Chapter 6

BIOCHEMISTRY
6.1 Introduction

The metabolic needs are dependent on nutritional and reproductive state and some phylogenetic factors. For maximizing the power of reproduction, individuals naturally devote their more energy for the reproduction. Studies have reported that much of the energy resources of molluscs are stored in the digestive gland and foot muscle (Sastry and Blake, 1971; Pazoc, et al., 1997 and Berthelin, et al., 2000). Earlier studies have also shown that the digestive gland is implicated not only in nutrient storage but also in the transfer of assimilated food to body tissues in molluscs (Sastry and Blake, 1971).

Most toxic substances exert their effects on basis level in the organism by reacting with enzymes or by affecting membrane and other functional compartments of the cell (Patel and Patel, 1985). The hepatopancreas has been found to be the major site of heavy metal accumulation in the molluscs (Berger and Dallinger, 1989; Nott and Nicolaidou, 1989 and Presing, et al., 1993). Alteration in enzyme activity can be correlated with molluscicide induced changes in the ultrastructure of the cells (Triebskorn, 1991). Ultrastructural response in the hepatopancreas can be used as biological markers to detect different levels of biological response to toxic conditions (Rubio, et al., 1993). Molluscicides greatly affect the metabolic activities of the snail intermediate host (Rawi, et al., 1995). Biochemical parameters in organism exposed to toxic contaminant have been used as biomarkers and can constitute an important diagnostic tool to assess the exposure and effects of xenobiotics (Forbes, et al., 1997 and McLoughlin, et al., 2000), in addition to, they act on glucose level, lipid and protein content in snail tissues and haemolymph (Abdel, 1999; Mantawy and Mahmoud, 2002; El-Moghazy and Abdel-Aziz, 2005 and Bakry, 2009). Changes in the physiological state of snail can affect the biochemical values of the haemolymph (Akinloye and Olorode, 2000). Molluscicides act on different enzymes, chiefly those of respiration and carbohydrate metabolism (Sakran and Bakry, 2005 and Sharaf, 2006). Biochemical parameters are sensitive index to monitor changes due to xenobiotics and can constitute important diagnostic tool in toxicological studies (Radwan, et al., 2008).
Lipids and glucose are rich energy source which can be stored as glycogen and glycerol in the body for later use. The concentration of protein is relatively higher than other metabolites. Land snails remain dormant for many months or even years, until favorable conditions allow them to resume vital processes of nutrition and reproduction (Herreid, 1977; Riddle, 1986 and Whitman and Storey, 1990). During these prolong periods of starvation previously stored materials are metabolized; glucose, a major source of energy, is the first of such materials to be used (De Silva and Zancan, 1994 and Rossi and De Silva, 1993;). When this metabolite is depleted, the animal mobilizes lipid reserves (Barclay, et al., 1983 and Piretti, et al., 1988), finally protein reserves are used as a last resort (Barclay, et al., 1983).

6.1.1 Significance of Albumin (ALB)

Bakry, (2009) found decrease in albumin content of the haemolymph of Biomaphalaria alexandrina infected with Schistosoma mansoni. The drop in albumin content may also reflect damage in the hepatic parenchyma which is considered the site of albumin origin (El-Husseini, et al., 1986 and Rawi, et al., 1995). Till date meager data was found related to albumin in molluscs.

6.1.2 Significance of Alkaline phophatase (ALP)

Alkaline and acid phosphatases are two phosphomonoesterases that catalyse transphosphorylation and hydrolysis on numerous orthophosphate esters.

