Malathion Toxicity: Effect on Some Metabolic Activities in *Oreochromis Niloticus*, the Tilapia Fish

Gehan H. Fahmy

***Abstract*—The toxic effect of the insecticide malathion on oxygen consumption and some biochemical characteristics (total protein, carbohydrate and cholesterol in liver, muscle, kidney and gills) of the tilapia fish (*oreochromis niloticus*) were estimated. The data shows that the rate of oxygen consumption was declined during all the exposure periods. On the other hand, all biochemical's parameters were found to be decreased in all tissues on comparison with control. The results indicated the toxic nature of the insecticide malathion.**

***Index Terms*—Insecticides, malathium, tilapia fish.**

I. Introduction

With the increasing industrialization human beings are continuously disturbing the delicate ecological balance in aquatic ecosystems. Pesticides are mainly synthetic organic compounds that are deliberately introduced into the environment to control selected organisms. Organophosphorous insecticides are used throughout the world for control of agricultural and domestic insect pests. Organophosphorous insecticides are employed in medicine and industry, because of their relatively low persistence due to biodegradability [1]-[2].

Saudi Arabia is the largest date producer in the world, with production amounting to 800,000 tonnes per year [3]. Recently, a number of acaricides were registered at the Ministry of Agriculture in Saudi Arabia to control the mite *oligonychus afrasiaticus*, which infest dates and causes severe damage. Malathion an organophosphate insecticide used in Saudi Arabia to control motile stages of mites and some other insects on fruits and vegetables and has limited plant systemic activity. Due to the large amount of dates consumed by Saudi residents (an average of 10 dates daily per person), the search for safe pesticides with negligible residual deposits has always been preferred [3].

Fish is highly nutritious, easily digestible and much sought after food. Nutritional value of fish depends on their biochemical composition which is affected by the water pollution. Alterations in biochemical components as response to environmental stress are authenticated by many authors; Tilak et al. [4] in *labeo rohita*, Alkhail, et al. [5] in *oreochromis niloticus*, arockia and mitton [6] in *oreochromis mossambicus*, venkateswara [7] in *oreochromis mossambicus*, sweilum [8] in Nile tilapia (*oreochromis niloticus* L.), and patnaik [9] in *clarias batrachus*. The aim of this study to investigate the effect of malathion on some biochemical aspects of Tilapia Fish (*oreochromis niloticus*) that may be used as environmental biological indicators of pollution.

Among organophosphorothioate (OPT) pesticides, malathion is considered relatively safe for use in mammals. Its rapid degradation by carboxylesterases competes with the cytochrome P450 formation of malaoxon, the toxic metabolite. However, impurities in commercial formulations are potent inhibitors of carboxylesterase, allowing a dramatic increase in malaoxon formation [10].

Malathion has been used in malaria eradication programs in Africa and Central America or in wide-scale pest control, including the Mediterranean fruit fly in the Unites States, through aerial applications. The reason for such widespread use lies in its relatively low toxicity to mammals and high selectivity toward insects, paralleled by a moderate persistence in the environment, when compared with other OPTs [11].

Malathion toxicity, in a manner similar to all OPTs, depends on its bioactivation to the toxic metabolite malaoxon [12]-[13], which inhibits acetylcholinesterase [14], causing the accumulation of acetylcholine within synapses and the consequent overstimulation of postsynaptic receptors. Impurities, such as isomalathion and various trimethylphosphorothioate esters, present in the technical grade malathion or formed during storage, can potentiate malathion-induced toxicity up to 10-fold [15]-[16] and have been considered responsible for other effects, including DNA lesions [17]-[18].

II. Materials and Methods

A commercial formulation of malathion (agrothion 57% EC 500gl^{-1}) was purchased from a local market in Saudi Arabia and was used in this study. The main structural characteristics and structural formula of Malathion were presented in Fig. 1 and TABLE I.

