Original Research Article

International Journal of Bioassays
ISSN: 2278-778X
www.ijbio.com
OPEN ACCESS

HISTOPATHOLOGICAL CHANGES IN THE OVARIES AND MUSCLE TISSUES OF FRESHWATER FAIRY SHRIMP *STREPTOCEPHALUS DICHOTOMUS* (BAIRD, 1860), EXPOSED TO MALATHION AND GLYPHOSATE

Arun Kumar MS* **and A Jawahar Ali**
Unit of Aquaculture and Aquatic Toxicology, P.G & Research Department of Zoology, The New College, Chennai-6000 14, India.

Received for publication: June 13, 2014; **Revised**: June 22, 2014; **Accepted**: July 07, 2014

Abstract: Possible impact of organophosphorus pesticides namely malathion and glyphosate on the ovaries and muscle tissues of the freshwater fairy shrimp *Streptocephalus dichotomus* were reported here for the first time. Preadult fairy shrimps were exposed to sub lethal concentration (1/5th of 96hrs LC50) of malathion (2.0 ppm) and glyphosate (0.0011 ppm) for a period of 30 days. Histological observations revealed a few marked pathological lesions such as mild destruction of epithelial layer, follicle cells, nurse cells, necrosis and degeneration of oocytes in the ovaries. Similarly degeneration of muscles, necrosis of muscle fibers, haemorrhages and appearance of pigmented cells in the muscle tissues were evident compared to control. The structural alterations observed in the ovaries and muscle tissues of the freshwater fairy shrimp are suggestive that malathion and glyphosate caused tissue damage at the tested concentrations. Therefore, the findings of this investigation can be taken as biomarkers for monitoring pesticides contamination in aquatic biota.

Key Words: Malathion, Glyphosate, *S. dichotomus*, Ovaries, Muscle, Necrosis, Haemorrhages.

INTRODUCTION

Freshwater ecosystems can be contaminated with pesticides by leaching, runoff, or direct or indirect spraying, this later occurring by action of the wind [1]. Most of the chemicals that are used as pesticides are generally toxic to many non-target species including humans, and other desirable forms of life that co-inhabitant the environment [2, 3].

During past few decades, the use of organophosphorus pesticides (Ops) has largely replaced organochlorine compounds in the agricultural activities. Ops have been widely used to control agricultural pests, but these are harmful to non-target aquatic organisms when frequently used, due to contamination of aquatic environment through run-off [4, 5]. The toxicity of organophosphates arises from their inhibition of the enzyme acetylcholinesterase (AChE E.C 3.1.1.7), which is essential for the transmission of nerve impulse and also plays a vital role in the development of neurons and network formation [6, 7, 8]. OPs are highly soluble in water and can therefore easily contaminate aquatic ecosystems, thereby increasing the exposure risk of aquatic flora and fauna [9]. Although other groups of insecticides with a shorter life and comparatively very low mammalian toxicity are available (e.g. pyrethroids), organophosphorus (OPs) compounds are still used frequently in agricultural practices. Therefore, extensive use and discharges of OPs in environment may impair biological communities and the accidental release into water sources may cause unexpected human intoxication.

Histopathological examination has been increasingly recognized as a valuable tool for the assessment of the impact of environmental pollutants on aquatic animals [10, 11, 12, 13, 14]. The fairy shrimp *Streptocephalus dichotomus* are commonly found in seasonal vernal pools. They are subjected to risks of exposure to agrochemicals especially OPs and other pollutants [15]. Hence, this study documents the possible impact of malathion and glyphosate on histological aspects of ovaries and muscle tissues of freshwater fairy shrimp, *S. dichotomus*.

MATERIAL AND METHODS

S. dichotomus were originally collected from the temporary ponds of Putlur, Thiruvallur District and maintained in laboratory for cyst production. Fairy shrimps were hatched from cysts by following the procedures of Ali and Dumont [16]. Toxicity tests were performed to determine the 96hrs LC_{50} values of malathion and glyphosate and were found to be 9.1 ppm and 0.0055 ppm, respectively to the preadults of *S. dichotomus* [15]. Based on the 96hrs LC_{50} value, a sub lethal concentration of each pesticide ($1/5^{th}$ of the 96hrs LC_{50}; malathion, 2.0 ppm; glyphosate, 0.0011 ppm) was chosen for histopathological studies. Preadults of *S. dichotomus* were exposed to sub lethal concentrations of malathion and glyphosate for a period of 30 days and at the end of the experimental period treated fairy shrimps were collected and preserved in 10% buffered formalin for histological studies. Preserved whole fairy shrimps were carefully processed by conventional method [17].

