E-ISSN: 2347-5129
P-ISSN: 2394-0506
(ICV-Poland) Impact Value: 5.62
(GIF) Impact Factor: 0.549
IJFAS 2019; 7(4): 280-284

www.fisheriesjournal.com
Received: 25-05-2019
Accepted: 28-06-2019

Saif M Al Ghais
Department of Environmental Sustainability, Environment Protection & Development Authority EPDA, United Arab Emirates

Sujatha Varadharajulu
Department of Environmental Sustainability, Environment Protection & Development Authority EPDA, United Arab Emirates

Pramod Kumbhar
Department of Environmental Sustainability, Environment Protection & Development Authority EPDA, United Arab Emirates

Correspondence
Sujatha Varadharajulu
Department of Environmental Sustainability, Environment Protection & Development Authority EPDA, United Arab Emirates

Effects of Abamectin on *Tilapia mossambica* peters changes in reduced glutathione (GSH) and protein content

Saif M Al Ghais, Sujatha Varadharajulu and Pramod Kumbhar

Abstract
The aim of this study was to assess the potential hazards of abamectin and to identify the toxicity of abamectin on tilapia fish. The fishes were exposed to abamectin (274.4 mg/20 L) for 96 hours. At the end of exposure period, fishes were sacrificed and liver, and gills were removed for estimation of reduced Glutathione (GSH), similarly liver, gills and brain were also analysed for protein content. The level of GSH in liver the fish grown in the control group was (1658 nmole GSH/g tissue), but decreased in treated group (1135 nmole GSH/g tissue). Notably, gills reduced glutathione content was lower than liver in both the control and treated group, (449 nmole GSH/g tissue and 235 nmole GSH/g tissue). The control group shows the protein content in liver was 17 µg/100mg of tissues in gills 12.67 µg/100mg of tissues and brain 18.33 µg/100mg of tissues, the protein content decreased in treated liver to 12.33 µg/100mg, gills 8.4 µg/100mg and brain 14.8 µg/100mg in compared with control group. The results showed decreased value of GSH and protein content in all the tissues when compared to control.

Keywords: Abamectin, *Tilapia*, reduced glutathione, protein

1. Introduction
For centuries, pesticides have been used in agriculture to improve food production by eliminating unwanted pests and controlling disease vectors [1]. Among common pesticides, organophosphorus (OP) compounds are broadly used in medicine, industry and cultivation [2]. The extensive and improper uses of pesticides cause pollution of water, air, soil, and food. The environmental pollution with pesticides results in detrimental effects on non-target organisms [3]. These insecticides have a long life period, which has resulted in the process of bioaccumulation and biomagnification in the environment and in the living organisms [4]. The toxicity of the insecticides has a greater impact on the aquatic environment via many pathways like, direct application for pest and disease vectors, surface runoff, rainfall and absorption from the vapor phase at the air-water inter phase etc. [5]. The wide spread usage of insecticides ultimately pollutes the aquatic environment there by affecting the aquatic fauna mainly fishes which constitute the major economy of the country and a valuable source of protein. It is observed that the structure and stability of natural fish populations would be adversely affected if the water where their development takes place is contaminated even at sublethal concentrations. It was documented that the exposure to low doses of pesticides produces various biochemical alterations leading to adverse health effects in the exposed organisms [6, 7]. Stress indicators at cellular and tissue levels have been developed in fish and other aquatic organisms to monitor environmental contamination [8-10]. Tissue cholinesterases and non-protein reduced glutathione (GSH), which protects cells against oxidative injury and detoxicates xenobiotics and/or their metabolites, have been validated as pollution biomarkers in fish and other aquatic animals [11]. A biomarker is defined as a change in biological response, ranging from molecular through cellular and physiological responses to behavioral changes, which can be related to exposure to toxic levels of environmental chemicals [12]. A number of pollutants or xenobiotic agents are present in our environment and exert various kinds of stress on ecosystem and can be proven to be deleterious to organisms [13]. Risk assessment of these pollutants to organisms and ecosystems is challenging because of diversities in chemical nature and mode of toxicity of the pollutants as well as variation in sensitivities of the organisms exposed to the pollutants. Even at low concentrations some pollutants produce deleterious effects on organisms which are difficult to predict, because measurable effects are expressed only after prolonged exposure.

