Recent Research in Science and Technology 2012, 4(11): 39-45
ISSN: 2076-5061
Available Online: http://recent-science.com/

Acute toxicity and behavioural responses of a freshwater mussel *Lamellidens marginalis* (Lamarck) to dimethoate exposure

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Abstract
Dimethoate is a commonly used organophosphate pesticide (OP) in agricultural practices, from where they reach into natural freshwater bodies through surface run-off, affecting the life of non-target aquatic organisms. Molluscs accumulate contaminants in their body tissues and thus are used as bio-indicator for evaluating water quality and habitat degradation. The mussels have great economic value, since they are consumed as food and have therapeutic significance. In this study 96h static acute toxicity test was carried out for dimethoate in freshwater mussel, *Lamellidens marginalis*. The mussels were exposed to 8 different concentrations of dimethoate (35.00, 37.00, 39.00, 41.00, 43.00, 45.00, 47.00, and 49.00 mg L^{-1}) and control (00.00 mg L^{-1}). The mortality data were subjected to EPA Probit analysis (version 1.5) statistical software based on Finney's method. The 24, 48, 72 and 96h LC_{50} values of dimethoate for freshwater mussel were determined as 45.09, 40.52, 38.71 and 36.35 mg L^{-1} respectively. Mussels show behavioural responses during exposure by exhibiting increase in duration for shell closure, increase in mucus secretion and decrease in oxygen consumption.

Keywords: Acute toxicity, Dimethoate, LC50, *Lamellidens marginalis*, Behavioural responses.

INTRODUCTION

Pesticides are the chemicals specially designed to kill unwanted organisms. Besides killing the target organisms these pesticides may be washed into water reservoirs and adversely affect the life of aquatic organisms (1, 2, 3). According to an estimate over 200 poisonous chemical pesticides are used in agriculture in different parts of the world (4). The ever increasing use of pesticides is reaching an alarming stage, throughout the world posing a great threat to the environment. Pesticide residues in food are global problem (5, 6, 7). According to WHO, developing countries use about 25% of pesticides in the world (7). India is the largest manufacturer of pesticides among South Asian and African countries and ranks twelfth in the world for the use of pesticides with exception of Japan (8).

Aquatic system is the major part of the globe and important base for survival of aquatic fauna. It's contamination by pesticides induce physiological, haematological, pathological and behavioral changes in inhabiting aquatic organisms and results in death (9, 10, 11, 12, 13, 14, 15, 16, 17 and 18).

Organophosphate (OP) pesticides are used preferably as insecticides in different parts of the world due to their biodegradability, high effectiveness and low persistence in the environment (19). In developed countries use of OP are restricted due to their high morbidity, but in developing countries like India, the use is still highly prevalent (20). OP are generally known to have increased toxicity with increase in temperature (9, 21) and directly inhibit acetyl cholinesterase activity, resulting in accumulation of acetylcholine in nerve tissue and effecter organs and are responsible for paralytic death of molluscs and fishes (9, 22, 23, 24, 25 and 26). Among the OP, dimethoate [IUPAC Name- O, O dimethyl S – (N-methyl carbamoylmethyl) phosphoro-dithioate : CAS No. 60-51-5] is an effective insecticide used on large scale in agriculture due to moderate toxicity in birds and mammals (27). Recently, however, European commission has banned the use of dimethoate for vegetable crops (28).

The molluscs are usually used as bio-indicator for monitoring and evaluating water quality and habitat degradation. These organisms take up and accumulate contaminants in their body tissue much above to the level found in their surroundings enabling the identification of "Hot Spot" in aquatic system (5, 6, 29, 30 and 31). Among the molluscs, freshwater mussel are widely employed in toxicity evaluation because they circulate large quantity of water through their body to obtain oxygen and food, their mantle acts as bio-filter in organic recycling or pesticide recycling and foot is sensitive to chemical composition of water (11, 32). Apart from these, the mussels have great economic value, since they are consumed as food in certain areas (33) and for treatment of arthritis in experimental animals (34).

There are few reports on the toxicity of some OP pesticides on freshwater mussels (9, 10, 35, 36, 37 and 38). To the authors knowledge there exist no published data on dimethoate toxicity on freshwater mussel, therefore, the present study was conducted to determine the static acute toxicity of dimethoate and its impact on physiological responses of the freshwater mussel, *Lamellidens marginalis*.

Received: June 08, 2012; Revised: Oct 19, 2012; Accepted: Nov 24, 2012.

