CHAPTER IV

DISCUSSION

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a) Mortality study :

Mortality is most noticeable effect of pollution and it plays an important role in the toxicological studies. It also helps in the determination of LC_{50} values of toxicant. In the present study of mortality, different concentrations (100,200,300,400 and 500ppm) of the plant toxins from *C. inerme, V. negundo* and *A.indica* are used to observe the mortality response in the fresh water snail, *V.bengalensis*.

It is observed that mortality of *V.bengalensis* was found altered in different concentrations of phytotxins at different time intervals. The results of present study forms the basis for the study of comparative toxicity of the plant toxins from *C. inerme, V.negundo* and *A.indica.*The results are also useful to calculate the LC_{50} values of each plant toxin in relation to the *V. bengalensis.*

In the present investigation, it is observed that some snails get died earlier to the effect of plant toxins while some try to survive little more time, when all others are dead. This natural variability in mortality responses is due to the resistance power of animal to each concentration. (Mouirhead - Thomson, 1971)

It is also observed that the mortality of snails under study was observed only in intoxicated sets at definite concentrations of the phytotoxins and not in the control set It is true and noticeable that the pH, temperature, salinity, hardness and dissolved oxygen of water in control and experimental set is normal even after addition of the toxicant.

This clearly indicated that no factors other than phytotoxins were responsible in this study for the mortality of snail *V- bengalensis.*

In concentration 100 ppm. of the plant toxins from *C. inerme, V. negundo* and *A. indica* no or less mortality of *V. bengalensis* was observed upto early 24 hrs. because of tolerance, mechanism.

Such type of tolerance phenomenon was observed in case of *T. mossambicus* (Patil *et al.*; 1988), in fish *Clarias batrachus* (Asfia Pareen and Vasantha, 1994), in Mollusc, *Lymnea stagnalis* (Bhide, 1998). in *V. bengalensis* from extract of *Eupatorium triplinerve* (Nanaware *et al.*; 2003, and Awati, 2004).

The intoxication data on moratality due to pesticides have been reported by many workers. The pesticide intoxication programme was successfully carried out by some workers by activating the high percentage of mortality as reported in *Achativa fulica* (Saxena and Dubey, 1970), *Lymnae stagnailis* (Bhide 1986 and 1989), in *Pila globosa* (Bhide 1987), *Antheraea agsama* (Bora and Handique 1990), in *Indonaia caeruceus* (Muley 1990) and in *Lymnaea stagnalis* (Bhide 1991).

Per cent mortality data recorded in Table No. 1,2 and 3 indicated that the rate of mortality is less in lower concentrations while it is more in higher concentrations of phytotoxins. The per cent mortality in the Experimental snails was also seen increased with increase in the exposure period. Therefore per cent mortality in the snails under study was found associated with the concentration of toxicant and intoxication period. This also suggests that the rate of mortality in snail, *V. bengalensis* is increased with increase in the concentration of the plant toxins *C. inerme, V. negundo* and *A. indica* and with increase in the intoxication period also.

This increased mortality is due to the decrease in resistance of the snails with increase in the intoxication period. This supports the previous observations on the high mortality in *Ariophants salata* (Bhat and Vishwanathan, 1972) after exposure to some molluscicides in, *Bellamya bengalensis,* (Sukumar and Rao, 1985) after exposure to Y – HCH, methyl parathion and carboryl. Lokhande and Kulkarni (1990) have reported the effect of pesticide toxicity in combination with temperature and pH in *Bellamya bengalensis*, with similar observations.

