Available Online at http://www.recentscientific.com

CODEN: IJRSFP (USA)

International Journal of Recent Scientific Research
Vol. 10, Issue, 06(E), pp. 33013-33016, June, 2019

ISSN: 0976-3031

International Journal of Recent Scientific Research

DOI: 10.24327/IJRSR

Research Article

ALTERATIONS IN BIOCHEMICAL CONTENT OF FRESH WATER BIVALVE *Lamellidens marginalis* AFTER CHRONIC EXPOSURE TO MALACHITE GREEN

Rupali S. Khandekar and Deepak V. Muley*

Department of Zoology, Shivaji University, Kolhapur, India

DOI: http://dx.doi.org/10.24327/ijrsr.2019.1006.3588

ARTICLE INFO

Article History:
Received 06th March, 2019
Received in revised form 14th April, 2019
Accepted 23rd May, 2019
Published online 28th June, 2019

Key Words:
Biochemical content, *Lamellidens marginalis,* sub lethal concentrations, Malachite green dye

ABSTRACT

The present study was carried out to investigate the effect of malachite green dye used , in dyeing and aquaculture, on the fresh water bivalve *Lamellidens marginalis*. The effects of sub lethal exposure i.e. 1/10th and 1/20th concentration of LC_{50}, were studied by changes in the bichemical constitutes like total glycogen, protein and lipid in different tissues like gill, hepatopancreas, gonad and mantle for 30 days exposure period. The glycogen content in selected tissues showed significant depletion ($p<0.001$) at both the concentration. The mantle showed maximum significant depletion in protein content at both i.e. 1/20th and 1/10th the concentration, (-28.77%) and (-37.77%) respectively compared to other tissues. The lipid showed significant decrease in gill (-21.31%) and mantle (-46.31%) at the 1/20th and 1/10th concentration.

INTRODUCTION

Dyes, the coloured substances, are soluble during application process which having affinity to the substrate to which they are being applied to the selective absorption of light (Pereira and Alves., 2012). Every year, hundreds of new coloured compounds produced into a series of different applications flooded in the market (Majcen-Le Marechal *et al*., 1997).

Malachite Green (Basic Green 4) is basic dye, easily soluble in water, used in dyeing wool, silk, leather, jute, cotton and acrylic industries; it is also used as food additive, food disinfectant, food colouring agent, medical disinfectant and antihelmenthic (Culp and Beland., 1996). It is also used in aquaculture industries as antiprotozoan, antibacterial, antifungal and antihelmenthic (Pointing and Vrijmoed, 2000; Campbell *et al*., 2001; Chang *et al*., 2001; Sudova *et al*., 2007). It is environmentaly persistent and causes serious health hazards. Both, clinical and experimental observations reported as MG is a multi-organ toxin. Due to its extreme toxicity and ecotoxicity hazards associated with MG, restriction has been implemented in many countries from 2000. It is still being used in many parts of the world due to its low cost, ready availability and efficacy.

Toxicological effects of this dye have been reported in cat fish, channel cat fish, Salmon, tilapia, snake head barb and carp (Sudova *et al*., 2007). Due to continuous exposure to this dye, the residues of this dye i.e. leucomalachite green accumulate in cell cytoplasm which will affect the biochemical content of that animal. This may change normal physiology of that animal. Bivalves have been used as sentinel organisms in pollution monitoring programmes (Moreira and Guilhermino, 2005). Many molluscan species such as *L. marginalis, L. corriance, B. bengalasis* are used as bioindicator depending on their tolerance power (Oehlmann *et al*., 2002; Druart *et al*., 2011). *Lamellidens marginalis* is considered as one of the best pollution bioindicator of pollution in aquatic environment (Kumar *et al*, 2017). It has been reported that the pollutants start exhibiting its negative exhibit toxicity on molluscs at lower environmental concentrations than other invertebrates or vertebrates (Chakraborty *et al*., 2010). In the view of the above the aim of the present study was to assess chronic toxicity of malachite green dye and its impact on biochemical prameters of *L. marginalis*.

MATERIALS AND METHODS

Chronic Toxicity Study

A total number of 100 healthy bivalves *L. marginalis* (7 – 8 cm) was used to determine 96 hrs LC_{50} value i.e. 0.9 ppm was determined by static bioassay. For chronic toxicity study, 30 *L. marginalis* were randomly divided into 3 equal group; treated group exposed to 1/20th and 1/10th concentration of LC50 of

Corresponding author:* **Deepak V. Muley
Department of Zoology, Shivaji University, Kolhapur, India

MG and control group. The experiment extended to 30 days and the water was renewed every day and there was no mortality was observed. in chronic study. The physicochemical parameters were measured during the exposure period.