Alkaline phosphatase breaks down ester compounds of orthophosphate acids under alkaline condition between pH 9.2 and 9.8 (Adolph and Lorenz, 1981). Alkaline and acid phosphatase proved to be good sensitive tools in the detection of any variations in the physiological process of living organisms (Tolpa, et al., 1991). Stimulation or inhibition of these enzymes will thus result in disturbances in metabolism. Alkaline phosphatase is brush border enzyme, which catalyses dephosphorylation of many molecules including nucleotides, proteins and alkaloids at alkaline pH. It is well known that phosphatases are involved in carbohydrate metabolism, growth and differentiation, protein synthesis, synthesis of certain enzymes, secretory activities and transport of phosphorylated intermediates across the cell membranes. Hydrolysis of phosphoesters, phosphate transferase activity, protein phosphatase activity, phosphate transport, modulation of organic cation transport and involvement in cell proliferation have been suggested as possible function of alkaline
phosphatase (Sarrouilhe, et al., 1992). The enhanced activity of alkaline phosphatase in the hepatopancreas might be due to activation of intracellular energy consuming process because the alkaline phosphatase facilitates breakdown of ATP to ADP and inorganic phosphate, thereby making free energy available for metabolic processes (Botham and Mayes, 2003). Alkaline and acid phosphatase are expressed in a constant pattern and help to identify the different zone of the adult shell forming tissue (Marxen, et al., 2003). Alkaline and acid phosphatases were recorded among the target enzymes which should be disturbed and may provide more accurate information on the molluscicide induced stress on molluscs. Impairment of these enzymes could be effective in affecting the feeding and reproductive competence at the mollusc population level (fertility, fecundity and reproductive rate) (Hasheesh, et al., 2011).

6.1.3 Significance of Glucose (GLU)

All living cells contain carbohydrate in different forms. These compounds are perhaps best defined as polyhydroxy aldehydes or ketones and their derivatives. The storage of carbohydrate in invertebrate and vertebrate body is important as they have reserves of calories (energy) for the body tissues. According to Clark (1975) the carbohydrates are considered to be the first organic reserve degraded under toxic stress condition imposed on animal. There is a causal connection between synthesis of glucose or glycerol and catabolism of glycogen (Storey, 1981; Steiner, 2000 and Li, et al., 2002). Carbohydrate is stored largely as glycogen and specialized galactogen and is transported and available from the blood as glucose, glycogen is distributed generally throughout the tissues i.e. hepatopancreas, foot and mantle. In order to promote energy production, gastropods categorize primarily carbohydrates, which are stored in certain tissues as glycogen and transported in the haemolymph as glucose (Livingstone and deZwaan, 1983). Glycogen level is one of the parameters that reflects the energetic and reserves status of organisms. Moreover, glycogen is used rapidly when organisms are under stress and levels of this energy reserve have been suggested as useful biomarker of general stress (Hugget, et al., 1992 and Vasseur and Cossu-Leguille, 2003). Carbohydrates are generally used as energy supply particularly in case of stress. It is well known that the sugar serve as energy reserve for the metabolic process. Carbohydrates are considered to be the first among the organic nutrients degraded in response to stress conditions imposed on an animal.
6.1.4 Significance of Total Protein (TP)

The proteins are the basic structural units present in all the biological systems. The physio-biochemical activities consist the life of the cell are catalyzed by the enzymes, which are almost proteins. Proteins form one of the most important and most complex groups of biological materials comprising the chief nitrogenous constituents of the body tissues.

The concentration of free amino acids in the tissues and extracellular fluid compartments of mollusc varies with the diet, season, temperature, reproduction and developmental stage and environmental stress related to desiccation, anaerobiosis, osmotic pressure, pollution and parasitism (Bishop, et al., 1983). Similarly, snails have been reported to be a good source of protein (Amusan and Omidiji, 1988 and Akinnusi, 2002). South (1992) observed that protein is the most abundant solute in the snail’s haemolymph. Meenakshi, (1995) observed the total remarkable increase in total free amino acids may also represent the proteolysis and a derangement of protein and release of amino acids from the liver to blood. Singh, et al. (1996) reported that the quantity of proteins depends on the rate of synthesis or its degradation. It is affected due to impaired incorporation of amino acids into polypeptide chain. Lipids and glucose are rich energy sources which can be stored as glycerol and glycogen in the body for later use. Depletion in protein level is due to either suppressed incorporation of protein synthesis or increased breakdown of proteins in to amino acids, which diffused out of cells (Gaur, 2011).
6.1.5 Significance of Uric Acid (UA)