The pesticides tested in the present study are abamectin (ABM) also, known as avermectin, a macrocyclic lactone derived from the soil microorganism *Streptomyces avermitilis*, is a mixture of B1a and B1b avermectins [14]. In agriculture, Abamectin is used as a systemic acaricide and insecticide worldwide; it is also used as a parasiticide for the lung worm and nasal bots and against gastrointestinal nematodes in cattle and sheep [15, 16]. Its mode of action is associated with its effect on the -aminobutyric acid (GABA) receptors and glutamate-gated chloride channels increasing the permeability of chloride ions, hyperpolarizing the nerve and muscle cells, and disturbing the neuromuscular transmission leading to death [17]. The abamectin is highly lipophilic and poorly soluble in water but readily soluble in most organic solvents [18]. In fish, the abamectin can also pass the blood, brain barrier and could cause toxic effects [19].

The current study was, therefore, undertaken to evaluate the status of GSH in the liver and Gills in *Tilapia mossambica*, a commercially important and relatively resistant species that is well adapted to aquaculture [20]. Furthermore, the study is carried out to determine the effect of abamectin on protein content of the Tilapia. During the stress, organisms need sufficient energy to encounter the stress. The instant energy required is supplied from the reserved materials which may be in form of glycogen, protein and lipid. Biochemical study gives an idea about the accumulation of toxicants in the tissue.

2. Materials and Methods

2.1 Pesticide used: Abamectin (Vertimec 1.8% EC) was supplied by Syngenta Co. The chemical name, 5-O-demethyl avermectin A1a (i) mixture with 5-O- demethyl- 25-de (1-methyl propyl-25- (1-methyl ethyl) avermectin A1a (ii).

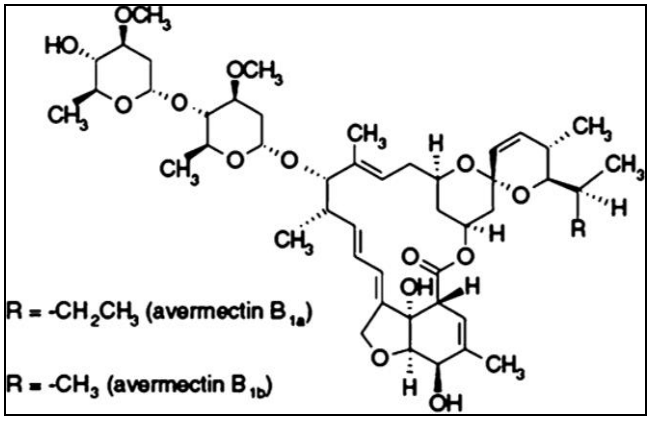

Fig 1: Chemical structure of abamectin (ABM).

2.2 Experimental fish and treatment

The *Tilapia mossambica* average body weight 150 ± 10 g. were obtained from local fish farm of Ras Al Khaimah. Fish were transferred to the laboratory in 20 liters well aerated fiberglass tanks, clinical examined to assure the absence of any abnormalities or external lesions on fishes. The fish were kept in identical glass aquaria supplied with continuous aerated dechlorinated water at 26 ± 2 ^{0}C, and pH was 7.5 ± 0.5. Fish were fed with commercial diet twice daily and kept seven days for acclimatization. After acclimatization the sample fishes were divided into two groups as control and treated (pesticide treatment). Treatment was prepared with Vertimec® pesticide (274.4 mg / 20 L) and after 96 h of aqueous exposure fishes were taken out for dissection. Liver, gills and brain tissues were washed with normal saline, dried and stored at a temperature of -36 ^{0}C for further biochemical estimations (n=12). Whole liver, gills and brain were dissected out, washed with ice-chilled normal saline, blotted dry and weighed. Similarly, fish samples from control group were processed. All experimental manipulations, unless otherwise stated, were conducted at 0-4 ^{0}C.