According to Malu and Kulkarni (1990) increased mortality is due to increased endosulfan and malthion residue with respect to pesticide exposure time. Higher accumulation of pesticides in mantle than foot and hepatopancreas showed increased mortality in the snail, *Viviparus bengalensis* by (1990) and it was more probably due to pesticide residue accumulation in *Lmnaea stagnalis* as reported by Bhide, 1986 and 1989 Similar observations were recorded by Bhide (1987) in *Pila globosa* and thiourea exposure in *Pila globosa* and *Lymnaea stagnailis* in *Lymnea acuminata* by Gupta (1995) in *Thiara lineata* by Chaturvedi *et al* (1990)

LC_{50} –

The present investigation provides the data derived form dose concentration and percent mortality for the determination of LC_{50} values and regression equation by probit analysis. The LC_{50} values of plant toxins for snail *V. bengalensis* were calculated with respect to the plant toxins derived from *C. inerme*, *V. negundo* and *A. indica*. The regression equation is useful for the calculation of lethal concentration.

Plant toxins	**LC_{50} values in ppm**
C. inerme	241.8
V. negundo	176.4
A indica	263.0

From LC_{50} values for 120 hrs. shown inabove table it is very clear that more lethal effect with less LC_{50} value shown by the phytotoxin from *V. negundo* indicated presence of more toxic compounds in it and the phytotoxin from *C. inerme* and *A. indica* had shown less lethal effect with more LC_{50} value. Therefore it also indicated that phytotoxin with lower lethal concentration has more toxicity and higher lethal concentration has less toxicity. From the data of LC_{50} values increasing order of toxicity of phytotoxins with respect to the snail under study (Table No. 22) is given as *A. indica*< *C. inerme*< *V. negundo*.

b) Oxygen consumption –

Oxygen consumption is most important phenomenon of life it is necessary for metabolic activities of animal to provide energy. When any toxic substance is present in the aquatic system reduce the oxygen content and hence indirectly affects the respiration of that aquatic animals. Due to this, one can assess the toxicological nature of that toxicant. In present investigation it was observed that the fresh water snails when exposed to 3 toxicants *C. inerme, V. negundo* and *A. indica,* reduction in the rate of oxygen consumption was noticed.

The oxygen consumption of experimental snail in control set was constant. By measuring oxygen consumption, it can be easy to detect the metabolic rate and state of stress condition in organisms. In present study it was observed that the oxygen consumption of snail, *V. begalensis* was gradually decreased with increase in time of exposure due to effect of above all three molluscicides used in this study. This indicated that rate of oxygen consumption was time dependant. It was also noticed that decrease in oxygen consumption was maximum at 96 and 120 hrs. of intoxication period and was reduced due to the effect phytotoxins *C. inerme, V. negundo* and *A. indica.*

Similar types of results were observed by Chaturvedi *etal.* (1990), Patil (1990); Rao *et al.* (1990); Lomate and Jadhav (1982); Mane and Muley (1984) and Choudhari *etal.* (1988).

Contradictory to above observations the rate of oxygen consumption was found increased in treated snails in earliear period. The activities of snails increased to surive from the toxic enviranment. Which incrases muscular activities and increased rate of respiration in the snail *V. bengalensis.* Some workers have recorded such increase in oxygen consumption, Mane and Muley (1987), Muley *et al.* (1981) Kulkarni *etal.* (1989) and Akarte *et al.* (1982), Mane *et al.* (1983) found initially increase in respiration but further it is decreased with increasing exposure period.

According Mane *et al.* (1984) and Muley (1986) the increase in the oxygen consumption might be due to increased physiological activities. According to Rice *et al.*; 1997 the consequent increase in mtetabolism of exposed animal is helpful to expel the pollutants from the body .

Many researchers have worked out the effects of heavy metals in relation to oxygen consumption in some molluscan species such as Mule and Lomate (1994), Saliba and Vella (1977), and Kapoor and Lomate (1987) reported that heavy metals affected the gill tissues and reduced the oxygen intake capacity and hence reduce the rate of oxygen consumption of *I. tuberculata, Monodonta articllata* and *Indonaia caeruleus*, respectively. Like wise muley and Mane (1989) and Alam and Lomate (1984) found similar results when *V. bengalensis* were exposed to mercurial salts and zinc sulphate respectively. Balavenkatsubhaiah *etal.* (1985) and Radhakrishnaia (1988) also found such type of results in *Lamellidens margnalis* after exposure to Hg and Cd respectively.