The nitrogenous products of degradation under physiological conditions of stress may be altered that would cause in the snail an intoxication by the excess of these products. Uric acid is produced and stored by species of *Pomacea* and *Pila*, both during period of activity and of aestivation (Meenakshi, 1995 and Little, 1968; 1981) and much has been written on the possible role of uric acid in nitrogen excretion in these contrasting physiological states (Lal and Saxena, 1952 and Little, 1968). Uric acid being a typical storage excretory product is often determined in molluscs in homogenates of the whole body or in certain organs such as hepatopancreas, kidney or foot (Becker and Schmale, 1975). The land snail *P. canaliculata* invest a large amount of nitrogen in the eggs perivitelline fluid, in the form of at least three perivitelline glycolipoproteins (Heras and Pollero, 2002; Dreon, *et al.*, 2006 and Heras, *et al.*, 2007), only a very low concentration of uric acid was found in the albumen gland and none was found in newly deposited eggs of *P. canaliculata*. Active *Pomacea canaliculata* does not excrete uric acid to any detectable extent, this purine is found in the circulation and that it accumulates in crystalloids within specialized cells of the midgut gland (Cueto, *et al.*, 2005 and Vega, *et al.*, 2007). Giraud-Billoud, *et al.* (2008) found that uric acid concentration in the albumen gland and in the newly deposited eggs of *P. canaliculata*. 
6.2  Material and Methods

6.2.1  Collection of test animal
As given in Chapter No. 5

6.2.2  Tested molluscicides
Two molluscicides Thiamethoxam and Diazinon belonging to two different chemical groups were tested.

6.2.3  Experimental Design
Five times less concentration of LC$_{50}$ concentration (0.10ppm and 0.12ppm) of both Thiamethoxam (0.51ppm) and Diazinon (0.64ppm) were taken for treating the test animal by spraying it on a food given to snail after starving it for 24hrs. This treatment was carried up to 7 days and then natural untreated food was provided to recover them up to 7 days again. Tissue samples were taken for biochemical studies after 7 days and 14 days to observe the treated and recovery effect of snails.

6.2.4  Sample preparation
After 7 days of treatment, shells of tested snails were removed by making a cut around the whorls in a continuous manner starting at the shell opening and the broken fragments of the shell were carefully removed. Foot and hepatopancreas tissues were dissected out and homogenized in distilled water (50mg/ml). The homogenates were centrifuged at 8000 rpm for 15 min. at 5°C in refrigerated centrifuge. The deposits were discarded and the supernatants were kept in a deep freezer till use to determine the activities of ALB, ALP, GLU, TP and UA.

Prepared homogenate was then processed as per the procedure according to the respective parameters. Readings were taken by using the Quantiamate turbidimetry chemistry, Auto Analyzer for biochemical tests of Tulip Diagnostics (P) Ltd. Goa, India.
6.2.5 Biochemical studies

Following parameters were studied to observe the effect of Thiamethoxam and Diafenthiuron on the pest snail *M.indica*.

6.2.5.1 Albumin (ALB) (BCG method), Bromocresol Green, End Point Assay (Liquid Gold), Span Diagnostics Ltd. India.

**Procedure**-

<table>
<thead>
<tr>
<th>Pipette into tube marked</th>
<th>Blank</th>
<th>Standard</th>
<th>Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample</td>
<td>-</td>
<td>-</td>
<td>10µl</td>
</tr>
<tr>
<td>Reagent 2</td>
<td>-</td>
<td>10µl</td>
<td>-</td>
</tr>
<tr>
<td>Reagent 1</td>
<td>1000µl</td>
<td>1000 µl</td>
<td>1000 µl</td>
</tr>
</tbody>
</table>

Mixed well and incubated at room temperature (15-30°C) for 1 minute.

Programmed the analyzer on 24 No. as per assay parameter.

1. Blanked the analyzer with reagent blank.
2. Measured absorbance of standard followed by the test.
3. Result calculated as per given calculation formula.

**Calculation**

\[
\text{Albumin (g/dl)} = \frac{\text{Absorbance of test}}{\text{Absorbance of Standard}} \times 4
\]
Globulins = Total Protein - Albumin.

**Conversion factor**

Albumin concentration in g/L = Albumin concentration in g/dl × 10.

6.2.5.2 Alkaline Phosphatase (ALP) Test Kit (pNPP-AMP (IFCC)), Kinetic assay (Autospan), Span Diagnostics Ltd., India.

**Procedure**-

<table>
<thead>
<tr>
<th>Pipette into tube marked</th>
<th>Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample</td>
<td>20 µl</td>
</tr>
<tr>
<td>Working ALP Reagent</td>
<td>1000 µl</td>
</tr>
</tbody>
</table>

Mixed well and aspirated immediately for measurement.