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MATERIAL AND METHODS

Mussels and maintenance conditions

Healthy freshwater mussel *Lamellidens marginalis* (Lamarck) were collected from river "Gomati" and carefully brought to the laboratory and acclimatized for 10 days under laboratory conditions in a 60 L capacity earthen tank. The crushed leaves of aquatic plants along with river water were provided as food on alternate days. Food was not given 24h before start of the toxicity test. The experiments were conducted during month of July-Aug 2008 (temp. 28 ± 0.5^0C and natural photoperiod of 12.51: 11.09±0.31, light: dark). The mussel of equal size (6.9 ± 0.29 cm length and 3.0 ± 0.30 cm width) and weight (33.4 ± 2.24 g) were selected for the toxicity test.

Physico-chemical characteristics of test water

The physico-chemical characteristics of test water was measured regularly during the experiment, following standard methods (40).The physico-chemical characteristics of test water are described in Table 1.

Toxicity test

The experiment was carried out in glass troughs of 15 L capacity in static laboratory condition. Before starting the test, all-experimental glass troughs were cleaned and filled with 14 L tap water. The stock solution was prepared by dissolving dimethoate (Rogor, 30% EC, Rallis India Ltd. Mumbai) in absolute alcohol. Pesticide was mixed well before ½h of transferring the animals. The range finding test prescribed by APHA (39) was used to determine the concentration range of dimethoate. The mortality was regularly assessed at 24, 48, 72 and 96h. The animals were considered dead, when the mantle edge no longer responsive to touch (Tapping response) and failed to close the shell valve. The dead mussels were removed immediately from the troughs. After determining the test range, eight acclimatized mussels in four replicates for each concentration (35.00, 37.00, 39.00, 41.00, 43.00, 45.00, 47.00, and 49.00 mg L^{-1}) were released in 15 L capacity water troughs for 24, 48, 72, and 96h acute toxicity test. A control set with equal number of mussels was also run simultaneously. LC_{50} values were calculated from the data obtained in acute toxicity tests by using EPA-Probit analysis version 1.5 statistical software, (http://www.epa.gov/nerleerd/stat2.htm#tsk) based on Finney's method (40).

Behavioral responses

Besides mortality data the behavioral responses (shell closure, mucus secretion and decrease in O_2 consumption) of the mussel were also taken into account. For this another set of experiment was conducted to evaluate these responses. Eight acclimatized mussels were subjected to 75% of 96 h LC_{50} concentration of dimethoate (27.27 mg L^{-1}) for 01, 24, 48, 72 and 96h exposure. The mussels usually keep the shell marginally open but when prodded by a rod shut their shell quickly. This closing response gradually decline in the exposed mussels. The time taken in shell closure was recorded in all groups at different intervals. During experiment the animals release large quantity of mucus and excreta which increases the quantity of suspended particles in the medium. This changes the color of water of treated aquaria to milky. To evaluate the change in medium the optical density (OD) of trough water was examined on 610_{nm} wavelength of digital spectrophotometer. The DO in trough water was measured at different intervals. The protocol of the research project has been approved by Animal Research Ethical Committee of the Kamla Nehru Institute of Physical & Social Sciences.

Statistical analysis

Statistical analysis in the present study was performed with the help of "Microsoft Excel data analysis program". The results were expressed as mean ± SD. Statistical differences between groups were analyzed using one and two way ANOVA. In addition, the data for control and treated groups of each time interval were subjected to t-test.

RESULTS

LC_{50} and Mortality

The LC_{50} values and their corresponding 95%upper and lower confidence limits for 24, 48, 72 and 96h are given in table 2. The LC_{50} values suggest that dimethoate is moderately toxic to freshwater mussel, *Lamellidens marginalis* and toxicity is time and dose dependent. For example when mussel were exposed to 35 mg L^{-1} concentration of dimethoate, 28% mortality occurred within 96h of exposure, whereas at 49 mg L^{-1} concentration mortality is 66% within 24h but 100% mortality does not appear at any concentration range in 24h of exposure. No mortality occurred below 35 mg L^{-1} concentration up to 96h of exposure and at 45 mg L^{-1} concentration mortality recorded is 50% at 24h, 72% at 48h, 81% at 72h and 100% at 96h. One way ANOVA of mortality data suggest significant differences in rate of mortality ($F=6.63$, $P<0.001$) with in sample along with gradual increase in concentration of dimethoate from 35 mg L^{-1} to 49 mg L^{-1}. The freshwater mussel when exposed against dimethoate exhibit certain physiological and behavioural responses, out of which O_2 consumption (respiratory rate), mucus release and shell closure time (tapping response) is considered as symptoms of toxicity in the present investigation.

