Pakistan J. Zool., vol. 54(3), pp 1435-1438, 2022 DOI: https://dx.doi.org/10.17582/journal.pjz/20201118201101

Short Communication

Effects of Pesticides on Total Protein Content of Different Organs of *Oreochromis niloticus* (Linnaeus, 1758)

Muhammad Amin[1]*, Masarrat Yousuf[1] and Naveed Ahmad[2]

[1]*Toxicology Laboratory, Department of Zoology, University of Karachi, Karachi*
[2]*Department of Maritime Sciences, Bahria University, Karachi, Karachi, 75260 Pakistan*

Article Information
Received 18 November 2020
Revised 30 November 2020
Accepted 04 December 2020
Available online 20 May 2021 (early access)
Published 04 March 2022

Authors' Contribution
MA, conceived the idea and wrote the manuscript. NA, helped in experimental work and data analysis. MY supervised the work.

Key words
***Oreochromis niloticus*, Pesticides, Biomarker, Toxicity, Bio-monitoring, protein**

ABSTRACT

In the present study, *Oreochromis niloticus* was exposed to one fourth of 24 h LC_{50} of malathion malathion (1.42 ppm), chlorpyrifos (0.125 ppm) and lambda-cyhalothrin (0.0039 ppm) for 24 and 48 h and then total protein was estimated in the brain, gills and muscle tissues. Significant (p< 0.005- 0.00) decreased in total protein was noted in response to all the pesticides as compared to control group and a slight increase was also noted during 48 h as compared to 24 h. The level of total protein was decreased for pesticides in order of chlorpyrifos>malathion> lambda-cyhalothrin during the exposure period.

Environmental pollution via toxicants has raised the most serious problem in the globe (Chandran *et al*., 2005; Lazartigues *et al*., 2013; Sthanadar *et al*., 2015). Pesticides contaminate aquatic system and their extensive use in agricultural and industrial processes during the past decades has posed serious threat to organisms (Dutta and Dalal, 2008; Fevery *et al*., 2016; Sharma and Singh, 2007). Fish are sentinel species of water quality and the effect of the pesticide can be monitored by analyzing the blood which is the pathological bioindicator of entire body (Sharma and Singh, 2004, 2006). Among pesticides organophosphates (OPs) and pyrethroids are non-persistent and less toxic compred to organochlorines (OC) (Andersen *et al*., 2002). OPs pesticides (chlorpyrifos and malathion) are used most commonly in garden and residential areas commercially and in the industries (Aspelin and Grube, 1999). Due to their comparatively non-persistent characteristics, OPs are widely used class of pesticides around the world. Therefore, several terrestrial and aquatic organisms in the environment are under threat due to exposure to these pesticides (Murphy, 1986; Stoytcheva *et al*., 2011; Naqvi *et al*., 2016). Synthetic pyrethroids are pesticides that were introduced during the past two decades for domestic and agricultural usage (Sanchez-Fortun and Barahona, 2005). Different pesticides are used to control agricultural pests and vectors which cause animal disease in Pakistan. According to Shah *et al*. (2007) pyrethroids have adverse effects on health for their continuous use in agriculture and livestock breeding (Ahmad *et al*., 2011; Burns and Pastoor, 2018; Bordoni *et al*., 2019). They are highly neurotoxic to non-targeted organisms especially to fishes (Reddy *et al*., 1991; Philip *et al*., 1995; Köprücü *et al*., 2006; Shoaib *et al*., 2013; Awoyemi *et al*., 2019; Ullah *et al*., 2019b), while birds and mammals are substantially less sensitive to these pesticides (WHO, 1992). Lambda cyhalothrin is one of the neurotoxic pesticides, causes complex symptoms of poisoning which are fatal to organisms.

Species of tilapia are highly recommended culturing fish as it exhibits rapid growth rates even on low- protein feeds, tolerate harsh environmental conditions and is widely accepted as food. Consequently, special attention was given to study the ecological conditions related to increase tilapia production (Barriga-Sosa *et al*., 2004).

