Original Research Paper

Volume-9 | Issue-7 | July - 2019 | PRINT ISSN No. 2249 - 555X

Environmental biology

STUDIES ON ACUTE AND BEHAVIOURAL TOXICITY OF TRIAZOPHOS ON FRESHWATER FISH *CYPRINUS CARPIO*

Pattar RM	Environmental Toxicology and Molecular Biology Laboratory, Department of PG Studies and Research in Zoology, Karnatak University, Dharwad- 580003
David M*	Environmental Toxicology and Molecular Biology Laboratory, Department of PG Studies and Research in Zoology, Karnatak University, Dharwad- 580003 *Corresponding Author

ABSTRACT The present study was undertaken to evaluate the median lethal concentration (LC_{50}), and changes in behavioural responses caused by commercial formulations of triazophos on freshwater fish *Cyprinus carpio*. The LC_{50} value was recorded to be 1.5mg/L and changes in behavioural responses were studied at sublethal concentration of 0.3mg/L ($1/5^{th}$), for a duration of 1, 10, 20 and 30 days, which revealed findings like, loss of fright response in addition to changes in swimming pattern that comprised of partial jerks, sinking at the bottom of the tank and whirling cork like movements. These changes were identified to be duration dependent thereby acknowledging the direct cause of damage due to triazophos. Based on the outcome of the present study, it is recommended that care must be taken when triazophos is used at agricultural sites especially nearby the aquatic ecosystems.

KEYWORDS : Acute toxicity, Aquaculture, Behavioural responses, Pesticide and Toxicity

INTRODUCTION

Pesticides belong to the only group of harmful chemicals that have been used as an effective weapon to protect agricultural products from the attack of pests (David et al., 2018). The increased usage of pesticides have posed a major threat to the aquatic habitats in the past few decades (Jones et al., 2009; David and kartheek, 2015). Pesticides comprise of different classes, among which, the organophosphates (OP's) form the largest and diverse group of insecticides, their toxicity to the non-target organisms range from extremely toxic to some of the least toxic pesticides known (David et al., 2016). Most OP's are not persistent and will break down to non-toxic materials in one to 30 days, depending on the compound, however, several OP's are known to persist in the environmental conditions for longer durations, posing a serious threat to the inhabiting fauna (David and Kartheek 2015).

Pesticide pollution in water affects fish and other aquatic animals which indirectly impact on the higher organisms through foodweb (Svensson et al., 1994; Kartheek and David, 2017). The preliminary reflection in terms of health of an aquatic environment is determined by the health status of fishes which are relatively sensitive to changes. Fish health may thus reflect, and give a good indication of the health status of a specific aquatic ecosystem, especially to those which are located under close proximity to agricultural landscapes (Burkepile et al., 2000; Kartheek and David, 2016).

The level of toxicity of any substance is determined by its acute toxicity value against a given experimental animal (Kartheek and David, 2018). The acute toxicity (LC_{50}) tests are generally carried out to determine the receptiveness and survival potential of organisms to particular toxic substances, such as pesticide, which usually diffuse in to water bodies impacting greatly on the surviving ability of the aquatic organisms (Halappa and David 2009). Higher LC_{50} values indicate less toxicity because greater concentrations are requisite to fabricate 50% mortality in organisms (Eisler and Gardner 1973; David and Kartheek, 2016). *Cyprinus carpio* (*C. carpio*) is an important species that constitutes nearly 10% of annual freshwater aquaculture production globally (Xu et al. 2014). The selected fish is known to dominate the freshwater aquaculture practice in terms of the production quantity and demand (Bostock 2010). *C. carpio* is one of the major species of freshwater fish that contributes to around 35% of inland aquaculture in certain areas of Karnataka, India (Yaraguntappa et al. 2007) where dynamic mining activities are evident since decades (Kolb et al. 2005). The pesticide induced responses include, reduced feeding, growth and swimming activity (Van Buskirk, 2002). Previously, many authors have reported the acute toxicity studies for different toxicants against various non-target organisms. Since the previously reported data validate the toxicity of various toxicants, the need for verifying the same for Triazhophos is important for evaluating toxicity levels. The present investigation was aimed to elucidate with the 96 h LC_{50} value of Triazhiophos and further elaborate investigations for the behavioural changes in the freshwater fish *Cyprinus carpio* following sublethal exposures.