Behavioral responses

To evaluate toxicity symptoms, mussels were exposed to a sub-lethal concentration (75% of 96h LC_{50}) of dimethoate (27.27 mg L^{-1}) after determining LC_{50} value. The control and test animals both release mucus during the experiment. In control, the secretion of mucus, usually started slowly and continued up to 96h causing a significant ($F=154.97$, $P<0.0001$, One way ANOVA) color change at 96h when compared to 01h. Whereas the water in the test solution becomes milky, immediately after dissolving the dimethoate, which is recorded as high OD (Significant, $P<0.0001$) at 01h (fig 1), when compared to control. Dimethoate exposed animals release comparatively large quantity of mucus, which get precipitated quickly and forms polythene like layer over the surface of water and gradually increases the milkiness with the exposure time causing decreased transparency, which is recorded as OD by spectrophotometer (fig. 1). A t-test values for 01h, 24h, 48h, 72h and 96h indicated statistical significant differences ($P<0.0001$) in the milkiness of test water in treated aquaria when compared to control. Results of two way ANOVA suggest significant differences between samples ($F=2422.02$, $P<0.0001$), within days of exposure ($F=878.09$, $P<0.0001$) and between the interaction of samples and days of exposure ($F=255.26$, $P<0.0001$).

The water of dimethoate exposed mussel troughs show a significant decrease in O_2 level in the first 24h, and this decrease continued slowly up to 96h. When this data is compared with the controls, it shows that DO level in the troughs records slow decrease indicating that the animals utilize less O_2 as compared to exposed mussels (fig. 2). A t-test values for 24h, 48h, 72h and 96h indicate statistically significant differences ($P<0.0001$) in the status of dissolved oxygen in dimethoate treated test water aquaria. Results of two way ANOVA suggests significant differences between samples ($F=1360.43$, $P<0.0001$), within days of exposure ($F=993.79$, $P<0.0001$) and between interaction of the samples and days of exposure ($F=87.02$, $P<0.0001$).

The mussels in tap water usually open their shell and extend their foot and close their shell valves quickly. Time taken to close the shell valve and foot retraction was recorded with the help of a digital stopwatch. The mussels up to 24h of exposure exhibit a quick response of shell closure and foot retraction. Thereafter, the time of shell closure response exhibits a gradual increase in dimethoate exposed mussels up to 96h, when compared to controls (fig. 3). A t-test values for 24h, 48h, 72h and 96h indicate statistical significant differences ($P<0.0001$) in the time taken to close the shell valve (Shell closure response) in treated mussels. Results of two way ANOVA suggests significant differences between samples ($F=213.00$, $P<0.0001$), within days of exposure ($F=321.13$, $P<0.0001$) and between interaction of the samples and days of exposure ($F=165.55$, $P<0.0001$).

Table 1. Physico-chemical characteristics of water used in the experiment.

Sl. No.	Characteristics of water	
1	Dissolved oxygen	7.85 ±0.2 (mg L^{-1})
2	Free CO_2	6.0 ±0.5 (mg L^{-1})
3	pH	7.4±0.11
4	Temperature	28±0.5 (^{0}C)
5	Total Hardness	274 ±3.74mg L^{-1}
6	Alkalinity (as $CaCO_3$)	180 ±4.50 mg L^{-1}

Table 2. LC_{50} values against dimethoate in *Lamellidens marginalis*

Sl No.	Duration	LC_{50} values (mg L^{-1})	Lower confidence limit (mg L^{-1})	Upper confidence limit (mg L^{-1})	Intercept	Slope
1.	24h	45.10	43.88	46.70	-19.90	15.05
2.	48h	40.52	39.56	41.41	-24.82	18.55
3	72h	38.71	37.86	39.46	-32.89	23.86
4.	96h	36.34	35.30	37.12	-36.29	26.45

