Alteration in Body Colour and Bioconcentration of an Organophosphate (Dimethoate) in Long Term Exposed Freshwater Mussel *Lamellidens marginalis* (Lamarck)

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ABSTRACT

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Freshwater mussel Lamellidens marginalis is a common bottom dweller bivalve known for the production of pearl, water purification and consumed as food. The ever increasing risk of pesticides contamination in freshwater bodies through agricultural runoff and municipal discharge causes adverse effects on life style of non-target economically important organisms, inhabiting there. In the present study L. marginalis were exposed on sub lethal concentration (25% of 96h LC_{50}) of an organophosphate pesticide (dimethoate) for 24days under static laboratory conditions. The exposed mussels show gradual change in body colour from pale-yellow to dark-brown and histological observations of vital tissues like gill, mantle, ganglia and hepatopancreas suggests bio-concentration of pesticides in the form of dark granules. Thus, dimethoate in water bodies may alter the body colour of non-target economically important organisms and gain access in the food chain through bioconcentration.

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I. INTRODUCTION

The mussel *L. marginalis* is commonly found in freshwater bodies like rivers, canals, ditches, ponds etc. throughout Indian subcontinent. They have great economic importance as produce precious stone pearl, bioconcentrate toxicants level above to the surroundings, consumed as food, their shell is used in production of button and lime (Raut and Biswas, 1989; Mishra and Mukhapadhyay 2008; Baby et al. 2010) and their body extract are antibacterial in nature (Ester et al. 2011) as well as effective in cure of arthritis and other similar disorders (Chakraborty et al. 2010). They are considered as purifier of aquatic environment due to their detritivorous and planktonic feeding nature as well as widely employed in toxicity evaluation because circulate large quantity of surrounding water through their body and respond quickly against toxicants (Kontreczky *et al.,* 1997; Mishra and Mukhapadhyay 2008). Rittschof and Mc-Clellan-Green (2005) recognized molluscs as multidisciplinary model in environmental toxicology.

Pesticide runoff in the nearer freshwater bodies from the site of application is the worldwide problem in present scenario (US-EPA 2005; Guibaud et al. 2007; Malik et al. 2009; Singh and Mishra 2009; IPIR, 2011). Aquatic system is the major part of the globe and important base for survival of aquatic fauna, its contamination from pesticides cause serious impact on lifestyle and biodiversity of non-target organisms inhabiting

there (Pereira *et al.,* 1996; Uno *et al.,* 2001). Among the pesticides, dimethoate [IUPAC Name- O, O dimethyl S – (N-methyl carbamoylmethyl) phosphoro-dithioate], CAS No. 60-51-5, is an effective organophosphate insecticide available in the market by the trade name of "Rogor" and commonly used by the people against insect pests. Dimethoate produces its action by inhibiting enzyme - Acetyl cholinesterase (AChE) necessary for degradation of neurotransmitter Acetylcholine (ACh). Inhibition of AChE leads to accumulation of Ach at nerve endings and caused over excitation. Affected, nervous system gradually lose the control over body activities and homeostasis (Bookhout *et. al.*, 1977; Rickwood and Galloway 2004; Adedji 2011; Dondero et al. 2011).

Every organism of the universe is known by their specific body colour and biological significance. A specific pattern of body colour of an animal is important because it play significant role in inter and intra species communication, protection against radiation and enemies as well as recognition of the sex (Svensson and Skold; 2011). This pattern of body colour in animals is controlled by endocrine and nervous system by control of chromatophores distribution (Oshama 2001). Change in body colour of fish after pesticide exposure was recorded by several workers (Pandey et al. 2009; Singh et al. 2009; Srivastava et al 2010). Akarte and Agnihotri (2013) reported chromatophores as bioindicator of arsenic trioxide poisoning when exposed freshwater fish *Channa punctatus* for 30days under laboratory conditions.

To the authors knowledge there is no published data on body colour change against pesticide exposure but some reports on bioconcentration of pesticides are available in *L. marginalis*. Therefore, in the present study, an effort was made to study the effect of commonly used pesticide dimethoate on body colour and bioconcentration in freshwater mussel *L. marginalis* under static laboratory conditions.

II. MATERIALS AND METHODS

Healthy freshwater mussel *L. marginalis* (Lamarck) were collected from river "Gomti" and carefully brought to the laboratory and acclimatized for 10 days under laboratory conditions in 60L capacity earthen tanks. The crushed leaf of aquatic plants along with river water was provided as food on alternate days. Food was not given 24h before the start of the toxicity test. The experiment was conducted during the month of July-Aug at room temperature (28.70±2.29°C) with natural photoperiod (12.51: 11.09±0.31h, Light: Dark period). The mussels of equal size (6.9±0.2cm long and 3.0±0.3cm wide) and weight (33.40±2.2g) were selected for the toxicity bioassay. The experiment of toxicity was carried out in glass troughs of 15L capacity under static laboratory conditions. The physico-chemical characteristics of test water were maintained, following standard methods of APHA (2005). The DO 7.8±0.2mg/l, free CO_2 6.0±0.5mg/l, temperature 28±0.5°C, pH 7.4±0.1, total hardness 274±3.74mg/l and alkalinity (as $CaCO_3$) 180±4.50mg/l.