Programmed the analyzer on 11 No. as per assay parameter.

1. Blanked the analyzer with purified water.
2. Read absorbance after 30 seconds. Repeated readings after every 30 seconds i.e. up to 120 seconds at 405nm wavelength.
3. Determined the mean absorbance change per minute (Δ A/minute).

**Calculation:**

ALP activity (U/L) = Δ A/minute × Kinetic factor

Where,

Δ A/minute = change in absorbance per minute

Kinetic factor (K) = 2712

Kinetic factor is calculated by using following formula

$$K = \frac{1}{M} \times \frac{TV}{ST} \times \frac{1}{P} \times 10^6$$

M = Molar extinction coefficient of p-Nitrophenol and is equal to 18.8×10³ lit at 405nm

TV = Sample volume + Working reagent volume

P = Optical path length

10⁶ = Constant

6.2.5.3 Glucose (GLU) (Enzymatic, GOD/POD method), End point assay and Kinetic assay (Autospan), Span Diagnostics Ltd. India.

**Procedure**-
Mixed well and incubated at 37°C for 10 minutes.

Programmed the analyzer on 13 No. as per assay parameter.

1. Blanked the analyzer with reagent blank.
2. Measured absorbance of standard followed by the test.
3. Result calculated as per given calculation formula.

**Calculation**

**For end point mode**

\[
\text{Sample Glucose (mg/dl)} = \frac{\text{Absorbance of test}}{\text{Absorbance of Standard}} \times 100
\]

**For fixed rate kinetic mode**

\[
\text{Sample Glucose (mg/dl)} = \frac{\text{AT2} - \text{AT1}}{\text{AS2} - \text{AS1}} \times 100
\]

Where,

AT1: Initial O.D. of Test
AT2: Final O.D. of Test
AS1: Initial O.D. of Standard
AS2: Final O.D. of Standard

**Conversion factor**

Glucose concentration in mmol/L = Glucose concentration in mg/dl × 0.05551

6.2.5.4 Total Protein (TP) Test Kit (Biuret method), Modified Biuret, End point assay (Liquid Gold), Span Diagnostics Ltd. India.

**Procedure**

<table>
<thead>
<tr>
<th>Pipette into tube marked</th>
<th>Blank</th>
<th>Standard</th>
<th>Test</th>
</tr>
</thead>
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<tr>
<td>Sample</td>
<td>-</td>
<td>-</td>
<td>10µl</td>
</tr>
<tr>
<td>Reagent 2</td>
<td>-</td>
<td>10µl</td>
<td>-</td>
</tr>
<tr>
<td>Working Glucose Reagent</td>
<td>1000µl</td>
<td>1000 µl</td>
<td>1000 µl</td>
</tr>
</tbody>
</table>
Mixed well and incubated at 37°C for 5 minutes.
Programmed the analyzer on 18 No. as per assay parameter.
1. Blanked the analyzer with reagent blank.
2. Measured absorbance of standard followed by the test.
3. Result calculated as per given calculation formula.

Calculation

Total Protein Concentration (g/dl) = \( \frac{\text{Absorbance of test}}{\text{Absorbance of Standard}} \times 6.5 \)

Globulins = Total Protein - Albumin

Conversion factor

Total protein concentration in g/L = Total protein concentration in g/dl \( \times 10 \).

6.2.5.5 Uric acid Test Kit (UA) (Uricase/POD method), End Point Assay (Liquid Gold), Span Diagnostics Ltd., India.

Procedure-

<table>
<thead>
<tr>
<th>Pipette into tube marked</th>
<th>Blank</th>
<th>Standard</th>
<th>Test</th>
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<tbody>
<tr>
<td>Sample</td>
<td>-</td>
<td>-</td>
<td>20µl</td>
</tr>
<tr>
<td>Reagent 2</td>
<td>-</td>
<td>20µl</td>
<td>-</td>
</tr>
<tr>
<td>Reagent 1</td>
<td>1000µl</td>
<td>1000 µl</td>
<td>1000 µl</td>
</tr>
</tbody>
</table>

Mixed well and incubate at 37°C for 5 minutes.
Programmed the analyzer on 21 No. as per assay parameter.
4. Blanked the analyzer with reagent blank.
5. Measured absorbance of standard followed by the test.
6. Result calculated as per given calculation formula.

