



**Advanced studies on some chemical pollutants
mostly detected in some fish farms**

by

Samaa Tarek Mohammed

(M.V.Sc., 2016, Suez Canal University)

Thesis submitted

**For the degree of Ph D of Vet. Medical
Science**

(Animal, Poultry and Environmental Hygiene)

To

**Department of Animal Hygiene, Zoonoses and Behavior,
Suez Canal University**

(2021)

Author	Samaa Tarek Mohammed Mohammed
Title	Advanced studies on some chemical pollutants mostly detected in some fish farms
Faculty	Veterinary Medicine
Location	Ismailia
Department	Animal Hygiene, Zoonoses and Animal Behavior
Degree	Ph.D.Sc.Degree(Animal, Poultry and Environmental Hygiene)
Date	2021
Language	English
Supervisor	Prof. Dr. Ahmed Mohamed Ahmed Hassan Prof. Dr. Mona Mohamed Abdel-Wahab Dr.Marwa Abd El- Moneim Mohamed Hassan
Abstract	
<p>The present study aimed to investigate the most popular pollutants (malathion and glyphosate) residue in water samples (84 sample) collected from different fisheries at summer season. Then, studying the effect of malathion and glyphosate on fish and role of dietary organic selenium in improving health status of exposed tilapia. Results during the field survey revealed higher levels of malathion than permissible limits in almost fish farms while glyphosate levels was within acceptable levels in all fish farms. A total of 210 apparently healthy fresh tilapia (<i>Oreochromis niloticus</i>) with average body weight 14 ± 0.5 g were adapted under laboratory conditions for two week and then they were randomly divided into 7 groups: CON: served as control, GLY: (glyphosate 2 mg /L and commercial diet), MAL: (malathion 0.5mg /L and commercial diet), GLY+ OSe: (glyphosate 2 mg /L and OS diet), MAL+ OSe: (malathion 0.5mg /L and OS diet), GLY+MAL+ OSe: (malathion 0.3 mg/L and glyphosate 1.6mg/L and OS diet) and GLY+MAL: (malathion 0.3 mg/L and glyphosate 1.6mg/L and commercial diet). The examined pollutants impact were investigated at different periods of the experiment (30, 45 and 60 days) by measuring the following parameters: Growth performance index (body weight gain and FCR), some hematological (Hb, RBCs, Ht, Thrombocytes and WBCs) and biochemical parameters (Total protein, Albumin, AST, ALT, Urea and Creatinine), anti-oxidant status (MDA, SOD and GPX) and finally fish were experimentally infected with <i>Aermonus hydrophilia</i>, then observed and examined for <i>A. hydrophilia</i> count in liver and kidney samples. The results revealed that the hazards caused by malathion were more prominent than that caused by glyphosate, malathion was more toxic to tilapia than glyphosate. Adding of organic selenium (0.8 kg^{-1} diet) was capable to enhance fish antioxidant status and relief the damage caused by malathion and /or glyphosate</p>	
<p>Key words : Malathion- Glyphosate-Organic selenium - Growth - Tilapia- Immunity- oxidative stress- <i>Aermonus hydrophilia</i>.</p>	

List of Contents

No.	Subject	Page No.
1	Introduction	1
2	Review of Literature	5
3	Materials and Methods	32
4	Results	53
5	Discussion	104
6	English Summery	148
7	Conclusion	157
8	References	158
9	Arabic Summery	

List of Abbreviation

Abbreviation	Expression
AChE	Acetylcholine esterase
Ag NO ₃	Silver nitrate
ALT	Alanine amino transferase enzyme
AST	Aspirate amino transferase enzyme
B. wt	Body weight
BWG	Body weight gain
CAT	Catalase
Cl ⁻	Chloride
CNS	Central nervous system
CON	Control
EC	Electric conductivity
EDTA	Ethylene Diamine Tetra Acetic acid
EPA	Environmental protection agency
FCR	Feed conversion ratio
GLY	Glyphosate
GPx	Glutathione peroxidase enzyme
GR	Growth rate
GRx	Glutathione Reductase
GSH	Glutathione
GT	Glutathione Transfease
Hb	Hemoglobin
HCL	Hydrochloric acid
HPI	Hypothalamic Pituitary Interrenal
K ₂ CrO ₄	Potassium chromate
LDH	Lactate dehydrogenase enzyme
MAL	Malathion

