Research Journal of Animal, Veterinary and Fishery Sciences _____ ISSN 2320 – 6535
Vol. **1(8),** 1-5, September **(2013)** *Res. J. Animal, Veterinary and Fishery Sci.*

Cypermethrin induced Respiratory and behavioural Responses of Estuarine clam, *Katelysia opima* (Gmelin)

Mukadam M.D. and Kulkarni A. S.
Department of Zoology, Gogate Jogalekar College, Ratnagiri, Maharashtra, 415612, INDIA

Available online at: www.isca.in
Received 8th August 2013, revised 20th August 2013, accepted 16th September 2013

Abstract

A static renewal bioassay method was conducted to determine the acute toxicity (LC_{50}) of technical grade pyrethroid insecticide, cypermethrin (25% EC) in estuarine clam, Katelysia opima. The clams were exposed to different concentrations of cypermethrin for 96 h. The acute toxicity value was found to be 2.79 µg/L . Behavioural patterns and oxygen consumption were studied in lethal and sub-lethal concentrations. During the experimental period, clams from higher concentration group isolated their body from the stressful environment by closing their shell valves for a considerable period. Mucilage secretion was observed in higher concentration groups. Variation in oxygen consumption was observed in both lethal and sub-lethal concentrations of cypermethrin respectively. Clams from LC_0 group showed an elevation in the rate of oxygen consumption after initial 12 hours and fluctuated up to 96 hours. Rate of respiration was severely hampered in LC_{50} group. Change in oxygen consumption may be due to respiratory distress as a consequence of impairment in oxidative metabolism.

Key words: Cypermethrin toxicity, oxygen consumption, behaviour, Katelysia opima.

Introduction

In recent years, human intervention has brought major changes in the aquatic ecosystem. One of such important intervention is that of pesticides. Indiscriminate use of different pesticides in agriculture to prevent crop damage from pests has increased over the years, especially in the developing countries[1]. Among the pesticides, pyrethroids are commonly used due to their high effectiveness, low toxicity to birds and mammals, and easy biodegradability[2]. Cypermethrin is a highly potent and broad spectrum synthetic pyrethroid which is used extensively for pest control. Although it is not persistent in the environment, the excess use of this pesticide may resultin its entry into natural waters through agricultural run-off and ultimately causes damage to non-target organisms such as fish[3-5].

Pollutant run-off into the ocean represents a potential threat to marine organisms, especially oysters and clams. These organisms have been postulated as ideal indicator organisms because of their wide geographical distribution and their sensitivity to environmental pollutants. They filter large volumes of seawater and thus accumulate and concentrate contaminants within their tissues[6]. Clams are known to be tolerant to pesticide accumulation and have a relatively long life span. Respiration is the most important and vital process of life for the derivation of energy in the form of ATP to perform different biological and physiological functions likes locomotion, feeding, reproduction, muscular contraction etc. Metabolic processes are the most sensitive parameters of stress as all enzymatic reactions on the substances and physiological responses are incorporated in a unique manner[7]. The metabolic response of an organism to a changing or stressful environment is an overall indicator of its adaptive ability. Therefore any change in the respiratory activities has been rightfully used as an indicator of stress in general and toxicant and chemical induced change in exposed animals in particular[8-10]. Oxygen consumption of animal is a very sensitive physiological process and changes in respiratory activity have been used the indicator of stress in pollutant exposed animals. However it is well known that respiration is a vital phenomenon of life and rate of oxygen consumption in turn control the metabolic activities. Oxygen consumption is generally taken as a measure of the intensity of metabolism.

Hence, in the present investigation rate of oxygen consumption and behavioural changes are considered as tool to evaluate the toxic effect of lethal and sub-lethal concentrations of technical grade cypermethrin in the estuarine clam, *Katelysia opima.*

Material and Methods

The experimental clams, *Katelysia opima* (4.0-4.4cm) used for the present study were collected from Bhatye estuarine region, Ratnagiri coast, Maharashtra during winter season (December-January, 2010) and were acclimatized to the laboratory conditions for 48 hours. Clams well acclimatized were grouped in tens and kept in plastic containers containing 5 liters filtered estuarine water. Static bioassay tests were conducted for 96 hours by using Cypermethrin (25% EC). The volume of the container was maintained at 5 L for each. Observations were made at 12, 24, 36, 48, 60, 72, 84 and 96 hours. While running the bioassay, the animals were closely observed for their general behaviour, health and number by considering clams from the

control group as a normal for the comparison with the behaviour of the experimental clams.