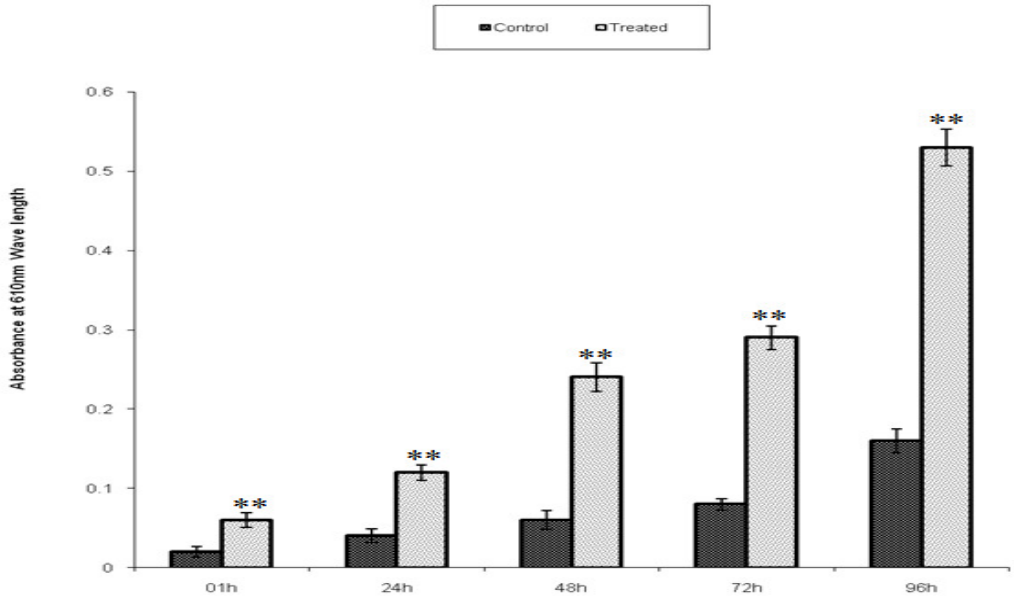


Fig 1. Optical density of test water at 610_{nm} wavelength for different times on exposure to sub lethal concentration (27.27 mg L^{-1}, 75% of 96h LC_{50}) of dimethoate. Values are mean ± SD of eight animals. Asterisks indicate insignificant (∗) and significant (**) differences at $P < 0.0001$ level from control.

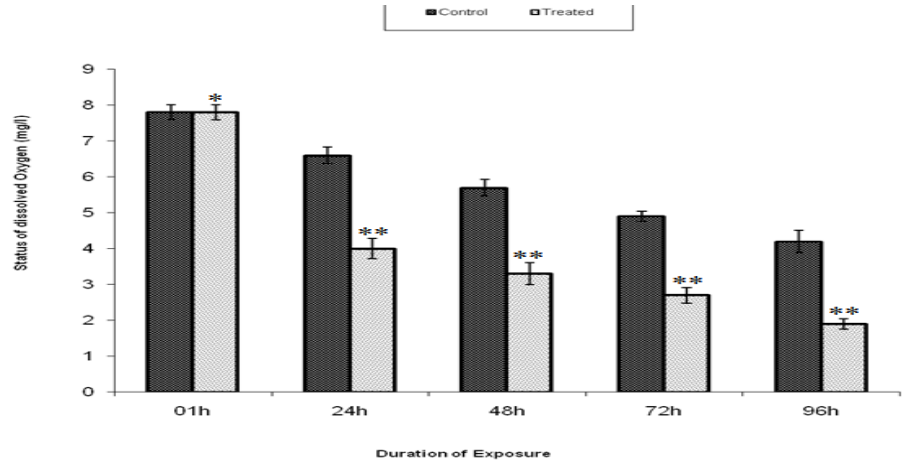


Fig 2. Status of dissolved oxygen in test water on different times on exposure to sub lethal concentration (27.27 mg L^{-1}, 75% of 96h LC_{50}) of dimethoate. Values are mean ± SD of eight animals. Asterisks indicate insignificant (∗) and significant (**) differences at P < 0.0001 level from control.