2.0 Materials and methods

2.1 Toxicant selected and test solutions

Commercial grade Triazophos (TAP) of 40% EC was selected as the toxicant for the present study and was procured from the local market under the trade name Trizocel. Stock solution of the concentration 1 g/L was prepared by dissolving 1 g of TAP (40% EC) in 1000 ml of double distilled water. The required test concentrations were freshly prepared by diluting the stock solution just before the initiation of acute toxicity studies.

2.2 Procurement and maintenance experimental fish

Healthy *C. carpio* of both sexes, weighing 7.0 ± 2.0 g with a length of 8.0 ± 1.0 cm, were procured from State fisheries department, Neersagar, Dharwad (Karnataka, India). The fish were allowed to acclimate in cement tanks (250 l) under laboratory conditions for 10 days. During acclimation, the water was aerated with a static system and the photoperiod of 12 h light and 12 h dark was maintained. Water was renewed daily and the physico-chemical characteristics were analysed following standard methods as mentioned in APHA (2005).

2.3 Grouping of Experimental Fish

For experimentation, the fish were divided into four groups namely, Control ©, 1 day exposure (E1), 10 day exposure (E2), 20 day exposure (E3) and 20 day exposure (E4). Each group was maintained in triplicate and consisted of 10 individual fishes each irrespective of their gender.

2.4 Exposure to sublethal concentrations

A single sublethal concentration of 0.3mg/l was selected. 10 healthy *C. carpio* were randomly selected and transferred to each glass aquaria containing 1000 ml water with its respective concentration of TAP solution. The tadpoles remained there for 96 h. However, before the tadpoles were transferred, it was made sure that the aquaria were clean enough and free from any kind of toxicant. For each concentration, including the control, 3 replicates were maintained and the mean values of these were taken into account for the present study. Further, the behavioral changes were observed and the body morphology of fish exposed to different sublethal concentrations of TAP was examined.

2.5 Behavioural Responses

The behavioural responses of fish under control and those under exposed group were monitored daily video recording.

2.6 Ethics statement

The present study was carried out at Department of PG Studies and Research in Zoology, Karnatak University, Dharwad (Karnataka, India) as per ethical committee regulations. The test animal used were maintained and subjected to experimentation process as well as disposed off upon completion of experiment, as per the guidelines issued by CPCSEA (Committee for the Purpose of Control and Supervision on Experiments on Animals).

3.0 RESULTS AND DISCUSSION

The results for physico-chemical parameters of water are presented in Table 1.

Table 1: showing values for quality assessment of water used for the present investigation

Parameter	Values obtained
Temperature,	25 ± 2 °C
pH,	7.3± 0.2
Dissolved oxygen,	7.7 ± 0.8 mg/L
Total hardness	30.4 ± 3.1 mg as CaCO3/L
Salinity,;	Nil
Specific gravity,	1.003
Conductivity	< 14 µS/cm
Calcium	17.31 ± 0.92 mg/L
Phosphate,	0.34 ± 0.004 µg/L
Magnesium	0.78 ± 0.3 mg/L

No mortality was recorded and fishes appeared active and healthy throughout the test periods under the control group. The 96 h LC_{50} value of commercial grade TAP for *C. carpio* fingerlings and was identified to be 1.5 mg/L and the results are represented in Table 2 and their respective figures are also provided (Figs. 1–2).

Table 2: Showing mortality of *Cyprinus carpio* exposed to different concentrations of commercial grade Triazophos at 96 hours.

Sl. No.	Concentration in mg/L	Log concentration	No of Fish exposed	No of Fish alive	No of Fish dead	Percent mortality	Probit mortality
1	0.25	-0.602	10	10	00	00	-
2	0.5	-0.301	10	09	01	10	3.72
3	0.75	-0.124	10	08	02	20	4.16
4	1.0	0.0	10	07	03	30	4.48
5	1.25	0.096	10	06	04	40	4.75
6	1.5	0.176	10	05	05	50	5.00
7	1.75	0.243	10	04	06	60	5.25
8	2.0	0.301	10	02	08	80	5.84
9	2.25	0.352	10	01	09	90	6.28
10	2.5	0.397	10	00	10	100	7.15

Figure 1: Showing concentration versus mortality for *Cyprinus carpio* exposed to commercial grade Triazophos