Calculation

Sample Uric acid (mg/dl) = \( \frac{\text{Absorbance of test}}{\text{Absorbance of Standard}} \times 6 \)

Conversion factor

Uric acid concentration in mmol/L = Uric acid concentration in mg/dl \( \times 0.059 \)
6.3 Observations
As per Chapter No. 5.

6.4 Results
The significant change in the content of ALB, ALP, GLU, TP and UA were observed and shown in the Table 6.1 and Chart 6.1-6.10.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Pesticide</th>
<th>Set</th>
<th>ALB</th>
<th>ALP</th>
<th>GLU</th>
<th>TP</th>
<th>UA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Foot</td>
<td>Thiamethoxam</td>
<td>Control</td>
<td>15.6±0.47</td>
<td>5.4±0.07</td>
<td>0.19±0.003</td>
<td>0.25±0.004</td>
<td>0.16±0.01</td>
</tr>
<tr>
<td></td>
<td>Treated</td>
<td></td>
<td>10.4±0.22</td>
<td>4.2±0.06</td>
<td>0.1±0.005</td>
<td>0.19±0.007</td>
<td>0.2±0.01</td>
</tr>
<tr>
<td></td>
<td>Recovery</td>
<td>*</td>
<td>12.5±0.28</td>
<td>4.5±0.16</td>
<td>0.13±0.003</td>
<td>0.21±0.005</td>
<td>0.2±0.01</td>
</tr>
<tr>
<td></td>
<td>Thiamethoxam</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Diafenthiuron</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Treated</td>
<td>**</td>
<td>11.1±0.40</td>
<td>4.6±0.08</td>
<td>0.12±0.006</td>
<td>0.2±0.003</td>
<td>0.19±0.02</td>
</tr>
<tr>
<td></td>
<td>Recovery</td>
<td>*</td>
<td>12.6±0.55</td>
<td>4.9±0.09</td>
<td>0.15±0.002</td>
<td>0.23±0.005</td>
<td>0.18±0.008</td>
</tr>
<tr>
<td>Hepato</td>
<td>Thiamethoxam</td>
<td>Control</td>
<td>16.3±0.33</td>
<td>6.2±0.10</td>
<td>0.2±0.002</td>
<td>0.26±0.013</td>
<td>0.2±0.003</td>
</tr>
<tr>
<td></td>
<td>Treated</td>
<td>12.6±0.2</td>
<td>4.9±0.40</td>
<td>0.15±0.013</td>
<td>0.18±0.004</td>
<td>0.26±0.008</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Recovery</td>
<td>*</td>
<td>14.0±0.38</td>
<td>5.2±0.08</td>
<td>0.19±0.003</td>
<td>0.21±0.003</td>
<td>0.24±0.01</td>
</tr>
<tr>
<td></td>
<td>Diafenthiuron</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Treated</td>
<td>**</td>
<td>13.7±0.28</td>
<td>5.6±0.10</td>
<td>0.17±0.009</td>
<td>0.2±0.008</td>
<td>0.25±0.009</td>
</tr>
<tr>
<td></td>
<td>Recovery</td>
<td>*</td>
<td>14.1±0.3</td>
<td>5.8±0.05</td>
<td>0.2±0.002</td>
<td>0.22±0.005</td>
<td>0.2±0.007</td>
</tr>
</tbody>
</table>

Table 6.1: Change in the content of parameters in foot and hepatopancreas.
Note: Thiamethoxam (LC50/5) and Diafenthiuron (LC50/5) were used and *, **, *** = significantly different from control at p<0.05, p<0.01 and p<0.001, respectively.
Graph 6.1: Changes in Albumin Content of Foot

Graph 6.2: Changes in Albumin Content of Hepatopancreas
Graph 6.3: Changes in Alkaline Phosphatase Content of Foot

Graph 6.4: Changes in Alkaline Phosphatase Content of Hepatopancreas
Graph 6.5: Changes in Glucose Content of Foot

Graph 6.6: Changes in Glucose Content of Hepatopancreas
Graph 6.7: Changes in Total protein Content of Foot

[Bar graph showing concentrations in mg/g for Control, Treated, Recovery, Treated, and Recovery for Thiamethoxam and Diazinon]