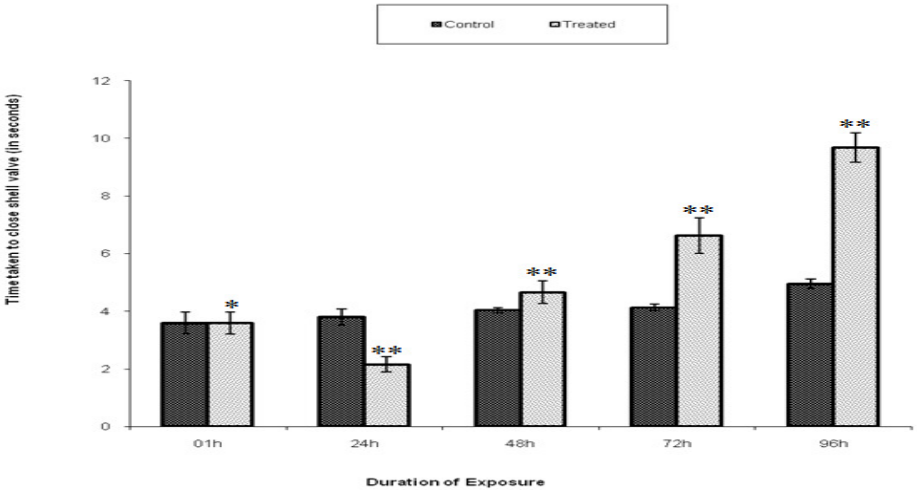
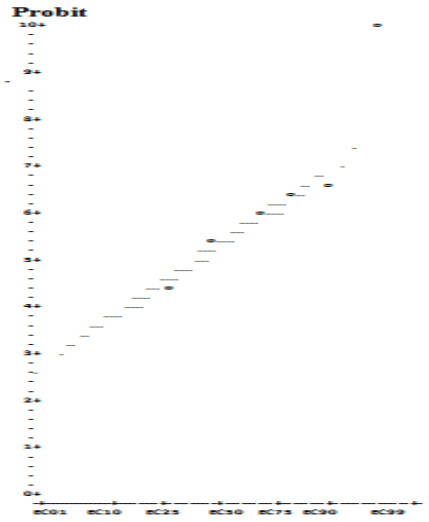


Fig 3. Shell closing response in freshwater mussel *Lamellidens marginalis* at different times on exposure to sub lethal concentration (27.27 mg L^{-1}, 75% of 96h LC_{50}) of dimethoate. Values are mean ± SD of eight animals. Asterisks indicate insignificant (∗) and significant (**) differences at P < 0.0001 level from control.



Parameters	Estimate	Std. Err.	95% Confidence limit
Intercept	-36.281982	6.024833	(-48.090656, -24.473310)
Slope	26.454550	3.811673	(18.983671, 33.925430)
Theoretical Spontaneous Response Rate = 0.0000			

Fig 4. Plot of adjusted probits and predicted regression line for dimethoate to freshwater mussel *Lamellidens marginalis* after 96h of exposure.

DISCUSSION

The toxicity tests of several mussel species are known with respect to different insecticides (9, 10, 35, 38, 40 and 41). The LC_{50} value of methyl parathion to *Parreysia favidens* were reported as 33.5, 30.5, 23.5, and 15.5 mg L^{-1} for 24, 48, 72 and 96h and for another species *Parreysia cearulea* the LC_{50} values recorded were 32.0, 24.0, 22.2 and 17.0 mg L^{-1} for 24, 48, 72 and 96h (38). In another study, Keller and Ruessler (10) reported variation in LC_{50} values of malathion in three different species of mussels with respect to environmental temperature at 25^0C. The LC 50 for *Elliptio icterina* record a variation from 61 mg L^{-1} for 24h to 32 mg L^{-1} for 96h, *Lampsilis straminea clibornensis* record a variation from 62 mg L^{-1} for 24h to 24 mg L^{-1} for 96h and *Lampsilis subangulatus* record a variation from 43 mg L^{-1} for 24h to 28 mg L^{-1} for 96h. For some other species (*Utterbackia imbecillis, Villosa villosa, Villosa lienosa*) the LC_{50} recorded, were very high (391 to 263 mg L^{-1} for 24h and 180 to 40 mg L^{-1} for 96h) at 32^0C (10). Moorthy et al. (35) reported 48h LC_{50} value for phosphamidon in freshwater mussel *Lamellidens marginalis* as 20 ppm. In present investigation the LC_{50} values for *Lamellidens marginalis* were recorded as 45.09, 40.52, 38.71 and 36.34 mg L^{-1} for 24, 48, 72 and 96h. These values indicate moderate toxicity of dimethoate towards the *Lamellidens marginalis.*

Koprucu and Seker (41) observed highly toxic effect of synthetic pyrethroid deltamethrin on *Unio elongatulus eucirrus* as 8.99, 8.09, 7.30 and 6.60 mg L^{-1} for 24, 48, 72 and 96h. Recently, Koprucu et al. (42) reported LC_{50} values for cypermethrin on *Unio e. eucirrus* as 96.50, 77.96 and 59.20 µg/l for 48, 72 and 96h respectively. This indicates that pyrethroids are more toxic when compared to OP insecticides reported in the above discussion.