Abbreviation	Expression
MCH	Mean corpuscular hemoglobin
MCHC	Mean corpuscular hemoglobin Concentration
MCV	Mean corpuscular volume
MDA	Malondialdehyde
Na₂CO₃	Sodium Carbonate
Ngg⁻¹	Nano gram per gram
NH₄	Ammonia
NO₃	Nitrate
O Se	Organic selenium
OC	Organo chlorines
OP	Organophosphate
PER	Protein efficiacy ratio
ppb	Part per billion
ppm	Part per million
RBCs	Red blood cells
ROS	Reactive oxygen species
rpm	Rotation per minute
SGR	Specific growth rate
SOD	Super oxide dismutase enzyme
SR	Survival rate
TDS	Total dissolved solids
TP	Total protein
USEPA	United state environmental protection agency
WBCs	White blood cells

List of Tables

		Page no.
Table 1	Topographical examination of the studied fish farms.	33
Table 2	Formulation and proximate analysis of the experimental diets (g/kg total diet).	41-42
Table 3	Phsico- chemical parameters of water in examined fish ponds at different fish farms	53
Table 4	Standard water quality parameters for aquaculture..	54
Table 5	Average of malathion and glyphosate ($\mu\text{g/l}$) in the examined fish ponds in the different fish farms.	55
Table 6	Component Matrix for different physicochemical parameters of fish farm water in relation to malathion.	56
Table 7	Component Matrix for different physicochemical parameters of fish farm water in relation to glyphosate.	57
Table 8	Probability for half lifetime of malathion and glyphosate mg/l in natural log days using Probit analysis.	58
Table 9	Probability probit for LC_{50} for malathion and glyphosate.	60
Table 10	Effect of malathion and /or glyphosate on Nile tilapia behavioral changes and ameliorative effect of organic selenium supplementation (Mean \pm SE).	61
Table 11	Effects of malathion and /or glyphosate on average growth performance parameters in Nile tilapia and ameliorative effect of organic selenium supplementation (Mean \pm SE).	62
Table 12	Evaluation of malathion and/or glyphosate on some growth performance parameters in Nile tilapia at Day 15and ameliorative effect of organic selenium supplementation (Mean \pm SE).	63
Table 13	Effects of malathion and/or glyphosate on some growth performance parameters in Nile tilapia at Day 30and ameliorative effect of organic selenium supplementation (Mean \pm SE).	64
Table 14	Effects of malathion and/or glyphosate on some growth performance parameters in Nile tilapia at Day 45and ameliorative effect of organic selenium supplementation (Mean \pm SE).	65
Table 15	Effects of malathion and/or glyphosate on some growth performance parameters in Nile tilapia at Day 60 and ameliorative effect of organic selenium supplementation (Mean \pm SE).	66

Table 16	Effects of malathion and/ or glyphosate on total protein ((g/dL)in Nile tilapia and ameliorative effect of organic selenium supplementation (Mean \pm SE).	74
Table 17	Effect of malathion and/ or glyphosate on albumin (g/dL)in Nile tilapia and the ameliorative effect of organic selenium. supplementation (Mean \pm SE).	75
Table 18	Effect of malathion and/ or glyphosate on globulin (g/dL) in Nile tilapia and ameliorative effect of organic selenium supplementation (Mean \pm SE).	76
Table 19	Effect of malathion and/ or glyphosate on A/G ratio in Nile tilapia and ameliorative effect of organic selenium supplementation (Mean \pm SE).	77
Table 20	Effect of malathion and/ or glyphosate on ALT and AST in Nile tilapia and ameliorative effect of organic selenium supplementation (Mean \pm SE).	78
Table 21	Effect of malathion and/ or glyphosate on creatinine and urea in Nile tilapia and ameliorative effect of organic selenium supplementation (Mean \pm SE).	80
Table 22	Effect of malathion and/ or glyphosate on average values of (RBCs, HB, HT, MCH, MCHC, MCV) in Nile tilapia and ameliorative effect of organic selenium supplementation (Mean \pm SE).	82
Table 23	Effect of malathion and/ or glyphosate on some hematological parameters in Nile tilapia at day 30and ameliorative effect of organic selenium supplementation (Mean \pm SE).	83
Table 24	Effect of malathion and/ or glyphosate on some hematological parameters in Nile tilapia at day 45and ameliorative effect of organic selenium supplementation (Mean \pm SE).	84
Table 25	Effect of malathion and/ or glyphosate on some hematological parameters in Nile tilapia at day 60and ameliorative effect of organic selenium supplementation (Mean \pm SE).	85
Table 26	Effect of a malathion and/or glyphosate on some hematological parameters (WBCs, neutrophils , lymphocyte and Monocyte) in Nile tilapia and ameliorative effect of organic selenium supplementation (Mean \pm SE).	87-88
Table 27	Hazard effects of malathion and/or glyphosate on blood platelets($10^3/mm^3$)in Nile tilapia and ameliorative effect of organic selenium supplementation (Mean \pm SE).	90
Table 28	Effect of malathion and/ or glyphosate on antioxidant status in liver of Nile tilapia and ameliorative effect of organic selenium supplementation (Mean \pm SE).	91