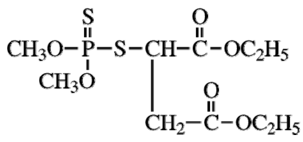

Fig. 1. Structural formula of Malathion

For the present study, Fresh water fish Tilapia (*oreochromis niloticus*) were collected from Al-Hassa spring channel and experiment was conducted in the laboratory at Department of Biological Sciences, College of Sciences, King Faisal University during summer 2010. A stock solution of 1000 ppm (mg/ml) malathion was prepared in

Manuscript received December 31, 2011.
Gehan H. Fahmy is with the Department of Biological Sciences, College of Science, King Faisal University, Saudi Arabia (Tel.: +966-564-761-703; fax: +966-3-588-6534; e-mail address: jfahmi@kfu.edu.sa).

acetone. Required dilutions of the acetone formulation were made with tap water. Acetone in the quantity used was not toxic to fish. The fishes were acclimated to the laboratory temperature (23±0.5) in large glass aquarium. The fish were fed twice daily with 38% protein commercial fish food (CP-Pet; Pokphand Animal Food Co. Ltd., Bangkok, Thailand). The period of acclimation lasted for 2 weeks. Batches of 10 healthy fishes were exposed to different concentrations of insecticide malathion to calculate the medium lethal concentration LC_{50} value using probit analysis method [19]. The fishes (Four groups) were exposed to the sublethal concentration (0.5 ppm) of malathion for 24, 48, 72 and 96 h respectively.

TABLE I: THE MAIN STRUCTURAL CHARACTERISTICS OF MALATHION.

Characteristics	Information
CAS Nomenclature	Diethyl[(dimethoxyphosphino -thioyl)thio]butanedioate
Common name	Malathion
Chemical formula	$C_{10}H_{19}O_6PS_2$
Molecular weight	330.36
Color	Colorless liquid Deep brown to yellow
Physical state	Liquid
Melting point	2.9 EC
Boiling point	156–157 EC
Density	at 25 EC 1.23 g/cm^3
Solubility: Water at 20 EC	145 mg/L

Another group was maintained as control. At the end of each exposure period, fishes were sacrificed and tissues such as liver, gill, muscle and kidney were dissected and removed. The tissues (10 mg) were homogenized in 80% methanol, centrifuged at 3500 rpm for 15 min and the clear supernatant was used for the analysis of total proteins, carbohydrates and cholesterol. Total Protein concentration was estimated by the method of Lowry [20]. Total carbohydrate in the tissue was estimated by the method described by Hedge and Horfreiter [21]. Cholesterol was estimated based on enzymatic method using cholesterol esterase, cholesterol oxidase and peroxides [22]. Changes in the rate of oxygen consumption of the fishes were evaluated at different exposure periods. The rate of oxygen consumption was estimated by Winkler's method [23]. Results were tested by one-way Analysis Of Variance(ANOVA). ANOVA effects and treatments differences were considered significant when $p \leq 0.05$.

III. RESULTS AND DISCUSSION

Environmental and chemical stress can interfere with physiological and biochemical functions such as growth, development, reproduction and circulatory system in fish. Numerous biochemical indices of stress have been proposed to assess the health of non-target organisms exposed to toxic chemicals in aquatic ecosystem [24]. However, it has been reported that apart from nervous tissue, tissues like blood, liver and gills also contribute information in the detection of toxic symptoms caused by certain groups of pesticides [25]. After exposing *Cyprinus carprio* to Malathion a decrease in protein content and an increase in free amino acid and protease activity levels were observed [26].

TABLE II: RATE OF OXYGEN CONSUMPTION (ML/G/HR) OF TILAPIA FISH (OREOCHROMIS NILOTICUS) EXPOSED TO 0.5 PPM OF MALATHION AT DIFFERENT TIMES OF EXPOSURE. MEANS + SD (N=4), *P ≤ 0.05

	Exposure Time (h)			
Treatment	**24**	**48**	**72**	**96**
Control	0.61± 0.01	0.64 ± 0.01	0.68 ± 0.01	0.60 ± 0.00
Malathion	0.342 ± 0.01*	0.341 ± 0.02*	0.344 ± 0.01*	0.322 ± 0.00*