Since protein is the main important component of fish as food, the present study describes the effect of malathion, chlorpyrifos and lambda cyhalothrin pesticides on total protein content of *Oreochromis niloticus*.

Materials and methods

Pesticides including OPs (malathion 57% EC and chlorpyrifos 40% EC) and synthetic pyrethroid (lambda- cyhalothrin 2.5% EC) of STEDEC Technology Commercialization Corporation of Pakistan (Pvt. Limited) were purchased from the market. For the preparation of different concentration of pesticides Charle's equation ($C_1 V_1 = C_2 V_2$) was used for the dilution of the stock solution,

* Corresponding author: aminmuhammad013@yahoo.com
0030-9923/2022/0003-1435 $ 9.00/0

where C_1 V_1 represent the initial concentration and volume of the pesticides (stock solution) and C_2 V_2 are the required concentration and volume of the pesticides.

Oreochromis niloticus (mean weight (17.8±0.2 g) and total length (12.12±0.6cm)) were purchased from the hatchery of Chilya Thatta and were transported to the Laboratory of Toxicology, University of Karachi in a plastic bag containing oxygenated water. An aerated glass aquarium (volume range: 57 x 30 x 30 cm) was used for the maintenance of the test fishes and were acclimatized for 15 days in dechlorinated tap water under the laboratory condition in natural light and dark period. Throughout the acclimatization period, the water in the aquarium was replenished and aerated regularly by an electric air pump. Fish were fed with commercial pellets of Oryza Pvt. Limited (40% protein) provided daily at 2% of body weight. The physicochemical properties of water were measured according to the methods mentioned in APHA (2005) and recorded as follows; temperature 26±0.7°C, dissolved oxygen 5.5 ± 0.5 mg/L, ammonia (NH3) 1± 0.1 mg/L, nitrate 1.2 ± 0.4 mg/L, and pH 7.08 ± 0.2. After the acclimatization period, ten fish were transferred to each aquarium (25 x 25 x 25 cm) containing 10 L of water so as to reduce the stress. The LC_{50} was determined by Finney (1971) method and then its 1/4th part was used for biochemical study. Fishes were exposed to the sub-lethal concentrations of malathion (1.425 ppm), chlorpyrifos (0.125 ppm) and lambda-cyhalothrin (0.0039 ppm) for 24h and 48h. Control groups were also run in water without the addition of pesticides. The experiment was performed in triplicates by using a renewal bioassay method where the exposure medium was exchanged every 24h to maintain the toxicant strength and the dissolved oxygen level and to minimize the levels of ammonia excretion throughout the experiment. Water was aerated with an electric air pump during fish exposure.

Five fish from each replicate of both control and treatment groups were taken and sacrificed. Muscle, brain and gill were carefully removed. One gram of each organ was taken and crushed in 5 mL of deionized water with the help of mortar and pestle. The whole each crushed sample was taken in a glass tube using Teflon coated mechanical tissue grinder for homogenization at the speed of 1000 rpm for 10 min. The homogenates were then centrifuged at 4500 rpm for 30 min. The supernatants were stored at 0 to -5°C and then used for estimations of protein according to the Biuret method (Gornall *et al*., 1949). The values were presented as Means ± SD of three replicates. For ANOVA and level of significance determination, Excel and Origin 9were used.The Tukey HSD test was used to determine the significant difference within the treated groups.

Results and discussion

Table I shows the protein contents in brain, gills and muscles of fish *Oreochromis niloticus.* A significant decrease in total protein content as compared to control was noted in all organs, total protein level was exceeded only in control 48h in brain (19.33±0.58) and muscle tissues (27.02±0.57). The significance decreased level ranged from $P < 0.01$ in brain to $P < 0.00$ in gill and muscle tissues. Thus, the order of toxicity recorded in the present study was chlorpyrifos>malathion> lambda-cyhalothrin.