For the selection of test concentrations, some pilot tests were performed to determine the range of toxicity of the pesticide. The range of concentrations selected was such that it resulted in zero to hundred percent mortality for short term exposures. The LC_{50} value for each time period was estimated by a regression analysis determined for the log of concentrations and percentage survival of the clam. The percentage mortality in various concentrations at particular period were converted into probit values and plotted against the log of concentrations[11].

The toxicity tests were repeated three times and LC_0 and LC_{50} values were determined. The regression equation between the log of concentration (X) and probit mortality (Y) were determined statistically for acute toxicity using the formula $Y= \alpha + \beta \log (x)$ and 95 % confidence limit was established[12].

Oxygen consumption experiments were performed in specially designed glass jars of one liter capacity fitted with rubber lid containing inlet and outlet rubber tubes. The marked clams were kept, one in each jar and immediately filled with filtered estuarine water through a siphon and then clamped at both the ends and were kept aside for one hour. Dissolved oxygen was determined by Winkler's method from the estuarine water[13]. The rate of oxygen consumption of the LC_0 and LC_{50} groups of the clams along with control after every 12 hours time interval was determined. All the values were subjected to statistical analysis. Rate of oxygen consumption was calculated in terms of ml/L/hr/gm wet weight. Comparing the results with control, the changes in the rate of oxygen consumption from LC_0 and LC_{50} concentration were statistically calculated[14]. The experiment was repeated three times for confirmation of the results.

Results and Discussion

Acute toxicity: The acute toxicity of Cypermethrin for the estuarine clam, *Katelysia opima* was found to be 2.79 µg/L. The upper and lower 95% confidence limits were found to be 1.7632 µg/L and 3.7368 µg/L, respectively (table- 1).

Oxygen consumption: Alteration in rate of respiration of *K. opima* after acute exposure to different concentrations of Cypermethrin during winter is presented in table 2 Control group of clam showed fluctuations in the rate of oxygen consumption between 0.217 ±0.0022 to 0.326±0.0022 ml/L/hr/gm from zero to 96 hours. In LC_0 (1.86ppm) group, clams exhibited fluctuations in the rate of oxygen consumption between 0.215±0.0027 to 0.376±0.0018 ml/L/hr/gm during zero to 96 hours. There was a considerable increase from 0.215±0.0027 to 0.376±0.0018 ml/L/hr/gm from zero to 60 hours. From 60 hours, there was a considerable decrease up to 0.297±0.0025 ml/L/hr/gm at the end of 96 hours. As compared to control, there was 6.44, 2.30, 1.26 and 0.92 % ($p<0.001$) decrease in oxygen uptake at the end of 84, 96, 72 and zero hours, respectively. There was an increase of 32.39, 22.29, 16.77,12 and 3.74 % at the end of 60, 48, 36, 24 and 12 hours, respectively. In LC_{50} (2.79ppm) group, the rate of oxygen consumption fluctuated between 0.174±0.0018 to 0.356±0.0018 ml/L/hr/gm from zero to 96 hours. There was a considerable increase from 0.210±0.0154 to 0.356±0.0018 ml/L/hr/gm at the end of 24 hours. It decreases from 0.247±0.0015 to 0.217±0.0022 from 36 hours to 48 hours and there was a decrease in the rate of oxygen consumption from 0.244±0.0031 to 0.174±0.0018 from 60 to 96 hours. As compared to control, 42.94, 42.76, 35.96, 26.68, 18.75, 14.08 and 3.22 % ($p<0.001$) decrease at the end of 84, 96, 72, 48, 36, 60 and zero hours, respectively. There was 9.53 % ($p<0.001$) and 1.70% ($p<0.01$) increase at the end of 24 and 12 hours respectively (table- 2).

Table- 1
96 h LC_{50}, Regression equation and 95% confidence limits of cypermethrin in the estuarine clam, *K. opima*.