Biochemical Estimations

After exposure period of 30 days bivalves from three groups i.e., control, 1/20th of LC_{50} and 1/10th of LC_{50} were sacrificed and different tissues like gill, mantle, hepatopancrease and gonad were separated to study various biochemical parameters. Glycogen was estimated by using anthron reagent (DeZwaan and Zandee, 1972), total protein content was estimated by the method of Lowry (1956) and lipid content was estimated by vanillin reagent (Barnes and Blackstock, 1973).

RESULT AND DISCUSSION

The physicochemial parameters like temperature, pH, O_2, CO_2, hardness, phosphate and nitrate content of test water were measured by standard method by APHA (2005). All physicochemical parameters of test water were relatively remains constant through out the experiment. The median lethal concentration (LC_{50}) of MG for 96 hours was 0.9 ppm. For chronic toxicity study, 1/20th of LC_{50} (0.045 ppm); 1/10th of LC_{50} (0.09 ppm) and control group contain only tap water was grouped.

Glycogen is the fuel for different metabolic and physiological processes. After chronic exposure for 30 days to sublethal concentrations of Malachite green showed significant decrease in glycogen content in all tissues ($p < 0.001$). At both 1/10th and 1/20th concentrations there was highly significant depletion. At both the (0.09 and 0.045 ppm) concentrations, the percent decrease was in the order of gill (-47.02%) > mantle (-32.4%) > hepatopancreas (-15.2%) > gonad (-12.38%) and gill (-28.2%) > mantle (-18.88%) > hepatopancreas (-8.77%) > gonad (-6.19%) respectively. The order of depletion was same and highly significant in both the concetrations. The decrease in glycogen (carbohydrate) content indicates the excessive utilization of carbohydrates to cope up with dye induced toxicity stress. The decreased amount of glycogen might be due to breakdown of glycogen in the digestive gland through glycogenolytic activity (Koundinya and Ramamurthi, 1979). Under stress condition, the stored form of glycogen was utilized through hexose monophosphate pathway. The decrease in glycogen content might be due to inhibition of hormonal and enzymatic response (Martinez *et al*., 2004; Patil *et al*., 2012). Srivastava (1995b) causes hepatic and muscle glycogenolysis in *H. fossilis*. Afaq (2010) showed decreased glucose serum level in *Cirrhinus mrigala* after lethal and sublethal exposure of Bismark brown. The anthraquinone dye after acute and chronic exposure to *Cyprinus carpio* also showed decreased glycogen content (Olaganathan *et al*., 2013).

Proteins are the major biochemical component, which act as source of energy for various physiological functions including reproduction. After 30 days of exposure period to malachite green dye more significant decrease in protein content was observed. At 0.045 ppm (1/20th) concentration the depletion of protein content was in the order of mantle (- 28.48%) > hepatopancreas (- 19.45%) > gill (-18.52%) > gonad (-17.04%). In case of 0.09 ppm (1/10th) concentration, the minimum decrease in protein content was found in gonad (-25.69%), while other tissues like gill (- 41.19%), hepatopancreas (- 40.2%), mantle (- 37.77%) showed highly significant ($p < 0.001$) decrease in protein content. The depletion in protein content may be due to increased catabolism and decreased anabolism of proteins or may be due to increased proteolysis and inhibition of protein synthesis (Muley *et al*., 2007). The different tissues like gills, foot and mantle of *Lamellidens corrianus* and *Lamellidens marginalis* showed decreased protein content after pesticidal stress (Kamble *et al*., 2010).

Lipid composition of molluscs is affected by external factors (environmental), or by internal factors such as metabolic and physiological activities. After 30 days exposure, at 0.045 ppm (1/20th) concentration, gonad showed less significant decrease ($p < 0.05$) in lipid content, while more significant decrease ($p < 0.001$) in lipid content was observed in gill. At 0.09 ppm (1/10th) concentration, the percent decrease was in the order of mantle (- 46.31%) > gill (- 30.17%) >hepatopancreas (-26.33%) > gonad (- 15.9%) in lipid content. At both the concentration, 0.09 ppm (1/10th) and 0.045 ppm (1/20th),less depletion was observed in lipid content in gonad. Decrease in total lipid content in animal tissue after exposure to various pollutants were reported by some investigators (Sonwane *et al*., 2015; Suryawanshi *et al*., 2016). The depletion of lipid content in mantle might be due to utilization of lipid to provide energy in the formation of gonad during gametogenesis (Pardeshi *et al*., 2015). Olaganathan (2013) noticed the chronic exposure of anthraquione dyes depleted the carbohydrate, protein and lipid content in *Channa punctatus* and *Cyprinus carpio*.