The decline in serum total protein content was in fish *Rhamdia quelen* also reported by Borges *et al.* (2007). According to Das *et al.* (2004), reduction in serum total protein content was either due to the haemolysis of RBCs creating plasma dilution or protein catabolism where proteins convert to energy. Sastry *et al.* (1982) have also reported depletion of total protein content in the plasma of fish when exposed to curacron. Radha *et al.* (2005) reported decline in protein level due to demethoate. Reduction in serum total protein content was observed in *Cyprinus carpio* (Maruthanayagam and Sharmila, 2004) and in *Clarias gariepinus* (Yekeen and Fawole, 2011) when exposed to monochrotophos and endosulfan, respectively. Ogueji and Auta (2007) observed the profound effects of

Table I. Effect of pesticides on total protein (mg/mL) content of brain, gills and muscles of *Oreochromis niloticus* after 24 h and 48 h exposure.

	Control		Malathion		Chlorpyrifos		Λ cyhalothrin	
	Mean± SD (mg/mL)		Mean± SD (mg/mL)		Mean± SD (mg/mL)		Mean± SD (mg/mL)	
	24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h
Brain	18.44±0.69[a]	19.33±0.58[a]	17.28±0.35[a]	15.8±0.90[b]	17.30±0.28[a]	18.13±0.64[a]	18.05±0.05[a]	17.19±0.25[a]
Gills	41.98±0.02[a]	22.01±0.55[b]	18.86±0.34[c]	17.54±0.50[c]	18.16±0.62[c]	19.03±0.37[c]	19.68±0.46[c]	20.87±0.11[c]
Muscles	26.43±0.30[a]	27.02±0.57[a]	23.57±0.54[b]	24.29±0.39[b]	25.91±0.42[a]	25.24±0.43[a]	23.46±0.35[b]	26.23±0.35[a]

Results are mean (X ± SD) of 3 observations shows the standard deviation values are significant at P < 0.001 when compared with control values; the values of control and pesticide treated groups are based on 24 h and 48 h. The same alphabet indicates that the mean values are not significantly different (P>0.05).

λ-cyhalothrin on serum total protein level in *Clarias gariepinus*. Similar findings indicated that protein is the major source of energy, there reduction resulted in high energy need increased under malathion stress in *Cyprinus carpio* (Malla-Reddy, 1987; Kale *et al*., 2006). Decreased trend in total protein level was also observed in Nile Tilapia (*Oreochromis niloticus*) when exposed to cypermethrin (Korkmaz *et al*.,2009). During stress condition in organisms proteins might be involved in the compensatory mechanism, when fish exposed to malathion (0.5 ppm) the protein level was decreased (Fahmy, 2012). Degradation in total protein level was also noted in fish response to deltamethrin (Ullah *et al*., 2019a). Hatami *et al*. (2019) reported a decrease in total protein level in fish when exposed to 25 and 50 mg/ L of chlorpyrifos as compared to control group. Ahmad *et al*. (2012) noted a decrease in total protein contents in zebra fish, *Danio rerio* (Hamilton) in response to λ-cyhalothrin. Mommsen and Walsh (1992) also suggested that fish can obtain energy during stress by the catabolism of protein. David *et al*. (2004) and Parthasarathy and Joseph (2011) also reported decreased] in protein levels in *C. carpio* and *Oreochromis mossambicus* in response to cypermethrin and λ- cyhalothrin, respectively.

Conclusion

The results of this study indicate that these pesticides (malathion, chlorpyrifos and λ-cyhalothrin) are extremely toxic to the fish *Oreochromis niloticus* and the total protein contents in the brain, gill and muscle tissues are decreased.

Acknowledgement

The laboratory facilities in the lab of Bahria University, Medical and Dental College provided by Assistant Professor Dr. Mehreen Lateef is gratefully acknowledged.

Animals ethics

The study was ethically approved by the University of Karachi, Pakistan.

Statement of conflict of interest

The authors have declared no conflict of interest.

References

Ahmad, L., Khan, A. and Khan, M.Z., 2011. *Pakistan Vet. J.,* **32:** 1

Ahmad, M., Sharma, D., Ansari, S. and Ansari, B., 2012. *Arch. Pol. Fish.,* **20**: 1 19-25. https://doi.org/10.2478/v10086-012-0003-5

Andersen, H.R., Vinggaard, A.M., Rasmussen, T.H., Gjermandsen, I.M. and Bonefeld-Jørgensen, E.C., 2002. *Toxicol. Appl. Pharmacol.,* **179**: 11-12.