Pesticide	96 h LC_{50} value (µg/L)	Regression equation Y = α + ßx	95% confidence limit	
			Upper limit	Lower limit
Cypermethrin	2.79 ± 0.04	0.6242 + 9.9484x	3.7368	1.7632

Table-2
Rate of oxygen consumption (ml/L/hr/gm. Wet wt.) in *K. opima* exposed to cypermethrin after acute exposure

Group	Exposure period								
	0Hrs	12Hrs	24Hrs	36Hrs	48Hrs	60Hrs	72Hrs	84Hrs	96Hrs
Control	0.217 ± 0.0022	0.294 ± 0.0025	0.325 ± 0.0027	0.304 ± 0.0015	0.296 ±0.0021	0.284 ± 0.0015	0.317 ± 0.0025	0.326 ±0.0022	0.304 ±0.0033
LC_0	0.215 ± 0.0027 (-0.92)	0.305 ± 0.0022 (3.74)	0.364 ± 0.0023 (12)	0.355 ± 0.0022 (16.77)	0.362 ±0.0027 (22.29)	0.376 ± 0.0018 (32.39)	0.313 ± 0.0028 (-1.26)	0.305 ±0.0015 (-6.44)	0.297 ±0.0025 (-2.30)
LC_{50}	0.210 ± 0.0154 (-3.22)	0.299 ± 0.0018 (1.70)	0.356 ± 0.0018 (9.53)	0.247 ± 0.0015 (-18.75)	0.217 ±0.0022 (-26.68)	0.244 ± 0.0031 (-14.08)	0.203 ± 0.0007 (-35.96)	0.186 ±0.0029 (-42.94)	0.174 ±0.0018 (-42.76)

Values in parenthesis are percent change, ± = S.D. of five animal. * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$

Behavioural observations: Clams from the control group closed their shell valves for a considerable period at the time of immersion in water, they opened their shell valves and protruded out their siphons after 5 - 10 minutes. Frequently, extension of siphons and foot occurred. Continuous circulation of water took place through the visceral body. The excreta with a little mucus appeared all the time in the plastic container. Extension of siphon and irrigation activity was prominent. It irrigated water for considerable distance away from the container.

Clams from LC_0 (1.86 ppm) and LC_{50} (2.79 ppm) group remained with the shell valves tightly closed with lapse of time. They slightly opened the shell valves to protrude pallial edges for initial 12 hours. 8 to 10 clams from LC_0 group opened the shell valves and protruded the foot up to 48 hours. From 48 to 96 hours, 4-5 clams react to gentle mechanical stimuli with the release of excreta and mucus secretion. In LC_{50} group, 8-10 clams opened the shell valve and protruded the foot from 12 to 36 hours, 5-7 clams react to mechanical stimuli with excessive mucus secretion and excreta up to 96 hours and five clams opened the shell valve and extended the foot with excesxive mucus secretion after 60, 72, 84 and 96 hours one after another. No excretion was seen but mucilage was observed and siphons were feeble in dying clams.

Respiration is a measure of animal's overall energy demand under particular conditions, at which the measurements are made. Oxygen consumption is a useful measure to assess the sub-lethal effects of xenobiotics, as energy processes serve as an indicator of the overall physiological state[15]. It is well known fact that the rate of oxygen consumption is used as an authentic tool for understanding the physiological state of metabolic activities of an organism[16]. Measurement of the rate of oxygen consumption is an important parameter to access the toxicant stress on aquatic organism since it is also an index of energy expenditure to fulfill the demands due to environmental and biological alterations.

In the present study, it was found that, control group of clams showed fluctuations in the rate of oxygen consumption. A decrease in oxygen consumption after pesticide stress was observed in *Corbicula regularis*[17,18], studied the impact of copper sulphate on the oxygen consumption in freshwater female crab, *Barytephsa guerini* and reported a significant decrease in the rate of oxygen consumption. In case of aquatic animals, there is no escape from toxicants and maximum part that is continuously exposed to pesticide is the respiratory surface *i. e*. Gill surface. High activity of gills and continuous exposure to a toxicant causes severe damage to gill surfaces and reduces the oxygen uptake capacity of the respiratory organs [19].

Clams close their shell valves for a considerable period to combat with unfavourable condition. In the present study, as compared to a control group of clams, LC_{50} group showed decreased oxygen uptake. The observed decrease is attributed to variation in the volume of water ventilated through the gills, caused by the intermittent closure and opening of the shell valves. Here the main factor responsible for decreased oxygen uptake was coagulation of mucus on gills due to Cypermethrin exposure. Coagulation of mucus causes reduction in the effective transfer of oxygen to internal tissues, adversely affects the absorption of oxygen from the ambient medium. In the present study, considerable mucus secretion was found in lethal concentration[20,21].

