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RESEARCH ARTICLE

EFFECT OF L-ASCORBIC ACID ON METHOMYL INDUCED ALTERATIONS IN THE LIPID CONTENT IN DIFFERENT TISSUES OF THE FRESHWATER BIVALVE, *LAMELLIDENS MARGINALIS* (LAMARCK)

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ABSTRACT

The present study investigates the effect of Methomyl induced alterations in lipid level of gills, gonads and digestive gland tissues and its possible recovery by treating with L-Ascorbic acid in the fresh water bivalve, *Lamellidens marginalis* after chronic exposure. The freshwater bivalve *Lamellidens marginalis* were exposed to chronic dose of Methomyl (35 PPM $LC_{50/2}$ values of 96 hours) alone and in combination with 50mg/L L-ascorbic acid for 21 days. Percent lipid contents in the gills, gonads and digestive gland tissues of control bivalve, *Lamellidens marginalis* were 4.5460±0.1560, 5.7463±0.0984, 6.2547±0.0758 respectively. Percent lipid contents in the gills, gonads and digestive gland of bivalve, *Lamellidens marginalis* on Methomyl intoxication were 4.5654±0.0831, 5.7658±0.1877 +10.63, 7.1566±0.0564 +18.42 respectively. Percent lipid contents in the gills, gonads and digestive gland of bivalve, *Lamellidens marginalis* on exposure to Methomyl intoxication with 50mg/L L-ascorbic acid were 4.9025±0.0440 +17.76, 5.9541±0.0365 +14.23, 8.098±0.2341 +34.43 respectively. Lipid contents in the gills, gonads and digestive gland of Methomyl and Methomyl with 50mg/L L-ascorbic acid exposed bivalve, *Lamellidens marginalis* showed remarkable increase in lipid content as compared to control. The increased level of lipid was observed in digestive glands as compared to other tissues. Exposure to pesticide Methomyl in combination with 50 mg/L of L-ascorbic acid showed considerable increase in the lipid levels. The pre-exposed bivalves for 21 days exposure to chronic dose of Methomyl showed recovery in normal water and the lipid contents were decreased to 4.2679±1.0255 +23.76, 5.2986±0.0286 +35.72, 6.4794±0.0469 +25.47 while percent lipid contents in presence of 50mg/L of L-ascorbic acid were decreased to 4.0077±1.0544 +17.65, 5.0457±0.3769 +27.48, 6.00652±0.1638 +25.66 respectively. Lipid content was found to be more in different tissues exposed to Methomyl without ascorbic acid as compared to Methomyl with ascorbic acid. Fast recovery of percent lipid contents was observed in presence of L-ascorbic acid than the recovery in the normal freshwater. This study indicates the protective and curative property of the L-ascorbic acid against the Methomyl induced damage.

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INTRODUCTION

The aquatic environment has always been subjected to different types of pollutants of industrial, domestic and agricultural wastes (Farkas *et al*., 2000). The aquatic ecosystems are very sensitive to pesticide pollution and this is a primary problem due to their persistence as they are not removed by biodegradation. The use of pesticides has resulted in increased crop production and other benefits and has raised concerns about potential adverse effects on the environment and human health. The chemicals which are used for pest management cause deleterious effects on the environment as they ultimately affect the non-target and useful organisms (Nimmo, 1985). Pesticide such as Methomyl is a potential problem for aquaculture in developing countries. Methomyl is highly toxic to aquatic invertebrates, when absorbed through the mucous membrane of the respiratory tract, resulting in systemic intoxication. Freshwater bivalves provides significant role in providing source of food for human being and other aquatic birds from all over the world (Malathi and Thippeswamy, 2013). Now-a-days, decline of freshwater mussels is observed due to several factors such as siltation, pollution, commercial harvest, and construction of dams. 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). Lipids are the most important source

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of energy in the absence of carbohydrates. This essential nutrient is drastically affected and altered by various environmental pollutants like pesticides. Pesticides are known to induce severe impairment in lipid transport and metabolism which may lead to change in lipid composition of tissues. Young (1982) showed due to stress condition the carbohydrate storage got quickly exhausted and lipid served as the next biochemical entity. It is broken down to meet the requirement of enhanced metabolism hence reduction in lipid occurs. Ascorbic acid has potential role to reduce the activity of free-radical induced reactions (Holloway and Peterson, 1984). Ascorbic acid prevents free radical induced protein damage (Halliwell and Gutteridge, 1999). Hence the present study was undertaken to elucidate the effect of pesticide methomyl on lipid content in vital tissues like gills, gonads and digestive gland and its possible attenuation by Ascorbic acid in fresh water bivalve *Lamellidens marginallis* after chronic exposure.