Lamellidens marginalis when exposed to dimethoate release huge quantity of mucus as compared to controls. Keller and Ruessler (10) also reported release of large quantity of mucus in bivalves when exposed to 150 mg L^{-1} of malathion. The release of mucus after insecticide exposure appears as a quick response of the animal to cover the soft body surface for protection.

The decrease in the DO content of water in troughs containing dimethoate exposed animals, clearly indicate more utilization of O_2 by the mussels present in the water. The data clearly indicate that exposed mussels are highly stressed due to insecticide and utilize more O_2 for their survival, when compared to control animals. Increased O_2 consumption in the initial exposure has been reported by several workers after pesticide exposure (36, 37, and 43) and exposure to metal (12).

Dimethoate exposed *Lamellidens* exhibit a decrease in closure time of gaped-shells up to 24h of initial exposure when compared to controls indicating more sensitivity by quick closure of their shells. However, their muscular response becomes gradually slow with the increase of exposure time. A similar response in muscle closing reflex was reported by earlier workers when exposed to pesticides (9, 10, 43 and 44). Moulten et al. (9) observed the decrease in muscle closure response was due to decline in cholinesterase activity in adductor muscle in dose related fashion to acephate and aldicarb. They also reported increase in cholinesterase activity when the exposed animals were subjected to recovery. They suggested that retarded shell closure response to shell-tapping may indicate a physiological dysfunction of the bivalves. Devi et al. (22) observed the inhibition in cholinesterase activity of clam after OP pesticide exposure in order of potency (chlorpyriphos > dichlorvas > methyl parathion).

The results indicate that insecticide, dimethoate stress was responsible for respiratory disorders, increased mucus secretion and decrease in shell closure responsiveness of the freshwater mussel. These parameters may be used as biomarker for the assessment of actual health of the organisms living in the polluted water.

ACKNOWLEDGEMENT

The authors are thankful to college administration for providing research facilities during the course of our research work.

REFERENCES

[1] Pereira, W.E., J.L. Domagalski, F.D. Hostettler, L.R. Brown and J.B. Rapp. 1996. Occurrence and accumulation of pesticide and organic contaminants in river sediments, water and clam tissue from the Jaquin river and tributaries California. *Environ. Toxicol. Chem.* 15(2):172-180.

[2] Toshiro, H. and S. Ytaka. 2004. Toxic examination of the fish and bottom fauna by chlorpyriphos: annual report of Fukuoka city. Ins. *Hyg. Environ.* 29.

[3] Chopra, A.K., M.K. Sharma and S. Chamoli. 2011. Bioaccumulation of organochlorine pesticides in aquatic system-an overview. *Environ. Monit. Assess.* 173:905-916

[4] Mishra, K.D. and K. Bohidar. 2005. Toxicity of pesticides, carbaryl and cartap hydrochloride on the freshwater teleost *Channa punctatus* (Bloch). *J. Adv. Zoo.* 26(1):20-23.

[5] Walsh, K., R.H. Dunstan, R.N. Murdoch, B.A. Conroy, T.K. Roberts and P. Lake. 1994. Bioaccumulation of pollutants and changes in population parameters in the gastropod mollusc *Austrocochlea constricta*. *Arch.Environ. Contam. Toxicol.* 26:367-373.

[6] Uno. S., H. Shiraishi, S. Hatakeyama, A. Otsuki and J. Koyama 2001. Accumulative characteristics of pesticide residue in organs of bivalves (*Anodonta woodiana* and *Corbicula leana*) under natural conditions. *Arch. Environ. Contam. Toxicol.* 40: 35-47.

[7] WHO 2003. Pesticide residue in food. In: International program on chemical safety FAO/WHO meeting on pesticide results evaluations 2002, Part 2 Toxicology.

[8] Abhilash, P. C. and N. Singh. 2009. Pesticide use and application: an Indian scenario. *J. Haz. Mat.* 165(1-3):1-12.

[9] Moulten, C.A., W.J. Fleming and C.E. Purnell. 1996. Effect of two cholinesterase-inhibiting pesticides on freshwater mussel. *Environ. Toxicol. Chem.* 15 (2):131-137.