In the present study, higher oxygen consumption rate exhibited by the clams of LC_0 group points out to a higher energy demand. As compared to the control group, clams treated with LC_0 concentration showed a slight elevation in oxygen consumption after initial 12 hours exposure. These findings relate with the findings of many workers[22] reported that oxygen consumption increased in *P. viridis* exposed to low concentrations of Ag, while concentrations above 0.01ppm, oxygen consumption sharply decreased. In the present study it was found that, control group of clams showed negligible fluctuations. Lethal (LC_{50}) group of clams showed significant decrease while in sub-lethal concentration (LC_0) group showed a significant increase in oxygen uptake. Significant increase in oxygen uptake was observed up to 48 and 60 hours[23]. They observed that the rate of oxygen consumption was found to be increased initially up to 48 hours and then decreased up to end of the experiment. The decrement may be due to the respiratory distress as a consequence of the impairment of oxidative metabolism [24].

Behaviour reflects the survival of aquatic animals and integration of many biochemical and physiological processes. Therefore, behaviour is an important area to examine when investigating the effect of toxicants on aquatic animals. Clams can isolate their tissues from the stressful external environment by closing their valves. Clams from lethal groups showed immediate valve closure after addition of Cypermethrin, but after a few hours they start opening the valves. While studying the acute toxicity of Metasystox on the intertidal bivalve mollusks, *Donax cuneatus* found that most of clams opened the shell valves and extended the foot at low concentrations (0.004 to 0.0052ppm) but this was slow and late at high concentrations (0.0056 to 0.008 ppm)[25]. It was also observed that, there was no protrusion of mantle edges in concentrations from 0.004 to 0.0052 ppm. Excreta were less in experimental groups than control groups.

Mucilage secretion was observed in lethal group of clams after 12 hours of acute exposure as compared to control and sub lethal groups. It was probably due to mucus secretion from mucus glands of mantle and gill to keep the body free from contaminated water. Mucus which is secreted as an induced action on exposure to Cypermethrin probably forms a protective layer on gill filaments and mantle, resulted in the reduction of gaseous exchange between the blood and water. All the changes result in depletion of oxygen and carbon dioxide accumulation leading to suffocation and death of clams. In the present study,

the failure in the rhythmic shell valve closing, mucus secretion, failure to respond to the external stimulant and permanent wide opening of the shell valve in the clams exposed to different concentrations of Cypermethrin gives an insight into dysfunction.

The behavioural responses of the mollusc varied in accordance with the test concentration of pesticides. Relatively reduced activity was exhibited during the early hours of exposure at all concentrations. The siphons were extended and food searching movements contributed but eventually the clams appeared to have been paralyzed as they could not retract their siphons even when mechanically stimulated. The pumping activity of the clams was also affected by the pesticide. When compared to controls, the surviving molluscs displayed excessive mucus secretion, sluggishness, gapping of shell valves and permanently extended siphons.

Conclusion

The data presented in this study demonstrated that Cypermethrin is highly toxic and had a profound impact on behaviour and clam physiology. Variation in the oxygen consumption in Cypermethrin exposed clams was probably due to impaired oxidative metabolism and pesticide induced stress. Clams exposed to Cypermethrin showed hyper excitability, extended siphon and secretion of excess amounts of mucus on the body and gills with eventual exhaustion and death.

References

1. Santhakumar M., Balaji M. and Ramude K., Effect of sublethal concentrations of Monocrotrophos on the ethological responses of an air breathing fish, *Anabas testudineus* (Bloch), *Ecology, Environment and Conservation,* **6(2),** 175-177 **(2000)**

2. Kale M.N., John Rathor S. and Bhatnagar D., Lipid peroxidative damage on pyrethroids exposure and alteration in antioxidant status in rat erythrocytes: a possible involvement of reactive oxygen species, *Toxicology Letters*, **105,** 197-205 **(1999)**

3. Stephanson R.R., Aquatic toxicology of Cypermethrin. I. Acute toxicity to some freshwater fish and invertebrates in laboratory tests, *Aquatic Toxicology*, **2,** 175-185 **(1982**)

4. Prashanth M.S. and Neelagund S.E., Impact of Cypermethrin on enzyme activities in the freshwater fish *Cirrhinus mrigala* (Hamilton), *Caspian Journal of Environmental Science*, **6(2),** 91-95 **(2008)**

5. Singh K.S., Singh S.K. and Yadav R.P., Toxicological and biochemical alterations of cypermethrin (Synthetic pyrethroids) against freshwater teleost fish, *Colisa fasciatus* at different seasons, *World Journal of Zoology*, **5(1),** 25-32 **(2010)**