APHA, 2005. *Standard methods for the examination of water and wastewater.* 21st Edition, American Public Health Association/American Water Works Association/Water Environment Federation, Washington DC.

Aspelin, A.L. and Grube, A.H., 1999. *Pesticide industry sales and usage 1996 and 1997 market estimates*. US Environmental Protection Agency, Office of Prevention, Pesticides, and Toxic Substances, Washington. DC 733–R–99–001.

Awoyemi, O.M., Kumar, N., Schmitt, C., Subbiah, S. and Crago, J., 2019. *Chemosphere*, **219**: 526-537. https://doi.org/10.1016/j.chemosphere.2018.12.026

Barriga-Sosa, I.D.L.A., Jiménez-Badillo, M.D.L., Ibáñez, A.L. and Arredondo-Figueroa, J.L., 2004. *J. Appl. Ichthyol.,* **20**: 7-14. https://doi.org/10.1111/j.1439-0426.2004.00445.x

Bordoni, L., Nasuti, C., Fedeli, D., Galeazzi, R., Laudadio, E., Massaccesi, L., López-Rodas, G. and Gabbianelli, R., 2019. *Exp. Gerontol.*, **124**: 110629. https://doi.org/10.1016/j.exger.2019.06.002

Borges, A., Scotti, L.V., Siqueira, D.R., Zanini, R., do Amaral, F., Jurinitz, D.F. and Wassermann, G.F., 2007. *Chemosphere*, **6**: 920-926. https://doi.org/10.1016/j.chemosphere.2007.05.068

Burns, C.J. and Pastoor, T.P., 2018. *Crit. Rev. Toxicol.,* **48**: 297-311. https://doi.org/10.1080/10408444.2017.1423463

Chandran, R., Sivakumar, A.A., Mohandass, S. and Aruchami, M., 2005. *Comp. Biochem. Physiol. Part C: Toxicol. Pharmacol.,* **140**: 422-426. https://doi.org/10.1016/j.cca.2005.04.007

Das, P.C., Ayyappan, S., Jena, J.K. and Das, B.K., 2004. *Indian J. Fish.,* **51**: 287-294.

David, M., Mushigeri, S.B., Shivakumar, R. and Philip, G.H., 2004. *Chemosphere*, **56**: 347-352. https://doi.org/10.1016/j.chemosphere.2004.02.024

Dutta, H.M. and Dalal, R., 2008. *Int. J. Environ. Res.,* **2**: 215–224.

Fahmy, G.H., 2012. *Int. J. Biosci., Biochem. Bioinf.*, **2**: 52-55. https://doi.org/10.7763/IJBBB.2012.V2.69

Finney, D.J., 1971. *Probit analysis (3rd ed.)*. Cambridge, England: Cambridge University Press.

Fevery, D., Houbraken, M. and Spanoghe, P., 2016. *Sci. Total Environ.,* **550**: 514-521. https://doi.org/10.1016/j.scitotenv.2016.01.123

Gornall, A.G., Bardawill, C.J. and David, M.M., 1949. *J. Biol. Chem.,* **177**: 751-766. https://doi.org/10.1016/S0021-9258(18)57021-6

Hatami, M., Banaee, M. and Haghi, B.N., 2019. *Chemosphere*, **21**: 981-988. https://doi.

org/10.1016/j.chemosphere.2018.12.077

Kale, M.K., Joshi, P.P. and Kulkarni, G.K., 2006. *Effect of cadmium toxicity on biochemical composition of freshwater fish Rasbora daniconicus*. Ecology and environment (BN Pandey and MK Joyti Eds.) Aph Publication, New Delhi, pp. 271-278.