Rao and Jaya Raju (1994) found that oxygen consumption and metabolic rate was lowered down in *Lymnea stagnalis* due to endosulfan and thiourea (Bhide 1986) and Bhide (1989, 1991,1998)) also shown reduction in oxygen consumption in same animal exposed to nuvan methyl, parathion and thimet, thiourea and BHC. They also found similar results in *Pila globosa* when exposed to thiourea and DDT (Bhide 1987).

The reduction of oxygen consumption might be the effect of toxic compounds on biochemical constituents of gills and also might be due to damage of gill architecture at cellular level (Awati 2004). The damaged cells of epithelium gill lamellae were recorded by Kulkarni *et al.* (1989) in *P. lateriscula* when exposed to endosulfan, by (Rao *et al.* 1990) in *T. lineata* when exposed to cythion, malathion and endosulfan by Lomate and Jadhav (1982) in *C. regularis* due to toxic pollutants. by Mane and Muley (1984) in two fresh water bivalves when exposed to endosulfan 35 EC; by Choudhari *et al.* (1988) in *V. bengalensis.*, when exposed to baseline; by Muley and Mane (1989) in *B. bengalensis* due to impact of Hg salts ; and by Radhakrishnaian (1988) in *L. marginalis* due to effect of Cd.

The present investigation reveals that the decrease in oxygen consumption was supported by the decreased level of organic constituents in gills. The decrease in oxygen consumption of snails after treatment of molluscicides also might be due to heavy secretion of thick mucus which was surrounded and accumulated on gills and also the damage of gill tissues might have affected the oxygen consumption.

C) Biochemical parameters :

In the present investigation, the effects of pesticides from *C. inerme, V.negundo* and *A. indica* on glycogen proteins and lipids in the gill of fresh water snail, *V.bengalensis* have been studied and recorded in table no. 26,27,and 28.

Snails exposed to stress condition, require more energy, this energy may be obtained from carbohydrate, proteins and lipids (Ganesen *etal.*1989). Among the carbohydrates,glycogen constitutes a rich and immediate source of energy which can be easily utilized (Parmal and Patel;1993). Therefore in stress condition, alteration in glycogen contents were prominently observed. The physical exhaution has been reported to produce such changes in different biochemical parameters of animals (Gupta and Tripati;1992)

The amount of glycogen ,proteins and lipids was found decreased after exposure to the plant toxins. The snails when exposed to the plant toxins, obtain energy from glycogen, proteins and lipids to fight with such stress condition and this may be the reason behind the depletion in the content of glycogen , proteins and lipids.

i) Glycogen:

The glycogen is the stored food material which is considered to be the major source of the energy in animal tissues, whereas maintaince of glycogen reserves is an essential feature of normal metabolism of organisms (Rao *etal.*;1995). In this investigation, the glycogen content of gills of snail, *V.bengalensis* was decreased , indicating the effect of phytotoxins from plants *C. inerme*, *V. negundo* and *A. indica*. These results are in agreement with the observations of Benegeri and Patil (1986) in fish *Labeo rohita*; Radhakarishnaiah *et al.* (1992) in *L. rohita*; Kabeer *et al.* (1978) in *Lamellidens marginalis* snail. The depletion was also observed by Khan *et al.* (2001) in gastropod; *Babylonia spirata* after effect of cadmium chloride; Bhagyalakshmi (1982) in crab, *Ozitelphusa senex senex*; Patil and Mane (1997) in fresh water bivalve after exposing to the toxicant; Khan *et al.* (2002) in gill of mussels of *Perina vivides.* after exposure to zinc chloride. Kulkarni and Choudhari (1995) in snail, *Cryptozona semirugata* due to monocrotophos.