2.3 Estimation of reduced glutathione (GSH)

According to Beutler [21] reaction mechanism involves oxidation of glutathione reduced (GSH) (glutathione, CAS no.: 1.04090.005, Merck) by 0.01 Mole DTNB (5,5 dithiobis-2-dinitro benzoic acid, CAS no.: 422592J, VWR, UK) to form glutathione disulfide and yellow derivative of 5, thio 2-nitrobenzoic acid and its measured at 412 nm by spectrophotometer. There is amount of GSH (glutathione, CAS no.: 1.04090.005, Merck) present in the solution (5%) is followed by oxidation with DTNB (5,5 dithiobis-2-dinitro benzoic acid, CAS no.: 422592 J, VWR, UK) produce Glutathione disulfide, and concentration depends on the amount of DTNB oxidized with glutathione. Ten percent sample solutions (liver, gills) were prepared by using tissue homogenizer (tissue homogenizer, REMI RQ-127A, India) mixed with 0.1M Tris HCl buffer with EDTA (pH 7.4). Pipetted 0.75 ml of gills solution (10%) and 0.5 ml of liver solution (10%) made final volume of both sample up to 1.0 ml with water. 0.25 ml of 20% v/v trichloacetic acid was added and samples were kept for incubation in refrigerator for about 45 min. 0.8 ml of water was added after incubation and was centrifuged at 2000 rpm for 20 min at room temperature. To the supernatant, 0.3 ml of 2M tris base and 0.1 ml of 0.01M DTNB (5,5 dithio bis 2-dinitrobenzoic acid, CAS no.:422592J, VWR, UK) were added. Absorbance was measured at 412 nm after 10 min by UV spectrophotometer, GENWAY 7315, UK.

2.4 Protein estimation

Protein was estimated by the method of Lowry *et al*, [22]. The liver, brain, and gill samples of fish muscle was taken out, washed with ice-cold normal saline, dried and weighed. Muscle homogenate (5% w/v) was prepared in ice-cold distilled water with the help of homogenizer. 0.2 ml of tissue homogenate was mixed with 1.3 ml of distilled water and 0.5 ml of 20% trichloroacetic acid to precipitates proteins. The tubes were allowed to stand at 4 °C for 30 min and centrifuged at 2500 rpm to sediment protein. The sediments were dissolved in 0.1 N sodium hydroxide solution. A suitable aliquot of protein solution thus obtained was taken out in another tube and made up to 0.5 ml with reagent A 2% Na2CO3 in 0.1 N NaOH. Then, 2.5 ml of reagent C 0.5% CuSO4.5 H2O in H2O was added and shaken. After 10 min, 0.25 ml of Folin and Ciocalteus Phenol reagent was added. After 30 min, the blue-colored solution at 660 nm by a UV spectrophotometer, GENWAY 7315, UK [23]

2.5 Statistical analysis

For comparing and maintaining the uniformity and homogeneity, all the data were transformed into the same units and the results were expressed as standard deviation.

3. Results and Discussion

Toxicology is the measurable study of the deleterious effects due to chemical or physical agents, which cause adverse effects on living organisms. The insecticide Vertimec was used as the source of abamectin for the present study. In order to assess the status of oxidative stress, in *T. mossambica*

exposed to abamectin present in the treated tank, the level of antioxidant GSH was found to be high in the liver and gills of fishes belonging to control. GSH content of liver tissues from abamectin treated fish (1135 nmole GSH/ g tissue) was found to be less than that of the control fish (1658 nmole GSH/ g tissue). Similarly, GSH content of gills of fish treated with abamectin were less than the control (235 nmole GSH/ g tissue & 449 nmole GSH/ g tissue respectively) Fig. 2. This suggested that elevated intracellular GSH content is probably a cellular adaptive response to protect against the deleterious effects of oxidative stress elicited by chemical pollutants present in the treated pesticides.

The results of this study clearly suggest that abamectin treatment significantly reduced intracellular GSH content and therefore increased its vulnerability to oxidative stress. This will place abamectin as a pesticide that is toxic to fishes at the level applied. This is in line with Maioli *et al*, who reported a toxic effect of abamectin [24]. Abamectin as a mixture of B1a and B1b avermectins, not its metabolites, is proved to be responsible for the toxic effect on isolated hepatocytes [25]. The ultrastructural examination showed swollen mitochondria and unclear structure of the inner membranes in treated cells from *Spodoptera frugiperda* (Sf9) [26]. The glutathione redox cycle plays a vital role in protecting cells from oxidative injury [27]. Alteration in the activity of glutathione redox cycle components is affected by the chemical structure and concentrations of pesticides that cells are exposed to endosulfan and abamectin [28]. Generally, the induction of toxicity may lead to further problems of mutagenic and carcinogenic activities [29]. The epidemiological studies demonstrated a relationship between pesticide exposure and the occurrence of cancer [30]. Therefore, the current awareness of the real or potential hazards of pesticides considering their toxic actions cannot be neglected. The present results indicate that abamectin significantly reduced the GSH activity compared with the control. Thus, the observations recommend the importance of *Tilapia* tissue GSH as potential biomarkers in monitoring the pesticides pollution and its impact on the physiology of fish. These results support that the restricted use of this pesticide might be a very effective practice for agriculture practices.