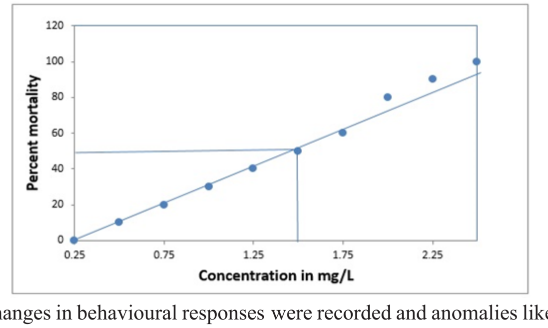
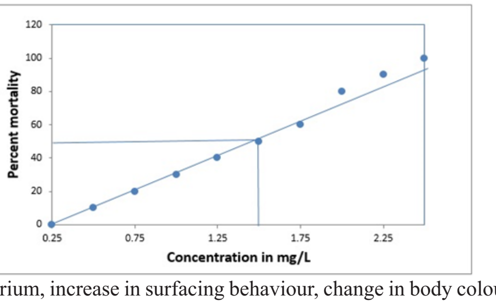


Figure 2: Showing percent mortality vs log concentration of *Cyprinus carpio* exposed to different concentrations of commercial grade Triazophos



Changes in behavioural responses were recorded and anomalies like, loss of equilibrium, increase in surfacing behaviour, change in body colour, increase secretion of mucus, irregular swimming activity, rapid jerk movement, whirling cork like movement, upside down, sinking to the bottom of the tank and partial jerks were observed in fish under the current study. However, the fishes under control group showed no changes. The Changes as recorded with respect to different duration of exposure under the current study have been presented in Table 3.

Days of exposure/ Groups	Anomalies observed									
	Loss of equilibrium	Surfacing behaviour	Change in body colour	Increase secretion of mucus	Rapid jerk movement	Whirling cork like movement	Upside down Swimming	Sinking to the bottom of the tank	Partial jerks	'S' jerks
Control	-	-	-	-	-	-	-	-	-	-
1	+	-	+	+	+ + +	-	-	-	+ + +	+ +
10	+ +	+	+	+ +	+ +	+	-	-	-	+
20	+ +	+ +	+	+ +	+	+	+ +	+	-	-
30	+ + +	+ +	+	+ +	+	-	+ +	+ +	-	-

Various authors have presented reports on the median lethal concentration studies by using different toxicants and their effects on aquatic animals. One such report, was given by Sailatha et al. (1981), who observed LC_{50} for 48h for technical and commercial grades of malathion on *Tilapia mossambica* as 5.52 and 0.337 ppm, respectively. It is to be noted that the declaration for commercial grade malathion for being more lethal as compared to technical grade was made under this investigation. Also acute for different organophosphate, malathion on other fishes like, *Ptychocheilus Lucius*, *Colisa fasciatus* and *Ctenopharyngodon idella* were found to be 3.71, 2.2 and 2.138 mgl/L, respectively. Comparing our results with that of the previous data, it could be inferred that, either *C. carpio* is comparatively susceptible to the other fishes, or the commercial grade TAP used in the current investigation could be more lethal in action as compared to malathion.
Changes in swimming patterns of fish are often considered as the first sign of toxicant induced stress in aquatic habitats. Additionally, the swimming performance is thought to be a valid parameter and a consistent index of the subletal toxicity that can easily be incorporated into the test protocols to increase the sensitivity of the pattern of the toxicity test (Wicks, et al., 2002). These changes are as the result of damage to central nervous system which alters the receptiveness of fish to the given environment (Zabin et al., 2017). Various speculations have been made in which decrease in antioxidant enzymes and abnormal haematological status are also ascertained to be a cause of death in different non target organisms (David and Kartheek, 2014; Kartheek and David, 2017; David et al., 2018).

Considering the reports of Rao et al., (2005) *G. affinis* fishes exposed to of 60 µg/L concentration of chlorpyrifos, were under stress and showed symptoms of reduced locomotory behavior like distance travelled per unit time (m/min) and swimming speed (cm/sec) with respect to the length of exposure. The possible attribution against alteration in locomotor behavior of fish may be due to an accumulation of acetylcholine (ACh), a neurotransmitter at synaptic junctions, due to the inhibition of AChE enzyme activity in brain and could be also due to the bioaccumulation of the toxicant in different parts of fish. Besides this, the other possible reasons could be the lowered oxygen uptake and transport abilities, and ion exchange capacities at gill surfaces (Tabche et al., 1990) which could have resulted in decreased swimming capacity. The likeliness of TAP interfering with selected muscarinic receptors in the brain cannot be ruled out for the behavioral alterations (Cory-Slechta and Pokora, 1995). While few reports have suggested that some neural pathways control instantaneous reactions to specific stimuli (Umeda and Shoji, 2017). Therefore, it can be stated that TAP was found to effect the neuronal mechanism in fish which could have led to altered behavioural responses. However, further studies, investigating the changes in biochemical factions, are nevertheless, necessary to ascertain the exact cause of behavioural disturbances (David and Kartheek 2014).