The rate of oxygen consumption (ml/g/hr) of tilapia fish (*oreochromis niloticus*) exposed to the sublethal concentrations of malathion for different exposure periods are shown in TABLE II. The data shows that the rate of oxygen consumption was declined all over the exposure periods. Several authors [27] reported that the disturbance in oxidative metabolism leads alteration in completely animal oxygen consumption in different species of fish exposed to pesticides. Total oxygen consumption is one of the indicators of the general health of the fish. It may also be useful to assess the physiological state of an organism. Respiratory activity of a fish is often the first physiological response to be affected by the presence of contaminants in the aquatic environment. Although many biological early warning systems monitor abnormal opercular movement as an indicator of respiratory stress, a more direct measurement of stress in this sense necessitates the quantification of oxygen consumed by the fish [27].

The changes in biochemical's parameters such as carbohydrates, proteins and lipids are important to indicate the susceptibility of organ systems to pollutants by altering their function. Proteins are important organic substances required by organisms in tissue building and play an important role in energy metabolism [28]. Proteins can be expected to be involved in the compensatory mechanism of stressed organisms. The result of the present study showed that when the fish were exposed to malathion (0.5 ppm) the protein content were found to have decreased (TABLE III). The present decrease was found to be greater in all exposures in liver tissue. The reduction of protein may be due to proteolysis and increased metabolism under

TABLE III: PROTEIN CONTENT (MG/G) IN TISSUES OF TILAPIA FISH (OREOCHROMIS NILOTICUS) EXPOSED TO 0.5 PPM OF MALATHION. MEANS + SD (N=4), *P ≤ 0.05

Exposure	**Tissues**	**Liver**	**Kidney**	**Gills**	**Muscles**
24 h	**Control**	6.62 + 0.03	5.54 + 0.02	4.42 + 0.02	5.12 + 0.01
	Malathion	3.67+ 0.01*	3.51 + 0.02*	4.65 + 0.02	4.87 + 0.05
48 h	**Control**	6.65 + 0.01	5.42 + 0.02	4.48 + 0.01	5.21 + 0.02
	Malathion	3.05 + 0.05*	2.89 + 0.02*	3.36 + 0.01	3.22 + 0.02*
72 h	**Control**	5.64 + 0.02	5.88 + 0.04	4.61 + 0.01	5.21 + 0.01
	Malathion	2.00 + 0.05*	2.32 + 0.02*	3.51+ 0.10	3.01 + 0.02*
96 h	**Control**	5.21 + 0.01	5.02 + 0.06	4.45 + 0.03	5.16 + 0.02
	Malathion	1.05 + 0.09*	1.21 + 0.02*	2.85+ 0.02*	2.45 + 0.03*

toxicant stress [28]. It was reported that reduction in protein content could be due to its utilization to mitigate the energy demand when the fish are under stress [29].

The results of the present findings showed a significant decrease in carbohydrate content in all the tissues studied (TABLE IV). The decrease in carbohydrates contents may result in impairment of carbohydrate metabolism due to toxic effect [30]. The carbohydrate reduction suggests the possibility of active glycogenolysis and glycolytic pathway to provide excess energy in stress condition. Many workers reported a similar trend of decrease in carbohydrate [29]-[30]. On the other hand, some other worker [31] reported that sublethal concentration of certain organophosphate pesticides caused glycogenolysis which produced hyperglycemia in the African food fish *Tilapia mossambica* and the Indian catfish, *heteropneustes fossilis*. On the contrary, it was reported that the elevation of carbohydrates might be due to the stress induced by the insecticides as physiology of organisms with the help of corticosteroids [28].

Cholesterol is an important normal body constituent used in the structure of cell membranes, synthesis of bile and steroid hormones. The results presented in TABLE V show a significant decrease in cholesterol content in the studied tissues of fish Tilapia (*oreochromis niloticus*). The decrease was found to be high in gills and less in muscles. Generally, the decrease in cholesterol contents in all tissues was found to be increased with the hours of exposure. The reduced cholesterol level may be due to the inhibition of cholesterol biosynthesis in the liver or due to reduced absorption of dietary cholesterol [32]. However, it was reported that the decline of cholesterol may be due to utilization of fatty deposits instead of glucose for energy purpose [28].