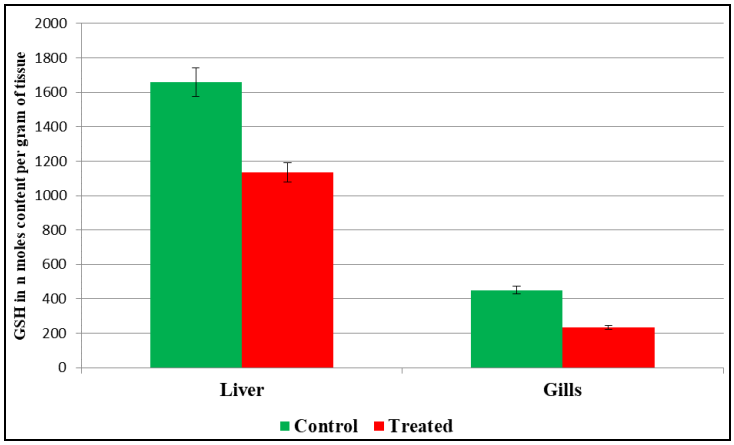

Fig 2: Average reduced glutathione level in liver and gills of *T. mossambica*. The data is shown as mean ± standard error of GSH content in control group and fish treated with abamectin, expressed as GSH content in nmole per gram for liver and gills.

The changes in biochemical compositions of liver, gills and brain of tilapia fish, *Tilapia mossambica* exposed to pesticide Abamaectin was studied along with experimental and control group with respect to the mg per gm of protein in wet tissue. The control fish showed the protein content in liver was 17 µg/100mg of tissues, in gills 12.67 µg/100mg of tissues and in brain 18.33 µg/100mg of tissues. The protein content decreased in treated fish liver to 12.33 µg/100mg, gills to 8.4 µg/100mg and brain to 14.8 µg/100mg in compared with control (fig. 3). Today the whole aquatic biota are facing a major problem of toxic contamination. We must pay attention towards serious problem because physiological function of the aquatic organisms is affected by these toxicants directly or indirectly.

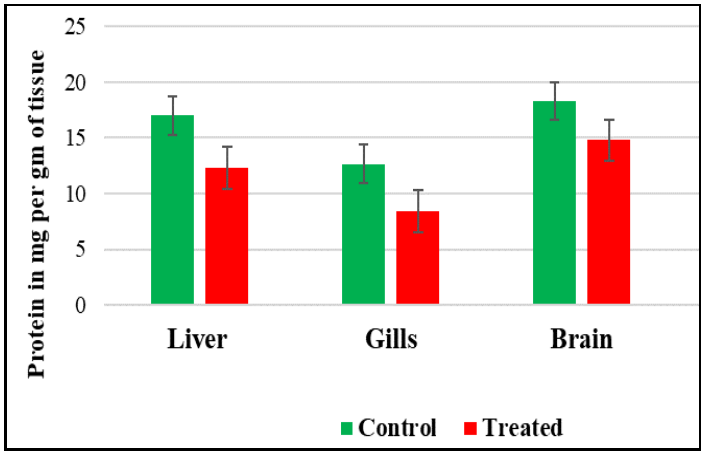

Fig 3: Average protein content in liver, gills and brain of *T. mossambica*. The data is shown as mean ± standard error of protein content in control and fish treated with abamectin, expressed as total protein content of different tissues (liver, gills and brain) in mg per gram of tissue.