***Corresponding Author:**
Arun Kumar MS,
Unit of Aquaculture and Aquatic Toxicology,
P.G & Research Department of Zoology,
The New College, Chennai 6000 14. India.

They were cut into 4 to 5µm thickness by a rotary microtome, stained with haematoxylin-eosin and examined under the Labomed light microscope.

RESULTS AND DISCUSSION

Chemical pollution in aquatic ecosystems especially seasonal vernal pools is a major environmental concern. Not only does this type of pollution cause a decrease in water quality, but subsequently affects all living organisms in the system [18, 19, 20, 21]. In the present investigation, the freshwater fairy shrimp *S. dichotomus* was exposed to sub lethal concentration of malathion (2.0 ppm) and glyphosate (0.0011 ppm).

Histopathological abnormalities in ovaries may be caused by several factors, viz., ionizing radiations, electric current, parasitic infections, mechanical injuries, xenobiotic toxicants [22] and by a variety of effluents [23, 24, 25]. The results of the present study revealed that *S. dichotomus* manifested histopathological changes in ovaries and muscles. In the control, ovaries showed normal histoarchitecture of epithelial layer, follicle cells, nurse cells and oocytes whereas the fairy shrimps exposed to sub lethal concentration of malathion and glyphosate had mild destruction of epithelial layer, follicle cells, nurse cells, necrosis and degeneration of oocytes (Figures 1-3). Similar histopathological alterations were reported in different aquatic organisms exposed to different pollutants by many investigators. Reddy *et al.,* [26] noticed destruction of epithelial layer, follicle cells, nurse cells, necrosis and degeneration of oocytes in the ovary of freshwater crab, *Barytelphusa guerini* when exposed to heavy metals. However, Sarojini *et al.,* [27] documented the deleterious effects of cadmium chloride on the ovarian development for the same species. Tehrani *et al.,* [28] postulated that the degree of damage in the ovaries of *Artemia urumiana* affected by carbamates pesticide was indicated by necrosis in ovarian nurse cells.