CONCLUSION

The results obtained in this study indicate that the malachite green dye which was used for number of purposes affect the metabolism in fresh water bivalve Lamellidens marginalis by reducing biochemical contents due to stress condition. The malachite green dye showed synergistic effects on physiology of the bivalves at sub lethal concentration after long exposure.

Table 1 Changes in glycogen content after chronic exposure of *L. marginalis* to malachite green (values are expressed in mg/100mg wet tissue)

Tissues	Control	1/20th	% change over control	1/10th	% change over control
Gonad	3.238 ± 0.0325	3.033 ±0.015***	-6.19	2.83 ± 0.053***	-12.38
Hepatopancreas	3.42 ± 0.037	3.127 ±0.027***	-8.77	2.904 ±0.042***	-15.2
Gill	2.02 ± 0.027	1.457 ± 0.04***	-28.2	1.077 ±0.022***	-47.02
Mantle	2.33 ± 0.018	1.895 ±0.021***	-18.88	1.575 ±0.031***	-32.4

Values are mean ± S.D., *** indicates significance level P<0.001, (n = 3)

Table 2 Changes in protein content after chronic exposureof *L. marginalis* to malachite green (values are expressed in mg/100mg wet tissue)

Tissues	Control	1/20th	% change over control	1/10th	% change over control
Gonad	3.93± 0.23	3.26 ± 0.039**	-17.04	2.929 ± 0.065**	-25.69
Hepatopancreas	3.683 ± 0.12	2.964 ± 0.13**	-19.45	2.201 ± 0.054***	-40.2
Gill	2.842 ± 0.133	2.314 ± 0.119**	-18.52	1.672 ± 0.152***	-41.19
Mantle	3.232 ± 0.128	2.31 ± 0.228**	-28.48	2.019 ± 0.039***	-37.77

Values are mean ± S.D., **, *** indicates significance level P<0.01, P<0.001 (n = 3)

Table 3 Changes in lipid content after chronic exposureof *L. marginalis* to malachite green (values are expressed in mg/100mg wet tissue)

Tissues	Control	1/20th (3.5 ppm)	% change Over control	1/10th (7 ppm)	% change over control
Gonad	3.578 ± 0.018	3.395 ± 0.081*	- 5.04	2.63 ± 0.0065***	- 15.9
Hepatopancreas	3.467 ± 0.024	2.81 ± 0.055***	- 18.67	2.43 ± 0.0038***	- 26.33
Gill	2.445 ± 0.011	1.92 ± 0.027***	- 21.31	1.32 ± 0.023***	- 30.17
Mantle	2.69 ± 0.036	2.438 ± 0.165*	- 9.66	2.269 ± 0.051***	- 46.31

Values are mean ± S.D., *, **, *** indicates significance level P < 0.05, P < 0.01, P < 0.001 (n = 3)

References

Afaq, S., Rana, K. S., & Lone, M. A. (2010). Toxicological effects of leather dyes on serum cholesterol of fresh water teleost, *Cirrhinus mrigala* (Ham.). *International Journal of Pharma and Bio Sciences*, *1*(2).

APHA (2005)Standard Methods for the Examination of Water and Wastewater

Barnes, H. and Blacksock, J. (1973):Estimation of lipids in marine animals and tissues. Detailed investigation of sulphophos-phovanillin method for total lipids. Journal of Experimental Marine Biology and Ecology, Vol. 12 (1): 103

Campbell, R.E., Lilley, J.H., Taukhid, , Panyawachira, V., Kanchanakhan, S., (2001). In vitro screening of novel treatments for Aphanomyces invadans. Aquacult. Res. 32 (3), 223–233.

Chakraborty, S., Ray, M., & Ray, S. (2010). Toxicity of sodium arsenite in the gill of an economically important mollusc of India. *Fish & shellfish immunology*, *29*(1), 136-148.

Chang, C.F., Yang, C.H., Shu, Y.O., Chen, T.I., Shu, M.S., Liao, I.C., (2001). Effects of temperature, salinity and chemical drugs on the in vitro propagation of the Dinoflagellate parasite, Amylodinium ocellatum. Asian Fish Soc. P31.

Culp, S. J. and Beland, F. A. (1996.) Malachite green: atoxicological review J. Am Coll. Toxicol.,15: 219-238.

De Zwaan, A. and Zandee, D. I. (1972):Body distribution and seasonal changes in the glycogen content of the common mussel, Mytilus edulis. Comparative Biochemistry and Physiology, 43 A: 53 - 58.

Druart, C., Millet, M., Scheifler, R., Delhomme, O., Raeppel, C., & De Vaufleury, A. (2011). Snails as indicators of pesticide drift, deposit, transfer and effects in the vineyard. *Science of the total environment*, *409*(20), 4280-4288.

Kamble, V. S. (2010).Effect of pesticides on the Lamellibranch Molluscs Lamellidens Corrianus found near Sangola and their impact on ecology.