Protein is an important organic constituent of animal tissue. It plays an important role in cellular metabolism. Protein regulates the process of interaction between intra and extra cellular media [23]. The tissue protein level has significant value, which may show changes as per the biochemical conditions of an organisms. The alternation in the tissue protein in the present study suggest disturbance in the physiological activity. Fall in protein content might be attributed to the divergence of energy routes/pathways to meet the impending energy demands when the fish is under stress or altered enzyme activities. The altered mobility and low content of proteins in abamectin treated groups reflects a change in the rate of synthesis and/or degradation of protein. In line the protein content decrease may be due to breakdown of proteins for meeting energy demands for organism [31]. Proteins are mainly involved in the architecture of the cell, which is chief source of nitrogenous metabolism and during chronic period of stress they are also a source of energy [23]. The depletion of protein fraction in various tissues may have been due to their degradation and possible utilization of degraded products for metabolic purposes [32] Under toxic stress, divergence of energy occurs to satisfy the impending energy demands. Protein content in all tissues of *Labeo rohita* was reduced as a result of its treatment with the insecticide Encounter (herbal plant extract) [33].

4. Conclusion

The toxicity of Abamectin on *Tilapia mossambica* under the experimental condition was expressed as a significant reduction in liver and gill content of GSH which is necessary as an antioxidant. GSH was notably higher, both in treated and control samples, of liver compared to gill samples. Protein content of liver, gills and brain were reduced in the tissues exposed to abamectin. This may be due to reduced synthesis or increased degradation of proteins. The results obtained underscore the need for stringent control over use of such as insecticide to avoid its adverse effects on non-target organisms in the terrestrial as well as aquatic ecosystem.

5. Conflict of interest

The authors declared that they have no conflict of interest.

6. References

1. Prakasam A, Sethupathy S, Lalitha S. Plasma and RBCs antioxidant status in occupational male pesticide sprayers, Clinica Chimica Acta. 2001; 103:107-112.
2. Storm E, Karl KR, Doull J. Occupational exposure limits for 30 organophosphate pesticides based on inhibition of red cell acetylcholinesterase, Toxicology. 2000; 150:1-29.
3. Gilden RC, Huffling K, Sattler B. Pesticides and health risks. Journal of Obstetric, Gynecologic, Neonatal Nursing. 2010; 39:103-110.
4. Sahai S. Accumulation and induced histopathological effect of pesticides in the testis of *Puntius ticto* (Teleosti). Journal of Ecobiology. 1992; 4:303-308.
5. Banerjee BD, Seth V, Bhattacharya A, Pasha ST, Chakraborty AK. Biochemical effects of some pesticides on lipid peroxidation and free-radical scavengers. Toxicology Letters. 1999; 107(1-3):33-47.
6. Al-Sarar AS, Abobakr Y, Al-Erimah GS, Hussein HI, Bayoumi AE. Hematological and biochemical alterations in occupationally pesticides-exposed workers of Riyadh municipality, Kingdom of Saudi Arabia. Research Journal of Environmental Toxicology. 2009; 3(4):179-185.
7. Al-Sarar AS, Abobakr Y, Bayoumi AE, Hussein HI, Al-Ghothemi M. Reproductive toxicity and histopathological changes induced by lambda-cyhalothrin in male mice. Environmental Toxicology. 2014; 29(7):750-762.
8. Al-Ghais SM, Ali B. Inhibition of glutathione S-transferase catalyzed xenobiotic detoxication by organotin compounds in tropical marine fish tissues. Bulletin of Environmental Contamination and Toxicology. 1999; 62:207-213.
9. Lam PKS, Gray J. The use of biomarkers in environmental monitoring Programmes. Marine Pollution Bulletin. 2003; 46:182-186.
10. Facey DE, Blazer VS, Gasper MM, Turcotte CL. Using fish biomarkers to monitor improvements in environmental quality. Journal of Aquatic Animal Health. 2005; 17:263-266.
11. Otto DME, Moon TW. Phase I and II enzymes and antioxidant responses in different tissues of brown bullheads from relatively polluted and non-polluted systems. Archives of Environmental Contamination and Toxicology. 1996; 31:141-147.
12. Stoliar OB, Lushchak VI. Environmental pollution and oxidative stress in fish, oxidative stress - Environmental Induction and Dietary Antioxidants, Dr. Volodymyr Lushchak (Ed.), 2012. ISBN: 978-953-51-0553-4. In Tech. http://dx.doi.org/10.5772/38094.
13. Van der oost R, Beyer J, Vermeulen NPE. Fish bioaccumulation and biomarkers in environmental assessment: A review. Environmental Toxicology and Pharmacology. 2003; 13(2):S1382-6689(02)00126-6.
14. Meister RT. Farm chemicals handbook Meister Publishing Company, Willoughby. 1992, 92.
15. Elbetieha A, Daas SI. Assessment of antifertility activities of abamectin pesticide in male rats. Ecotoxicology and Environmental Safety. 2003; 55(3):307-313.
16. Novelli A, Vieira BH, Cordeiro D, Cappelini LTD, Vieira EM, Espíndola ELG. Lethal effects of abamectin on the aquatic organisms *Daphnia similis*, *Chironomus xanthus* and *Danio rerio*. Chemosphere. 2012; 86(1):36-40.
17. Cully DF, Vassilatis DK, Liu KK, Paress PS, van der Ploeg LHT, Arena JP *et al*. Cloning of an avermectin-sensitive glutamate-gated chloride channel from Caenorhabditis elegans. Nature. 1994; 371(6499):707-11.
18. Roth M, Richards RH, Sommerville C. Current pesticides in the chemotherapeutic control of sea lice infestations in aquaculture, a review. Journal of Fish Diseases. 1993; 16:1-26.
19. Hoy T, Horsberg TE, Nafstad I. The disposition of ivermectin in Atlantic salmon (Salmo solar). Pharmacology and Toxicology. 1990; 67:307-312.
20. De Silva SS, Subasinghe RP, Bartley DM, Lowther A. Tilapias as alien aquatics in Asia and the Pacific: A review. FAO Fisheries Technical Paper. 2004; 453:65.
21. Beutler E, Duron O, Kelly MB. Improved method for the determination of blood glutathione. The Journal of Laboratory and Clinical Medicine. 1963; 61:882-888.
22. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein estimation with Folin Phenol reagent. Journal of Biological Chemistry. 1951; 193:265-275.
23. Saif Al Ghais, Sujatha Varadharajulu. Variations of