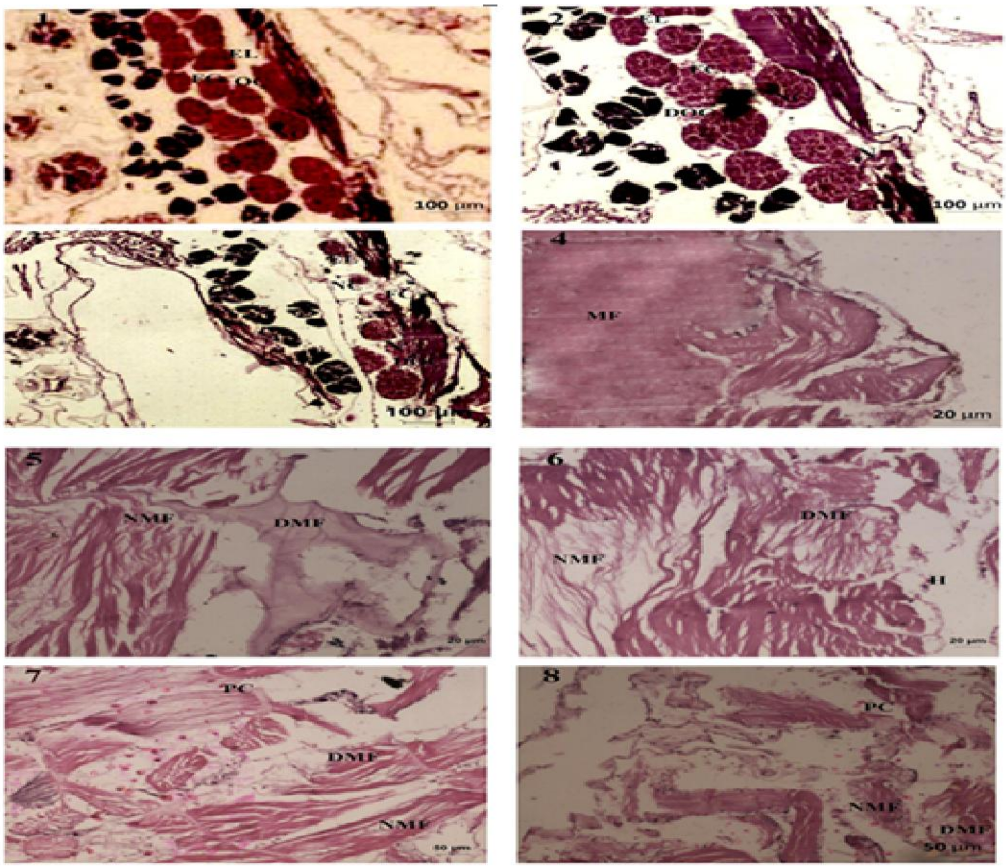

Figure 1: Photomicrograph of L.S through the ovaries of control fairy shrimp showing normal epithelial layer (EL), follicle cells (FC), nurse cells (NC) and oocytes (OC). **Figures 2-3:** Photomicrographs of L.S through the ovaries of test fairy shrimps exposed to malathion and glyphosate, 2-3 the ovary showing mild destruction of epithelial layer (EL), follicle cells (FC), nurse cells (NC), necrosis (N) and degeneration of oocytes (DOC). **Figure 4:** Photomicrograph of L.S through the muscles of control fairy shrimp showing normal histoarchitecture of muscle, muscle fibers (MF) and absence of Haemorrhages (H) and Pigmented cells (PC). **Figures 5-8:** Photomicrographs of L.S through the muscle tissue of test fairy shrimps exposed to malathion and glyphosate, 5-8 the muscle showing degeneration (DMF), Necrosis of muscle fibers (NMF), Haemorrhages (H) and Pigmented cells (PC).

Kharat *et al., [29]* recorded the effects of tributyltin chloride induced histopathological insult of ovarian tissue of freshwater prawn, *Macrobrachium kistnensis*. They reported marked damages in epithelial layer, degeneration of oocytes, vacuolization appearance in cytoplasm and nucleoplasm. Similarly, Jadhav and Sheikh [30] observed exposure and concentrated mediated changes in ovaries of freshwater crab, *Barytelphusa cunicularis* treated with endosulfan. Likewise, Rani *et al.,* [31] reported degenerative changes in ovaries of mud crab, *Scylla olivacea* when exposed to cadmium nanoparticle. Damage to the ovarian tissue may be due to the direct effects of organophosphorus pesticides on developing oocytes interfere with the enzyme system in metabolism or destroying the function of hormone that controlling the ovarian growth and leads to decline reproductive activity.

In the present study, several histopathological alterations were noticed in the muscles of *S. dichotomus* when exposed to sub lethal concentration of malathion and glyphosate. The pathological findings include degeneration of muscles, necroses of muscle fibers with haemorrhages and RBC like pigmented cells (Figs 4-8). As muscle tissue is the primary site of exposure, pollutants affected the muscle epidermis abruptly. Pigmented cells are prominent feature of chronic inflammatory response. The present investigation closely agreed with a similar report by Tehrani *et al.,* [28] in the muscle tissues of *Artemia urmaiana* in response to carbamates pesticide resulting in degeneration, Zenkers necrosis of muscle fiber with haemorrhages and RBC like cells. Exposure of *Labeo rohita* to hexachlorocyclohexane was found to induce separation of muscle bundles and intracellular oedema in the muscle tissues [32]. Moreover, Fatma [33] observed degeneration of muscle bundles with aggregations of inflammatory cells and focal areas of necrosis in the muscle tissues of *Tilapia zillii* and *Solea vulgaris* exposed to heavy metal. Such observations were also made in muscle tissues of *Oreochromis mossambicus* on exposure to dimethoate [34]. Histopathological alterations in the muscle tissues of *Heteropneustes fossilis* exposed to polluted river water were also recorded by Rakhi *et al.,* [35].

In this study, malathion and glyphosate caused histopathological alterations in *S. dichotomus* at the tested concentration of respective pesticides. No studies were conducted so far to document the histopathological effect of pesticides in freshwater fairy shrimp. Moreover, the findings of present study serve as "biomarkers" for assessing pesticide toxicity in the aquatic biota. The use of pesticides in various agricultural and non-agricultural landscapes should be minimized. Instead of synthetic chemicals, biological methods could be employed for controlling pests in agricultural and non-agricultural areas.