[10] Keller, A.E. and D.S. Ruessler. 1997. The toxicity of Malathion to unionid mussel: relationship to expected environmental concentrations. *Environ. Toxicol. Chem.* 16(5) : 1028-1033.

[11] Kontreczky, C.S., A. Farkas, , J. Nemesok and J. Salanki. 1997. Short and long term effects of deltamethrin on filtering activity of freshwater mussel (*Anodonta cygnea L.*). *Ecotoxicol Environ. Saf.* 38:195-199.

[12] Das, S. and B.B. Jana. 2003. Oxygen uptake and filtration rate as

animal health biomarker in *Lamellidens marginalis* (Lamarck). *Ind. J. Exp. Biol.* 41:1306-1310.

[13] Arab, N., C. Minier, S. Lemaire, E. Unruh, P.D. Hansen, B.K. Larsen, O.K. Anderson and J.F. Narbone. 2004. Biochemical and histological responses in mussel (*Mytilus edulis*) exposed to North Sea oil and alkyl phenols. *Mar. Environ. Res.* 58, (2-5):437-441.

[14] Gagnair, B., M. Gay, A. Huvet, J.Y. Daniel, D. Saulnier, and T. Renault. 2007. Combination of a pesticide exposure and bacterial challenges : in vivo effects on immune responses of pacific oyster, *Crassostrea gigas* (Thunberg). *Aqua. Toxicol.* 84 (1):92-102.

[15] El-shenawy, N.S., T.I.S. Moawad, M.E. Mohallal, I.M. Abdel-Nabi and I.A. Taha. 2009. Histopathological biomarker response of clam *Ruditapes decussates* to organophosphate pesticide reldan and roundup : a laboratory study. *Ocean Scie. J.* 44(1):27-34.

[16] Pandey, R.K., A. Malviya and V.K. Das. 2009. Toxicity of Cypermethrin and its effect on serum electrolytes (Ca^{2+}, Mg^{2+} and Pi) levels and recovery response in freshwater catfish *Heteropneustes fossilis* (Bloch). *Int. J. Biol. Chem Scie*. 3(5):1183-1191.

[17] Singh, N.N., V.K. Das and A.K. Srivastava. 2002. Insecticides and ionic regulation in teleosts: *A review. Zool. Polo.* 47 (3-4):49-64.

[18] Singh, N.N. and A.K. Srivastava. 2010. Haematological parameters as bioindicators of insecticide exposure in teleosts. *Ecotoxicol* 19:838-855.

[19] Fulton, M.H. and P.B. Key. 2001. Acetylcholinesterase inhibition in estuarine fish and invertebrates as an indicator of an organophosphorus insecticide exposure and effects. *Environ. Toxicol. Chem* 20:37-45.

[20] Balaram, P. 2003. Pesticides in the environment. *Curr. Scie.* 85:561-562.

[21] Pandey, R.K., R.N. Singh and V.K. Das. 2008. Effect of temperature on mortality and behavioural response in freshwater catfish *Heteropneustes fossilis* (Bloch) exposed to dimethoate. *Global J. Environ. Res.* 2(3):126-132.

[22] Devi, K.P., S.K. Pandian, and N.S.S. Kumar. 2005. Cholinesterase activity in clam Meretrix casta: possible biomarkers of organophosphate pesticide pollution. *Bulletin of Environ. Contam. Toxicol.* 74:250-255.

[23] De, mel, G.W.J.L.M.V.T.M. and A. Pathiratne. 2005. Toxicity assessment of insecticides commonly used in rice pest management to the fry of common carp *Cyprinous carpio* a food fish culturable in rice fields. *J. Appl. Ich.* 21:146-150.

[24] Rao, J.V., G. Begum, R. Pallela, P.K. Usman and R.N. Rao. 2005. Changes in behavior and brain acetylcholinesterase activity in mosquito fish *Gambusia affinis* in reference to the sub-lethal exposure of chlorpyrifos. *Int. J. Environ. Res. Pub. Health.* 2(3-4):478-83.

[25] Canty, M.N., , J.A. Hagger, R.T.B. Moore, L. Cooperand, T.S. Galloway. 2007. Sub-lethal impact of short term exposure to the organophosphate pesticide azamethiphos in the marine mollusc *Mytilus edulis. Mar. Poll. Bull.* 54 (4):396-402.

[26] Amanullah, B., A. Stalin, P. Prabu and S. Dhanapal. 2010. Analysis of AchE and LDH in mollusc, Lamellidens marginalis after exposure to chlorpyrifos. *J. Environ. Biol.* 31, 417-419.