Protein Content in Commercially Important Fishes of Southern Arabian Gulf. International Research Journal of Engineering and Technology. 2019; 06(01):417-420.
24. Maioli MA, de Medeiros HCD, Guelfi M, Trinca V, Pereira FTV, Mingatto FE. The role of mitochondria and biotransformation in abamectin-induced cytotoxicity in isolated rat hepatocytes. Toxicology *in Vitro*. 2013; 27:570-579.
25. Meister RT. Farm Chemicals Handbook '92, Meister Publishing Co., Willoughby, Ohio, 1992.
26. Huang JF, Tian M, Lv CJ, Li HY, Muhammad R, Zhong GH. Preliminary studies on induction of apoptosis by abamectin in Spodoptera frugiperda (Sf9) cell line. Pesticide Biochemistry and Physiology. 2011; 100(3):256-263.
27. Meister A. Selective modification of glutathione metabolism. Science. 1983; 220:472-477.
28. El-Shenawy NS. Effects of insecticides fenitrothion, endosulfan and abamectin on antioxidant parameters of isolated rat hepatocytes. Toxicology *in Vitro*. 2010; 24(4):1148-57.
29. Valko M, Rhodes CJ, Moncol J, Izakovic M, Mazur M. Free radicals, metals and antioxidants in oxidative stress induced cancer. Chemico Biological Interactions. 2006; 160(1):1-40.
30. Dich J, Zahm SH, Hanberg A, Adami HO. Pesticides and cancer. Cancer Causes Control. 1997; 8(3):420-443.
31. Tulasi G, Jayantha Rao K. Effect of chromium on protein metabolism in different tissues of fish, *Cyprinus carpio*, Research Journal of Pharmaceutical, Biological and Chemical Sciences. 2013; 4(1):143-148.
32. Nagaraju B, Venkata Rathnamma V. Effect of Profernophos an organophosphate on protein levels in some tissues of fresh water fish, Labeo rohita, International Journal of Pharmacy and Pharmaceutical sciences. 2013; 5(1):276-279.
33. Neff JM. Use of biochemical measurements to detect pollutant-mediated damage to fish. American Society for Testing and Materials, 1985, 155-1881.