6. Bernal-Hernández Y.Y., Medina-Díaz I.M., Robledo-Marenco M.L.,Velázquez-Fernández J.B., Girón-Pérez M.I., Ortega-Cervantes L., Maldonado-Vázquez W.A. and Rojas-García A.E., Acetylcholinesterase and metallothione in in oysters (*Crassostrea corteziensis*) from a subtropical Mexican Pacific estuary, *Environmental Monitoring Assessment*, **169(1),** 473-485 **(2010)**

7. Dillon D.M. and Lynch M.P., Physiological responses of stress in marine and estuarine organisms, Edit. Gary. W.Barrel and Rutger Rosenberg, John Wiley and Sons Ltd. **(1981)**

8. Schaumberg T., Howard E. and Walden C.C., A method to evaluate the effects of water pollutants on fish respiration, *Water Res*., **13,** 731-737 **(1967)**

9. Anderson J.M., Assessment of the effects of pollutants on the physiology and behavior, *Proc. Royal Soc.* London, **177** B, 307-320 **(1971)**

10. Sharp, J. W., Sitts, R. M. and Knight, A. W., Effect of kelthane on the estuarine shrimp, *Crangon franciscarum*. *Ind. J. Mar. Sci*., **13,** 196-198 **(1979)**

11. Ghosh T.K., Nuvan induced physiological, biochemical and behavioral changes in *B. stigma, Poll. Res*., **5(3) (1986)**

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

13. Welsh J.H. and Smith R.I., Laboratory Exercise in Invertebrate Physiology, Burgess Publishing Co., Minneapolis, **(1961)**

14. Doudeswell W.H., Biochemical Physiology, **40B,** 58 **(1959)**

15. Sigmon C., Oxygen consumption in *Daphnia pulex* exposed 2, 4-Dor 2, 4, 5 – T, Bulletin of ***Environmental*** Contamination and ***Toxicology***, **21,** 822-825 **(1979)**

16. Kulkarni K.M., Accumulation of copper, zinc and cadmium in clam, *Paphia textile Environment and Ecology,* **9,** 197-200 **(1983)**

17. Lomte V.S. and Jadhav M.L., Effect of toxic compounds on oxygen consumption in the freshwater bivalve *corbicula regularis, Compartive Physiology and Ecology*, **7,** 31-33 **(1982)**

18. Mali R.P. and Ambore N.E., Impact of copper sulphate on the oxygen consumption in the freshwater female crab *Barytephasa guerini, Journal of Comparative Toxicology and Physiology*, **1(1),** 14-18 **(2003)**

19. Roberts D., The assimilation and chronic effects of sublethal concentrations of endosulfan on condition and spawning in common mussel*, Mytilus edulis. Marine Biology*, **16,** 119**-**129 **(1979)**

20. Muley D.V., Pesticide toxicity to freshwater *lamellibranch* molluscs from Maharashtra state, India, *Proceedings of*

*10th International Malacol. Congress (*Tiibinger F.R.G., 1989), 207-211 **(1991)**

21. Savant K.B**.** and Amte G.K., Respiratory response of estuarine crab, *Ilyoplax gangetica* exposed to three metallic chemicals, *Journal of Ecotoxicology and Monitoring*, **2(4),** 259-262 **(1992)**

22. Mathew R. and Menon N.R., Oxygen consumption in tropical bivalves *Perna viridis* and *Meretrix casta* (Chem.) exposed to heavy metals, *Indian Journal of Marine Sciences*, **12,** 57-59 **(1983)**

23. Rathod D.S. and Shembekar V.S., Dimethoate Induced toxicity in oxygen consumption of freshwater fish *Arias dussumeri*, UGC sponsored National conference in "Recent Trends in Biological Sciences", organized by Deptt. Of Zoology, Dr. Babasaheb Ambedkar College, Mahad **(2009)**

24. Prashanth M.S., David M. and Kuri R.C., Effect of Cypermethrin on toxicity and oxygen consumption in the freshwater fish, *Cirrhinus mrigala, Journal of Ecotoxicology and Environmental Monitoring*, **13(4),** 271-277 **(2003)**

25. Muley D.V., Akarte S.R., Kulkarni D.A. and Rao K.R., Acute toxicity of Metacystoxto Wedge clam, Donax cutaneus from west c oast of India, *Fishery Technology*, **24(1),** 27-30 (**1987)**