ACKNOWLEDGEMENTS

The authors are thankful to the Management, Principal, The New College Chennai-14 for providing necessary facilities. Thanks are also due to Dr. N. Munuswamy (Professor, Department of Zoology, University of Madras) and Dr. M. Asrar Sheriff (Associate Professor and Head, Department of Zoology, The New College, Chennai-14) for their comments and positive criticisms on the manuscript.

REFERENCES

1. WHO. The WHO recommended classification of pesticides by hazard and guidelines to classification: 2009, 2010, ISBN: 9789241547963.
2. Martinez CBR, IMS, Colus, Biomarcadores em peixes neotropicais para monitoramento da poluição aquática na bacia do rio Tibagi. In: (Medri, M.E., Pimenta, J. A., Shibatta, O. A. eds.), A bacia do Rio Tibagi. Editora dos Editores, Londrina, PR, Brazil. 2002, PP -551-577.
3. Yazhini JS, Darcus, Insecticide (Profenofos) induced biochemical changes in the freshwater fish *Catla catla.* Int J Curr Sci., 2012, pp.120-124.
4. Joseph B, SJ, Raj, Impact of pesticide toxicity on selected biomarkers in fishes. Int. J. Zool. Res., 2011. 7:212-222.
5. Patil KT, BS, Yadav, Effect of sub lethal concentration of Metasystox pesticide on freshwater crab *Barytelphusa cunicularis.* Int J Sci Inn. & Disc. 2012, 2(1), 197-200.
6. Ozmen MS, Sener A, Mete H, Kucukbay, In vitro and in vivo acetylcholinesterase- inhibiting effect of new classes of organophosphorus compounds. Environ Toxicol Chem., 1999, 18: 241-246.
7. Fulton MH, PB Key, Acetylcholinesterase inhibition in estuarine fish and invertebrates as an indicator of organophosphorus insecticides exposure and effects. Envion Toxicol Chem., 2001, 20: 37-45.
8. Paraoanu LE, PG Layer, Acetylcholinesterase in cell adhesion, neurite growth and network formation. FEBS J., 2008, 275:618-624.
9. Agdi KA, Bouaid AM, Esteban PF, Hernando A, Azmani C, Camara, Removal of atrazine and four organophosphorus pesticides from environmental waters by diatomaceous earth remediation method. J. Environ Monit 2000, 2(5), 420-423.
10. Peebua P, M, Kruatrachue Pokethitiyook S, Singhakaew, Histopathological alterations of Nile tilapia *Oreochromis niloticus* in acute and sub chronic Alachlor exposure. J Envi Bio., 2008, 29(3) pp.325-331.
11. Saravana Bhavan PP, Geraldine, Manifestation of Carbaryl toxicity on soluble protein and histopathology in the heptopancreas and gills of the prawn, *Macrobrachium malcolmsonii.* J Envi Bio., 2009, 30(4) pp.533-538.

12. Manosathiyadevan M, Selvisabhanayakam V, Divya, Morphological alterations and biochemical contents of the testis of adult male freshwater prawn *Macrobrachium malcolmsonii.* Ind J Fun App Lif Sci., 2012, 2(4) pp.104-113.

13. Maryam SM, Khalili V, Abbasi A, Hedayati, Sub-lethal effect of copper toxicity on liver lesions of Roach (*Rutilus rutilus caspicus*) juveniles. JNASCI. 2013, 2(4): 119-123.

14. Chourpagar AR, GK, Kulkarani, Effect of mercuric chloride on gill structure of a freshwater female crab, *Barytelphusa cunicularis* (Westwood). J Glob Biosci., 2014, 3(2): 423-427.

15. Arun Kumar MS, A, Jawahar Ali, Toxic impacts of two organophosphorus pesticides on the acetylcholinesterase activity and biochemical composition of freshwater fairy shrimp *Streptocephalus dichotomus*. Int J Pharm & Bio Sci., 2013, 4(2):966-972.

16. Ali AJ, HJ, Dumont, Larviculture of the fairy shrimp, *Streptocephalus proboscideus* (Crustacea: Anostraca): effect of food concentration and physical and chemical properties of the culture medium, Hydrobiologia, 1995, 298: 159-165.

17. Bancroft D, A, Stevens R, Turner, Theory and practice of histological techniques. 4th edition, Churchil Livingstone, Edinburgh, London, Melbourne, 1996.

