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22. Evaluation of Methomyl Pesticide Effect on Glycogen Content in Different Tissues of the Freshwater Bivalve, Lamellidens Marginalis (Lamarck) from Nashik District (M. S.)

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Abstract

The present study investigates the effect of carbamate pesticide Methomyl induced alterations in glycogen 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 50 mg/L L-ascorbic acid for 21 days. Glycogen contents in the gills, gonads and digestive gland of Methomyl and Methomyl with 50 mg/L L-ascorbic acid exposed bivalve, *Lamellidens marginalis* showed remarkable increase as compared to control. The marked increase in glycogen level 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 glycogen levels.

Key words: Bivalve, *Lamellidens marginalis*, Methomyl, L- Ascorbic acid, glycogen content.

Introduction

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

provide significant role in providing source of food for human being and other aquatic birds from all over the world. (Malathi and Thippeswamy, 2013). Carbamate pesticides are reported to alter the carbohydrate metabolism (Sastry and Siddique,1982; Rao et.al.,1984; Chaudhari,1988). Now-a-days, decline in freshwater mussels' population is observed due to several factors such as siltation, pollution, uncontrolled/ nonregulated 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). Glycogen, a polymer of glucose is an energy reserve of animal tissues and maintenance of glycogen reserves is an official feature of normal organism. Many toxicants were reported to cause abnormalities in cellular carbohydrate metabolic pathways resulting in much hardship to organism for survival. The average glycogen content in Gill, digestive gland and gonads of *Lamellidens marginallis* decreased after acute and chronic treatment of Methomyl. 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 glycogen 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 maintained as control.

B. Group 'B' animals were exposed to acute dose of Methomyl (35 PPM $LC_{50/2}$ values of 96 hours) up to 96 hours.

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

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

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 maintained 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 50 mg / l of L-ascorbic acid in fresh water up to 21days.

III. Animals pre-treated to Methomyl were allowed to cure in 100 mg / 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 ^{0}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 glycogen. The glycogen content was estimated by (Anthrone method). 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 glycogen is useful to know the effects of 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 vigor of an organism.

Results and Discussion: The data obtained regarding the glycogen contents in different tissues after chronic exposure to Methomyl with and without L - ascorbic acid and during recovery are depicted in the Table 1. In the present study, the decrease in the glycogen level in Gill and Gonads is less than that of the digestive gland. This suggests that, these tissues do not contribute much to the anoxia or hypoxia resulting from pesticide stress, since anoxia and

hypoxia are known to increase carbohydrate consumption (Dezwaan and Zandee, 1972). As digestive gland, contributes much to the anoxia it results in the increase of carbohydrate consumption. This might be due to greater glycolytic activity in the gland than other tissues. The greater depletion of glycogen level in digestive gland indicates that the digestive gland is the principle metabolic centre for various metabolic functions. Decreasing glycogen content indicates disrupted carbohydrate metabolism which might be due to enhanced glycogen breakdown to meet high energy demands during toxic stress (Lomte and Alam, 1982). The decrease in glycogen content noted in the present study in pesticide treated bivalve, *Lamellidens marginallis* is in agreement with the above cited work. In the present investigation, greater elevation in the glycogen 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 glycogen content is less as compared to Methomyl intoxication. In present study, it was also observed that glycogen content, 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 glycogen content was brought about in all the animals pre-exposed to pesticide 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 trend 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.

Conclusion

Glycogen content in gill, gonad and digestive gland was found to be decreased. Much glycogen decreases occurred in the digestive gland after acute and chronic treatment by pesticides and recovery occurred in the glycogen content after treatment with ascorbic acid and glycogen content was found in increased amount in digestive gland than in gills and gonads of fresh water bivalve *lamellidens marginalis.*

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Table No. 1: Glycogen content in selected tissues of *Lamellidens margianlis* 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	8.5901± 0.8164	7.904±0.7950				
	Gonad	8.4678±0.8124	7.6520±0.7632				
	D. Gland	9.945±0.5345	8.976±0.5423				
Methomyl	Gill	7.725±1.0924 - 11.11	4.635±0.7164 - 46.87				
	Gonad	7.9106±0.950 - 4.44	4.7332±0.9704 - 44.80				
	D. Gland	8.1676±0.1634 - 17-35	4.9623±0.0665 - 50.09				
Methomyl 50 mg / L A. A.	Gill	7.935±1.0120 - 3.45	6.935±0.6120 - 43.45				
	Gonad	8.0243±0.730 - 3.45	6.1232±0.9654 - 42.80				
	D. Gland	8.9342±0.1453 - 16-54	6.1226±0.07545 - 45.69				
Methomyl 100 mg / L A. A.	Gill	8.298±.0120 - 2.15	7.125±0.4120 - 23.44				
	Gonad	8.1674±0.8256 - 3.145	6.9278±0.94546 - 40.60				
	D.	9.3956±0.	6.9872±0.5				

		Gland	1253 - 16.23	623 - 40.69				
After 96 hrs exposure to Acute Methomyl	Normal water	Gill			4.965±0.8434	5.673±0.7632	6.246±0.6548	6.9873±0.0683
		Gonad			7.586±0.0673	8.1980±0.4560	8.284±0.5463	8.352±0.395
		D.Gland			9.3989±0.1897	9.3032±0.4328	9.5098±0.8743	9.684±0.5437
	Normal water ±50mg/L A.A.	Gill			5.658±0.8434	6.376±0.7263	7.642±0.6954	7.9975±0.0863
		Gonad			8.658±0.0967	8.0189±0.6540	9.483±0.6354	9.523±0.5687
		D.Gland			9.3889±0.1677	9.3952±0.4328	9.6078±0.7853	9.8764±0.5677
	Normal water ±100 mg /L A.A.	Gill			6.986±0.8434	7.687±0.8763	7.865±0.7864	8.0975±0.0983
		Gonad			8.988±0.0989	9.0389±0.7640	9.676±0.6453	9.884±0.6587
		D.Gland			9.4756±0.1787	9.4582±0.5438	9.7967±0.8753	9.9789±0.6578

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.

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