The Bioscan 8(3): 1053-1056, 2013 (Supplement on Toxicology)
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES
www.thebioscan.in

MONOCROTOPHOS INDUCED BEHAVIORAL STRESS, BIOCHEMICAL AND HISTOLOGICAL ALTERATIONS IN *LAMELLIDENS MARGINALIS* (LAMARCK)

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KEYWORDS
Lamellidens marginalis
Monocrotophos
Mantle
Recovery

Received on :
21.03.2012

Accepted on :
07.07.2012

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ABSTRACT

The present study aimed to investigate the possible effects of acute exposure of monocrotophos on behavioural response, inhibition of protein and glycogen activity, and histopathological changes in mantle and muscle tissues of freshwater bivalve, *Lamellidens marginalis*. Animals were exposed to sub lethal LC_5 concentration (47.45 ppm) of monocrotophos. The glycogen and protein content decreased significantly ($p<0.05$) in mantle (28.27%, 35.96%) gill, (16.59%, 53.05%) foot (28.05%, 71.41%) and adductor muscle (27.37%, 64.21%) respectively after 96 hours exposure to monocrotophos. Decrease in glycogen content shows greater utilization of glycogen for metabolic purposes and to combat with monocrotophos stress. Fragmentation of muscle fibre and hypertrophy in mucous cells of mantle was observed after acute exposure to monocrotophos. Protein and glycogen content recovered significantly ($p<0.05$) in mantle and muscle after 14 days, while all the tissues recovered significantly ($p<0.05$) after 28 days. These results revealed that there was a significant recovery in biochemical parameters in bivalve after a recovery period of 28 days.

INTRODUCTION

Mussels are sedentary, widely available, distributed in a variety of habitats, easy to sample, sufficiently long-lived to allow the sampling and, have a high filtration rate which favours the uptake and bio-concentration of toxic chemicals (Fisher *et al.,* 1993). Sessile lifestyle, resistance to stress, filter feeding mechanism , high accumulation potential of a wide range of contaminants, and suitability as a model for toxicity testing are useful characteristics of fresh water bivalves for bio-monitoring studies (Bernal Hernandez *et al.,* 2010).

Now-a-days, decline of freshwater mussels is observed due to several factors such as siltation, pollution, commercial harvest, and construction of dams. Bivalve molluscs represent interesting specimens in eco-toxicological studies since they exist in direct contact with contaminated aquatic sediments and they are exposed to water-borne contaminants. Exposure assessment is essential in understanding the potential effects of contaminants to non-target animal populations, like mussels which are considered to be excellent indicator organisms for reflecting bio-available concentrations of environmental contaminants (Jayakumar *et al.,* 2008).

According to the 'Stockholm Convention on Persistent Organic Pollutants', 9 of the 12 most dangerous and persistent organic chemicals are pesticides (Gilden *et al.,* 2010). In agricultural land, pesticides are widely used for pest control. The runoff from treated area enters the river which gets contaminated. Wide use of pesticides has resulted in their widespread distribution in the environment and increased toxicity risk to non-targeted organisms. Large scale use of pesticides not only constitutes a loss of money but also causes a lot of undesirable side effects on human health, environment, food quality, and biodiversity (Rathore and Nollet, 2012; PAN Europe, 2010). It is known that less than 0.1% of the applied pesticide actually reaches the targeted pests, while the rest 99.9% has the potential to move into the environment, including ground water and surface water (Racke, 2003; Younos and Weigmann, 1988).). The exposure of aquatic organisms to even very low levels of pesticides in their environment may result in various bio-chemical, physiological and histological alterations in vital tissues of aquatic organisms (Bayne *et al.,* 1979; El-Shenawy *et al.,* 2009; Dash *et al.,* 2011).

Monocrotophos may induce stress and interfere with the behaviour and biological activity of *Lamellidens marginalis*. In the gill of *L. marginalis*, sodium arsenite induced histological alterations were observed by Chakraborty *et al.,* (2010). However, scanty information is available on the histopathological effects of monocrotophos on the mantle and muscle tissue of bivalve *L. marginalis*.