Köprücü, S.Ş., Köprücü, K. and Ural, M.S., 2006. *Bull. Environ. Contam. Tox.,* **76**: 59-65. https://doi.org/10.1007/s00128-005-0889-3

Korkmaz, N., Cengiz, E.I., Unlu, E., Uysal, E. and Yanar, M., 2009. *Environ. Tox. Pharmacol.,* **28**: 198-205. https://doi.org/10.1016/j.etap.2009.04.004

Lazartigues, A., Thomas, M., Cren-Olivé, C., Brun-Bellut, J., Le Roux, Y., Banas, D. and Feidt, C., 2013. *Environ. Sci. Pollut. Res.,* **20**: 117-125. https://doi.org/10.1007/s11356-012-1167-7

Malla-Reddy, P., 1987. *Biomed*., **7**: 21-24.

Maruthanayagam, C. and Sharmila, G., 2004. *Nat. Environ. Pollut. Technol.,* **3**: 491-494.

Mommsen, T.P. and Walsh, P.J., 1992. *Experientia*, **48**: 583-593. https://doi.org/10.1007/BF01920243

Murphy, S., 1986. Toxic effects of pesticides. In: *Casarett and Doull's toxicology: the basic science of poisons* (eds. C. Klaasen, M. Amdur, J. Doul). Macmillan, New York, NY, USA, pp. 519-558.

Naqvi, G.Z., N. Shoaib and A.M. Ali. 2016. *Pakistan. J. Zool.*, **48**: 1643-1648.

Ogueji, E.O. and Auta, J., 2007. *J. Fish. Int*., **2**: 86-90.

Parthasarathy, R. and Joseph, J., 2011. *Afr. J. Environ. Sci. Technol.,* **5**: 98-103.

Philip, G.H., Reddy, P.M. and Sridevi, G., 1995. *Ecotoxicol. Environ. Saf.,* **31**: 173-178. https://doi.org/10.1006/eesa.1995.1059

Radha, G., Logaswamy, S. and Logankumar, K., 2005. *Nat. Environ. Pollut. Technol.,* **4**: 307-310.

Reddy, A.T., Ayyanna, K. and Yellamma, K., 1991. *Biochem. Int.,* **23**: 959-962.

Sánchez-Fortún, S. and Barahona, M.V., 2005. *Chemosphere*, **59**: 553-559. https://doi.org/10.1016/j.chemosphere.2004.12.023

Sastry, K.V., Siddiqui, A.A. and Singh, S.K., 1982. *Chemosphere*, **11**: 1211-1216. https://doi.org/10.1016/0045-6535(82)90035-2

Shah, M.K., Khan, A., Rizvi, F. and Siddique, M., 2007. *Pak. Vet. J.,* **27**: 171-175.

Sharma, G. and Singh, S., 2004. *Bionotes,* **6**: 20.

Sharma, G. and Singh, S., 2006. *Bionotes*, **8**: 21. https://doi.org/10.1007/BF02913099

Sharma, G. and Singh, S., 2007. *J. Environ. Res. Dev.,* **1**: 261-263.

Shoaib, N., Siddiqui, P.J. and Khalid, H., 2013. *Pakistan J. Zool*., **45**: 1160-1164.

Sthanadar, I.A., Begum, B., Sthanadar, A.A., Nasar, M.J., Ahmad, I., Zahid, M., Muhammad, A. and Ullah, S., 2015. *J. Biodivers. Environ. Sci.,* **6**: 74-80.

Stoytcheva, M., Zlatev, R., Velkova, Z. and Valdez, B., 2011. *Pestic. Strateg. Pestic. Anal. In. Tech., Croatia*., pp. 359-372.

Ullah, S., Li, Z., Arifeen, M.Z.U., Khan, S.U. and Fahad, S., 2019a. *Chemosphere*, **214**: 519-533. https://doi.org/10.1016/j.chemosphere.2018.09.145

Ullah, S., Li, Z., Zuberi, A., Arifeen, M.Z.U. and Baig, M.M.F.A., 2019b. *Environ. Chem. Lett*., pp. 1-29.

World Health Organization, 1992. *Alpha-cypermethrin. Environmental Health Criteria 142*. World Health Organization, Geneva, Switzerland

Yekeen, T.A. and Fawole, O.O., 2011. *Afr. J. Biotechnol.,* **10**: 14090-14096. https://doi.org/10.5897/AJB10.2468