Various authors studied similar reduction of lipids in various tissues. Srinivas et al. [33] has showed decreased lipid content in *T. mossambica* on exposed to atrazine. Gradual depletion in lipid content of liver and muscle when exposed to malathion was analysed by Mishra et al. [34]. Significant decrease was observed in cholesterol content in different tissues of the freshwater fish, *Tilapia mossambica* when the fish was exposed to pesticide metribuzin [35]. Generally, the present results indicated the toxic nature of the insecticide malathion.

TABLE IV: CARBOHYDRATES CONTENT (MG/G) IN TISSUES OF TILAPIA FISH (OREOCHROMIS NILOTICUS) EXPOSED TO 0.5 PPM OF MALATHION. MEANS + SD (N=4), *P ≤ 0.05

Exposure	Tissues	Liver	Kidney	Gills	Muscles
24 h	**Control**	5.15 + 0.02	2.52 + 0.02	2.12 + 0.02	3.16 + 0.02
	Malathion	2.88 + 0.02*	2.00 + 0.05	1.32 + 0.02*	2.88 + 0.02
48 h	**Control**	5.25 + 0.05	2.77 + 0.04	2.01 + 0.01	3.65 + 0.10
	Malathion	3.05 + 0.01*	1.52 + 0.02*	1.15 + 0.03*	2.62 + 0.10
72 h	**Control**	5.32 + 0.02	2.50 + 0.02	1.98 + 0.02	3.65 + 0.10
	Malathion	2.72 + 0.03*	1.42 + 0.10*	1.00 + 0.01	2.48 + 0.05*
96 h	**Control**	5.75 + 0.15	2.52 + 0.10	1.98 + 0.02	4.00 + 0.05
	Malathion	1.73 + 0.01*	1.32 + 0.05*	1.05+ 0.01	2.14 + 0.02*

TABLE V: CHOLESTEROL CONTENT (MG/G) IN TISSUES OF TILAPIA FISH (OREOCHROMIS NILOTICUS) EXPOSED TO 0.5 PPM OF MALATHION. MEANS + SD (N=4), *P ≤ 0.05

Exposur	Tissues	Liver	Kidney	Gills	Muscles
24 h	**Control**	15.08 ± 0.01	10.02 ± 0.05	2.23 ± 0.02	12.55 ± 0.15
	Malathion	10.98 ± 0.02*	9.55 ± 0.04	1.98 ± 0.02	12.03 ± 0.10
48 h	**Control**	15.22 ± 0.04	10.21 ± 0.02	2.24 ± 0.02	12.58 ± 0.05
	Malathion	10.88 ± 0.05*	9.21 ± 0.10	1.86 ± 0.00	11.55 ± 0.10
72 h	**Control**	15.32 ± 0.10	10.31 ± 0.10	2.20 ± 0.00	12.48 ± 0.10
	Malathion	9.55 ± 0.05*	7.85 ± 0.10*	1.42 ± 0.02*	10.21 ± 0.02*
96 h	**Control**	15.40 ± 0.02	10.11 ± 0.02	2.30 ± 0.01	12.02 ± 0.02
	Malathion	9.32 ± 0.05*	9.00 ± 0.05	1.22 ± 0.02*	9.88 ± 0.10*

ACKNOWLEDGMENTS

We are grateful to Prof. Dr. Adel A. Fathi (Department of Biological Sciences, College of Science, King Faisal University, Saudi Arabia) for helpful comments on the manuscript.

REFERENCES

[1] F. G. Uzun, S. Kalender, D. Durak, F. Demir, and Y. Kalender. Malathioninduced testicular toxicity in male rats and the protective effect of vitamins C and E. *Food Chem. Toxicol*. 47:1903–1908,2009.