4. CONCLUSION

Our study finds TAP to be highly toxic to freshwater fish *C. carpio*, with an acute toxicity concentration of 1.5mg/L. Upon the investigations under the sublethal studies, effect was evident to be duration dependent. The overall outcome suggests that TAP can alter the behavioural patterns at concentrations as low as 0.3mg/L. Hence, care is to be taken when TAP is used under agricultural landscapes with close proximities to aquatic bodies.

5. ACKNOWLEDGEMENTS

Financial support and sponsorship: The authors are thankful to University Grants Commission and Department of Science and Technology for providing financial assistance through UGC Major Research Project scheme (F. No. 41-103/2012 (SR); UGC SAP scheme [No. F.4-18/2015/DSA-I (SAP-II)] and DST Purse Phase-II program.

6. Conflict of Interests: The authors hereby declare that, there are no conflicts of interest.

7. REFERENCES

1. APHA Standard methods for the examination of water and waste water. 21st Edn. Washington DC. 2005.
2. Bostock J, McAndrew B, Randolph R, Jauncey K, Trevor T, Kai L, Little D, Ross L, Handisyde N, Gatward I, Corner R. Aquaculture: global status and trends. Philosophical transactions of royal society of London b biological sciences. 2010; 27; 365(1554): 2897-912.
3. Burkepile DE, Moore MT, Holland MM. Susceptibility of five non target organisms to aqueous diazinon exposure. Bulletin of environmental contamination and toxicology 2000; 64: 114-121.
4. Cory-Slechta DA, Pokora MJ. Lead-induced changes in muscarinic cholinergic sensitivity. Summer. Neurotoxicology. 1995; 16(2): 337-47.
5. David M, Kartheek RM, Manjunath GP. Acute and sublethal toxicity of chlorpyrifos on developmental stages of Dattaphrynus melanostictus. Journal of applied pharmaceutical science. 2018; 8(06): 087.
6. David M, Kartheek RM. 2015. Malathion acute toxicity in tadpoles of Duttaphrynus melanostictus, morphological and behavioural study. The journal of basic and applied zoology. 2015; 72: 1–7.
7. David M, Kartheek RM. Biochemical changes in liver of freshwater fish Cyprinus carpio exposed to sublethal concentration of sodium cyanide, Indo American journal of pharmaceutical research. 2014; 4(09): 3669-3675.
8. David M, Kartheek RM. Histopathological alterations in spleen of freshwater fish Cyprinus carpio exposed to sublethal concentration of sodium cyanide, Open veterinary journal. 2015; 5(1):1-5.
9. David M, Kartheek RM. In vivo studies on hepato-renal impairments in freshwater fish Cyprinus carpio following exposure to sublethal concentrations of sodium cyanide. Environmental science and pollution research international. 2016; 23(1):722-33.
10. David M, Kartheek RM. Sodium cyanide induced biochemical and histopathological changes in fresh water fish Cyprinus carpio under sublethal exposure. International journal of toxicology and applied pharmacology. 2014; 4(4): 64-69.
11. David M, Manjunath GP, Kartheek RM. Ameliorative Effect of Mentha spicata on dichlorvos-induced oxidative stress in the rat brain. Saudi journal of medical and pharmaceutical science. 2018; 4(4): 468-476.
12. David M, Shrinivas J, Kartheek RM. Biochemical Modulations in Duttaphrynus melanostictus tadpoles, following exposure to commercial formulations of cypermethrin: an overlooked impact of extensive cypermethrin use. Journal of applied biology and biotechnology 2016; 4(6): 32-037.
13. Eisler R, Gardner GR. Acute toxicology to an estuarine teleost of mixtures of cadmium, copper and zinc salts. Journal of fisheries biology. 1973; 5: 131–142.
14. Halappa R, David M. Behavioural responses of the freshwater fish, Cyprinus carpio (Linnaeus) following sublethal exposure to chlorpyrifos. Turkish journal of fisheries and aquatic sciences 2009; 9: 233-238.