Therefore, the present study aims to determine the protein and glycogen content as well as to observe histopathological effects on mantle and muscle tissues in *L. marginalis* exposed to sub lethal concentration of monocrotophos and to assess recovery in biochemical estimations, after transfer of organisms, to pesticide free water for 14 and 28 days

MATERIALS AND METHOD

Experimental organism and their maintenance

The freshwater mussels *L. marginalis* with shell- length 7-9 cm were collected from reservoirs around Pune. Animals were acclimatized at laboratory condition for seven days in de-chlorinated stored water. Animals were fed with 1 gm of Spirulina powder per 10 litres of stored water every day (Vaughn and Hakenkamp, 2001; Nath, 2007). The water was renewed after every 24 hours (Jayakumar *et al.,* 2008). Behavioural changes of test animals were closely followed and recorded. The results were evaluated by comparing the mean durations of the open and closed positions of the valves before the treatment and during the treatment.

Experimental design

The pesticide toxicant selected for exposure was monocrotophos (Phoskill 36%). Toxicant exposure was done by following a 24 hours renewal bioassay system (Jayakumar *et al.,* 2008). Animals were maintained without feeding during exposure period. Fifteen animals were exposed to sub lethal (47.45 ppm) concentration of monocrotophos for 96 hours. The test solution was prepared by mixing the required quantity of formulated monocrotophos in stored water having dissolved oxygen 8.5mg/L, pH 7.6 ± 0.1, Total alkalinity 75 to 150 ppm (Janakiram, 2003) and water temperature 27 ± 3ºC. Parallel control groups were also maintained without addition of pesticide.

At the end of the exposure period (96h), shells were cleaned with distilled water and sacrificed to collect foot, gill, mantle and muscle. The tissues were used for glycogen estimation, protein estimation and histology. Glycogen estimation was done with the help of De-Zwaan and Zandee's (1972) method. Protein estimation was done with the help of Lowry *et al.* (1951) method.

Histopathology

Animals were dissected. Mantle and muscle tissues were removed with the help of fine, sharp and sterile blade. The tissues were immediately fixed in Bouin's solution and later they were dehydrated through standard alcoholic gradation and embedded in paraffin. The tissue sectioning was done with a rotary microtome with an average tissue thickness of 5μ. Staining was done with Harris haematoxylin and eosin stain. Stained slides were observed under a light microscope. For the purpose of recovery, six animals after 96hr of exposure were kept in pesticide free water for 14 and 28 days. The conditions during the recovery experiment were the same as those in the exposure experiment. At the end of the recovery period, tissues were isolated and processed for further analysis. For statistical analysis one way ANOVA was used.

RESULTS AND DISCUSSION

Behavioural observations

The toxicant causes stress on the organisms; the behavioural changes are the immediate responses to the toxicant and are indicators of possible stress (Ait *et al.,* 2011). All shells in the control were shut firmly and required a scalpel to open and to keep shell open. At higher concentrations the shells were easily opened, even by hand, and remained open for sustained periods consistent with a decline in neuromuscular control. The impact of environmental pollution results in a significant change in the behaviour pattern of non target organisms (Surwase, 2009). Present study showed that, with increasing concentration of pesticide, animals become lethargic, same trend was observed by Bharathi, 1994. Increased mucus secretion and decrease in shell closure responsiveness of the freshwater mussel may be used as biomarker for the assessment of actual health of the organisms living in the polluted water (Kumar *et al.,* 2012).

Behavioural observations of *L. marginalis* when exposed to monocrotophos are explained in Table 1

Protein and carbohydrates are the primary sources for various metabolic processes. Carbohydrates in the tissues of the aquatic animals exist as glycogen. It is well-known that the

Table 1: Behavioural pattern of *L. marginalis* in different exposure concentrations