18. Lahr J, Ecotoxicology of organisms adapted to life in temporary freshwater ponds in Arid and Semi-Arid regions. Arch Environ Contam Toxicol., 1997, 32, 50-57.

19. Lahr J, An ecological assessment of the hazard of eight insecticides used in desert Locust control, to invertebrates in temporary ponds in the Sahel. Aquatic Ecology, 1998, 32:153-162.

20. Lahr J, A, Badji S, Marquenie E, Schuiling KB, Ndour AO, Diallo, and JW, Everts, Acute toxicity of Locust insecticides to two indigenous invertebrates from Sahelian temporary ponds. Ecotox & Environ Safety, 2001. 48, 66-75.

21. Ripley BJ, KC, Davis BJ, Carter M, Simovich, Toxicity of Malathion and Roundup to the San Diego fairy shrimp. *Trans West Sect Wildl Soc.,* 2003, 38/39 13-21.

22. Sarojini A and B Victor, Toxicity of mercury on the ovaries of the caridean prawn. Curr Sci., 1985, 54, 398-400.

23. Saxena PK and M Grag, Effect of insecticidal pollution on ovarian recnidesence in the freshwater teleost, *Channa punctatus*. Ind J Exp Biol., 1978, 16: 690-691.

24. Farmer D, IR, Hill SJ, Maund, A comparison of the fate and effects of two pyrethroid insecticides (Lambda - cyhalothrin and cypermethrin) in pond Mesocosm. Ecotoxicol., 1995. 4, 219-244.

25. Kumar M, SP, Trivedi I, Benerjee A, Soni, Influence of anionic surfactant linear alkyl benzene sulphonatase on ovarian phosphatase activity in *Heteropneustes fossilis* (Bloch). *Himalayan* J Environ Zool., 2000, 14, 53-58.

26. Reddy SLN, K, Shankaraiah, KV, Raman Rao, Time course alteration in protein metabolism of freshwater field crab, *Barytelphusa guerini.* In proceedings of the all India symposium on physiological responses of animals to pollutants, Dr. B. A. M. U Aurangabad, 1982,

27. Sarojini R, PR, Machale AK, Khan, R, Nagabhushanam, Effect of cadmium chloride on histology and biochemical contents of the hepatopancreas of the freshwater crab, *Barytelphusa guerini*. Enviro. Series, 1990, 6: 91-97.

28. Tehrani AAG, Sadeghi Z, Badamchi NH, Sanjou Mansoub A, Azhari, Effect of Carbamates pesticides on Instar I-II larvae and Adult *Artemia urumiana.* Annals of Biological research, 2011, 2(3):515-525.

29. Kharat PS, KB, Shejule BC, Ghoble, Histopathological changes in ovary of freshwater prawn, *Macrobrachium kistnensis* exposed to TBTCl. World J Zoo., 2011, 6(3): 296-300.

30. Jadhav TJ, JD, Shaikh, Histopathological changes in the ovary of freshwater crab, *Barytelphusa cunicularis* exposed to Endosulphan. Int J Sci., 2012, 1(2) pp.139-140.

31. Rani DS, R, Kavitha M, Padmaja. Histological and biochemical changes in reproductive organs of Mud crab *Scylla Olivacea* (Herbst, 1796) exposed to Cadmium nanoparticle. *J Acad & Indus Resea.,* 2013, Vol.2 (7): 391-396.

32. Das BK, SC, Mukherjee, A histopathological study of carp *Labeo rohita* exposed to Hexachlorocyclohexane. Veterinarski Arhiv., 2000, 70(4), 169-180.

33. Fatma ASM, Histopathological studies on *Tilapia zillii* and *Solea vulgaris* from Lake Qarun, Egypt. World J Fish & Mar Sci., 2009, 1(1):29-39.

34. Parikh H, P, Rangrez A, Bagchi BN, Desai, Effect of Dimethote on some histoarchitecture of freshwater fish *Oreochromis mossambicus* (Peters, 1852). The Bioscan, 2010, 5(1): 55-58.

35. Rakhi SF, AHMM, Reza MS, Hossen Z, Hossain, Alterations in histopathological features and brain acetylcholinesterase activity in stinging catfish *Heteropneustes fossilis* exposed to polluted river water. *Int Aqua Resea.,* 2013, 5 (7): 1-18.

Source of support: Nil
Conflict of interest: None Declared