Graph 6.8: Changes in Total protein Content of Hepatopancreas

[Bar graph showing concentrations in mg/g for Control, Treated, Recovery, Treated, and Recovery for Thiamethoxam and Diazinon]
6.5 Discussion

6.5.1 Albumin (ALB)

Esmaeil (2009) found decrease in total proteins and albumin in *B. alexandrina* snails treated with the fungicide Topas to degeneration of the snail’s ovotestis and digestive glands cellular structure. Mohamed, *et al.* (2012) observed reduction of total proteins and albumin concentration in tissues of snails treated with the pesticides Basudin and Selecron and the phytoalkaloid Colchicine due to structural damages of their internal organs (Tolpa, *et al.*, 1997).

In present study the significant decrease in the content of albumin (ALB) was noted in the foot and hepatopancreas at 10.4±0.22 mg/g (*P*<0.01), 11.1±0.40 mg/g (*P*<0.01) Chart 6.1 and 12.6±0.20 mg/g (*P*<0.001), 13.7±0.28 mg/g (*P*<0.01) Chart 6.2 compared to control 15.6±0.47 mg/g and 16.3±0.33 mg/g and significant changes are observed after 7 days of treatment at 12.5±0.28 mg/g (*P*<0.05), 12.6±0.55 mg/g (*P*<0.05) Chart 6.1 and 14.04±0.38 mg/g (*P*<0.001), 14.1±0.30 mg/g (*P*<0.01) Chart 6.2 treated with both Thiamethoxam and Diafenthiuron. This result correlates with findings of Esmaeil (2009) and Mohamed, *et al.* (2012).

6.5.2 Alkaline phosphatase (ALP)

Saxena and Malhendru, (2004 and 2005) found that alkaline phosphatase activity decreased with increasing dichlorvos activity and duration of exposure. The mucous cells had acid and alkaline phosphatase activities and the mucous released by the activity of both the enzymes in the mucus cells (Ning, *et al.*, 2005). Sangita, *et al.* (2006) observed that the combinations of few plant derivatives were more effective against acid and alkaline phosphatase in the snail tissue as compared to treated alone. Jaiswal, *et al.* (2010) studied the effect of molluscicidal components of *Myristica fragrans* trinymristin and myristicin activity on *Lymnaea acuminata* which showed significant decrease in the acid and alkaline phosphatase and acetylcholinesterase activities in the nervous tissue of treated snails. Akinpelu, *et al.* (2012) observed the effect of sub-lethal concentration of saponin on *Lanistes lybicus* and found that decrease in activity of muscle alkaline phosphatase but increased enzyme activity in hepatopancreas. Increase in protein concentration with increasing saponin concentration.
In present findings the significant decrease in the content of Alkaline phosphatase (ALP) was noted in the foot and hepatopancreas at 4.24±0.06 mg/g (P<0.001), 4.62±0.08 mg/g (P<0.01) Chart 6.3 and 4.92±0.40 mg/g (P<0.05), 5.68±0.10 mg/g (P<0.01) Chart 6.4 compared to control 5.42±0.07 mg/g and 6.26±0.10 mg/g and significant changes are observed after 7 days of treatment while feeding normally at 4.56±0.16 mg/g (P<0.01), 4.94±0.09 mg/g (P<0.001) Chart 6.3 and 5.24±0.08 mg/g (P<0.01), 5.84±0.05 mg/g (P<0.01) Chart 6.4 treated with both Thiamethoxam and Diafenthiuron. These results are in agreement with the results of Saxena and Malhendru (2004 and 2005); Jaiswal, et al. (2010) and Akinpelu, et al. (2012).

6.5.3 Glucose (GLU)

Effects in snails under starvation and trematode infected with reduction in the glucose level in the hemolymph and depletion of the carbohydrates reserves (Cheng and Lee, 1971; Friedle, 1971; Lee and Cheng, 1972; Manohar and Rao, 1976; Teng, et al., 1979 and Joosse and Van Elk, 1986). Ishak, et al. (1975) reported depletion of glycogen in infected snails and the reason suggested was the low energy efficiency of anaerobic metabolism leading to fast depletion of carbohydrate store.