MATERIALS AND METHODS

The adult fresh water bivalve molluscs *Lamellidens marginalis* were collected from the banks of Darna river at Chehedi water works (pumping station, Latitude 19 ^{0} 55.873'and Longitude 73 ^{0} 51.429'), village Chehedi near Nashik (M.S.) during summer season. After collection the animals were brought to the laboratory, the shells of the bivalves were brushed and washed with water to remove the mud and fouling algal and fungal biomass. The bivalves were acclimatized in the laboratory condition at room temperature for 4-5 days in dechlorinated tap water. The active acclimatized bivalves of approximately same size were selected for experiment.

Set –I Experimental Design

For the experimental studies the animals were divided into four groups.

A. Group 'A' was maintaining as control.
B. Group 'B' animals were exposed to acute dose of Methomyl (35 PPM $LC_{50/2}$ values of 96 hours) up to 96hours.
C. Group 'C' animals were exposed to acute dose of Methomyl (35 PPM $LC_{50/2}$ values of 96 hours) along with 50mg /1 of L-ascorbic acid up to 96hours.
D. Group 'D' animals were exposed to acute dose of Methomyl (35 PPM $LC_{50/2}$ values of 96 hours) along with 100mg /1 of L-ascorbic acid up to 96hours.

Acute exposure was carried over up to four days. Every day the solutions were changed.

Set- II : Experimental Design for Recovery Studies

1) Group 'A' animals were maintains as control.
2) Group 'B' animals from set –I were divided into three groups for recovery study.

I. Animals pre-treated to Methomyl were allowed to self cure normally in untreated fresh water up to 21days.
II. Animals pre-treated to Methomyl were allowed to cure in 50mg /l of L-ascorbic acid in fresh water up to 21days.
III. Animals pre-treated to Methomyl were allowed to cure in 100mg /l of L-ascorbic acid in fresh water up to 21days.

After 24 and 96 hours of interval animals from set-I and after 4, 7,14 and 21 days, animals from control and set-II were taken out, dissected and their gills, gonads and digestive glands were taken out and dried at 80^o C in an oven till constant weight was obtained. The dried powders of different tissues of control and experimental animals were used for estimation of lipids. The total lipids were estimated by A. Okabe (1975). All the values expressed in terms of mg/100 mg of dry weight of tissue powder. Qualitative and quantitative study of changes in major biochemical components of organisms such as lipids is useful to know different toxicants and defensive mechanism of the body against toxic effects of pesticides. These biochemical components are indices of pollution as they determine nutritional status, health and vigour of an organism.

RESULTS AND DISCUSSION

The data obtained regarding the lipid contents in different tissues after chronic exposure to Methomyl with and without L- ascorbic acid and during recovery are given in the Table 1. The lipids constitute the vital organic constituents and are important in energy metabolism. So changes in lipids are studied in the present investigation. Gills, gonads and digestive gland are of vital and metabolic importance and any stress on the animals is depicted by changes in the constituents of these tissues. In the present investigation, total lipid content was found to be increased in gill, gonads and digestive gland of bivalve *Lamellidens marginallis* after acute and chronic treatments of Methomyl. The increase in total lipid content after toxicants stress in the bivalve *Lamellidens marginallis* can be explained on the basis of observation made by Coley and Jensen (1973), who detected the inhibition of lipase activity following organophosphate pesticide administration. The lipase reacts differently with the different pesticides in different organisms. There was a drop in lipase activity indicating lesser lipolysis and unhampered esterification of glycerol by fatty acids which could cause elevation of free fatty acids by pesticidal impact. Voogt, (1983) stated that lipids in bivalves are multifunctional, one or more of the functions during the maturation of gametes, drastic environmental conditions, starvation, pollution and stress etc. can be more noticeable. Such a role of liquid in the body maintenance and metabolism during pesticides stress can be seen in the present study in *Lamellidens marginallis.* In lamellibranch molluscs the conversion of glycogen into fatty acids or triglyceride reserves, via triose phosphate entry in the glycolytic sequence and to the production of pentose sugar for nucleic acid synthesis as well as the necessary intermediates for lipogenesis is well-documented. Swami, *et al*. (1983), suggested a shift in carbohydrate and protein metabolism to lipid synthesis in the freshwater mussel, *Lamellidens marginallis*, exposed to flodit and metacid. The degradation of amino acids, gives rise to keto acids which provide the acetate units for lipogenesis.