According Patil *et al.* (1992) the concentration of glycogen, proteins and lipids was affected in *Thiara lineata* after exposure to zolone. Kulkarni *et al.* (1984) observed decreased value in carbohydrate after impact of endosulfan in *Pila globosa.* Shrivastava *et al.* (1992) observed significant decrease in gills of infected snails, *Melanoides tuberculatus, Lymnea luteola* and *V. bengalensis.* Nanaware *et al.* (2003) revealed depletion in *V. bengalensis* after exposure to phytotoxin from *A. sinuata* and *S. laurifolius.*

In present investigation when snails exposed to the plant toxins from *C. inerme, V. negundo* and *A. indica*, the adverse stess condition is formed resulting in rapid movements and the increased respiration rate in which glycogen content in gill is declined. This depletion in glycogen content suggests the possibility of glycogenolysis which in turn produces energy by anaerobic glycolysis to cope up with the adverse condition. The stress also results in the break down of glycogen in other tissues to meet high demands for energy.

ii) Proteins –

Proteins is an essential organic constituent and building blocks of animal tissue and it plays a significant role in cellular metabolism. Protein is also an energy source to spare during the period of stress condition (Sastry and Dasgupta 1991). In present study, when the snail, *V. bengalensis* were exposed to the molluscicides from plant *C. inerme V. negundo* and *A. indica* the protein contents of its gills were found slightly depleted.

Other results are coincided with the results obtained by Somanath (1991) in *L. rohita*; Gupta and Tripati (1992) in *C. batrachus* when exposed to DDVP. methyl parathion, malathion, environmental acidity, tannic acid and thiourea respectively. Nanaware *et al.* (2004) in *V. bengalensis* due to phytotoxins form *A. sinuata* and *S. laurifolius* and Awati (2005) in *V. bengalensis* after exposure to thimet 10 G and sodium pentachloride.

A decrease in proteins was also supported by Ranarayan and Sathyanesan (1984) in *C. punctatus* after exposing to mercuric chloride; Vijaya Lakshmi *et al.* (1991) in *Cyprinus carpio* due to environmental acidity; Jadhav Sunita *et al.* (1995) in *Corbicula striatella* after effect of carboyl and Choudhari *et al.* (1998) in *Indoplanorbis exustus* after effect of sodium pentachlorophenate.

From the results of present study it is concluded that depletion in the proteins in gill of fresh water snail is due to the increased proteolysis and decreased protein synthesis due to effect of the plant toxins from *C. inerme, V. negundo* and *A. indica*. It is also concluded that due to the stress conditions metabolism of snails was increased during which proteins were also degraded resulting in the depletion of the contents in the gills.

iii) Lipids –

Lipids are one of the structural constituents of animal tissues and are the reservior or potential source of chemical energy (Sharma and Gupta 1991). In the present investigation when snail *V. bengalensis* was exposed to the plant toxins from *C. inerme, V. negundo* and *A. indica*, the lipid contents were found decreased in the gills.

A decrease in the lipids in gills was also observed by Pant *et al.* (1991) in *Cyprinus carpio* due to environmental acidity and Katti and Sathyanesan (1984) in *Clarias batarachus* exposed to toxicant.

This type of depletion is well agreed with Kulkarni *et al.* (1995); Nanaware (2003); Bhoite (2003); Choudhary *et al.*(1991); Choudhary *et al.* (1994) and Choudhary *et al.* in (1998).

On the basis of results of the present study, it is concluded that when the snails exposed to the sublethal concentration of plant toxins from *C. inerme*, *V. negundo* and *A. indica*. To avoid the stress condition snails require additional energy which is obtained from the utilization of lipids. Consequently depletion in lipids of gill occurs due to increased metabolism.

From above discussion it is concluded that the molluscicide from *C. inerme*, *V. negundo* and *A. indica* is a cause behind the depletion of the glycogen, proteins and lipids.