Pinheiro and Amato (1994) found that in the snail B. similaris infected with E. coelomaticum the glucose content in the hemolymph was reduced in more than 60% and the deposits of glycogen were reduced in 90% at the end of pre-patent period. Pinheiro (1996) observed that under starvation B. similaris has reduction more than 80% in its carbohydrate reserves of cephalopedal mass, digestive gland and albumen gland. Chemical stress causes rapid depletion of stored carbohydrates primarily in liver and other tissues (Jyothi and Narayan, 2000). Hamlet, et al. (2012) observed the effect of Thiamethoxam on hepatopancreas carbohydrate level of H. aspersa, the insecticide formulation was 0, 25, 50, 100, 200 mg/L after six weeks of treatment biochemical study showed that the content of total carbohydrate was not significantly reduced at 25 and 50 mg/L of Thiamethoxam but very significant reduction in total carbohydrate level was observed in H. aspersa exposed at 100 mg/L and highly significant depletion in the concentration at 200 mg/L as compared to control value.

In present findings the significant depletion in the content of Glucose (GLU) was noted in the foot and hepatopancreas at 0.11±0.005 mg/g (P<0.001), 0.12±0.006 mg/g (P<0.001) Chart 6.5 and 0.15±0.13 mg/g (P<0.001), 0.17±0.009 mg/g (P<0.001)
Chart 6.6 compared to control 0.19±0.003 mg/g and 0.23±0.002 mg/g and significant changes are observed after 7 days of treatment while feeding normally at 0.13±0.003 mg/g (P<0.001), 0.15±0.002 mg/g (P<0.001) Chart 6.5 and 0.19±0.003 mg/g (P<0.001), 0.20±0.002 mg/g (P<0.001) Chart 6.6 treated with both Thiamethoxam and Diafenthiuron. The present findings agreed with Pinheiro (1996) and Hamlet, et al. (2012).

### 6.5.4 Total protein (TP)

Reduction in protein level may be due to the inhibition of alkaline phosphatase activity, as it plays an important role in protein synthesis (Pilo, et al., 1972) and other secretary activities (Ibrahim, et al., 1974). The synthesis of protein in any of a tissue can be affected due to RNA synthesis at the transcription stage and affects the uptake of amino acids in the polypeptide chain both possibilities may account for the lowering the protein content in the affected tissues (Tariq, et al., 1977; Shrivastava, et al., 2010 and Singh, et al., 2010). Similar observations were also noted in Pila globosa (Rao Ramana and Ramamurthy, 1980). Rao, et al. (1981) reported the decrease in total proteins in snail tissues exposed to methyl parathion suggest the possible utilization of these compounds for metabolic purposes. The decrease of proteins in tissues of mollusc is due to their degradations and possible utilization of degraded products for metabolic purposes. Enhanced protease activity and decreased protein level have resulted in marked increase of free amino acids in snail tissue. The accumulation of free amino acids can also be attributed to their involvement in the maintenance of an acid-base balance (Moorthy, et al., 1984) and the less use of amino acids (Rao, et al., 1987). The decline in protein content may be related to impaired food intake, the increased energy cost of homeostasis, tissue repair and the detoxification mechanism during stress (Neff, 1985). Rao, et al. (1993) observed decrease in protein content of fresh water gastropod, Indoplanoribis exustus due to toxicity. Patil, et al. (1995) reported the depletion protein level in fresh water snail, Thiara lineate due to pesticidal stress. The quantity of protein depends on the rate of protein synthesis or its degradation. It also affected due to impaired incorporation of amino acids into polypeptide chains (Singh, et al., 1996). Reduction in the total protein level was observed in Eobania vermiculata snails treated with carbamate pesticides (Radwan, et al., 2008). Kulkarni, et al. (2005) observed significant decrease in total protein content in foot, hepatopancreas and gills of freshwater snails.
mussel, *Lamellidens corrianus* on exposure to organo chlorine insecticide, lindane. Yadav, *et al.* (2007) studied that the animals exposed to chemicals obtain extra energy requirement from the tissue protein. Singh and Gupta (2007) reported the decrease in protein level in foot and mantle, while an elevation in hepatopancreas of *Pila globosa* under stress of azodyes. Gupta, *et al.* (2010) observed significant inhibition in protein content in foot, mantle and gill, while an elevation in hepatopancreas of *Lamellidens marginalis* exposed to congored. The depletion of cellular proteins might be caused by inhibition of amino acid incorporation, breakdown of proteins into amino acids and diffusion out of the cells. The synthesis of RNA plays an important role in protein synthesis. The inhibition of RNA synthesis at transcription level may affect the protein level. The decrease in the RNA concentration may have been a cause of protein depletion. The increase in protease activity may be the cause of increased protein degradation (Singh and Singh, 2010). Glycogen is available for instant energy source while proteins are important organic constituents of the animal cells playing a vital role in the process of interactions between intra and extracellular media. The active depletion of these metabolites might be due to their mobilization to liberate energy during pesticidal stress (Ahirrao and Kulkarni, 2011).

