid tilapia were used as biomarkers to examine the consequences of glyphosate herbicide via behavioural, morphological, histopathological and biochemical alterations in fish, which is very useful for environmental pollution monitoring. The information generated from this study would be essential for aquaculture and fisheries, and environmental development in Malaysia and the world in general because glyphosate and its formulations are now sprayed globally in order to improve agriculture.

1.3 Hypothesis

This research aims at testing the following hypothesis;

- Glyphosate affects the behaviour of red hybrid tilapia
- Glyphosate affects the haematological profile and serum enzymes of red hybrid tilapia
- Glyphosate affects the cholinesterase enzyme activity and protein content of red hybrid tilapia
- Glyphosate affects the histopathological profiles of tissues of red hybrid tilapia
- Glyphosate affects growth performance and somatic indices of red hybrid tilapia.

1.4 Research questions

- Does glyphosate exposure affect the behaviour of red hybrid tilapia, and how do the effects are different from or similar to other fish species from the literature?
- Does glyphosate exposure affect the haematological profiles and serum enzyme levels of red hybrid tilapia, and how do the effects are different from or similar to other fish species from the literature?
- Does glyphosate exposure affect the cholinesterase enzyme activity and protein content of red hybrid tilapia, and how do the effects are different from or similar to other fish species from the literature?

- Does glyphosate exposure affect the histopathological profiles of tissues of red hybrid tilapia, and how do the effects are different from or similar to other fish species from the literature?
- Does glyphosate exposure affect the measured growth performance and somatic indices of red hybrid tilapia, and how do the effects are different from or similar to other fish species from the literature?
- Are these effects observed can be a justification for the use of red hybrid tilapia as a sentinel organism?

1.5 Objectives

Given the need for information of side-effects of glyphosate herbicide, this study aims to ascertain the effects of glyphosate on tissues of hybrid red hybrid tilapia.

The objectives of this study were;

1. To determine the lethal concentration (LC_{50}) and resulted in behavioural changes induced by glyphosate to red hybrid tilapia
2. To assess the effects of glyphosate on haematological profile and some serum enzymes of red hybrid tilapia
3. To determine the effects of glyphosate on cholinesterase enzyme (ChE) activity and protein content in tissues of red hybrid tilapia
4. To evaluate the effects of glyphosate herbicide on the histopathological profile of tissues of red hybrid tilapia
5. To ascertain the effects of glyphosate exposure on growth performance and somatic indices of red hybrid tilapia

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Umar Abubakar Muhammad was born into the family of Malam Umar Mahdi and Amina Muhammad in 1981, at Kumo town Bauchi State, Federal Republic of Nigeria. He started primary education at Akkoyel Primary school Kumo in 1987 and completed in 1993. He proceeded to Government Science Secondary School Gombe in 1993 and successfully completed in 1999. He attended a one-year remedial science programme in the University of Maiduguri between 2000 and 2001 and passed all the prescribed courses to enable him admitted into a Bachelor of Science programme in the Department of Biological Sciences in 2002. He completed the bachelor program in 2007 and awarded BSc. (Hons) in Environmental Biology. He attended a one-year compulsory National youth service corps in Akwa Ibom State in 2008. He secured a temporary appointment as a graduate assistant in Gombe State University in 2009. He was admitted into Modibbo Adama University of Technology Yola, Adamawa State in 2010 and awarded a Master of Technology (M. Tech) in Applied Hydrobiology and Fisheries in 2014. Upon completing a master programme in 2014, his appointment was confirmed by the governing council of Gombe State University before joining the Universiti Putra Malaysia in 2016. Umar is happily married.

LIST OF PUBLICATIONS

Abubakar M. Umar, Tham Lik Gin, Natarajan Perumal, Hassan Mohd Daud, Nur Adeela Yasid and Mohd Y. Shukor. Assay for Heavy Metals Using Inhibitive Assay Based on the Acetylcholinesterase from Clarias batrachus. 2016. Journal of Bioremediation Science and Technology Research. Vol. 4, No 2. 6-10.

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