15. Jones DK, Hammond JI, Relyea RA. Very highly toxic effects of endosulfan across nine species of tadpoles: lag effects and family-level sensitivity. Environmental toxicology and chemistry. 2009; 28: 1939–1945.
16. Kartheek RM and David M. Assessment of renal toxicity in rats exposed to commercial formulations of fipronil. International journal of pharmaceutical, chemical and biological sciences. 2017; 7(3): 296-303
17. Kartheek RM, David M. Assessment of fipronil toxicity on wistar rats: a hepatotoxic perspective. Toxicology reports. 2018; 5: 448-456.
18. Kartheek RM, David M. Fipronil induced modulations in biochemical and histopathological aspects of male Wistar albino rats: A subchronic study. Journal of environmental biosciences. 2016; 5(2):26-32.
19. Kartheek RM, David M. Modulations in haematological aspects of wistar rats exposed to sublethal doses of fipronil under subchronic duration. International journal of pharmacy and pharmaceutical sciences. 2017; 5(3): 177-194.
20. Kolb J, Rogers A, Meyer FM. Relative timing of deformation and two-stage gold mineralization at the hutti mine, dharwar craton india. mineralium deposita. 2005; 40: 156–174.
21. Rao JV, Begum G, Pallela R, Usman PK and Rao RN. Changes in behavior and brain acetylcholinesterase activity in mosquito fish, Gambusia affinis in response to the sublethal exposure to chlorpyrifos. International journal of envirnonmental research and public health. 2005; 2(4): 474-483.
22. Sailatha BD, Ahammad Sahib JK, Rao KVR. Toxicity of technical and commercial grade malathion to the fish, Tilapia mossambica (Peters). Proceedings of Indian academy of sciences (animal science). 1981; 90(1): 87-92.
23. Svensson BG, Akesson B, Nilsson A, Paulsson K. Urinary excretion of methylamines in men with varying intake of fish from the Baltic Sea. . Journal of toxicology and environmental health. 1994; 41(4): 411-20.
24. Tabche LM, Martinez CM, Sanchez-Hidalgo E. Comparative study of toxic lead effect on gill and haemoglobin of tilapia fish. Journal of applied toxicology. 1990; 10(3): 193-195.
25. Umeda K, Shoji W. From neuron to behavior: Sensory-motor coordination of zebra fish turning behaviour. Development, growth and differentiation. 2017; 59(3): 107-114.
26. Van Buskirk, J. The costs of an inducible defense in anuran larvae. Ecology. 2000; 81: 2813–2821.
27. Wicks BJ, Joensen R, Tang Q, Randall DJ. Swimming and ammonia toxicity in salmonids: the effect of sub lethal ammonia exposure on the swimming performance of coho salmon and the acute toxicity of ammonia in swimming and resting rainbow trout. Aquatic toxicology. 2002; 10(59): 55-69.
28. Xu P, Zhang X, Wang X, Li J, Liu G, Kuang Y, Xu J, Zheng X, Ren L, Wang G, Zhang Y, Huo L, Zhao Z, Cao D, Lu C, Li C, Zhou Y, Liu Z, Fan Z, Shan G, Li X,Wu S, Song L, Hou G, Jiang Y, Jeney Z, Yu D,Wang L, Shao C, Song L, Sun J, Ji P,Wang J, Li Q, Xu L, Sun F, Feng J, Wang C, Wang S, Wang B, Li Y, Zhu Y, Xue W, Zhao L, Wang J, Gu Y, Lv W, Wu K, Xiao J, Wu J, Zhang Z, Yu J, Sun X. Genome sequence and genetic diversity of the common carp, Cyprinus carpio. Nature genetics 2014; 46(11):1212–1221.
29. Yaraguntappa B, Doddanajappa TP, Kumuda R, Shankarnyarayana PK, Naika UD, Jahageerdar S, Srivastava PP, Penman DJ, Mair GC. Genetic diversity in common carp stocks assayed by random-amplified polymorphic DNA markers. Aquaculture research. 2007; 38(2): 47-155.
30. Zabin SB, Kartheek RM and David M. Studies on effect of fipronil on behavioural aspects and protein metabolism of freshwater fish Oreochromis mossambicus. International journal of fisheries and aquatic studies. 2018; 6(3): 221-226.