In present findings the significant depletion in the content of Total Protein (TP) was noted in the foot and hepatopancreas at 0.19±0.007 mg/g (P<0.001), 0.20±0.003 mg/g (P<0.001) Chart 6.7 and 0.18±0.004 mg/g (P<0.001), 0.20±0.008 mg/g (P<0.001) Chart 6.8 compared to control 0.25±0.004 mg/g and 0.26±0.013 mg/g and significant changes are observed after 7 days of treatment while feeding normally at 0.21±0.005 mg/g (P<0.001), 0.23±0.005 mg/g (P<0.001) Chart 6.7 and 0.21±0.003 mg/g (P<0.001), 0.22±0.005 mg/g (P<0.001) Chart 6.8 treated with both Thiamethoxam and Diafenthiuron. These findings correlate with the results of Tariq, *et al.* (1977); Rao Ramana and Ramamurthy (1980) Rao, *et al.* (1993); Patil, *et al.* (1995); Kulkarni, *et al.* (2005); Radwan, *et al.* (2008) and Ahirrao and Kulkarni, (2011).

### 6.5.5 Uric acid (UA)

Becker and Schmale (1975) observed alteration in nitrogenous products of degradation metabolism in snails in response to the alteration that occurred during starvation. Bishop, *et al.* (1983) observed that in terrestrial species of molluscs the most of nitrogenous products of degradation were excreted as urea or uric acid and
other purine compounds. In the terrestrial snails under normal conditions, the urea excretion is a process more advantageous than uric acid excretion, once that the urea can be excreted highly diluted avoiding the intoxication of the snail. Becker (1983) studied the alteration in uric acid and guanine in the tissues of *Biomphalaria glabrata* under starvation and *Schistosoma mansoni* infected and observed an increase of these purines concentration. Circulating uric acid may serve as a free radical scavenger and antioxidant (Becker, 1993), particularly during the tissue reoxygenation that occurs after aestivation in snails (Hermes-Lima, *et al.*, 1998).

In present findings the significant increment in the content of Uric acid (UA) was noted in the foot and hepatopancreas at 0.21±0.01 mg/g (P<0.001), 0.19±0.02 mg/g (P<0.001) Chart 6.9 and 0.26±0.008 mg/g (P<0.001), 0.25±0.009 mg/g (P<0.001) Chart 6.10 compared to control 0.16±0.01 mg/g and 0.23±0.003 mg/g and significant changes are observed after 7 days of treatment while feeding normally at 0.20±0.01 mg/g (P<0.001), 0.18±0.008 mg/g (P<0.001) Chart 6.9 and 0.24±0.01 mg/g (P<0.001), 0.23±0.007 mg/g (P<0.001) Chart 6.10 treated with both Thiamethoxam and Diafenthiuron. These results are in agreement with the results of Becker (1983).

### 6.6 Conclusion

In present study both the pesticides caused an alteration in some biochemical aspects, which could lead to serious metabolic and cellular damage. The magnitude of the stress was more in Thiamethoxam than Diafenthiuron. Data in Table 6.1 showed that pesticide Thiamethoxan (LC$_{50/5}$) (0.51ppm) and Diafenthiuron (LC$_{50/5}$) (0.64ppm) shows significant changes in the ALB, ALP, GLU, TP and UA of foot and hepatopancreas of the *M. indica*. Thiamethoxam and Diafenthiuron treated *M. indica* shows significant decrease in ALB, ALP, GLU and TP while slightly increase in UA content.
6.7 References


34) Heras, H.; Dreon, M.S.; Ituarte, S. and Pollero, R.J. (2007). Egg carotenoproteins of Neotropical Ampullariidae (Gastropoda:


(*- original not cited)