[2] A. M. Al-Othman, S. A. Khaled, E. E. Gaber, Y. Kareem, A. A. Zeid, A. M. Mourad, and P. G. John. Protection of α-tocopherol and selenium against acute effects of malathion on liver and kidney of rats. *Afr. J. Pharm. Pharmacol*. 5(10):1263-1271, 2011.

[3] A. A. Kamel, S. Al-Dosary, S. Ibrahim, and A. M. A. Asif. Degradation of the acaricides abamectin, flufenoxuron and amitraz on Saudi Arabian dates. *Food Chemistry*. 10:1590–1593, 2007.

[4] K. S. Tilak, K. Veeraiah, and G.V. Ramanakumari. Toxicity and effect of chloropyriphos to the freshwater fish *Labeo rohita* (Hamilton). *Rol. Res*. 20(3):438-445, 2001.

[5] A. R. A. A. Alkhail, A. I. Askar, L. K. Younis, K. S. El-Gendy, M. M. Abbas, and A. S. M. Marei. Risk assessment of tributyltin oxide in aquatic environment: A. toxicity and sublethal effects on brain AChE and gill ATPases activity of tilapia fish, *oreochromis niloticus*. *Pak. J. Biol. Sci*. 7:1117-1120, 2004.

[6] J. J Arockia and John M. C. Mitton. Effect of carbamate pesticide lannate (methomy1) on the biochemical components of the freshwater cichlid *Oreochromis mossambicus* (Peters). *Indian J. Environ & Ecoplan*. 12 (1):263-268, 2006.

[7] J. R. Venkateswara. Toxic effects of novel organophosphorus insecticide (RPR-V) on certain biochemical parameters of euryhaline Wsh, *Oreochromis mossambicus*. *Pest. Biochem. Physiol.* 86:78-84, 2006.

[8] M. A. Sweilum Mohamed. Effect of sublethal toxicity of some pesticides on growth parameters, haematological properties and total production of Nile tilapia (*Oreochromis niloticus* L.) and water quality of ponds. *Aquacul. Res*. 37(11):1079–1089, 2006.

[9] L. Patnaik. Biochemical Alterations Induced by Sevin in *Clarias batrachus*. *Asian J. Exp. Biol. Sci*. 1(1): 124-127, 2010.

[10] F. M. Buratti, A. D'Aniello, M. Volpe, A. Meneguz, and E. Testai. Malathion Bioactivation in the human Liver: The Contribution of Different Cytochrome P450 Isoforms. *DMD*, 33:295-302, 2005.

[11] R. D. Wauchope, T. M. Butler, A. G. Hornsby, P. M. Augustin-Beckers, and J. P. Burt. The SCS/ARS/CES pesticide properties database for environmental decision making. *Environ. Contam. Toxicol*. 123:1-155, 1992.

[12] C. W. Thompson, J. A. Frick, B. C. Natke, and L. K. Hansen. Preparation, analysis and anticholinesterase properties of O,O-dimethyl phosphothioate isomerides. *Chem. Res. Toxicol*. 2:386-391, 1989.

[13] L. G. Sultatos. Mammalian toxicology of organophosphorus pesticides. *J. Toxicol. Environ. Health*, 43:271-289, 1994.

[14] C. S. Forsyth and J. E. Chambers. Activation and degradation of the phosphorothionate insecticides parathion and EPN by rat brain. *Biochem. Pharmacol*. 38:1597-1603, 1989.

[15] G. Pellegrini and R. Santi. Potentiation of toxicity of organophosphorus compound containing carboxylic ester functions towards warm-blooded animals by some organophosphorus impurities. *J. Agric. Food Chem*. 20:944-950, 1972.

[16] W. N. Aldridge, J. W. Miles, D. L. Mount, and R. D. Verschoyle. The toxicological properties of impurities in malathion. *Arch. Toxicol*. 42:95-106, 1979.

[17] P. Flessel, P. J. E. Quintana, and K. Hooper. Genetic toxicity of malathion: a review. *Environ. Mol. Mutagen*, 22:7-17, 1993.