Sr. No.	Conc. (ppm/lit)	Behavioural responses of mussels during 96h exposure to different concentrations of monocrotophos
1	0	Initially, the shell valves were closed .After 14 hours, Bivalves began to open the shell valves. Two pallial edges were extended out of the valves.After 48 hours, extended Pallial edges, foot as well as siphons were observed. After 48, 72 and 96 hours, the gentle mechanical stimulus made the extended organs to retract in shell valves immediately (Duration of retraction response- 10 seconds).
2	20	After 24 hours, Bivalves began to open the shell valves, Pallial edges, foot and siphons were extended out of the valves. After 24, 48, 72 and 96 hours, the gentle mechanical stimulus made the extended organ to retract in shell valves immediately (Duration of response -10seconds).
3	40	The shell valves were closed up to 36 hrs. After 48 hours, Extended foot was observed and gentle stimulus made the extended foot to retract in shell valves slowly (Duration of response- 30 seconds).After 72 hours, Pallial edges were at the border of shell valves but siphons were protruded outside the shell valves. Slow retraction of organs was observed after stimulus(Duration of response- 70 seconds)
4	60	After 48 hours, Swollen foot was observed extending through the shell valves and gentle stimulus made the extended organ to retract in shell valves slowly as compared to earlier concentrations (Duration of response- 100 seconds).After 72 hours, Shell remained open slightly, as valves could not close tightly because of swollen foot. mucous secretion was observed
5	80	After 48 hours, The shell valves trapped the swollen foot slightly along with the Pallial edges and siphon outside the shell valves, Shell remained open slightly. (Duration of response- 300 sec) Large secretion of mucous was observed
6	100	After 48 hours, Bivalves were inactive and shells remained widely open with well extended swollen foot. After 72 hours, Large secretion of mucous was observed.(Duration of response-15 minutes)
7	120	After 96 hours, 100% mortality was observed. Open shell valves with extended swollen foot. (No response at all.)

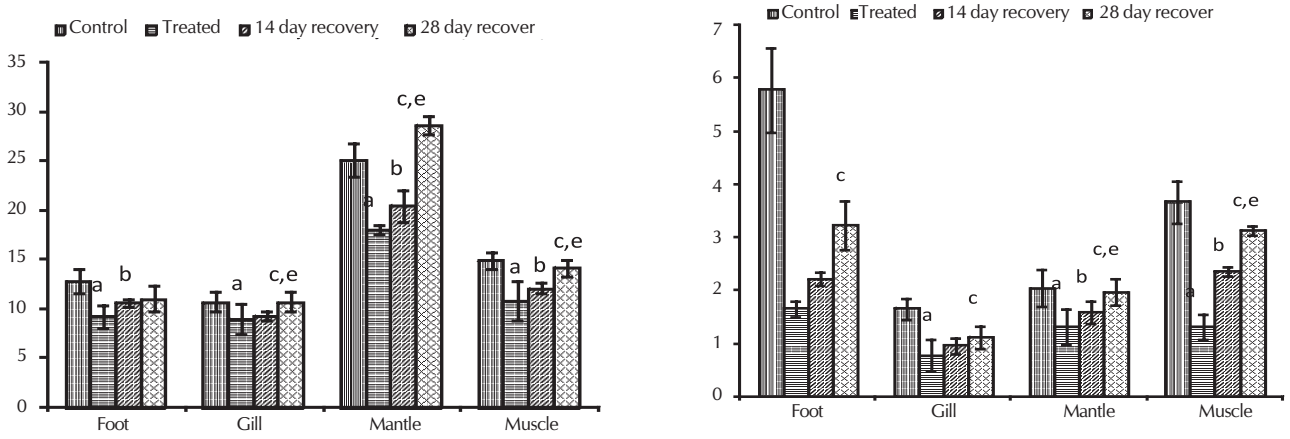
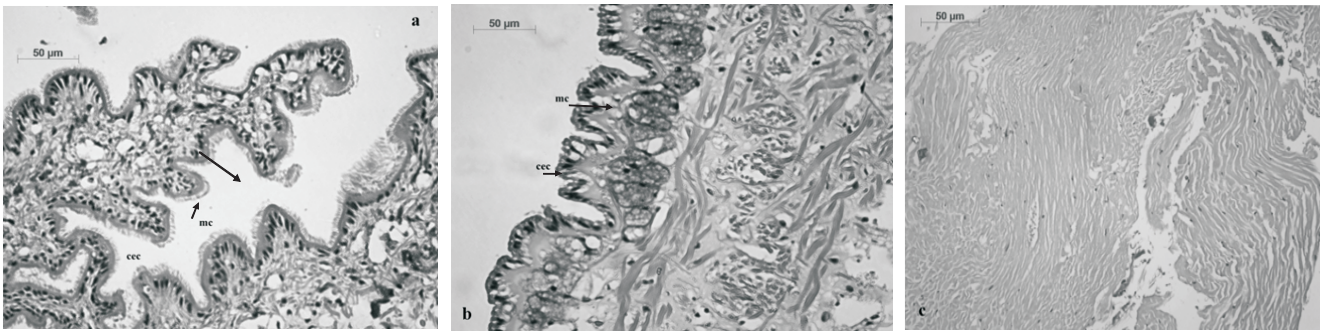