Koundinya, P. R., & Ramamurthi, R. (1979). Effect of organophosphate pesticide Sumithion (Fenitrothion) on some aspects of carbohydrate metabolism in a freshwater fish, Sarotherodon (Tilapia) mossambicus (Peters). *Experientia*, *35*(12), 1632-1633.

Kumar M, Kumari S, Sujila T, Drishya S. and Gopinathan S., (2017). Impact of dye effluent on Enzymological changes in freshwater fish *Oreochromis mossambicus* Journal of Biotechnology and Biochemistry (IOSR-JBB) Volume 3, Issue 6 (Nov.- Dec. 2017), PP 34-38.

Lowry, O. H., Rosenbrough, N. J., Farr, A. L. and Randall, R. J. (1956): Protein measurement using the Folin phenol reagent. Journal of Biochemistry193, 265

Majcen-Le Marechal., Slokar Y.M., and Taufer T., (1997). "Decoloration of Chlorotriazine Reactive Azo Dyes with H2O2/UV," Dyes Pigments, vol. 33, pp. 281-298.

Martinez, C. B. R., Nagae, M. Y., Zaia, C. T. B. V., & Zaia, D. A. M. (2004). Acute morphological and physiological effects of lead in the neotropical fish *Prochilodus lineatus.* Brazilian Journal of Biology, 64(4), 797-807.

Moreira, S. M., & Guilhermino, L. (2005). The use of Mytilus galloprovincialis acetylcholinesterase and glutathione S-transferases activities as biomarkers of environmental contamination along the northwest Portuguese coast. *Environmental monitoring and Assessment*, *105*(1-3), 309-325.

Muley, D. V., Karanjkar, D. M., & Maske, S. V. (2007).Impact of industrial effluents on the biochemical composition of fresh water fish *Labeo rohita*. Journal of environmental biology, 28(2), 245-249.

Oehlmann, V. D., Korte, H., Sterner, C., & Korsching, S. I. (2002). A neuropeptide FF-related gene is expressed selectively in neurons of the terminal nerve in Danio rerio. *Mechanisms of development*, *117*(1-2), 357-361.

Olaganathan, R., & Patterson, J. (2013). Effect of anthraquinone dyes on the carbohydrate, protein and lipid content in the muscle of *Channa punctatus* and *Cyprinus carpio*. International Journal of Pharmaceutical Applications, 4, 11- 19

Pardeshi and Gapat (2012): Ascorbate effect on protein content during nickel intoxication in the freshwater bivalve, *Lamellidens corrianus* Bioscience Discovery 3(2): 270-274 27.

Patil, I. and A.M. Malwadkar.(2012). Glycogen profile in Puntiussaranasarana from Pavana, a Tributary of river Bhima, Maharashtra.Bionano Frontier ;Vol. 5 (2) 250-252

Pereira, L., Alves, M. (2012). Dyes-environmental impact and remediation. In: Malik A, Grohmann E (eds) Environmental protection strategies for sustainable development, strategies for sustainability. Springer, New York

Pointing, S.B., Vrijmoed, L.L.P., (2000). Decolorisation of azo and triphenylmethane dyes by Pycnoporus sanguineus producing laccase as the sole phenol oxidase. World J. Microbiol. Biotechnol. 16, 317–318.

Sonawane, S. M. (2015).Effect of heavy metals on gills of fresh water bivalve *Lamellidens marginalis*. IOSR J Environ Sci Toxicol Food Technol, 9, 5-11.

Srivastava, A. K., Sinha, R., Singh, N., Roy, D., & Srivastava, S. (1995). Malachite green-induced changes in carbohydrate metabolism and blood chloride levels in the freshwater catfish Heteropneustes fossilis [Bloch]. *Acta Hydrobiologica*, *2*(37).

Sudova, J. Machova (2007). Negative effects of malachite green and possibilities of its replacement in the treatment of fish eggs and fish: a review: Veterinarni Medicina, 52, 2007 (12): 527–539.

Suryavathi, V., Sharma, S., Sharma, S., Saxena, P., Pandey, S., Grover, R., ...& Sharma, K. P. (2005). Acute toxicity of textile dye wastewaters (untreated and treated) of Sanganer on male reproductive systems of albino rats and mice. *Reproductive Toxicology*, *19*(4), 547-556.

How to cite this article:

Rupali S. Khandekar and Deepak V. Muley., 2019, Alterations in Biochemical content of Fresh water Bivalve Lamellidens Marginalis after Chronic Exposure to Malachite Green. *Int J Recent Sci Res.* 10(06), pp. 33013-33016.
DOI: http://dx.doi.org/10.24327/ijrsr.2019.1006.3588

****