[18] J. Blasiak, P. Jaloszynski, A. Trzcciak, and K. Szyfter. In vitro studies on the genetoxicity of the organophosphorus insecticide malathion and its two analogues. *Mutat. Res*. 445:275-283, 1999.

[19] D. J. Finney. Probit Analysis, 3rd edition, London: Cambridge University Press. pp. 20, 1971.

[20] O. H. Lowry, N. J. Rosenbrough, and R. J. Randall. Protein measurements with folin phenol reagent. *J. Biol. Chem*. 193:265-267, 1951.

[21] J. E. Hedge, and B. T. Hofreiter. Determination of reducing sugars, Method in carbohydrate chemistry. M. L., Academic Press, New York, 1962.

[22] W. Richmond. Preparation and properties of a cholesterol oxidase from *Nocardia sp*. and its application to the enzymatic assay of total cholesterol in serum. *Clin. Chem*. 19:1350-1356, 1973.

[23] J. H. Welsh and R. Smith. Laboratory exercise in invertebrate physiology. Minneapolis, Burgers Publishing Company, 1960.

[24] A. J. Nimmi. Review of biochemical methods and other indicators to assess fish health in aquatic ecosystem containing toxic chemicals. *J. Great Lakes Res.* 16:529-541, 1990.

[25] G. V. Venkataramana, P. N. Sandhya Rani, and P. S. Murthy. Impact of malathion on the biochemical parameters of gobiid fish, *Glossogobius giuris* (Ham). *J. Environ. Biol*. 27(1):119-122, 2006.

[26] P. M. Reddy and G. H. Philip. Hepato toxicity of malation on the protein metabolism in Cyprinus carprio. *Acta Hydrochim. Hydrobiol*. 19(1):127-130, 1991.

[27] P. N. Dube and B. B. Hosetti. Behaviour surveillance and oxygen consumption in the freshwater fish *Labeo rohita* (Hamilton) exposed to sodium cyanide. *Biotech. Anim. Husb*. 26 (1-2):91-103, 2010.

[28] K. M. Remia, S. Logaswamy, K. Logankumar, and D. Rajmohan. Effect of an insecticide (Monocrotophos) on some biochemical constituents of the fish *Tilipia Mossambica*. *Poll. Res*. 27(3):523-526, 2008.

[29] G. V. Venkatramana Sandhya, P. N. Rani, and P. S. Moorthy. Impact of Malathion on the biochemical parameters of gobiid fish, *Glossogobius giuris* (Ham). *J. Environ. Biol*. 27(1):119-122, 2006.

[30] C. Thenmozhi, V. Vignesh, R. Thirumurugan, and S. Arun. Impacts of malathion on mortality and biochemical changes of freshwater fish *labeo rohita*. *Iran. J. Environ. Health. Sci. Eng*. 8(4):189-198, 2010.

[31] S. Logaswamy and K. M. Remia. Impact of Cypermethrin and Ekalux on respiratory and some biochemical activities of a fresh water fish, *Tilapia mossambica*. *Curr. Biot*. 3(1):65-73, 2009.

[32] M. K. Kanagaraj, M. Ramesh, K. Sivakumari, and R. Manavalaramanujam. Impact of acid pollution on the serum haemolymph cholesterol of the crab, *Paratelphusa hydrodromous*. *J. Ecotoxicol. Environ. Monit*. 3(2):99-102, 1993.

[33] T. Srinivas, T. A. V. Prasad, G. M. D. Rafi, and D. C. Reddy. Effect of atrazine on some aspects of lipid metabolism in freshwater fish. *Bio. Inter*. 23(3):603-609, 1991.

[34] S. K. Mishra, J. Padhi, and L. Sahoo. Effect of malathion on lipid content of liver and muscles of *Anabas testudineus*. *J. Appl. Zool. Res*. 15(1):81-82, 2004,

[35] N. Saradhamani and B. J. Selvarani. A study on the effect of herbicide metribuzin on the biochemical constituents of the freshwater fish, *Tilapia mossambica* Peters. *Curr. Biot*. 3(2):220-231, 2009.