Figure 1: Alteration in the glycogen (a) and protein (b) content of *L. marginalis* when exposed to monocrotophos for 96 hr
a. ($p < 0.05$) between the control and treated groups, b. ($p < 0.05$) between the treated and 14 day recovery, c. ($p < 0.05$) between the treated and 28 day recovery, e. ($p < 0.05$) between the 14 and 28 day recovery



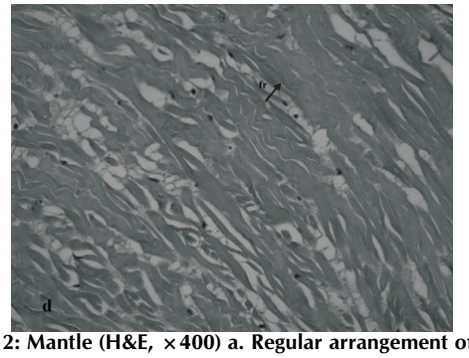


Figure 2: Mantle (H&E, ×400) a. Regular arrangement of columnar epithelial cells, mucus cells in control b. swelling due to hypertrophy of mucous cells in treated animals, cec: columnar epithelial cells, mc: mucous cells; Muscle (H&E, ×400) Muscle tissues of *L. marginalis* c. normal arrangement of muscle in control d. muscle fragmentation in treated animal, fr: fragmentation

glycogen serves as energy reserve for various metabolic processes.

Glycogen was estimated from foot, gill, mantle and muscle (Fig.1a). Mantle showed maximum storage of glycogen compared to other tissues in control condition. Glycogen reduction in foot (28.05 %) mantle (28.27%), gill (16.59%) and muscle (27.37%) in treated animals is significant ($p < 0.05$) when compared with control. Similar reduction in the stored tissue glycogen content has been reported in *L. marginalis* when exposed to copper sulphate (Satyaparameshwar, 2006).

The observed reduction in glycogen content indicates the utilization of stored glycogen possibly through anaerobic glycolysis to meet the extra energy requirement under hypoxia or because of toxic stress of pesticide. Under chemical or toxicant stress *L. marginalis* reduces the oxygen consumption (Chati, 2009). Decrease in glycogen content indicates greater utilization of glycogen for metabolic purposes and to combat with stress (Tendulkar and Kulkarni, 2012).

After 14 days of recovery period, foot, muscle and mantle recovered significantly ($p < 0.05$). While after 28 days, Mantle, muscle and gill showed significant ($p < 0.05$) recovery when compared with treated group.

Protein was estimated from foot, gill, mantle and muscle (Fig.1b). Foot showed maximum content of protein compared to other tissues in control condition. Reduction in Protein content is observed in foot (71.41%), gill, (53.05%) mantle(35.96%) and muscle (64.21%)tissues in treated animals as compared to control. Vijayavel (2006), Waykar and Pulate (2012), Shandilya *et al.,* (2010) also observed same trend of reduction in protein content, which suggests an increase in proteolytic activity and possible utilization of its products for metabolic purpose. Fall in protein level during exposure may be attributed to increased catabolism and decreased anabolism of protein due to toxic stress of monocrotophos (Patil, 2011).

Mantle and muscle showed significant ($p < 0.05$) recovery of protein after 14 day. All tissues showed significant ($p < 0.05$) recovery after 28 days.

Many investigators have reported toxicant induced

histopathological abnormalities and degenerative changes in certain tissues of various animals (Chakraborty *et al.,* 2010; Shaikh and Mane, 2013)

In T.S. of mantle of control animal (Fig. 2a) regular arrangement of mucous cells, columnar epithelial cells with cilia and muscle filament was observed.

In T. S. of mantle of the treated animal hypertrophy was observed in mucous cells. Vacuoles were observed in columnar epithelial cells. Fragmentation of muscle was also observed (Fig. 2b).

T.S. of muscle of control animal showed normal arrangement of muscle fibres (Fig. 2c). While Fragmentation of muscle fibres was observed in muscle when treated with sub lethal concentration of monocrotophos (Fig. 2d).

All the histopathological observations indicated that exposure to sub lethal concentrations of monocrotophos caused degenerative changes to some extent in mantle and muscle tissues of animal.

ACKNOWLEDGEMENT

The authors are grateful to 'Board of College and University Development' and 'University Grant Commission- Career Advancement Scheme' for the financial assistance and grateful to Head, Department of Zoology for laboratory facilities and encouragement.

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