Table 1. Lipid content in selected tissues of *Lamellidens marginallis* after acute exposure to Methomyl without and with Ascorbic acid during recovery (Value represent percentage in dry weight)

Treatment	Tissue	24 hrs	96 hrs	Recovery			
				4 days	7 days	14 days	21 days
Control	Gill	4.2348 ±0.1885	4.5460 ±0.1560				
	Gonad	5.2588 ±0.0674	5.7463 ±0.0984				
	D gland	6.1242 ±0.1335	6.2547 ±0.0758				
Methomyl	Gill	4.2548 ±0.1245 +2.37	4.5654 ±0.0831 +9.565				
	Gonad	5.2677 ±0.056 +1.61	5.7658 ±0.1877 +10.63				
	D.gland	6.2870 ±0.1329 +4.10	7.1566 ±0.0564 +18.42				
Methomyl 50 mg/L A.A.	Gill	4.3563 ±0.0128 +4.45	4.9025 ±0.0440 +17.76				
	Gonad	5.4258 ±0.1188 +4.12	5.9541 ±0.0365 +14.23				
	D. gland	6.4451 ±0.0773 +7.09	8.098 ±0.2341 +34.43				
Methomyl 100 mg/L A.A.	Gill	4.4934 ±0.0563 +7.878	4.7168 ±0.0691 +12.88				
	Gonad	5.6453 ±0.1727 +8.125	5.9863 ±0.1987 +10.98				
	D. gland	6.7456 ±0.0656 +11.78	6.9653 ±0.9874 +15.45				
After 96 hrs exposure to Acute methomyl	Normal water Gill			4.1276 ±0.0287 +13.55	4.2331 ±0.0456 +13.55	4.2443 ±0.0676 +18.98	4.2679 ±1.0255 +23.76
	Gonad			4.763 ±0.2535 +18.39	4.9832 ±0.2765 +20.41	5.3120 ±0.3059 +33.98	5.2986 ±0.0286 +35.72
	D. gland			6.2162 ±0.1072 +22.65	6.2343 ±0.1123 +22.65	6.357 ±0.0564 +17.65	6.4794 ±0.0469 +25.47

Continue…….

Normal water ±50mg/L AA	Gill	4.0517 ±0.0365 +8.0176	4.1974 ±0.0593 +10.76	4.0568 ±0.1351 +12.39	4.0077 ±1.0544 +17.65
	Gonad	4.7256 ±0.0283 +10.72	4.7560 ±0.0673 +12.43	5.2656 ±0.6976 +17.91	5.0457 ±0.3769 +27.48
	D. gland	6.1816 ±0.0158 +8.154	6.1656 ±0.0190 +10.54	6.0454 ±0.0674 +19.57	6.0052 ±0.1638 +25.66
Normal water ±100mg/ L AA	Gill	4.6532 ±0.0264 +9.10	4.9843 ±0.0764 +12.75	5.1293 ±0.1351 +14.63	5.3241 ±1.0983 +18.64
	Gonad	6.1096 ±0.0283 +10.79	6.2356 ±0.0673 +12.43	6.4532 ±0.6976 +17.91	6.8757 ±0.3769 +27.48
	D. gland	6.7832 ±0.0201 +12.98	6.8752 ±0.0263 +15.54	7.4832 ±0.0287 +21.76	7.8973 ±0.2901 +27.98

1. Values expressed as mg/100 mg dry wt. of tissue.
2. (+)or (-) indicate percent variation over control.
3. ± indicate Standard deviation of three observations.
4. Values are significant at *=P<0.05;**=P<0.01;***=P<0.001; NS= Not Significant.

The acetyl coenzyme A condenses with the existing fatty acids which may continue to increase the chain length of fatty acids and also the consequent esterification with glycerol mostly resulting in the formation of lipids, as was suggested by Chaudhari and Lomte (1990) in *Bellamya bengalensis* after pesticidal impact. Thus, the possible mechanism for elevation in lipid contents in different tissues might be due to increased lipid synthesis, diminished degradation of lipid, inhibition of lipase activity, diminished transport of lipids away from these body parts. In the present investigation greater elevation in the lipid content of digestive gland is observed as compared to other tissues. In presence of 50 mg / l &100 mg / l of L- ascorbic acid, elevation of total lipids level is less as compared to Methomyl intoxication. In present study, it was also observed that, total lipids levels in different tissues are closely comparable with control in case where 50 mg /l and 100 mg / l of L- ascorbic acid were used. It was noticed that, a significant restoration of lipids level was brought about in all the animals pre-exposed to pesticides and were allowed for recovery in 50 mg / l and 100 mg / l of L- ascorbic acid as compared to animals allowed to cure in pesticide free water. The result indicates that L- ascorbic acid plays an important role in the cure of pesticide-induced damage and 50 mg /l to 100 mg / l of L- ascorbic acid dose is appropriate. It may be concluded that the physiological disturbances arising in animals after exposure to pesticides exhibits a trends towards normalization and this rate of recovery from pesticide induced damage is faster on exposure to L-ascorbic acid indicating the preventive and curative property of the L-ascorbic acid against the pesticide induced damage.

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