[27] EHC, 90-1989, 1990. Environmental health criteria world health organization. 1-51.

[28] Horticulture Week Bulletin. New residue level rules mean dimethoate now banned for vegetable crops (Cited 9 April 2010) Available from URL: http://www.hortweek.com/resources/PestsAndDiseases/995126/New-residue-level-rules-mean-dimethoate-banned-vegetable-crops/

[29] Simkiss, K., M. Taylor and A.Z. Mason. 1982. Metal detoxification and bioaccumulation in molluscs. *Mar. Biol. Lett.* 3:187-201.

[30] Livingstone, D.R. 1991. Towards a specific index of impact by organic pollution for marine invertebrates: mini review. *Comp. Biochem. Physiol.* (c) 100:151-155.

[31] Serrano, R., F. Hernandez, J.B. Pena, V. Dosda and J. Canales. 1995. Toxicity and bioconcentration of selected organophosphate pesticides in *Mytillus galloprovincialis* and *Venus gallina*. Arch. Environ. *Contam. Toxicol.* 29(3):284-290.

[32] Nadamuni Chetty, A. and K. Indira. 1994. Protection against free radical toxicity in mantle and foot of a freshwater bivalve exposed to ambient ammonia. *Proc. Nat. Aca. Scie., India* (B) 64(1):35-39.

[33] Baby, R.L., I. Hasan, K.A. Kabir and M.N. Naser. 2010. Nutrient analysis of some commercially important molluscs of Bangladesh. *J. Scie. Res.* 2, (2):390-396.

[34] Cakraborthy, M., S. Bhattacharya, P. Bhattacharjee, R. Das and R. Mishra. 2010. Prevention of the progression of adjuvant induced arthritis by oral supplementation of Indian freshwater mussel (*Lamellidens marginalis*) aqueous extract in experimental rats. *J. Ethonophormacol.* 132 (1):316-320.

[35] Moorthy, K.S., M.D. Naidu, C.S. Chetty and K.S. Swamy. 1983. Changes in carbohydrate metabolism in tissue of freshwater mussel (*Lamellidens marginalis*) exposed to phosphamidon. *Bull. Environ. Contam. Toxicol.* 30:219-222.

[36] Moorthy, K.S., B.K. Reddy, K.S. Swamy and C.S. Chetty. 1984. Changes in respiration and ionic content in tissue of freshwater mussel exposed to methyl parathion toxicity. *Toxicol. Lett.* 21:287-291.

[37] Basha, S.M., K.S. Swami and A. Puspanjali. 1988. Ciliary and cardiac activity of freshwater mussel *Lamellidens marginalis* (Lamark) as an index of evaluating organophosphate toxicity. *J. Environ. Biol.* 9 (3):313-318.

[38] Koppar, B.J., R.S. Kulkarni and S.A.T. Venkatachari. 1993. Application of static bioassay procedure in determining the comparative relative toxicity of pesticide methyl parathion on two freshwater mussels. *J. Environ. Biol.*14 (3):183-193.

[39] APHA, 2005. Standard methods for the examination of water and waste water. 20th edn. APHA, AWWA, WPCF, Washington DC, USA.

[40] Finney, D.J. 1971. Probit analysis. University Press Cambridge.

[41] Koprucu, K. and E. Seker. 2008. Acute toxicity of deltamethrin for freshwater mussel, *Unio elongatulus eucirrus* Bourguignat. *Bull. Environ. Contam. Toxicol* 80:1-4.

[42] Koprucu, K., S.M. Yonar and E. Seker. 2010. Effect of cypermethrin on antioxidant status, oxidative stress biomarkers, behaviour and mortality in the freshwater mussel *Unio elongatulus eucirrus*. *Fish Sci.*76:1007–1013.

[43] Koprucu, S.S., E.Yonar and E. Seker 2008. Effect of deltamethrin on antioxidant status and oxidative stress biomarkers in freshwater mussel *Unio elongatulus eucirrus*. *Bull. Environ. Contam. Toxicol.* 81:253-257.

[44] Ayad, M.A., M.A. Fdil, and A. Mouabad. 2011. Effects of cypermethrin (Pyrethroid insecticide) on the valve activity behaviour, byssal thread formation and survival in air of the marine mussel *Mytillus galloprovincialis. Arch. Environ. Contam. Toxicol.* 60(3):462-470.