Table 29	Effect of malathion and/ or glyphosate on antioxidant status in kidney of Nile tilapia and ameliorative effect of organic selenium supplementation (Mean \pm SE).	93
Table 30	Cumulative survival of Nile tilapia exposed to malathion and /or glyphosate in absence and presence of organic selenium supplementation.	95
Table 31	Effect of malathion and/ or glyphosate on fish morbidity and mortality challenged with <i>Aeromonas hydrophilia</i> and ameliorative effect of organic selenium supplementation.	96
Table 32	Bacterial count (log CFU/ml) in liver and kidney of experimental challenged fish with <i>Aeromonas hydrophilia</i> (Mean \pm SE).	98
Table 33	Physico-chemical parameters of water in experimental fish groups (Mean \pm SE).	100

List of Figures

Figure (1)	Average of nitrite (NO₂) and nitrate (NO₃) mg/l in the examined fish ponds at different farms.
Figure (2)	Average of malathion and glyphosate (µg/l) in the examined fish ponds in the different fish farms
Figure (3)	Principal component plot for different physico-chemical parameters of fish farm water in relation to malathion.
Figure (4)	Principal component plot for different physico-chemical parameters of fish farm water in relation to glyphosate.
Figure (5)	Probability for the half lifetime of malathion and glyphosate mg/l in natural log days using Probit analysis.
Figure (6)	Probability probit for LC₅₀ for malathion and glyphosate.
Figure (7)	Initial weight and Final body weight (FBW) for fish in all experimental groups.
Figure (8)	Body weight for fish in all experimental groups at different experimental periods.
Figure (9)	Body Weight Gain (BWG) for fish in all experimental groups during different experimental periods.
Figure (10)	Feed Intake (FI) for fish in all experimental groups at different experimental periods.
Figure (11)	Specific Growth Rate (SGR) for fish in all experimental groups during the different experimental periods.
Figure (12)	Feed Conversion Ratio (FCR) for fish in all experimental groups at 15, 30, 45, and 60 days and overall mean.
Figure (13)	Protein Efficiency Ratio (PER) for fish in all experimental groups at 15, 30, 45, and 60 days.
Figure (14)	Total protein in Nile tilapia exposed to malathion and/ or glyphosate intoxication with or without organic selenium supplementation.

Figure (15)	Albumin in Nile tilapia exposed to malathion and/ or glyphosate with or without organic selenium supplementation.
Figure (16)	Globulin in Nile tilapia exposed to malathion and/ or glyphosate with or without organic selenium supplementation.
Figure (17)	Albumin: Globulin ratio in Nile tilapia exposed to malathion and/ or glyphosate with or without organic selenium supplementation.
Figure (18)	ALT and AST in Nile tilapia exposed to malathion and/ or glyphosate with or without organic selenium supplementation.
Figure (19)	Creatinine and urea in Nile tilapia exposed to malathion and/ or glyphosate with or without organic selenium supplementation.
Figure (20)	Role of organic selenium as a feed additive in reducing hazard effects of malathion and /or glyphosate in Nile tilapia RBCs and Hb
Figure (21)	Differential leucocytic count in Nile tilapia and role of organic selenium as a feed additive in reducing hazard effects of a malathion and/or glyphosate.
Figure (22)	Blood Platelets in Nile tilapia and role of organic selenium as a feed additive in reducing hazard effects of a malathion and/or glyphosate.
Figure (23)	Organic selenium as an antioxidant in liver MDA, SOD and GPX of Nile tilapia exposed to malathion and/or glyphosate.
Figure (24)	Organic selenium as an antioxidant in kidney MDA, SOD and GPX of Nile tilapia exposed to malathion and/or glyphosate.
Figure (25)	Cumulative survival Rate for fish in all experimental groups exposed to malathion and /or glyphosate in absence and presence of organic selenium supplementation.
Figure (26)	Effect of malathion and / or glyphosate on fish morbidity % challenged with <i>Aeromonas hydrophilia</i> with the role of organic selenium supplementation.

Figure (27)	Effect of malathion and/ or glyphosate on fish survival and mortality % challenged with <i>Aeromonas hydrophilia</i> with the role of organic selenium supplementation.
Figure (28)	Bacterial count (log CFU/ml) in liver and kidney of experimental challenged fish with <i>Aeromonas hydrophilia</i>.

List of photos:

Photo (1):	Effect of malathion in <i>Oreochromis niloticus</i>.
Photo (2):	Experimentally infected <i>Oreochromis niloticus</i> with <i>Aeromonas hydrophilia</i> exposed to malathion combined with glyphosate.
Photo (3):	Glyphosate effect on post mortum lesion in experimentally infected fish with <i>Aeromonas hydrophilia</i>.