The LC_{50} of dimethoate for *L. marginalis* were recorded as 45.10, 40.52, 38.71 and 36.34mg/l for 24h, 48h, 72h and 96h respectively following EPA-Probit analysis version 1.5 statistical software, (http://www.epa.gov/nerleerd/stat2.htm#tsk) based on Finney's (1971) method (Kumar et el. (2013). Before the start of the bioassay, all-experimental glass troughs were cleaned and filled with 14L tap water. The stock solution of required concentrations was prepared by dissolving dimethoate (Rogor, 30% EC, Rallis India Ltd. Mumbai) in absolute alcohol. The 25% concentration (9.08mg) of 96h LC_{50} of dimethoate was considered as sub-lethal and mussels were exposed for 24days period (long term). After preparation of stock solution test concentration was maintained in 4 different troughs and 4 animals were transferred in each. All the animals of a trough were removed and dissected regularly on 3d, 6d, 12d and 24d for the morpho-histological observations. Histological observation was done following techniques of

Kierrnan (1990). A control set with equal number of troughs and mussels was also run simultaneously. Results are presented in comparison to control.

II. RESULTS AND DISCUSSION

The body colour of untreated *L. marginalis* is shiny yellow in colour and surrounded by a layer of slimy mucus throughout the period of exposure. In contrast, dimethoate treated mussels show remarkable change in body colour from pale-yellow to dark-brown (Photo Plate 1 and Fig. 2-4) and almost prominent dark colour are seen in 24days treated mussels (Photo Plate 1 and Fig. 4). Histopathological changes were reported earlier in gills (Kumar et al (2012) and hepatopancreas (Kumar et al 2011) after 24 hrs pesticide exposure but without any remarkable change in body color. Fahmy and Sayed (2017) also studied toxicological perturbations of zinc oxide NP in gills and digestive gland of *Coelatura aegyptiaca*. Benjamin *et al* (2019) very recently reported histopathological changes in soft tissues of *Corbicula fluminea* after Bisphenol A (an endocrine disruptor) exposure. The histopathogical changes were similar in the above studies. It included hypertrophy and hyperplasia in glandular cells of hepatopancreas and irregularity and swelling of gill filaments (Kumar *et al* 2011, 2012, Fahmy and Sayed 2017, Benjamin *et al* 2019). Dimethoate is a neuro-toxic pesticide interferes with neurotransmitters then endocrine system. The affected nervous and endocrine system leads to disturbances in chromatophores distribution which results in body colour change of treated mussels. Change in body colour of fish after dimethoate exposure was reported by several workers (Pandey *et al.* 2009; Singh *et al.* 2009; Srivastava *et al.* 2010).

The histological observations of slides of treated mussel's vital organs (viz. mantle, pedal ganglia hepatopancreas and gill) in comparison of control showing dark granule deposited between connective and muscular tissues. These granules are well prominent in the histological slides of 24 days treated mussels and indicated as PD in photo plate 2: fig. 5-8. The dark granules are more prominent in hepatopancreas and colour of this metabolically active organ was comparatively more darken than other. Serrano *et al* (1995) reported bio concentration of organophosphate pesticides in two different bivalve species under laboratory conditions. EI-Shenawy et *al.* (2009) recorded similar granules deposition in clam *Ruditapes decussatus* when exposed under laboratory condition for long term.

Therefore, the present study is concluded that ever increasing risk of pesticide contamination and concentration in freshwater bodies leads morpho-histological changes in non-target economically important organisms and cause danger of food chain contamination. Thus, dimethoate use should be avoided, if necessary used with caution and applied different pesticide management procedures.

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Photo Plate 1:- Change in body colour of *L. marginalis* after dimethoate exposure. Fig.1: Control (Untreated) mussel body colour, fig. 2: Body colour of mussel after 6d of exposure, Fig. 3: Body colour of mussel after 12d of exposure and fig. 4: darkened body colour of mussel after 24d of exposure.

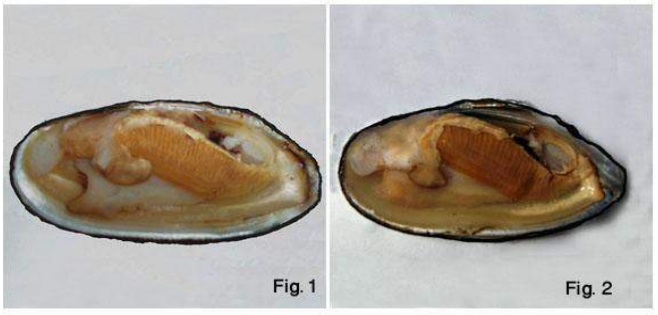

Photo Plate 2:- Different tissues of *L.marginalis* showing pesticide accumulation and disint egration after 24d of dimethoate exposure. Fig. 5:

T.S of Mantle. Fig. 6: T.S. of pedal ganglia. Fig. 7: T.S. of hepatopancreas and Fig. 8: T.S. of gill. PD=pesticide deposits, TD=tissue disintegration.